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The Pharmacological Basis of Therapeutics

14TH EDITION

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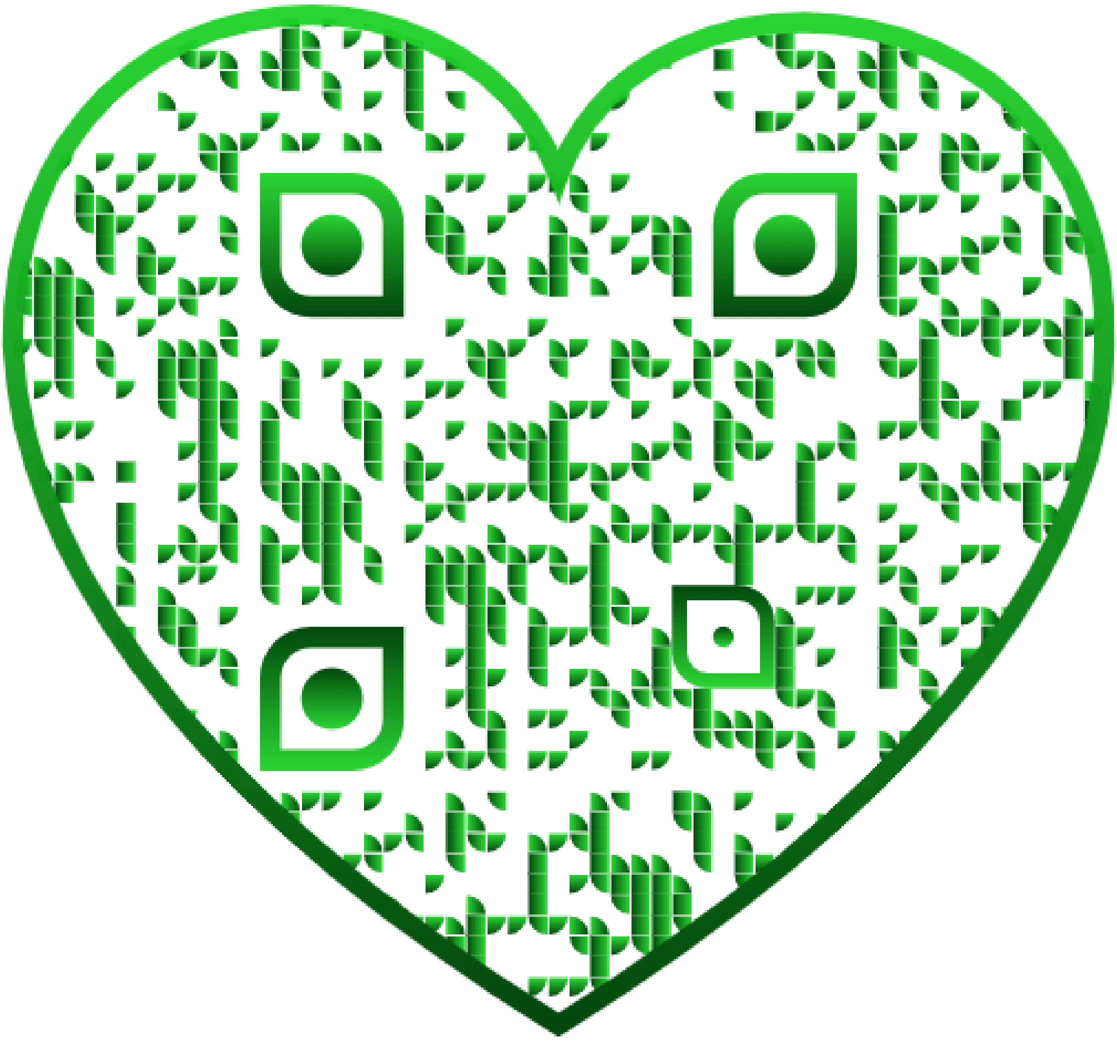

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THE PHARMACOLOGICAL BASIS OF THERAPEUTICS

FOURTEENTH EDITION

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FOURTEENTH EDITION

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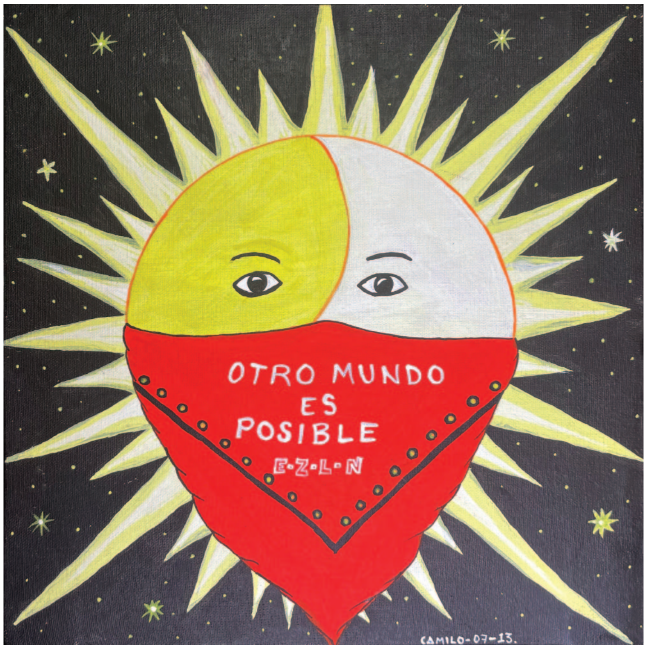
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Preface

This is the 14th edition of book that began as a collaboration between two friends and professors at Yale, Louis Goodman and Alfred Gilman. Over the years, “G&G” has been acclaimed as the “blue bible” of pharmacology. Surely much of that acclaim reflects the book’s purpose, delineated by the original authors and steadily adhered to over 81 years: to correlate pharmacology with related medical sciences, to reinterpret the actions and uses of drugs in light of advances in medicine and the basic biomedical sciences, to emphasize the application of pharmacodynamics to therapeutics, and to create a book that would be useful to students of pharmacology and to healthcare practitioners.

Following these principles is demanding: the sheer volume and unremitting growth of knowledge in the basic biomedical sciences and their clinical applications continue to amaze, challenging editors and contributors who are trying to produce a one-volume work, and surely challenging students. To create a book that reflects our times, we have updated all chapters and have added five new chapters: drug response and the gastrointestinal biome, pharmacovigilance, the blood-brain barrier (it is not simply a lipid sheath), cannabinoids, and immunotherapies for cancer, plus a novel appendix on drug-drug interactions. Advances in immunomodulation are presented in most sections. In addition, we have continued to reach out to younger contributors who are on the forefront of pharmacological investigation and clinical practice. As a result, we have, in this edition, 56 new contributors, drawn from diverse backgrounds, who will ensure the book’s vigor into the future.

A multi-authored work such as *Goodman & Gilman* grows by accretion, deletion, addition, replacement, and repair. The current text reflects over eight decades of such activity, with wisdom, memorable pearls, new material, and flashes of wit, hopefully edited to meet the present and to be forward looking. End-of-chapter notes acknowledge retired contributors to the 13th edition, but I am happy to acknowledge that several generations of editors and contributors have helped to bring this 14th edition to its present form. As in the 13th edition, we have used a larger page size, no extract type, and more mechanistic figures as we attempt to explain the pharmacodynamics of new agents. Some readers have complained that the book is getting too complex. We believe that a thorough understanding of a drug’s actions and interactions at multiple physiological sites and with other drugs is essential to modern therapeutics. However, we also prominently summarize the mechanisms of action, ADME, and clinical use of individual agents and drug classes. Not wanting to favor one manufacturer’s product over that of another, we continue generally to avoid using trade names except as needed to distinguish multiple formulations of the same agent that have distinct pharmacokinetic or pharmacodynamic properties or that are known only by a trade name. The full text is available online at many medical, pharmacy, and nursing schools by institutional subscription to *AccessMedicine.com* and *AccessPharmacy.com*, where we publish regular updates. Feel free to contact the editors by email if you have comments on the book or the websites.

Editing this book brings to mind a number of larger issues, both positive and negative, relating to health care; among them: the remarkable explosion of molecular genetic techniques, the proliferation of therapeutic agents affecting the immune system, and the power of computer-aided drug design; antibiotic resistance promoted by the continuing misuse and overuse of antibiotics in healthcare and animal husbandry; the adverse environmental effects of human activity to life on Earth; the effects of global warming and the sheer size of the human population on global health and nutrition; the ease with which infectious diseases can spread around the world; the fragility of truth and fact, and the difficulty of promoting health based on science and data in the face of determined conspiracy theories and political ideology. A better world is possible.

A number of people have contributed to the preparation of this edition of *Goodman & Gilman*. Many thanks to: my co-editor, Bjorn Knollmann, and to the clinical pharmacology fellows at Vanderbilt whom he recruited to read the first drafts of chapters as they honed their editorial skills; our attentive publisher at McGraw Hill, Michael Weitz, and his colleagues Christina Thomas and Melinda Avelar; consulting pharmacist Nelda Murri; Nitesh Sharma at KnowledgeWorks Global Ltd, who tirelessly oversaw the transformation of Word documents into a printed book; Jason McAlexander of MPS North America, whose rapid-response artwork brightens the pages; and the eagle-eyed Becky Hainz-Baxter, who saw what the editors had missed.

My special thanks to Lynne Larson, a novelist, artist, and grants management specialist who managed this enterprise and kept the editors organized. Lynne managed the production of the 11th edition of *Goodman & Gilman* when I first became the editor, when everything was done with hard copy and Word files submitted by mail, when galley proofs were actual long sheets of paper on which corrections were handwritten and then transcribed to new Word files. I was delighted when Lynne agreed to manage this all-electronic project. We would not have this 14th edition without her.

Laurence L. Brunton
San Diego, CA
14 July 2022

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Chapter 1

Drug Discovery: From Medicinal Plants to Computer-Aided Drug Design

Michael K. Gilson and Laurence L. Brunton

FROM MEDICINAL PLANTS TO COMPUTER-AIDED DRUG DESIGN
- Early Experiences With Plants
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The first edition of *Goodman & Gilman*, published in 1941, helped to organize the field of pharmacology, giving it intellectual validity and an academic identity. That edition began: "The subject of pharmacology is a broad one and embraces the knowledge of the source, physical and chemical properties, compounding, physiological actions, absorption, fate, and excretion, and therapeutic uses of drugs. A *drug* may be broadly defined as any chemical agent that affects living protoplasm, and few substances would escape inclusion by this definition." In practice, of course, a chemical or biological agent is considered a legal drug only if it has been approved as such by a national regulatory agency, such as the U.S. Food and Drug Administration (FDA) or the European Medicines Agency; these approved compounds are the focus of this book.

This first nine chapters of this book, ***General Principles***, provide the underpinnings for these definitions of pharmacology and drugs by exploring the physiological, biochemical, and molecular mechanisms of drug action. This section covers drug invention, development, and regulation, as well as how drugs act in biological systems, i.e., *pharmacodynamics*, *pharmacokinetics* (including *drug transport* and *metabolism*), *the influence of the gastrointestinal microbiome*, and *pharmacogenetics*, with brief forays into *pharmacovigilance* and *drug toxicity and poisoning*. Subsequent sections deal with the use of specific classes of drugs as therapeutic agents in human subjects. The present chapter is an introduction to pharmaceuticals, their development, and the activities of the pharmaceutical industry and government surrounding the discovery, production, and use of therapeutic agents. The processes of discovery and invention of drugs have changed substantially with the general progress of biomedical sciences, the advent and improvement of computer-aided drug design, and technical advances in biochemistry and molecular biology. Some of these new capabilities are reviewed below.

From Medicinal Plants to Computer-Aided Drug Design

Early Experiences With Plants

The human fascination—and sometimes infatuation—with chemicals that alter biological function is ancient and begins with our long experience with and dependence on plants. Because most plants are root-bound, many produce defensive compounds that animals learn to avoid and humans to exploit or abuse. Thus, the prior of an Arabian convent came to appreciate coffee (caffeine) after noting the behavior of goats that gamboled and frisked through the night after eating the berries of the coffee plant; women sought to enhance their beauty by using an extract of the deadly nightshade plant, *Atropa belladonna* ("beautiful lady"), enriched in *atropine*, to produce pupillary dilation; the Chinese herb *ma huang* (*ephedrine*) was used as a stimulant; indigenous people of South American used curare to paralyze and kill animals hunted for food; and poppy juice (opium), containing *morphine* (from the Greek *Morpheus*, the god of dreams), has long been used for pain relief and control of diarrhea. *Morphine*, of course, has well-known addicting properties, as do other psychoactive natural products, such as nicotine, cocaine, and ethanol. Note that these drugs did not derive from a search for a druggable target or any knowledge of a target. Rather, drug discovery in the past often resulted from serendipitous observations of the effects of plant extracts or individual chemicals on animals or humans. Drugs were selected based on effect, with no understanding of mechanism as we use the term today. In the 20th century, the hunt for natural products broadened, driven in part by the discovery of antibiotics, such as *penicillin* and the cephalosporins, which fungi and microbes make to compete with each other.

Abbreviations

ADME: absorption, distribution, metabolism, and excretion
BLA: Biologics License Application
CADD: computer-aided drug discovery
DEL: DNA-encoded compound library
DHHS: U.S. Department of Health and Human Services
DMPK: drug metabolism and pharmacokinetics
FBDD: fragment-based drug discovery
FDA: U.S. Food and Drug Administration
GPU: graphics processing unit
HCV: hepatitis C virus
HDL: high-density lipoprotein
HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A
HTS: high-throughput screening
IND: Investigational New Drug
LDL: low-density lipoprotein
mRNA: messenger RNA
NDA: New Drug Application
NIH: National Institutes of Health
NMEs: new molecular entities
PDUFA: Prescription Drug User Fee Act
SBDD: structure-based drug design
siRNA: small interfering RNA

Drug Discovery or Drug Invention?

The conventional phrase *drug discovery* makes sense for therapeutic compounds obtained from plants and other organisms. Today, however, only a fraction of the new drugs introduced each year are discovered in nature. Instead, most drugs are not discovered, but are totally new compounds, painstakingly optimized against many criteria through an interplay of design and experimentation. In that sense, today's new drugs are more invented than discovered.

The current paradigm for drug development grew out of synthetic organic chemistry, which arose as the dye industry in the late 19th century and has continued to flourish. Dyes are colored compounds with selective affinity across various biological tissues. Study of these interactions stimulated Paul Ehrlich to postulate the existence of chemical receptors in tissues that interacted with and "fixed" the dyes. Similarly, Ehrlich thought that unique receptors on microorganisms or parasites might react specifically with certain dyes and that such selectivity could spare normal tissue. Ehrlich's work culminated in the invention of *arsphenamine* in 1907, which was patented as "salvarsan," suggestive of the hope that the chemical would be the salvation of humankind. This and other organic arsenicals were used to treat syphilis until the discovery of *penicillin*. Gerhard Domagk demonstrated that another dye, *prontosil* (the first clinically useful sulfonamide), was dramatically effective in treating streptococcal infections, thereby launching the era of antimicrobial chemotherapy. The collaboration of pharmacology with chemistry on the one hand and clinical medicine on the other has been a major contributor to the effective treatment of disease, especially since the middle of the 20th century.

Early on, new compounds could be tested for their activities only in whole organisms. This is how the nonsteroidal anti-inflammatory drug *indomethacin* was discovered, for example (Brune and Hinz, 2004). In the past 70 years, researchers have begun to understand in considerable detail the cellular and molecular mechanisms of disease. As a result of this basic biomedical research, it is possible to do initial testing of compounds *in vitro* ("in glass"), using cellular and molecular assays. For example, one could look for the cellular responses due to inhibition of a protein involved in a disease process. In this scenario, by testing enough appropriately chosen compounds, one could develop at least a partial understanding of which types of compounds are most likely to be active and then use this information to steer the program of chemical synthesis and testing toward increasingly potent compounds.

In the 1980s, it became practical to determine high-resolution three-dimensional structures of complex organic molecules and even larger molecules such as proteins, using and refining the techniques of X-ray crystallography pioneered by Hodgkin, Kendrew, and Perutz in the mid-20th century. It was already known that many drugs worked by binding tightly to a disease-related protein and thereby modulating (e.g., inhibiting or activating) its biological function, but the atomic details of these interactions had remained mysterious. As a consequence, the only way to advance a drug discovery project had been by synthesizing and testing one compound after another. Now, with the protein's three-dimensional structure in hand, one could finally hope to design a compound that would bind with high affinity by fitting snugly into a pocket in the protein, such as an enzyme's active site. Thus, protein crystallography enabled *structure-based drug design* (SBDD), where the three-dimensional structure of the drug target is used to guide creation of tight-binding compounds, often called *ligands*.

Around the same time, computer technology began to advance rapidly. This accelerated the data processing needed to go from X-ray diffraction patterns to protein structures (i.e., three-dimensional atomic coordinates) and enabled interactive visualization of complex protein structures comprising thousands of atoms. It also opened new vistas in *computer-aided drug discovery* (CADD), including the use of molecular simulations to model the physical interactions of compounds and proteins, and the development of tools to encode, archive, share, and analyze chemical and pharmacological data. In parallel, automation and miniaturization have dramatically increased experimental throughput, notably through robotic *high-throughput screening* (HTS), in which hundreds of thousands of compounds can be tested rapidly and at relatively low cost in cellular or molecular activity assays. Today, excitement about the power of artificial intelligence motivates wide-ranging efforts to apply these technologies to drug discovery.

The following section goes into more detail regarding the process of drug discovery, focusing on so-called *small-molecule* drugs, organic compounds with molecular weights typically less than 500 Da, which have traditionally been the most common type of drug. Subsequent sections introduce biological drugs, such as antibodies and other engineered biomolecules.

Target Identification

Today, most small-molecule drug discovery projects grow out of basic research that implicates a specific macromolecule, usually a protein, as a key player in a disease and, further, suggests that a small molecule which binds this macromolecule could be used to treat the disease. The macromolecule thus becomes a candidate *drug target*. Many small-molecule drugs are inhibitors (antagonists), which work by reducing the activity of their macromolecular target. Examples include the statins, which reduce cholesterol synthesis by binding and inhibiting the enzyme 3-hydroxy-3-methylglutaryl (HMG) coenzyme A (CoA) reductase, and β-lactam antibiotics, which kill bacteria by inhibiting enzymes involved in the synthesis of bacterial cell walls. However, some small molecules are activators (agonists) rather than inhibitors. Activators frequently target proteins whose normal role involves cell signaling, such as hormone receptors. For example, the asthma medication *albuterol* dilates bronchi by binding and activating β adrenergic receptors on bronchial smooth muscle, thereby mimicking the effect of adrenaline (epinephrine; see Chapter 10).

Candidate drug targets have been identified in many ways (Hughes et al., 2011). For example, the enzymes targeted by the β-lactam antibiotics were unknown in advance and were discovered precisely because they are bound by these naturally occurring antibiotics. In contrast, the target of the statins, HMG-CoA reductase, was identified by elucidation of the pathways of cholesterol synthesis (Tobert, 2003), and this information was used to help discover the first statins. Similarly, as researchers have determined the regulatory functions of human protein kinases—enzymes that change the activities of other proteins by covalently attaching phosphate groups to their hydroxyl-containing side chains—specific kinases have been targeted for small-molecule drug discovery (Cohen et al., 2021). Many kinase inhibitors are anticancer agents that work by inhibiting protein kinases that accelerate cell proliferation. Some of these targeted kinases carry abnormal, cancer-associated mutations that make them hyperactive, so inhibiting them returns their regulatory activities toward normal. The pioneering example of this scenario is the drug *imatinib*, which inhibits a cancer-associated mutant protein kinase, the Bcr-Abl tyrosine kinase, and is used to treat chronic myelogenous leukemia (Buchdunger et al., 2002).

In recent years, technological advances enabling genome-wide experimentation (*omics*) have opened new approaches to identifying candidate targets (Lindsay, 2003; Paananen and Fortino, 2020). Fast, inexpensive genome sequencing facilitates genome-wide association studies, in which variations in the susceptibility to a disease across many people are correlated with variations in specific genes, leading to suggestions for gene products (i.e., proteins), that may be suitable drug targets. The growing availability of patient genomic data in the context of patients' electronic medical records will likely open new opportunities for data mining in support of target discovery in the coming years. It has also become routine to measure the quantities of messenger RNA (mRNA) transcribed from thousands of genes simultaneously (the *transcriptome*) and to quantify thousands of translated proteins (*proteomics*). By comparing such data between, for example, cancer cells and normal cells, one can identify proteins transcribed or present at elevated or depressed levels in the disease state. Mining data about these proteins from sources such as biomedical databases, scientific articles, and patents, and integrating it with the omics data, may suggest certain proteins as candidate drug targets.

A totally different approach starts with the use of high-throughput instrumentation and robotics to test a large collection of small molecules (a *chemical library*) for biological activity in a *phenotypic screen* (Swinney and Lee, 2020), which might use automated microscopy and image analysis to determine which compounds produce desired biological effects, such as the activation of a desired gene in cultured human cells or the death of a parasitic microorganism in culture. Various methods may then be used for *target deconvolution* (i.e., to determine how the active small molecules work). For example, candidate targets of compounds found to kill the malarial parasite *Plasmodium falciparum* were identified by cultivating these organisms in gradually increasing concentrations of the compound to select for resistant protozoa and then using omics methods to determine which genes had changed. The proteins encoded by these genes may then become candidate drug targets (Flannery et al., 2013).

Target Validation

After a candidate drug target has been identified, additional research is usually warranted to *validate* it by seeking stronger evidence that a small molecule that binds and modulates it will actually treat the disease (Jones, 2016; Lansdowne, 2018; see Box 1–1). For example, the fact that a protein is more abundant in cancer cells than normal cells by no means proves that it is a suitable drug target. Instead, this might be a correlate rather than a cause, so further research is needed to assess its role. Accordingly, *target validation* aims to "de-risk" a project by lowering the probability that a compound carefully developed to hit the targeted protein will fail in clinical trials, whether because hitting the target does not influence the disease as expected or because the compound generates unanticipated toxicity, termed *on-target* or *mechanism-based* toxicity.

BOX 1–1 ■ Target Validation: The Lesson of Leptin

Biological systems frequently contain redundant elements or can alter expression of drug-regulated elements to compensate for the effect of the drug. *In general, the more important the function, the greater the complexity of the system.* For example, many mechanisms control feeding and appetite, and drugs to control obesity have been notoriously difficult to find. The discovery of the hormone leptin, which suppresses appetite, was based on mutations in mice that cause loss of either leptin or its receptor; either kind of mutation results in enormous obesity in both mice and people. Leptin thus appeared to be a marvelous opportunity to treat obesity. However, on investigation, it was discovered that obese individuals have high circulating concentrations of leptin and appear insensitive to its action.

There are no absolute criteria for target validation, nor is there a single method. One approach is to use a *chemical probe*, a small molecule that binds the target, and study its biological effects (Quinlan and Brennan, 2021). This approach requires that such a probe be available, and the fields of *chemical genetics* (Stockwell, 2000) and *chemogenomics* (Bredel and Jacoby, 2004) aim to create selective chemical probes for as many proteins in the human genome as possible. Alternatively, one may use gene silencing via small interfering RNA (siRNA) to block production of the target protein, thereby mimicking the effect of an inhibitor of the protein's activity. Additional insight into the biological role of a candidate drug target may sometimes be obtained by studying genetically modified mice, including *knockout mice,* in which the gene coding for the target has been disabled entirely, and *transgenic mice*, in which expression of the target's gene is placed under the control of a promoter that can be turned on by feeding the animals a specific compound, such as *tetracycline* (Lindsay, 2003).

Target Druggability

It is important to know whether the candidate target is *druggable*, that is, whether it can, in principle, bind a small molecule with sufficient affinity. If the protein has been the target of a prior drug discovery effort, there may be informative small-molecule binding data in a public database, such as BindingDB (Gilson et al., 2016), PubChem (Kim et al., 2021), or ChEMBL (Gaulton et al., 2012), or in an article or patent not yet curated by one of these databases. One may also check the Protein Data Bank (Berman et al., 2000; Berman and Gierasch, 2021) for a crystal structure of the target, which may assist in locating a suitable binding pocket for the small molecule to be developed as a drug. This is frequently true for metabolic enzymes and receptors that have evolved to bind small substrate and transmitter molecules. Many proteins belong to families, such as the protein kinases, whose members have similar properties (e.g., an ATP binding pocket), so that if one member of a family is druggable, then the others probably are also. In contrast, receptors for proteins often have large, relatively flat binding surfaces, rather than small binding pockets suitable for a small-molecule drug, and are thus less likely to be druggable and influenced by small molecules. Efforts are under way to systematically search for all druggable targets encoded by the human genome (Nguyen et al., 2017; Finan et al., 2017; Hopkins and Groom, 2002) and to gain traction against targets hitherto considered *undruggable* (Dang et al., 2017).

The ultimate validation of a candidate target is the successful development of a novel drug that works by binding to it. Such a novel drug is termed *first-in-class*. A first-in-class drug is a true innovation and may represent a medical breakthrough, so one might expect first-in-class to be the goal of every drug discovery project. In fact, however, pharmaceutical companies often engage in less innovative, more predictable projects by developing *me-too drugs* against old targets that are already fully validated by a first-in-class drug. Such projects aim to improve on the first-in-class drug through, for example, greater potency, reduced side effects, or more convenient dosing (e.g., oral instead of intravenous), and ideally to produce a new drug considered *best-in-class*. For example, Merck's *lovastatin* broke ground as the first statin, the first in a class of drugs that lower cholesterol by inhibiting the enzyme HMG-CoA reductase (see Chapter 37); but other statins, such as *atorvastatin*, have also achieved enormous commercial success.

Beyond Single-Protein Drug Targets

A number of drugs, whether by accident or by design, hit multiple protein targets, a phenomenon termed *polypharmacology* (Peters, 2013). This phenomenon is particularly common when the target is a member of a family of proteins with similar binding sites. For example, the full physiological effect of an adrenergic antagonist is determined by its actions across the family of adrenergic receptor types and subtypes. Similarly, many protein kinase inhibitors inhibit multiple kinases, each to a different degree. There are instances where hitting multiple targets is fruitful, such as inhibiting sequential reactions in a series. Modulating multiple proteins in a single biochemical pathway or signaling network overcomes the evolved redundancy of a robust biological system and hence leads to greater efficacy than modulating only one protein. A single compound may, alternatively, hit two entirely different targets in different pathways, although this is more challenging to achieve without going to larger compounds. The analysis of complex molecular systems in relation to drug action is termed *systems pharmacology*.

Polypharmacology is not always beneficial, and indeed, it can lead to toxicity. Some of the unintended effects of a drug will be termed side effects or even major adverse drug responses. For example, a number of initially promising compounds have proven to bind and inhibit hERG, the K^+ channel in the heart that mediates repolarization (the I_{Kr} current; see Chapter 34); inhibition of hERG can lead to potentially fatal arrhythmias. The hERG channel has, therefore, become a notorious *anti-target* that must be scrupulously avoided by drug discovery projects (Garrido et al., 2020).

Some small-molecule drugs do not bind to proteins at all. For example, platinum anticancer drugs, such as *carboplatin*, kill cancer cells by binding covalently to DNA; the aminoglycoside antibiotics block bacterial protein synthesis by binding to RNA within the bacterial ribosome; and antiviral nucleoside analogues are incorporated into viral DNA in place of normal nucleosides and then block DNA replication. The drug *sugammadex* has both an unusual purpose and an unusual mechanism. Surgical patients often receive not only general anesthesia but also the nondepolarizing neuromuscular blocking agent *rocuronium*, which prevents involuntary movements of skeletal muscle during surgical procedures (see Chapter 13). *Sugammadex*, a larger, cup-shaped molecule, binds and sequesters *rocuronium*. Thus, injection of *sugammadex* rapidly reduces the concentration of unbound *rocuronium* in the blood and promptly reverses paralysis when a procedure is complete.

Protein-Drug Binding: Affinity and Allostery

A successful drug with a protein target must bind to its target with high affinity so that even a small dose of the drug will yield a blood concentration high enough to bind a large fraction of the targeted protein. If the affinity were low, then a high concentration of drug would be needed for a substantial fraction of the target sites to be occupied, and a large dose of drug would need to be administered, leading to inconvenience and an increased risk of side effects. The affinity of a small molecule for a protein is generally given as the dissociation constant, the concentration of free drug molecules in solution at which 50% of the targeted protein has bound drug; the lower this concentration, the higher the affinity (see Figure 3–3). Drug design projects typically aim for a dissociation constant on the order of 10^{-9} mol/L (1 nM); such a "nanomolar drug" is typically dosed in milligrams to grams per day. A successful drug should also exhibit a high degree of specificity for its target protein, meaning that the drug does not interact with other proteins that could lead to undesired side effects and toxicity. In some cases, the effectiveness of a drug may be influenced by not just the affinity but also the kinetic rate constants for drug-protein binding and dissociation, which determine the drug's residence time at its receptor (Copeland, 2016).

Most drugs bind their targeted proteins via attractive, intermolecular interactions that do not involve a covalent chemical bond. These *noncovalent interactions* typically include:

- Hydrogen bonding, in which an electronegative atom with a bound hydrogen atom, such as a hydroxyl group, partly shares its hydrogen with an electronegative atom on the other molecule
- Attractive electrostatic interactions between atoms of opposite charge, such as between a negatively charged carboxylic acid belonging to the drug and a positively charged arginine side chain of the protein
- The hydrophobic effect, in which nonpolar or "greasy" parts of the drug and protein associate with each other to reduce their energetically unfavorable exposure to water, much as oil droplets coalesce in salad dressing
- Dispersion forces—the attractive part of van der Waals interactions—short-ranged attractive interactions between the instantaneous electrical dipoles that result from the constant fluctuations of negatively charged atomic electron clouds around positively charged atomic nuclei

These attractive forces need to overcome the entropic tendency of the drug and protein to wander apart, due to thermal energy. There are also, inevitably, forces that oppose binding and that must be overcome by the attractive ones. For example, there is an energy penalty for stripping water from polar chemical groups of the ligand and protein as they come together to bind. Thus, the overall affinity of a drug-protein interaction reflects a delicate and hard-to-predict balance of attractive and repulsive interactions.

Small-molecule drugs do not bind to the relatively smooth, exterior surfaces of their protein targets, but instead are enfolded by binding pockets in the protein (see Figure 1–4). This structural arrangement makes it possible to form the extensive, short-ranged, physical interactions that are needed to hold the two molecules together tightly. Druggable binding pockets (i.e., ones that enable small-molecule binding) usually are available in enzymes whose substrates are small molecules and in receptors that bind small-molecule hormones and transmitters. However, many proteins lack a concave pocket and therefore are difficult or impossible to drug with a small molecule. In such cases, one may instead consider developing a protein therapeutic, such as an engineered antibody that targets the protein of interest. Because proteins are large, they can form extensive, short-ranged, physical interactions even with the relatively flat exterior surface of a targeted protein, and thus can achieve adequate binding affinity where a small-molecule drug cannot. These considerations also help explain why it is difficult to develop a small-molecule drug that will block a protein-protein interaction: protein-protein binding usually involves a large number of interactions on a relatively flat binding interface between the two proteins, and a small molecule cannot get sufficient purchase on such a flat surface.

Note that a drug must not only bind to its target but also have the desired effect upon it. If the goal is to inhibit an enzyme, then a drug that binds in the active site should easily accomplish this by simply blocking association of the enzyme with molecules of substrate. In contrast, when a cell-surface receptor is the target, a small molecule might interact at the agonist binding site but without inducing an activating conformational change and thus might function as an antagonist or inverse agonist (see Chapter 3). A drug may also inhibit the function of a protein by binding in a pocket outside the active site, and thereby modifying the three-dimensional conformation of the targeted protein; this is an allosteric effect. Such a drug must not only bind in a suitable pocket but also induce the desired conformational change. *Efavirenz* and *nevirapine*, used in treating HIV-AIDS, are nonnucleoside reverse transcriptase inhibitors that act allosterically to inhibit viral transcription of viral RNA to DNA (see Figure 65–5). Similarly, a number of ligands interact with allosteric sites on $GABA_A$ receptors (see Figure 16–11) and other Cys-loop receptors to modulate receptor/channel function. Allostery can also offer a sophisticated strategy to target a single enzyme from among a family of similar enzymes. Thus, in designing a drug, one might take advantage of the fact that, even within a family of related proteins with similar active sites, the members will likely have other regions of their structure that are more variable and possibly unique. Designing a small ligand that binds to such a site might produce an agent that is a quite selective allosteric modifier of enzyme function. This approach is being used to target selected protein phosphatases (Mullard, 2018).

A few small-molecule drugs react chemically with their protein targets to form *irreversible*, *covalent* bonds, rather than relying entirely on the noncovalent attractions discussed above. Such covalent drugs bond to a

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specific chemical group of the protein target, often a relatively reactive amino acid side chain within an enzyme's catalytic site. In principle, covalent drugs should require smaller, less frequent dosing, because a covalently bound drug will not dissociate from the protein as the concentration of free drug dwindles over time following a dose (but note that some boron-containing compounds form *reversible* covalent bonds [Diaz and Yudin, 2017]). Drug developers have tended to avoid covalent drugs because they necessarily possess chemically reactive groups that risk reacting not only with the desired target but also with other proteins and biomolecules, with the potential for causing undesired biological effects. However, selectivity can be achieved by specific noncovalent interactions between the drug and the protein that pull the compound into a location and conformation where it is poised to form the desired covalent bond.

Covalent binding has been used to successfully target and inhibit a member of the RAS GTPase family, KRAS G12C, which had been viewed as virtually undruggable. As a result of such targeted positioning, the cancer drug *sotorasib* gains both potency and specificity by forming a covalent bond with a cysteine side chain present in an oncogenic mutant form of KRAS but not in normal KRAS (Lanman et al., 2020).

Experimental Approaches to Drug Discovery

Given a validated target, the next major milestone in a drug discovery project is arrival at a *clinical candidate*, a small molecule that binds the target with high affinity and specificity, has the desired effect on it, and meets a range of other criteria for a safe, efficacious drug (Hefti, 2008). Some of these criteria relate to *pharmacokinetics*: How well will the compound be absorbed if given orally? How well does it distribute to the targeted organs and tissues? How rapidly and by what mechanisms is it eliminated? Is it metabolized to an active metabolite? These properties are often lumped together as absorption, distribution, metabolism, and excretion (*ADME*) or drug metabolism and pharmacokinetics (*DMPK*).

It is also essential to confirm that the compound does not show evidence of toxicity. Both pharmacokinetics and toxicity can be initially studied *in vitro*. For example, there are *in vitro* methods that examine the ease with which the compound enters cells (see Chapter 4) and the likelihood that liver enzymes (see Chapter 5) will chemically modify the compound. Compounds also can be evaluated *in vitro* for evidence of toxicity and mutagenicity. However, *in vitro* studies cannot fully model the complexities of a living organism; animal studies are still required to minimize the chances that a compound will be problematic when first given to human subjects. For example, toxicity is usually assessed by long-term monitoring of the health of two species of animals, generally one rodent (usually mouse) and one nonrodent (often rabbit), when dosed with the compound. A good clinical candidate should also meet some nonbiological criteria. In particular, it must be amenable to large-scale synthesis and high-grade purification at acceptable cost, and it should be possible to create a formulation (e.g., a tablet or injection) that is sufficiently water soluble and stable.

Sophisticated technologies have been developed to speed the process of generating a clinical candidate. These mainly focus on the discovery or design of compounds that will bind the protein target with high affinity (*potent ligands*). Less progress has been made toward designing in safety and favorable pharmacokinetics. These properties pose more complex challenges, because they go far beyond how a small molecule and a protein interact with each other and instead involve the interactions of the small molecule with thousands of different biomolecules in a living system. The technologies for ligand discovery are both experimental and computational, and different methods are applicable in different settings. The following subsections touch on broad approaches but are not comprehensive. Note, too, that various approaches can be used in combination, so the distinctions made here are ultimately somewhat artificial.

Medicinal Chemistry

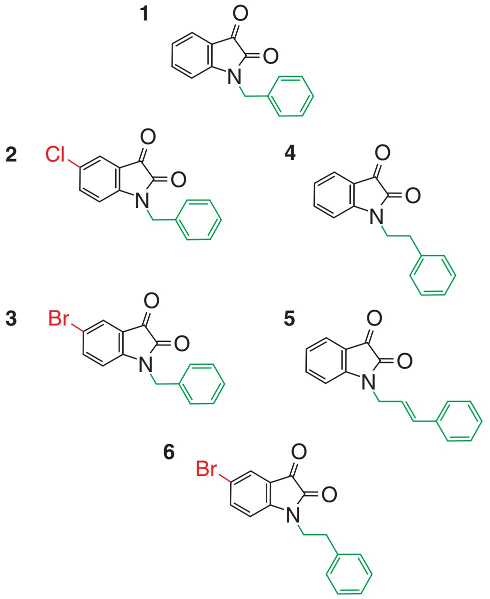
Synthetic organic chemistry remains at the heart of small molecule drug discovery, where it is specialized and known as medicinal chemistry. Medicinal chemists typically are part of a project team that includes, among others, biologists, assay specialists, and computational chemists; their role is to reduce chemical concepts to practice by synthesizing and purifying compounds that may ultimately lead to a new drug. In addition to providing the expertise needed to synthesize compounds of interest, they also help guide the design and selection of the compounds to be made. A key consideration is the complexity of a compound's synthesis, or "synthetic accessibility", which must be balanced against the level of interest in the compound. For example, it can be difficult to generate pure stereoisomers of compounds with multiple chiral carbon atoms, and certain chemical structures can by synthesized only via demanding, multi-step syntheses. A compound that is too difficult to make or purify will not only slow down the research effort but may also lead to a drug that is too costly to manufacture.

Medicinal chemists also inform the drug design process by providing insights into the properties of various chemical groups that might be incorporated into a drug, such as the attractive or repulsive interactions they may form with the targeted protein, their susceptibility to metabolic changes following administration, their potential to spontaneously form undesired covalent bonds with biomolecules, and their influence on the compound's ability to cross the blood-brain barrier (which may be desirable or undesirable, depending on the goal of the project). This expertise comes into play, for example, when a compound binds the target well but is rapidly metabolized by the liver into an inactive product. In this setting, the medicinal chemist may try substituting the part of the compound that is metabolized with a "bioisostere", a different chemical group with a similar shape and ability to interact with the protein but with reduced susceptibility to metabolic modification. More broadly, decades of experience have led to a number of rules of thumb for what makes a compound "drug-like", such as the "rule of five" (Lipinski, et al., 2001). These may be useful guides during drug discovery projects, but there are also many exceptions to the rules (Zhang et al., 2007).

High-Throughput Screening

If nothing is known about the structure of the target protein and what small molecules can bind it, it is common to turn to HTS, in which thousands or millions of compounds are tested using automation and robotics (Wildley et al., 2017). Tiny samples of each compound are drawn from a stored *chemical library* and deposited into multiwell plates for testing. Substantial effort often must be invested to devise an assay that works reliably in miniature and without user intervention. Most provide an optical readout, such as a change in luminescence, fluorescence, or color, as these can be efficiently measured with an optical plate reader. The compounds screened can range from part of the vast, in-house compound collection that a major pharmaceutical company has assembled over the years to a smaller set purchased from a commercial vendor. A screening library is often designed for the particular application. For example, one can purchase libraries tuned for activity against protein kinases, libraries with reactive groups that can form covalent bonds to the protein, and libraries designed to sample a wide range of compounds through high *chemical diversity*. A compound chosen at random from a screening library has a very low probability, typically 0.1% or less, of being active against a given target (Shun et al., 2011), and HTS measurements are subject to experimental error. Therefore, many of the compounds that appear active on an initial screen (*hit compounds*) are false positives, so careful data analysis and confirmatory testing are essential.

Even the confirmed hits from a high-throughput screen are far from being drugs. Their affinity for the target usually is orders of magnitude too weak, they may lack the desired specificity, and they do not meet DMPK or safety criteria. However, they offer an initial toehold on the challenge of finding a potent drug candidate. The next step is to purchase (*analogue by catalog*) and/or synthesize (*medicinal chemistry*) similar compounds that ultimately give a picture of how various changes in



Compound	ALDH1A1	ALDH2	ALDH3A1
1	0.02	82	7.7
2	0.06	2.1	16
3	0.58	2.1	69
4	0.07	3.5	0.45
5	0.07	>100	0.31
6	2.0	0.05	18

Figure 1–1 *Structure-activity relationship: scaffolds and substituents.* Five inhibitors of the aldhyde dehydrogenase family of enzymes have a common chemical scaffold (black) while having different chemical substituents at two positions (red, green). The table lists the IC_{50} (μM) of each compound for three members of the aldehyde dehydrogenase family of enzymes: ALDH1A1, ALDH2, and ALDH3A1; i.e., the concentration of compound needed to provide 50% inhibition of each enzyme. The lower the IC_{50}, the more potently the compound inhibits the enzyme. Focusing first on compounds **1**, **2**, and **3**, one can see that adding an increasingly bulky halogen atom (Cl, Br) on the six-membered ring tends to reduce the compound's potency against ALDH1A1 and ALDH3A1 but to increase it against ALDH2. Focusing next on compounds **1**, **4**, and **5**, one can see that adding increasingly bulky, nonpolar, aromatic substituents at the nitrogen modestly reduces the potency against ALDH1A1, initially improves but then destroys potency against ALDH2, and consistently improves potency against ALD3A1. Such patterns can guide the design of new compounds with desired potency and selectivity. For example, the substituents in compounds **3** and **4** each reduce potency against ALDH1A1 while increasing potency against ALDH2, so it is not surprising that compound **6**, which combines both substituents, has particularly low potency against ALDH1A1 and high potency against ALDH2. Note, however, that this kind of reasoning can only offer guidelines; its predictions are not always borne out by experiment. Data drawn from Kimble-Hill et al., 2014.

the chemical structure influence activity against the target (*structure-activity relationships, or SAR*) and other properties (Figure 1–1). This information is used to guide the synthesis of often hundreds of compounds with gradually improving properties. The most promising early molecules (*lead compounds*) serve as starting points for further improvement (*lead optimization*), ultimately generating, hopefully, a clinical candidate, potentially accompanied by several *backup compounds* in case the leading candidate fails.

Fragment-Based Drug Discovery

Even a large-scale screen can fail to provide useful hits (Keserü and Makara, 2009). This result becomes understandable when one recognizes that the number of stable, drug-sized, organic compounds is on the order of 10^{60} (Reymond et al., 2010), so a screen of even 10^6 compounds scarcely touches the vastness of *chemical space*. This vastness results from the combinatorial explosion of ways of connecting various chemical substructures, such as benzene rings, hydroxyl groups, and cycloalkanes. To be a good binder, a compound has to get multiple substructures positioned so they all form favorable interactions with complementary groups in the targeted binding pocket. If it has two chemical components suitable for binding the target but a third that is inappropriate or in the wrong place on the compound, it may fail to bind the target. This perspective motivates another method of discovering binders, *fragment-based drug discovery* (FBDD) (Erlanson, 2012; Lamoree and Hubbard, 2017). In FBDD, one conceptually breaks down drug-sized compounds into their substructures (*fragments*) and tests simple substructures against the target. Although such fragment-like molecules can bind only very weakly, such studies can, nonetheless, identify a small set of chemical substructures that are suitable for the target, and one can then buy or synthesize larger compounds assembled from these components. When either X-ray crystallography (Patel et al., 2014) or nuclear magnetic resonance spectroscopy (Shuker et al., 1996) is used to detect or analyze fragment binding, specific information is usually available about where each fragment binds to the protein. This information can be used to stitch together designed compounds that place the appropriate fragments at the right places in the protein's binding pocket (*fragment linking*) or to optimize and expand one selected fragment (*fragment growing*). In this way, FBDD avoids the combinatorial explosion of possible compounds made from various chemical components and allows researchers to focus quickly on compounds made from only a productive subset of chemical components. The drug *vemurafenib*, which targets an oncogenic mutation of B-Raf kinase and was developed with a fragment-growing strategy, is usually referenced as the first FBDD success story (Bollag et al., 2012).

Emerging Experimental Technologies

The difficulty and cost of drug discovery, coupled with the market and human need for new medications, have driven ongoing innovation in drug discovery technologies. For example, DNA-encoded compound libraries (DELs) dramatically expand the number of compounds that can be tested, relative to conventional HTS (Halford, 2017). Unlike a traditional HTS compound library, where each compound is kept in its own separate container or well, a DEL is a mixture of compounds in a single container and can include far more compounds—into the billions and even trillions. Each unique compound in the mixture is covalently bound to a corresponding unique short DNA molecule, which serves as an identification tag. Such libraries can be synthesized and tagged with the methods of *combinatorial chemistry*, where a mixture of compounds is split into multiple portions, each portion is modified with a different chemical step and its DNA tags modified accordingly, and the portions are mixed again. This process is iterated until the synthesis is complete. To screen the DEL for active compounds, one may immobilize the target of interest on a solid surface, expose the surface to the DEL mixture, and then wash the surface to remove all the DEL compounds that have not bound tightly to the target. The binders are then removed from the target by more aggressive washing, and the active compounds in the wash are identified by sequencing the DNA tags they carry.

Another emerging technology, sometimes termed *clinical trials in a dish* (Alpeeva et al., 2017; Fermini et al., 2018; Strauss and Blinova, 2017), aims to predict the effects of a compound in humans more accurately than is possible with standard cell culture or animal models. This approach involves creating specific cell types of interest from human pluripotent stem cells and using them to create three-dimensional organoids in culture (Fligor et al., 2018; Liu et al., 2021; Sato and Clevers, 2013) or artificial tissue architectures via three-dimensional bioprinting (Ferrer and Simeonov, 2017). These relatively intricate *in vitro* constructs promise to better recapitulate the properties of the corresponding *in vivo* tissues and may be used to test compounds for activity, DMPK properties, compound metabolism, and toxicity.

Computer-Aided Drug Discovery

The rise of information technology has enabled the research community to store and move large quantities of information, to write and maintain complex software, and to do calculations at unprecedented speed and scale. These continually improving capabilities are used in a variety of ways to support and accelerate drug discovery. Thus, chemical informatics enables compact databasing of information on hundreds of millions of compounds and rapid recovery of chemical data for a specific compound and/or chemically similar compounds (Willett et al., 1998), while the Internet makes chemical (Gaulton et al., 2012; Gilson et al., 2016; Kim et al., 2021), macromolecular (Benson et al., 1994; Berman et al., 2000; Berman and Gierasch, 2021; UniProt Consortium, 2015), biomolecular pathway (Croft et al., 2014; Ogata et al., 2000; Oughtred et al., 2021; Wishart et al., 2020), and other databases readily accessible to researchers worldwide. These data are useful in their own right and also support the development and evaluation of computer models used in drug discovery.

In parallel, exponential increases in computer speed, measured as the number of mathematical operations executed per second, have made more and more detailed molecular simulations feasible. Ideally, a computational chemist could design a compound, hand the design to a medicinal chemist to synthesize, and the compound would prove to bind the target with nanomolar affinity. When this level of accuracy becomes feasible, one might go further and compute the affinity of a candidate drug to all known human proteins in order to check for unwanted interactions. This level of accuracy is not possible today, but existing methods have predictive value, and growing computer power may make this vision achievable in the coming years.

Approaches to predicting the interactions of a small molecule with a protein may be broadly divided into *ligand-based* and *structure-based approaches*, as explained below.

Using Chemical Similarity to Discover Targeted Ligands

If the targeted protein is an enzyme with a small-molecule substrate or a receptor for a small-molecule transmitter (e.g., histamine), then compounds chemically similar to the substrate or transmitter may be active against the target and thus useful starting points for drug design (Figure 1–2). For some targets, more extensive information about ligands for the target may be available from prior drug discovery efforts and may be used to guide a new project. As noted above, even if a drug has already been developed against the target, there may still be room for a me-too drug with better properties, such as less frequent oral dosing or reduced side effects. Large quantities of data to support this ligand-based drug discovery approach are available in the scientific literature, patents, and public databases (Gaulton et al., 2012; Gilson et al., 2016; Kim et al., 2019).

Metrics of chemical similarity abstract the detailed chemical structures of compounds into characteristics that can be computed and compared across molecules. One approach computes a compound's molecular fingerprint, which indicates whether various molecular substructures are present (Muegge and Mukherjee, 2016). Other similarity metrics jettison such details and, instead, compute and compare the overall shapes of the two molecules and the electrical fields they generate (Bajorath, 2017). In a third approach, even molecular shape is set aside, and one instead computes tens or hundreds of quantitative *descriptors* for each compound. Examples include simple descriptors, such as molecular weight or number of aromatic rings, and more complex descriptors such as electrical dipole and quadrupole moments. If one imagines descriptors as Cartesian coordinates in a multidimensional space, one can then quantify the similarity of two molecules in terms of how close they are in this *descriptor space* (Wale et al., 2008).

Similarity metrics such as these enable *virtual screening*, a fast, inexpensive, computational alternative to experimental HTS (Figure 1–3). In this approach, every compound in a chemical library—a large set of compounds that are available or synthesizable—is assessed for its similarity to one or more known ligands of the protein target. The most similar compounds are tested in an experimental assay, and confirmed hits become candidates for further chemical optimization. This approach is most relevant when the three-dimensional structure of the targeted protein has not been determined. When the structure is known, powerful structure-based methods become applicable.

Structure-Based Drug Design

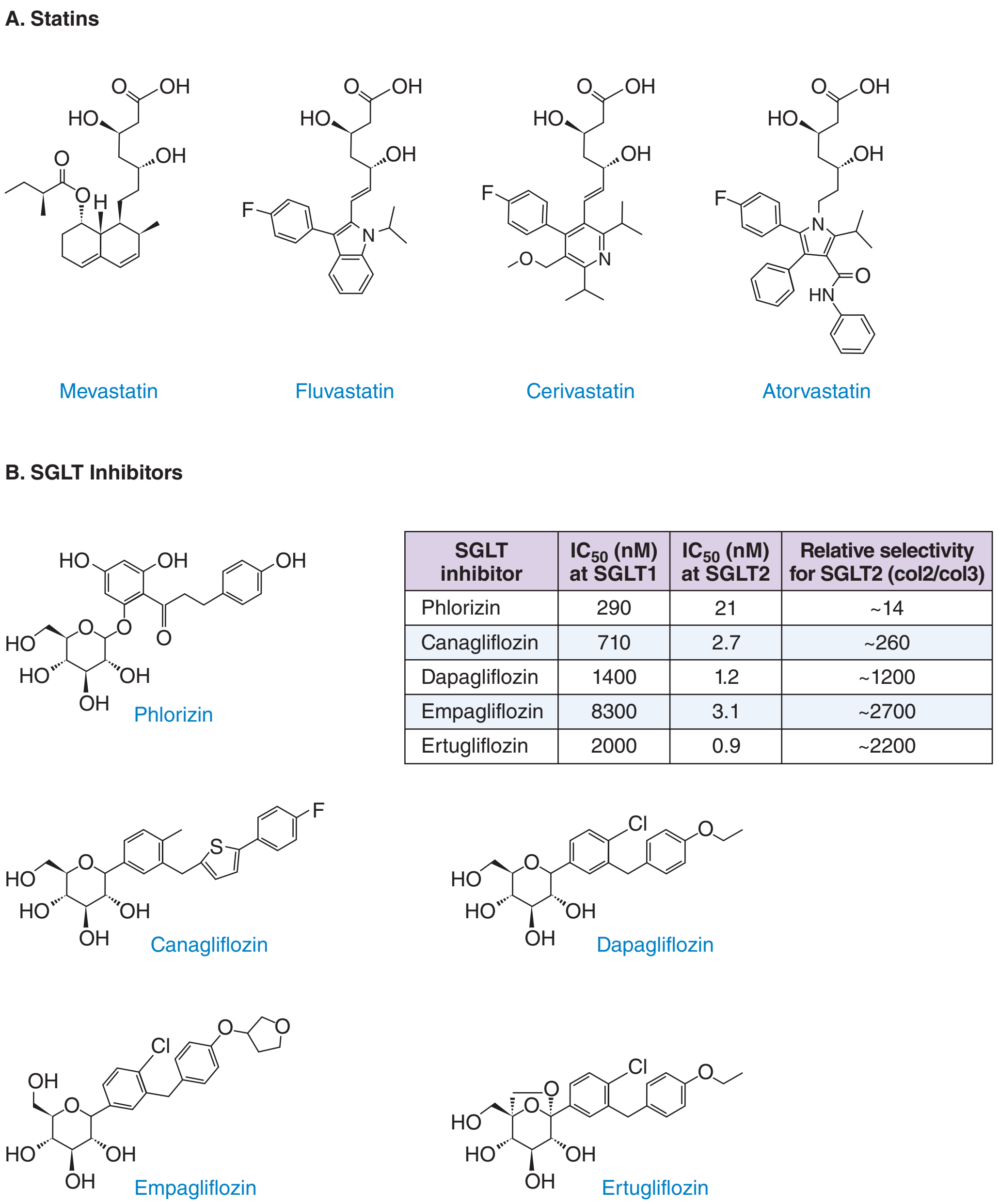
The detailed three-dimensional structure of a targeted protein opens up a range of additional computational methods for designing a small molecule that binds the target with high affinity (Figure 1–4). The applicability of such SBDD methods has grown continually, due to rapid increases in computer power and the development of technologies that make determining protein structures easier and faster. One example is the use of synchrotrons (e.g., the Advanced Photon Source at Argonne National Laboratory) to generate high-quality X-ray beams for use in protein X-ray crystallography. Another is the development of methods to solve the structures of membrane-bound proteins, such as ion channels and cell-surface receptors. These can be high-quality drug targets because a drug does not need to enter the cell to access them and because they regulate many cellular processes. However, their structures were virtually impossible to solve until methods were developed in recent years to grow three-dimensional crystals of them. Since at least the 1980s, the promise of advances in SBDD methods has inspired the founding of multiple companies.

The field of physical chemistry tells us how to compute the binding affinity of two molecules in water (Gilson and Zhou, 2007). Ideally, one could use numerical solutions of Schrödinger's equation to obtain the electronic wave function for the compound, the target protein, and the aqueous solvent, for any given conformation of the system (i.e., given the Cartesian coordinates of all atoms). From the wave function, one could then compute the instantaneous force on every atom. Given this method of computing atomic forces, one could simulate the system at atomistic detail, computing the reversible work of gradually pulling the compound out of the protein binding site as all the atoms wiggled, jiggled, and shifted due to thermal motion (Feynman et al., 1963). This reversible work would equal the free energy of binding, ΔG^o, which is directly related to the dissociation constant, K_D:

$$\Delta G^o = RTlnK_D \qquad \text{(Equation 1–1)}$$

This would be a prohibitively massive calculation with existing computer technology. However, researchers have created fast approximations to such an ideal calculation, each with its own strengths and weaknesses in terms of accuracy, range of applicability, and the computer power required (Figure 1–5).

An important approximation used in molecular modeling is the force field or potential function, a mathematical model for the atomic forces that can be evaluated orders of magnitude faster than solving Schrödinger's equation (Dauber-Osguthorpe and Hagler, 2019). Force fields often contain adjustable parameters fitted to give agreement with reference solutions of Schrödinger's equation. With a force field in hand, it becomes practical to use molecular simulations to estimate protein-ligand binding free energies (Tembe and McCammon, 1984; Kollmann, 1993; Gilson et al., 1997; Simonson et al., 2002). Such *free energy methods* are among the most accurate approaches available to predict protein-ligand binding affinities (Schindler et al., 2020), and their use by the drug



A. Statins

B. SGLT Inhibitors

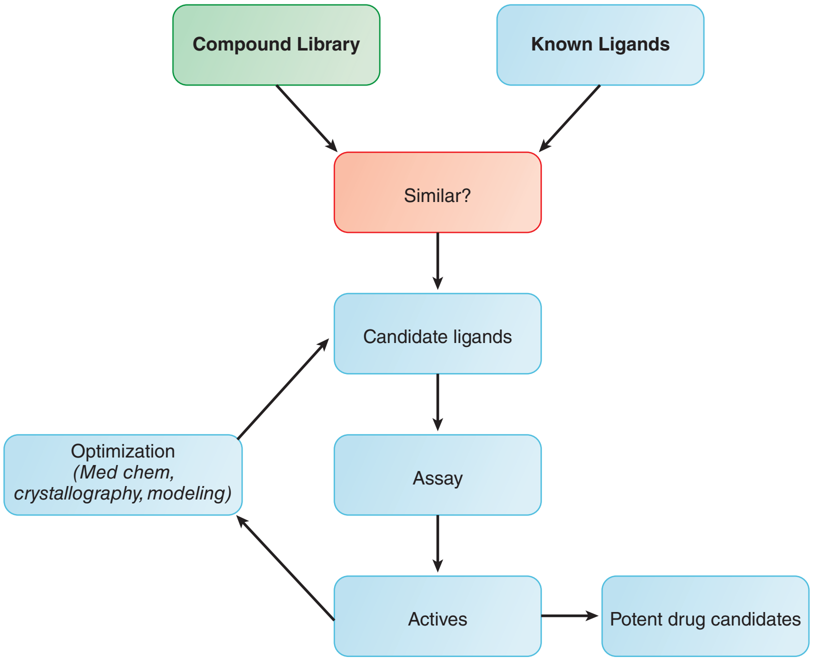
SGLT inhibitor	IC$_{50}$ (nM) at SGLT1	IC$_{50}$ (nM) at SGLT2	Relative selectivity for SGLT2 (col2/col3)
Phlorizin	290	21	~14
Canagliflozin	710	2.7	~260
Dapagliflozin	1400	1.2	~1200
Empagliflozin	8300	3.1	~2700
Ertugliflozin	2000	0.9	~2200

Figure 1–2 *Using chemical similarity to develop ligands.* **A.** *Statins.* Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), the rate-limiting enzyme in cholesterol synthesis. These inhibitors are widely used to lower blood levels of cholesterol (see Chapter 37). Mevastatin is a natural product that inspired development of the three FDA-approved statins shown here. Each compound has a polycyclic lower part linked to a common hydroxyacid moiety, which can also exist as a cyclic lactone. **B**. *SGLT inhibitors.* Sodium-glucose cotransporters (SGLTs) facilitate glucose ingress in the gastrointestinal tract (SGLT1) and the kidney (SGLT2). The natural product phlorizin inhibits both SGLTs to varying extents. Modifications of the phlorizin structure led to the four FDA-approved relatively specific SGLT2 inhibitors, the gliflozins, shown here. Gliflozins reduce renal reabsorption of glucose, thereby lowering blood sugar concentrations, and thus are used to treat type 2 diabetes (see Chapter 51). Each compound has a glucose moiety (except *ertugliflozin*, which has a glucose-similar moiety), sensible for compounds that interact with transporters that bind glucose. Phenyl-containing moieties endow each inhibitor with varying activities against each of the two protein forms, as shown in the table. Activities are given as IC$_{50}$, the concentration of drug (nM) that reduces the transporter's activity by 50%. Data adapted from Fediuk et al. (2020) and Wright (2021).

discovery community has been enabled by the acceleration of molecular simulations on graphics processing units (GPUs) (Salomon-Ferrer R, et al., 2013). Even with GPUs, though, the simulations are too slow to replace an experimental high-throughput screen of millions of compounds. Instead, simulations are most commonly used to help medicinal chemists decide which chemical variations on a promising starting compound are worth synthesizing and testing. Fast molecular simulations also are used to explore the various conformations that a protein can adopt. For example, if a simulation shows that a new binding pocket could form as a result of thermal protein motions, it may be possible to design a drug that will bind this hitherto unrecognized site.

Another computational approach, *molecular docking* (Guedes et al., 2014; Huang, 2010; Meng, 2011), is fast enough to substitute for (or supplement) a large-scale experimental high-throughput screen. In docking, most or all of the protein is held rigid, and the software tries a vast number of different locations and conformations—*poses*—of a small molecule in the target's binding site, searching for the one that is lowest in energy and hence most stable. Because docking leaves out so many known contributions to the free energy of binding (e.g., protein flexibility and entropy), the energy model usually must be tuned against experimental binding data to make it more predictive. The resulting model is often called a docking *score*, to differentiate

A. Screening based on concepts of chemical similarity


Compound Library
Known Ligands
Similar?
Candidate ligands
Optimization
(Med chem, crystallography, modeling)
Assay
Actives
Potent drug candidates

B. Screening based on protein-ligand docking

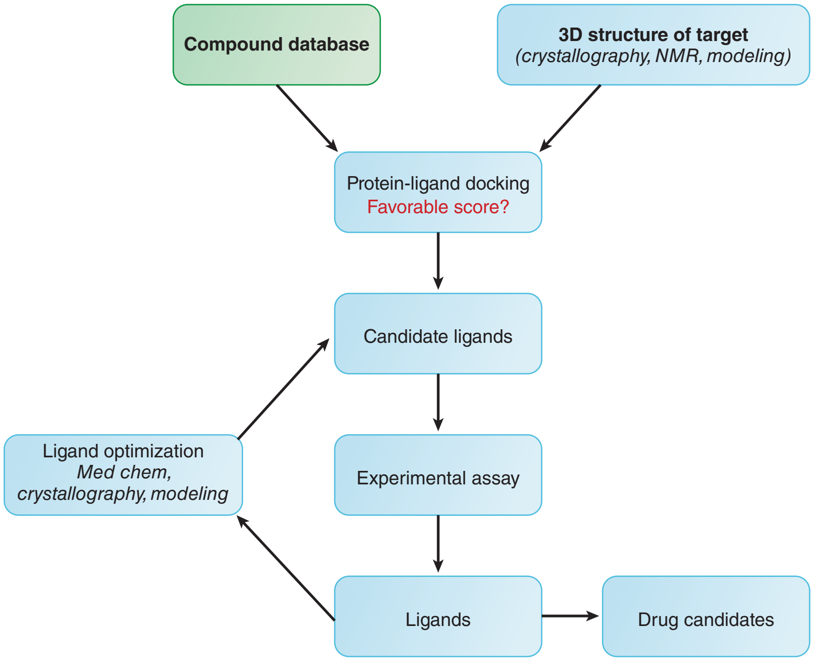

Compound database
3D structure of target
(crystallography, NMR, modeling)
Protein-ligand docking
Favorable score?
Candidate ligands
Ligand optimization
Med chem, crystallography, modeling
Experimental assay
Ligands
Drug candidates

Figure 1–3 *Virtual screening.* **A.** *Virtual compound screening based on concepts of chemical similarity.* Using available similarity metrics, the compounds in a database (green) are computationally tested for chemical similarity to the known ligands (binders) of the targeted protein. Compounds that are above some threshold similarity are considered candidate ligands and so are experimentally assayed for binding to the protein target. Those found to be inactive are set aside, while “actives” are subjected to iterative rounds of ligand optimization where structure-activity relationships are defined and used to guide the design of new compounds by medicinal chemists. When sufficiently active compounds are found, these become early-stage drug candidates. **B.** *Virtual compound screening based on protein-ligand docking.* The compounds in a database (green) are computationally docked; i.e., optimally fitted into the binding site of a target protein of known three-dimensional structure. Compounds whose computed stabilizing interactions with the binding site are above a threshold similarity are considered candidate ligands and so are experimentally assayed for binding to the protein target. Those found to be inactive are set aside, while “actives” are subjected to iterative rounds of ligand optimization. This typically involves using the protein structure to design new compounds that can form better interactions with the binding site and solving crystal structures of the protein with selected compounds to determine whether the designed compounds bind as hoped and to guide further rounds of chemical design and synthesis. Advanced computational methods, such as simulation-based free energy calculations, may also be used at this stage. When sufficiently active compounds are found, these become early-stage drug candidates.

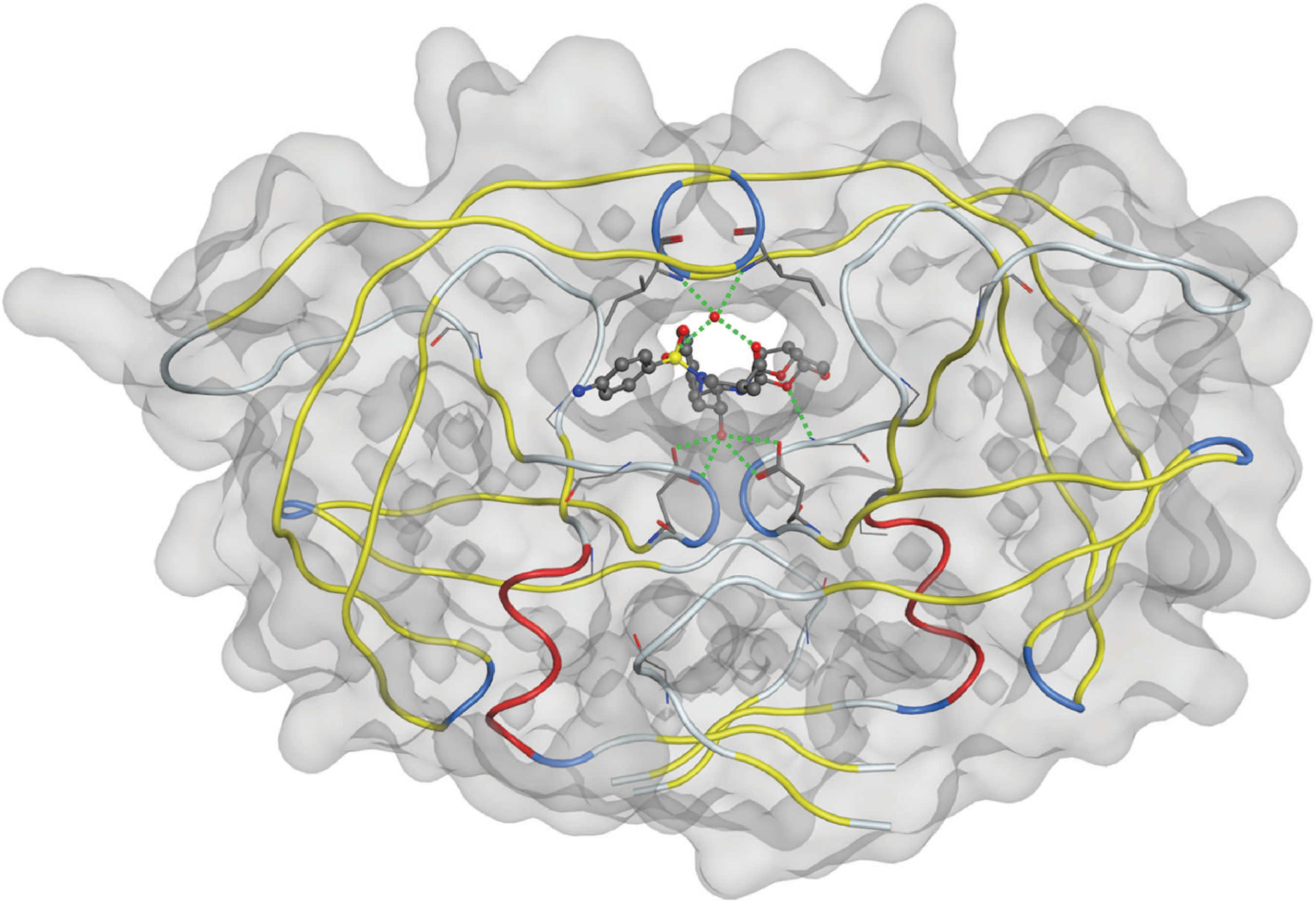


Figure 1–4 *Crystal structure of the human immunodeficiency virus 1 protease (HIV-1 protease) with the protease inhibitor darunavir bound in the active site.* Colored tubes: protein backbone of the enzyme, a symmetric dimer made up of two identical subunits, where color indicates secondary structure (yellow, β-sheet; red, α-helix; blue, turn; white, none). Translucent gray: overall surface of the protein, including both side-chain and backbone atoms. Ball and stick: *darunavir* in the tunnel-shaped active site, with atoms colored by element (gray, carbon; red, oxygen; blue, nitrogen), with hydrogen atoms omitted for simplicity. Key hydrogen bonds are shown as dashed green lines, and the oxygen of a water molecule that bridges between the drug and the protein is shown as a red ball. Atomic coordinates from Protein Data Bank (Wang et al., 2011).

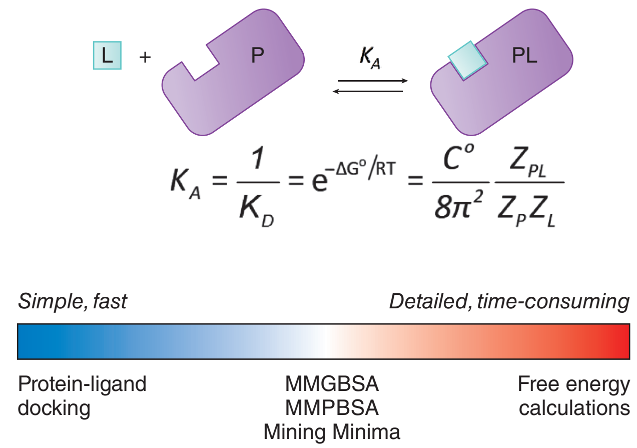
it from a true force field. Docking calculations are typically used for *virtual HTS* (see Figure 1–3), in which thousands or millions of compounds in a chemical library are rapidly fitted into the binding site of the targeted protein. Tens or hundreds of the top-scoring compounds may then be subjected to more detailed calculations or tested experimentally. Although not all of the top-scoring compounds will be good binders, the fraction of binders will normally be enriched relative to the chemical library as a whole. In addition, the predicted binding poses may provide mechanistic insight and serve as starting points for molecular simulations (Guest et al., 2022; Heinzelmann and Gilson, 2021). The empirically tuned scoring functions used in docking can also be used to guide manual chemical editing of a known binder with graphical molecular modeling software. For example, one may manually edit an existing compound in the context of a three-dimensional rendering of the binding pocket to design a new compound that reaches into a neighboring subpocket and forms stabilizing hydrophobic and hydrogen-bonding interactions with the protein. This interactive work may be aided by immersive visualization and manipulation technologies, such as virtual reality.

Artificial Intelligence in Drug Discovery

Deep neural networks have proven their power in wide-ranging artificial intelligence tasks such as image recognition and language translation, and researchers are now exploring their use in drug discovery. These methods may be *trained* on existing data, such as on existing collections of protein–small-molecule binding data, the results of DELs, and protein structures, to enable direct prediction of protein–small-molecule binding and automated design of ligands for a targeted protein. They may also support drug discovery in other ways, such as by predicting the three-dimensional structures of proteins (AlQuraishi, 2021; Baek et al., 2021; Jumper et al., 2021), the energies of molecules as a function of conformation (Smith et al., 2017), and molecular properties such as whether a compound is water soluble (Francoeur and Koes, 2021). Artificial intelligence and machine learning will undoubtedly play an expanding role in drug discovery in the coming years.

Designing Large Molecules as Drugs: The Rise of Biopharmaceuticals

Large molecules are increasingly important as therapeutic agents. For example, antisense oligonucleotides are used to block gene transcription or translation, as are siRNAs and modified mRNAs (as in several vaccines for SARS-CoV-2 [severe acute respiratory syndrome coronavirus 2]). Important proteins used therapeutically include monoclonal antibodies, enzymes, and peptide hormones. Protein therapeutics were uncommon before the advent of recombinant DNA technology except for the few peptide hormones that could be isolated and purified in bulk. Insulin was



$$K_A = \frac{1}{K_D} = e^{-\Delta G^o/RT} = \frac{C^o}{8\pi^2}\frac{Z_{PL}}{Z_P Z_L}$$

Figure 1–5 *Physics-based computational methods for estimating protein-ligand binding affinities.* These methods provide an estimate of the association constant, K_A, for binding of a ligand, L, to a protein of known three-dimensional structure, P, to form a protein-ligand complex, PL, held together typically by noncovalent interactions such as hydrogen bonding and the hydrophobic effect. The equation relates K_A to the standard free energy of binding ΔG^o, the gas constant R, and the absolute temperature T, and further relates the binding free energy to the standard concentration C^o, and the configuration integrals of the protein-ligand complex (Z_{PL}), the unbound protein (Z_P), and the unbound ligand (Z_L). As more low-energy conformations are accessible to each molecular species (PL, P, L), the corresponding value of Z increases. Therefore, if the protein-ligand complex can access more low-energy conformations than the separate protein and ligand, the equilibrium constant will be large, favoring binding. Direct calculation of these configuration integrals is a computational challenge; however, researchers have created a spectrum of computational methods, ranging from fast, approximate methods that are expected to be less accurate, to more detailed, more computationally demanding methods that are typically more accurate (Gilson and Zhou, 2007). Docking, discussed in the text, is at the fast end of the spectrum; it treats the protein as mainly rigid, along with other approximations. Free energy calculations, also discussed in the text, are at the slow end of the spectrum; they treat the protein and ligand as fully flexible. In the middle of the spectrum are the molecular mechanics generalized Born/surface area (MMGBSA) (Srinivasan et al., 1998), molecular mechanics Poisson-Boltzmann/surface area (MMPBSA) (Gouda et al., 2003), and Mining Minima methods (Chen et al., 2010). These use various approaches to directly estimate the configuration integrals, Z_{PL}, Z_P, and Z_L. For example, Mining Minima searches for low-energy conformations of the protein, the ligand, and the complex; estimates their individual contributions to Z; and sums these contributions to provide an overall estimate of the configuration integral.

introduced into clinical medicine for the treatment of diabetes following the experiments of Banting and Best in 1921. Insulins purified from porcine or bovine pancreas are active in humans, although antibodies to the foreign proteins are occasionally problematic. Growth hormone, used to treat pituitary dwarfism, exhibits more stringent species specificity. Only the human hormone could be used after purification from pituitary glands harvested during autopsy, and such use had its dangers—some patients who received the human hormone developed Creutzfeldt-Jakob disease (the human equivalent of mad cow disease), a fatal degenerative neurological disease caused by prion proteins that contaminated the drug preparation.

Thanks to gene cloning, expression of the cloned gene in bacteria or eukaryotic cells, and large-scale production techniques, protein therapeutics now use highly purified preparations of human (or humanized) proteins. Rare proteins can be produced in quantity, and immunological reactions are minimized. Proteins can be designed, customized, and optimized using genetic engineering techniques.

Proteins used therapeutically include hormones, growth factors (e.g., erythropoietin, granulocyte colony-stimulating factor), cytokines, and a number of monoclonal antibodies used in the treatment of cancer and autoimmune diseases (see Chapters 38–40, 45, and 72). Murine monoclonal antibodies can be "humanized" (by substituting human for mouse amino acid sequences). Alternatively, mice have been engineered by replacement of critical mouse genes with their human equivalents, such that they make completely human antibodies. Protein therapeutics are administered parenterally, and their receptors or targets must be accessible extracellularly.

Using some of the strategies outlined above, nonantibody therapeutic proteins and peptides can now be optimized for stability, activity, and targeting to particular cell types. Peptides are being developed as therapeutics, especially in the area of interrupting protein-protein interactions where the large contact surfaces may defy small-molecule action. Computational methods are proving very useful in the design of peptide therapeutics (Belvisi et al., 2021). Therapeutic proteins are usually close copies of naturally occurring proteins that are optimized for high stability (both during manufacture and after administration) and optimized to avoid rapid degradation, to have low immunogenicity, and to have high potency when administered to a patient. Strategies include optimizing expression of a protein's gene sequence in multiple hosts, exploring close relatives of the protein of interest and mutations (random and rational), introduction of posttranslational modifications, and exploring biological modifications such as fusion with macromolecules (Dellas et al., 2021). Conjugation strategies (e.g., PEGylation) can be used to improve pharmacokinetic properties of therapeutic proteins (Moncalvo et al., 2020). The roster of recently engineered proteins that are not antibodies includes agents for cancers, gout, clotting disorders and hemophilia, inherited metabolic diseases, lysosomal storage disorders, pancreatic exocrine deficiency, insufficiencies of hormones and growth factors, and macular degeneration, among others. The number of nonantibody FDA-approved therapeutic proteins and peptides is growing rapidly (see a database of FDA-approved proteins and peptides at https://webs.iiitd.edu.in/raghava/thpdb/index.html; Usmani et al., 2017). A few protein therapeutics are administered topically or orally, but most are administered by injection. However, this is changing with the development of liposomal drug delivery systems, which are administered parenterally but are proving amenable to inhalation, ocular, and topical routes.

The Investigational New Drug Application

Before the drug candidate can be administered to human subjects in a clinical trial, the sponsor must file an Investigational New Drug (IND) application, a request to the FDA for permission to use the drug for human research (see Clinical Trials, below). The IND describes the rationale and preliminary evidence for efficacy in experimental systems, as well as pharmacology, toxicology, chemistry, manufacturing, and so forth. It also describes the plan (protocol) for investigating the drug in human subjects. The FDA has 30 days to review the IND application, by which time the agency may disapprove it, ask for more data, or allow initial clinical testing to proceed.

Clinical Trials

Role of the FDA

The FDA, a federal regulatory agency within the U.S. Department of Health and Human Services (DHHS), is responsible for protecting the public health by ensuring the safety, efficacy, and security of human and veterinary drugs, biological products, medical devices, our nation's food supply, cosmetics, and products that emit radiation (FDA, 2018). The FDA also is responsible for advancing public health by helping to speed innovations that make medicines and foods more effective, safer, and more affordable and by helping people obtain the accurate, science-based information they need to use medicines and foods to improve their health.

The first drug-related legislation in the U.S., the Federal Pure Food and Drugs Act of 1906, was concerned only with the interstate transport of adulterated or misbranded foods and drugs. Motivations for federal regulation included the prominence of "patent medicines" and their adulteration, the journalism of S. H. Adams (via articles in *Colliers Weekly*), and Upton Sinclair's novel *The Jungle* (Law, 2004). In the 1906 act, there were no obligations to establish drug efficacy or safety. This act was amended in 1938 after the deaths of over 100 children from "elixir sulfanilamide," a solution of *sulfanilamide* in *diethylene glycol*, an excellent but highly toxic solvent and an ingredient in antifreeze. The enforcement of the amended act was entrusted to the FDA, which began requiring toxicity studies as well as approval of a New Drug Application (NDA) (see The Conduct of Clinical Trials, below) before a drug could be promoted and distributed. Although a new drug's safety had to be demonstrated, no proof of efficacy was required.

In the 1960s, *thalidomide*, a hypnotic drug with no obvious advantages over others, was introduced in Europe. Epidemiological research eventually established that this drug, taken early in pregnancy, was responsible for an epidemic of what otherwise is a relatively rare and severe birth defect, phocomelia, in which limbs are malformed. In reaction to this catastrophe, the U.S. Congress passed the Harris-Kefauver amendments to the Food, Drug, and Cosmetic Act in 1962. These amendments established the requirement for proof of efficacy as well as documentation of relative safety in terms of the risk-to-benefit ratio for the disease entity to be treated (the more serious the disease, the greater the acceptable risk). Today, the FDA faces an enormous challenge, especially in view of the widely held belief that its mission cannot possibly be accomplished with the resources allocated by Congress. Moreover, harm from drugs that cause unanticipated adverse effects is not the only risk of an imperfect system; harm also occurs when the approval process delays the approval of a new drug with important beneficial effects.

The Conduct of Clinical Trials

Clinical trials of drugs are designed to acquire information about the pharmacokinetic and pharmacodynamic properties of a candidate drug in humans and to establish the efficacy and safety of the drug prior to its sale in the U.S. The U.S. National Institutes of Health (NIH) identifies seven ethical principles that must be satisfied before a clinical trial can begin (NIH, 2021):

1. Social and clinical value
2. Scientific validity
3. Fair selection of subjects
4. Informed consent
5. Favorable risk-benefit ratio
6. Independent review
7. Respect for potential and enrolled subjects

The FDA-regulated clinical trials typically are conducted in four phases. Phases I to III are designed to establish safety and efficacy. Phase IV postmarketing trials and surveys gather additional data from larger populations and increasing numbers of administered doses. This phase provides information regarding new indications, risks, and optimal doses and schedules, as presented in Chapter 8. Table 1–1 and Figure 1–6 summarize the important features of each phase of clinical trials; note the attrition at each successive stage over a relatively long and costly process. When initial phase III trials are complete, the sponsor (usually a pharmaceutical company) applies to the FDA for approval to market the drug; this application is called either an NDA or a BLA (Biologics License Application). These applications contain comprehensive information, including individual case report forms from the hundreds or thousands of individuals who have received the drug during its phase III testing. Applications are reviewed by teams of specialists, and the FDA may call on the help of panels of external experts in complex cases.

Under the provisions of the Prescription Drug User Fee Act (PDUFA; enacted in 1992 and renewed every 5 years, most recently in 2017), pharmaceutical companies now provide a significant portion of the FDA budget via user fees, a legislative effort to expedite the drug approval review process by providing increased resources. The PDUFA also broadened the FDA's drug safety program and increased resources for review of television drug advertising. Under PDUFA, review typically takes 6 to 10 months after an NDA is submitted to the FDA. During this time, numerous review functions are usually performed, including advisory committee meetings, amendments, manufacturing facility inspections, and proprietary name reviews (FDA, 2013). Before a drug is approved for marketing, the company and the FDA must agree on the content of the "label" (package insert)—the official prescribing information. This label describes the approved indications for use of the drug and clinical pharmacological information, including dosage, adverse reactions, and special warnings and precautions (sometimes posted in a "black box"). Promotional materials used by pharmaceutical companies cannot deviate from information contained in the package insert. Importantly, the physician is not bound by the package insert; a physician in the U.S. may legally prescribe a drug for any purpose that he or she deems reasonable. However, third-party payers (insurance companies, Medicare, and so on) generally will not reimburse a patient for the cost of a drug used for an "off-label" indication unless the new use is supported by a statutorily named compendium (e.g., the American Hospital Formulary Service–Drug Information [AHFS-DI]). Furthermore, a physician may be vulnerable to litigation if untoward effects result from an unapproved use of a drug.

TABLE 1–1 ■ TYPICAL CHARACTERISTICS OF THE PHASES OF CLINICAL TRIALS REQUIRED BY THE FDA BEFORE THE MARKETING OF NEW DRUGS*

PHASE I FIRST IN HUMAN	PHASE II FIRST IN PATIENT	PHASE III MULTISITE TRIAL	PHASE IV POSTMARKETING
10–100 participants	50–500 participants	A few hundred to a few thousand participants	Many thousands of participants
Usually healthy volunteers; occasionally patients with advanced or rare disease	Patient-subjects receiving experimental drug	Patient-subjects receiving experimental drug	Patients in treatment with approved drug
Open label	Randomized and controlled (can be placebo controlled); may be blinded	Randomized and controlled (can be placebo controlled) or uncontrolled; may be blinded	Open label
Safety and tolerability	Efficacy and dose ranging	Confirm efficacy in larger population	Adverse events, compliance, drug-drug interactions
1–2 years	2–3 years	3–5 years	No fixed duration
U.S. $10 to 15 million	U.S. $20 to 40 million	U.S. $50–150 million	Variable
Success rate: 50%	Success rate: 30%	Success rate: 25%–50%	—

*Costs of clinical trial phases vary widely with a drug's therapeutic area, size and complexcity of trial, whether trial must prove non-inferiority to existing agents, etc. Overall cost to develop a new molecular entity (NME) from laboratory to FDA approval is estimated at $1 billion to $4 billion.

AL GRAWANY

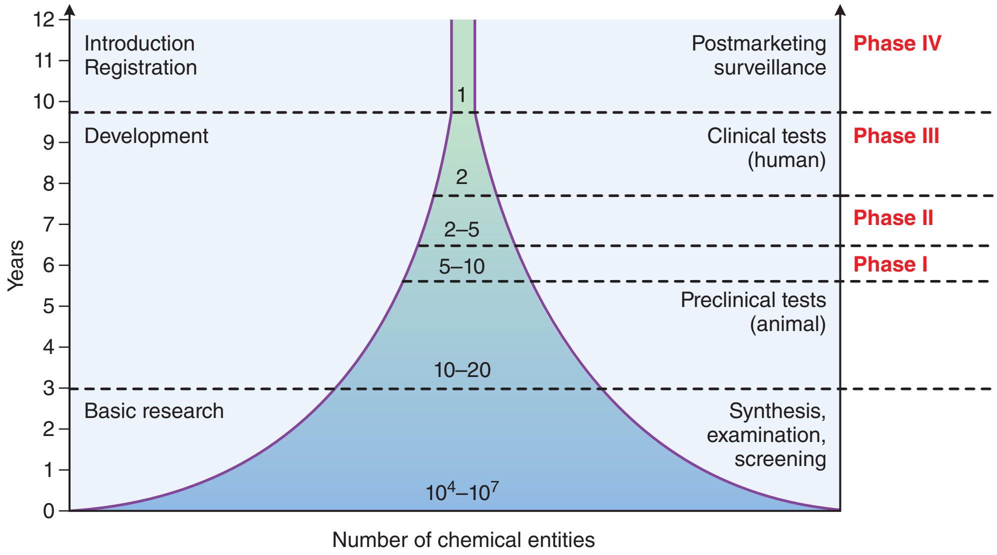


Figure 1–6 *The phases, timelines, and attrition that characterize the development of new drugs.* See also Table 1–1.

Determining "Safe" and "Effective"

Demonstrating efficacy to the FDA requires performing "adequate and well-controlled investigations," generally interpreted to mean two replicate clinical trials that are usually, but not always, randomized, double-blind, and placebo (or otherwise) controlled. Is a placebo the proper control? The World Medical Association's Declaration of Helsinki (World Medical Association, 2013) discourages use of placebo controls when an alternative treatment is available for comparison because of the concern that study participants randomized to placebo in such a circumstance would, in effect, be denied treatment during the conduct of the trial. What must be measured in the trials? In a straightforward trial, a readily quantifiable parameter (a secondary or surrogate end point), thought to be predictive of relevant clinical outcomes, is measured in matched drug- and placebo-treated groups. Examples of surrogate end points include low-density lipoprotein (LDL) cholesterol as a predictor of myocardial infarction, elevated high-density lipoprotein (HDL) cholesterol as a predictor of reduced risk of myocardial infarction (see Box 1–2), bone mineral density as a predictor of fractures, or hemoglobin A_{1c} as a predictor of the complications of diabetes mellitus. More stringent trials would require demonstration of reduction of the incidence of myocardial infarction in patients taking a candidate drug in comparison with those taking an HMG-CoA reductase inhibitor (statin) or other LDL cholesterol–lowering agent or reduction in the incidence of fractures in comparison with those taking a bisphosphonate. Use of surrogate end points significantly reduces cost and time required to complete trials, but there are many mitigating factors, including the significance of the surrogate end point to the disease that the candidate drug is intended to treat.

Some of the difficulties are well illustrated by experiences with *ezetimibe*, a drug that inhibits absorption of cholesterol from the gastrointestinal tract and lowers LDL cholesterol concentrations in blood, especially when used in combination with a statin. Lowering of LDL cholesterol was assumed to be an appropriate surrogate end point for the effectiveness of *ezetimibe* to reduce myocardial infarction and stroke, and the drug was approved based on such data. Surprisingly, a subsequent clinical trial (ENHANCE) demonstrated that the combination of *ezetimibe* and a statin did not reduce intima media thickness of carotid arteries (a more direct measure of subendothelial cholesterol accumulation) compared with the statin alone, despite the fact that the drug combination lowered LDL cholesterol concentrations substantially more than did either drug alone (Kastelein et al., 2008). Critics of ENHANCE argued that the patients in the study had familial hypercholesterolemia, had been treated with statins for years, and did not have carotid artery thickening at the initiation of the study. Should *ezetimibe* have been approved? Must we return to measurement of true clinical end points (e.g., myocardial infarction) before approval of drugs that lower cholesterol by novel mechanisms? The costs involved in such extensive and expensive trials must be borne somehow (see below). A follow-up 7-year study involving over 18,000 patients (IMPROVE-IT) vindicated the decision to approve *ezetimibe* (Jarcho and Keaney, 2015). Taken in conjunction with a statin, the drug significantly reduced the incidence of myocardial infarction and stroke in high-risk patients.

BOX 1–2 ■ A Late Surprise in the Search for a Blockbuster

Torcetrapib elevates HDL cholesterol (the "good cholesterol"). Higher levels of HDL cholesterol are statistically associated with (are a surrogate end point for) a lower incidence of myocardial infarction. Surprisingly, clinical administration of *torcetrapib* caused a significant *increase* in mortality from cardiovascular events, ending a development path of 15 years and $800 million. In this case, approval of the drug based on this secondary end point would have been a mistake (Cutler, 2007). A computational systems analysis suggested a mechanistic explanation of this failure (Xie et al., 2009).

No drug is totally safe; all drugs produce unwanted effects in at least some people at some dose. Many unwanted and serious effects of drugs occur so infrequently, perhaps only once in several thousand patients, that they go undetected in the relatively small populations (a few thousand) in the standard phase III clinical trial (see Table 1–1). To detect and verify that such comparatively rare effects are, in fact, drug-related would require administration of the drug to tens or hundreds of thousands of people during clinical trials, adding enormous expense and time to drug development and delaying access to potentially beneficial therapies. In general, the true spectrum and incidence of untoward effects become known only after a drug is released to the broader market and used by a large number of people (phase IV, postmarketing surveillance). Drug development costs and drug prices could be reduced substantially if the public were willing to accept more risk. This would require changing the way we think about a pharmaceutical company's liability for damages from an unwanted effect of a drug that was not detected in clinical trials deemed adequate by the FDA. Would the public accept a drug with extremely severe unwanted effects, including death, if its therapeutic effect were sufficiently unique and valuable? Such dilemmas are not simple and can become issues for great debate.

Several strategies exist to detect adverse reactions after marketing of a drug. Formal approaches for estimation of the magnitude of an adverse drug response include the follow-up or cohort study of patients who are receiving a particular drug; the case-control study, in which the frequency of drug use in cases of adverse responses is compared to controls; and meta-analysis of pre- and postmarketing studies. Voluntary reporting of adverse events has proven to be an effective way to generate an early signal that a drug may be causing an adverse reaction (Aagard and Hansen, 2009). The primary sources for the reports are responsible, alert physicians and third-party payers (pharmacy benefit managers, insurance companies); consumers also play important roles. Other useful sources are nurses, pharmacists, and students in these disciplines. In addition, hospital-based pharmacy and therapeutics committees and quality assurance committees frequently are charged with monitoring adverse drug reactions in hospitalized patients. In the U.S., the FDA sponsors MedWatch, a drug safety information and adverse event reporting program. The website for MedWatch (http://www.fda.gov/Safety/MedWatch/default.htm) provides simple forms for reporting and lists recent safety alerts about specific medications. In addition to online access, the service may be reached by calling 800-FDA-1088. Health professionals also may contact the pharmaceutical manufacturer, who is legally obligated to file reports with the FDA.

Personalized (Individualized, Precision) Medicine

Drug inventors strive to "fit" the drug to the individual patient. To realize the full potential of this approach, however, requires intimate knowledge of the considerable heterogeneity of both the patient population and the targeted disease process. Why does one antidepressant appear to ameliorate depression in a given patient, while another drug with the same or very similar presumed mechanism of action does not? Is this a difference in the patient's response to the drug; in patient susceptibility to the drug's unwanted effects; in the drug's ADME; or in the etiology of the depression? How much of this variability is attributable to environmental factors and possibly their interactions with patient-specific genetic variability? Recent advances, especially in genetics and genomics, provide powerful tools for understanding this heterogeneity. The single most powerful tool for unraveling these myriad mysteries is the ability to sequence DNA rapidly and economically. The cost of sequencing a human's genome is now less than $1000, about six orders of magnitude lower than at the start of the 21st century (National Human Genome Research Institute, 2021). The current focus is on the extraordinarily complex analysis of the enormous amounts of data now being obtained from many thousands of individuals, ideally in conjunction with deep knowledge of their phenotypic characteristics, especially including their medical histories. Readily measured biomarkers of disease are powerful adjuncts to DNA sequence information. Simple blood or other tests can be developed to monitor real-time progress or failure of treatment; many such examples already exist. Similarly, chemical, radiological, and genetic tests may be useful not only to monitor therapy but also to predict success or failure, anticipate unwanted effects of treatment, or appreciate pharmacokinetic variables that may require adjustments of dosage or choice of drugs. Such tests already play a significant role in the choice of drugs for cancer chemotherapy, and the list of drugs specifically designed to "hit" a mutated target in a specific cancer is growing. Such information is also becoming increasingly useful in the choice of patients for clinical trials of specific agents, thereby reducing the time required for such trials and their cost, to say nothing of better defining the patient population who may benefit from the drug. These important subjects are discussed in detail in Chapter 7, Pharmacogenetics, and in Chapter 8, Postmarketing Drug Safety.

Public Policy Considerations

The Pharmaceutical Industry Operates in a Capitalist Economy

Drugs can save lives, prolong lives, and improve the quality of people's lives. However, in a free-market economy, access to drugs is not equitable. Not surprisingly, there is tension between those who treat drugs as entitlements and those who view drugs as high-tech products of a capitalistic society. Supporters of the entitlement position argue that a constitutional right to healthcare should guarantee access to drugs, and they are critical of pharmaceutical companies and others who profit from the business of making and selling drugs. Free-marketeers point out that, without a profit motive, it would be difficult to generate the resources and innovation required for new drug development. Clearly, drug development is both a scientific process and a political one, and also a process about which attitudes can change quickly. Critics of the pharmaceutical industry frequently begin from the position that people (and animals) need to be protected from greedy and unscrupulous companies and scientists (Angell, 2015; Kassirer, 2005; Ryan and DeSanctis, 2005). In the absence of a government-controlled drug development enterprise, our current system relies predominantly on investor-owned pharmaceutical companies that, like other companies, have a profit motive and an obligation to shareholders. The price of prescription drugs causes great consternation among consumers, especially as many health insurers seek to control costs by choosing not to cover certain "brand-name" products (see below). Further, a few drugs (especially for treatment of cancer) have been introduced to the market in recent years at prices that greatly exceeded the costs of development, manufacture, and marketing of the product. Many of these products were discovered in government laboratories or in university laboratories supported by federal grants. The U.S. is the only large country that places no controls on drug prices and where price plays no role in the drug approval process. Many U.S. drugs cost much more in the U.S. than overseas; thus, U.S. consumers subsidize drug costs for the rest of the world, and they are irritated by that fact. The example of newer agents for the treatment of hepatitis C infection brings many conflicting priorities into perspective (Box 1–3).

BOX 1–3 ■ The Cost of Treating Hepatitis C

Infection with hepatitis C virus (HCV) is a chronic disease afflicting millions of people. Some suffer little from this condition; many others eventually develop cirrhosis or hepatocellular carcinoma. Who should be treated? The answer is unknown. Until recently, the treatment of choice for people with genotype 1 HCV involved year-long administration of an interferon (by injection) in combination with *ribavirin* and a protease inhibitor. Unwanted effects of this regimen are frequent and severe (some say worse than the disease); cure rates range from 50% to 75%. A newer treatment involves an oral tablet containing a combination of *sofosbuvir* and *ledipasvir* (see Chapter 63). Treatment usually requires daily ingestion of one tablet for 8 to 12 weeks; cure rates exceed 95%, and side effects are minimal.

Controversy surrounds the price of the treatment, about $1000/day. Some insurers refused to reimburse this high cost, relegating many patients to less effective, more toxic, but less expensive treatment. However, these third-party payers have negotiated substantial discounts of the price, based on the availability of a competing product. Is the cost exorbitant? Should insurers, rather than patients and their physicians, be making such important decisions?

Continued and excessive escalation of drug and other healthcare costs will bankrupt the healthcare system. The question of appropriate cost involves complex pharmacoeconomic considerations. What are the relative costs of the two treatment regimens? What are the savings from elimination of the serious sequelae of chronic HCV infection? How does one place value to the patient on the less toxic and more effective and convenient regimen? What are the profit margins of the company involved? Who should make decisions about costs and choices of patients to receive various treatments? How should we consider cases (unlike that for HCV) for which the benefits are quite modest, such as when a very expensive cancer drug extends life only briefly? One astute observer (and an industry critic of many drug prices) summarized the situation as follows: "great, important problem; wrong example."

The drug development process is long, expensive, and risky (see Figure 1–6 and Table 1–1). Consequently, drugs must be priced to recover the substantial costs of invention and development and to fund the marketing efforts needed to introduce new products to physicians and patients. Depending on the methods of accounting for sales and dispensing, prescription drugs account for 9% to 13% of total U.S. healthcare expenditures (Conti et al., 2021).

Although the increase in prices is significant in certain classes of drugs (e.g., anticancer agents), the total price of prescription drugs is growing at a slower rate than other healthcare costs. Even drastic reductions in drug prices that would severely limit new drug invention would not lower the overall healthcare budget by more than a few percent. Are profit margins excessive among the major pharmaceutical companies? There is no objective answer to this question. Pragmatic answers come from the markets and from company survival statistics. The U.S. free-market system provides greater rewards for particularly risky and important fields of endeavor, and many people argue that the rewards should be greater for those willing to take the risk. The pharmaceutical industry is clearly one of the more risky:

- The costs to bring products to market are enormous.
- The success rate of drug development is low (accounting for much of the cost).
- Accounting for the long development time, effective patent protection for marketing a new drug is only about a decade (see Intellectual Property and Patents).
- Regulation is stringent.
- Product liability is great.
- Competition is fierce.
- With mergers and acquisitions, the number of companies in the pharmaceutical world is shrinking.

Many feel that drug prices should be driven more by their therapeutic impact and their medical need, rather than by simpler free-market considerations; there is movement in this direction. There are many components and points of contention in estimating a drug's value (Schnipper et al., 2015), and, as a consequence, there is no well-accepted approach to answer the question of value.

Who Pays?

The cost of prescription drugs is borne by consumers ("out of pocket"), private insurers, and public insurance programs such as Medicare, Medicaid, and the State Children's Health Insurance Program (SCHIP). Some major retailers and mail-order pharmacies run by private insurers offer consumer incentives for purchase of generic drugs, and these initiatives have helped to contain the portion of household expenses spent on pharmaceuticals; however, more than one-third of total retail drug costs in the U.S. are paid with public funds—tax dollars. Healthcare in the U.S. is more expensive than everywhere else, but it is not, on average, demonstrably better than everywhere else. One way in which the U.S. system falls short is with regard to healthcare access. Although the Patient Protection and Affordable Care Act of 2010 ("Obamacare") has reduced the percentage of Americans without health insurance to a historic low, practical solutions to the challenge of providing healthcare for all who need it must recognize the importance of incentivizing innovation.

Intellectual Property and Patents

Drug invention produces intellectual property eligible for patent protection, protection that is enormously important for innovation. As noted by Abraham Lincoln, the only U.S. president to ever hold a patent (for a device to lift boats over shoals; never commercially produced):

> *Before* [patent laws]*, any man might instantly use what another had invented; so that the inventor had no special advantage from his own invention. The patent system changed this; secured to the inventor, for a limited time, the exclusive use of his invention; and thereby added the fuel of interest to the fire of genius, in the discovery and production of new and useful things.* (Lincoln, 1859)

The U.S. patent protection system provides protection for 20 years from the time the patent is filed. During this period, the patent owner of a drug has exclusive rights to market and sell the drug. When the patent expires, equivalent nonproprietary products can come on the market. A generic product must be therapeutically equivalent to the original, contain equal amounts of the same active chemical ingredient, and achieve equal concentrations in blood when administered by the same routes. These generic preparations are sold much more cheaply than the original drug and without the huge development costs borne by the original patent holder. The long time course of drug development, often more than 10 years (see Figure 1–6), reduces the time during which patent protection functions as intended. The Drug Price Competition and Patent Term Restoration Act of 1984 (Public Law 98-417, informally called the Hatch-Waxman Act) permits a patent holder to apply for extension of a patent term to compensate for delays in marketing caused by FDA approval processes; nonetheless, the average new drug brought to market now enjoys only about 10 to 12 years of patent protection. Some argue that patent protection for drugs should be shortened, so that earlier generic competition will lower healthcare costs. The counterargument is that new drugs would have to carry even higher prices to provide adequate compensation to companies during a shorter period of protected time. If that is true, lengthening patent protection would actually permit lower prices. Recall that patent protection is worth little if a superior competitive product is invented and brought to market.

Bayh-Dole Act

The Bayh-Dole Act (35 U.S.C. § 200) of 1980 created strong incentives for federally funded scientists at academic medical centers to approach drug invention with an entrepreneurial spirit. The act transferred intellectual property rights to the researchers and their respective institutions (rather than to the government) to encourage partnerships with industry that would bring new products to market for the public's benefit. While the need to protect intellectual property is generally accepted, this encouragement of public-private research collaborations has given rise to concerns about conflicts of interest by scientists and universities (Kaiser, 2009).

Biosimilars

The path to approval of a chemically synthesized small molecule that is identical to an approved compound whose patent protection has expired is relatively straightforward. The same is not true for large molecules (usually proteins), which are generally derived from a living organism (e.g., eukaryotic cell or bacterial culture). Covalent modification of proteins (e.g., glycosylation) or conformational differences may influence pharmacokinetics, pharmacodynamics, immunogenicity, or other properties, and demonstration of therapeutic equivalence may be a complex process. The Biologics Price Competition and Innovation Act was enacted as part of the Affordable Care Act in 2010. The intent was to implement an abbreviated licensure pathway for certain "similar" biological products. Biosimilarity is defined to mean "that the biological product is highly similar to a reference product notwithstanding minor differences in clinically inactive components" and that "there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product." In general, an application for licensure of a biosimilar must provide satisfactory data from analytical studies, animal studies, and one or more clinical studies. However, the interpretation of this language has involved endless discussion, and hard-and-fast rules seem unlikely.

Drug Promotion

In an ideal world, physicians would learn all they need to know about drugs from the medical literature, and good drugs would sell themselves. Instead, we have print advertising, visits from salespeople directed at physicians, and extensive direct-to-consumer advertising aimed at the public (in print, on the radio, and especially on television). There are roughly 80,000 pharmaceutical sales representatives in the U.S. who target about 10 times that number of physicians. The number of salespeople is down from about 100,000 in 2010, a decline possibly related to increased attention to conflicts of interest. The amount spent on promotion of drugs approximates or perhaps even exceeds that spent on research and development. Pharmaceutical companies have been especially vulnerable to criticism for some of their marketing practices (Angell, 2015). Promotional materials used by pharmaceutical companies cannot deviate from information contained in the FDA-approved package insert. In addition, there must be an acceptable balance between presentation of therapeutic claims for a product and discussion of unwanted effects. Such requirements notwithstanding, direct-to-consumer advertising of prescription drugs remains controversial and is permitted only in the U.S. and New Zealand. Canada allows a modified form of advertising in which either the product or the indication can be mentioned, but not both. The efficacy of direct televised marketing is measurable (Gray et al., 2020; Sullivan et al., 2021), and physicians frequently succumb, albeit with misgivings, to patients' advertising-driven requests for specific medications.

The counterargument to such marketing is that patients are educated by such marketing efforts and are thereby encouraged to seek medical care, especially for conditions (e.g., depression) that they may have been denying (Avery et al., 2012). A major criticism of drug marketing involves some of the unsavory approaches used to influence physician behavior. Gifts of value (e.g., sports tickets) are now forbidden, but dinners where drug-prescribing information is presented by non–sales representatives are widespread. Large numbers of physicians are paid as "consultants" to make presentations in such settings. The acceptance of any gift, no matter how small, from a drug company by a physician is now forbidden at many academic medical centers and by law in several states. In 2009, the board of directors of the Pharmaceutical Research and Manufacturers of America (PhRMA) adopted an enhanced *Code on Interactions With Healthcare Professionals* that prohibits the distribution of noneducational items, prohibits company sales representatives from providing restaurant meals to healthcare professionals (although exceptions are granted when a third-party speaker makes the presentation), and requires companies to ensure that their representatives are trained about laws and regulations that govern interactions with healthcare professionals.

Concerns About Global Injustice

Drug discovery is expensive (see Table 1–1), and economic realities influence the direction of pharmaceutical research. For example, investor-owned companies generally cannot afford to develop products for rare diseases or for diseases that are common only in economically underdeveloped parts of the world. Funds to invent drugs targeting rare diseases or diseases primarily affecting developing countries (e.g., malaria, schistosomiasis, and other parasitic diseases) often come from taxpayers or wealthy philanthropists.

Because development of new drugs is so expensive, private-sector investment in pharmaceutical innovation has focused on products that will have lucrative markets in wealthy countries such as the U.S., which combines patent protection with a free-market economy. Accordingly, there is concern about the degree to which U.S. and European patent protection laws have restricted access to potentially lifesaving drugs in developing countries. To lower costs, pharmaceutical companies increasingly test their experimental drugs outside the U.S. and the E.U., in developing countries where there is less regulation and easier access to large numbers of patients. According to the U.S. DHHS, there has been a 2000% increase in foreign trials of U.S. drugs over the past 25 years. When these drugs are successful in obtaining marketing approval, consumers in the countries where the trials were conducted often cannot afford them. Some ethicists have argued that this practice violates the justice principle articulated in the Belmont Report (DHHS, 1979, p. 10), which states that "research should not unduly involve persons from groups unlikely to be among the beneficiaries of subsequent applications of the research." A counterargument is that the conduct of trials in developing nations also frequently brings needed medical attention to underserved populations. This is another controversial issue.

Product Liability

Product liability laws are intended to protect consumers from defective products. Pharmaceutical companies can be sued for faulty design or manufacturing, deceptive promotional practices, violation of regulatory requirements, or failure to warn consumers of known risks. Failure-to-warn claims can be made against drug makers even when the product is approved by the FDA. With greater frequency, courts are finding companies that market prescription drugs directly to consumers responsible when advertisements fail to provide an adequate warning of potential adverse effects. Although injured patients are entitled to pursue legal remedies, the negative effects of product liability lawsuits against pharmaceutical companies may be considerable. First, fear of liability may cause pharmaceutical companies to be overly cautious about testing, thereby delaying access to the drug. Second, the cost of drugs increases for consumers when pharmaceutical companies increase the length and number of trials they perform to identify even the smallest risks and when regulatory agencies increase the number or intensity of regulatory reviews. Third, excessive liability costs create disincentives for development of so-called orphan drugs, pharmaceuticals that benefit a small number of patients. Should pharmaceutical companies be liable for failure to warn when all of the rules were followed, and the product was approved by the FDA but the unwanted effect was not detected because of its rarity or another confounding factor? The only way to find "all" of the unwanted effects that a drug may have is to market it—to conduct a phase IV "clinical trial" or observational study. This basic friction between risk to patients and the financial risk of drug development does not seem likely to be resolved except on a case-by-case basis, in the courts. The U.S. Supreme Court added further fuel to these fiery issues in 2009 in the case *Wyeth v. Levine*. A patient (Levine) suffered gangrene of an arm following inadvertent arterial administration of the antinausea drug *promethazine*, subsequently losing her hand. The healthcare provider had intended to administer the drug by so-called intravenous push. The FDA-approved label for the drug warned against, but did not prohibit, administration by intravenous push. The state court and then the U.S. Supreme Court held both the healthcare provider and the company liable for damages. Specifically, the Vermont court found that Wyeth, the drug's maker, had inadequately labeled the drug. This means that FDA approval of the label does not protect a company from liability or prevent individual states from imposing regulations more stringent than those required by the federal government.

"Me Too" Versus True Innovation: The Pace of New Drug Development

As noted previously, the term *me-too drug* describes a pharmaceutical that is usually structurally similar to a drug already on the market. Other names used are derivative medications, molecular modifications, and follow-up drugs. In some cases, a me-too drug is a different molecule developed deliberately by a competitor company to take market share from the company with existing drugs on the market. When the market for a class of drugs is especially large, several companies can share the market and make a profit. Other me-too drugs result coincidentally from the simultaneous development of products by several companies without knowing which drugs will be approved for sale (Box 1–4). There are

BOX 1–4 ■ A Not-So-New Drug

Some me-too drugs are only slightly altered formulations of a company's own drug, packaged and promoted as if really offering something new. An example is the heartburn medication *esomeprazole*, marketed by the same company that makes *omeprazole*. *Omeprazole* is a mixture of two stereoisomers; *esomeprazole* contains only one of the isomers and is eliminated less rapidly. Development of *esomeprazole* created a new period of market exclusivity, although generic versions of *omeprazole* are marketed, as are branded congeners of *omeprazole/esomeprazole*. Both *omeprazole* and *esomeprazole* are now available over the counter—narrowing the previous price difference.

valid criticisms of me-too drugs. First, an excessive emphasis on profit may stifle true innovation. Second, some me-too drugs are more expensive than the older versions they seek to replace, increasing the costs of healthcare without corresponding benefit to patients. Nevertheless, for some patients, me-too drugs may have better efficacy or fewer side effects or promote compliance with the treatment regimen. For example, the me-too drug that can be taken once a day rather than more frequently is convenient and promotes compliance. Some me-too drugs add great value from a business and medical point of view. For instance, *atorvastatin* was the seventh statin to be introduced to market; it subsequently became the best-selling drug in the world. Critics argue that pharmaceutical companies are not innovative and do not take risks and, further, that medical progress is actually slowed by their excessive concentration on me-too products.

Figure 1–7 summarizes a few of the facts behind this and other arguments. Clearly, only a modest number of new molecular entities (NMEs), about two dozen a year, achieved FDA approval between 1980 and 2016. In recent years (2017 through 2021), the annual number of approved NMEs has averaged 38 (and approval of biologicals has averaged 13 a year). Thus, there is a recent uptick in FDA-approved NMEs and BLAs. However, from 1980 to 2021, the industry's annual investment in research and development grew 45-fold, from $2 billion to $91 billion. This disconnect between research and development investment and new drugs approved occurred at a time when combinatorial chemistry was blooming, the human genome was being sequenced, highly automated techniques of screening were being developed, and new techniques of molecular biology and genetics were offering novel insights into the pathophysiology of human disease. A continued increase in productivity will be needed to sustain today's pharmaceutical companies as they face waves of patent expirations. There are strong arguments that development of much more targeted, individualized drugs, based on a new generation of molecular diagnostic techniques and improved understanding of disease in individual patients, will improve both medical care and the survival of pharmaceutical companies. Many of the advances in genetics and molecular biology are still new, particularly when measured in the time frame required for drug development. Moreover, the techniques of computational chemistry and computerized drug design described earlier in this chapter are still advancing and being integrated into the process. One can hope that modern molecular medicine will sustain the development of more efficacious and more specific pharmacological treatments for an ever-wider spectrum of human diseases.

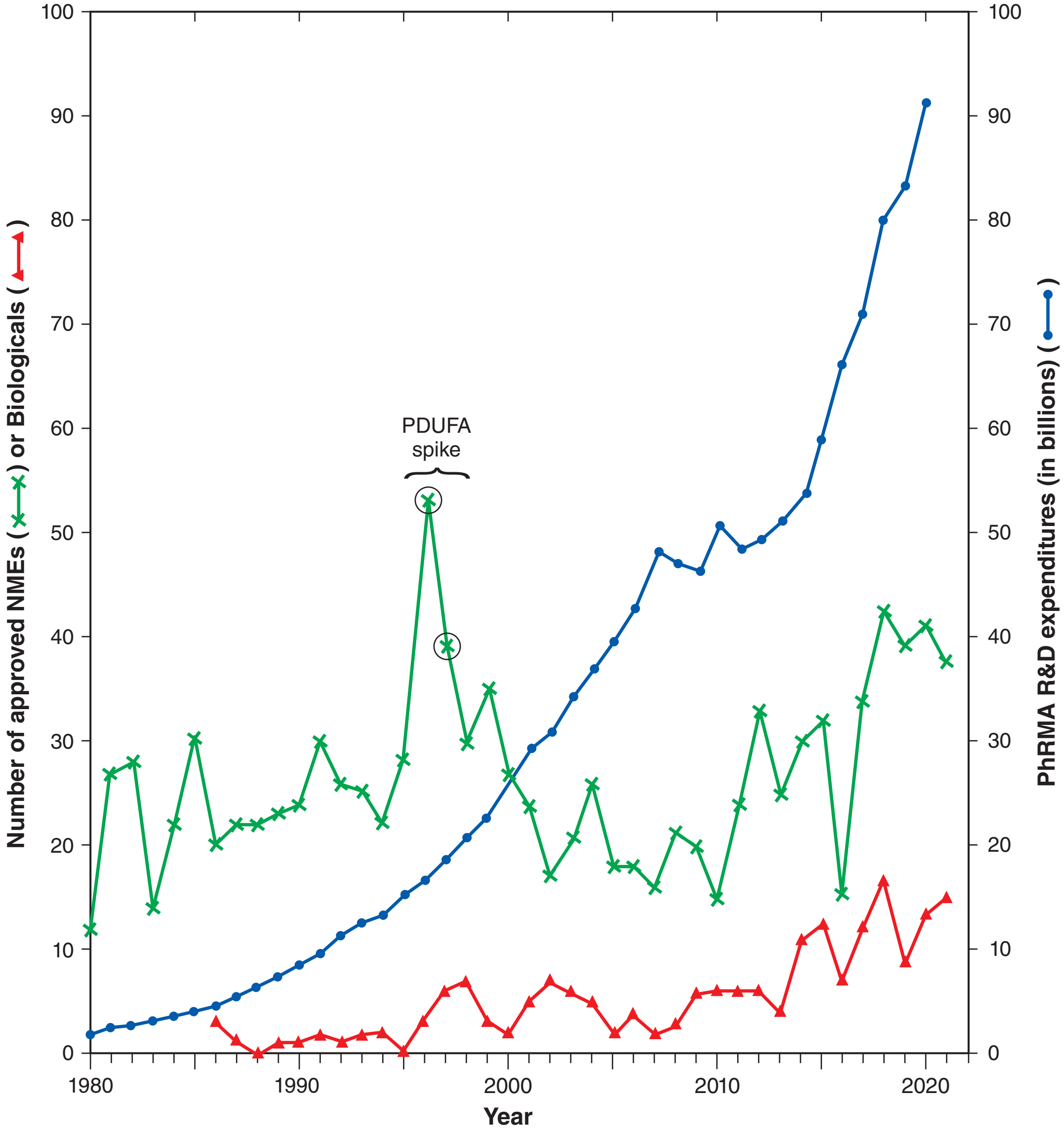


Figure 1–7 *The cost of drug invention is rising. Is productivity rising accordingly?* Tally includes enzymes, antibodies, peptides, and small molecules and excludes vaccines and blood products. *Sources*: Center for Drug Evaluation and Research, 2022; Congressional Budget Office, 2021; McClung, 2021; Mullard, 2022.

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Chapter 2

Pharmacokinetics: The Dynamics of Drug Absorption, Distribution, Metabolism, and Elimination

Iain L. O. Buxton

PASSAGE OF DRUGS ACROSS MEMBRANE BARRIERS

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THERAPEUTIC DRUG MONITORING

The human body restricts access to foreign molecules; therefore, to reach its target within the body and have a therapeutic effect, a drug molecule must cross several restrictive barriers en route to its target site. Following administration, the drug must be absorbed and distributed, usually via vessels of the circulatory and lymphatic systems. In addition to crossing membrane barriers, the drug must survive metabolism (primarily hepatic) and elimination (by the kidney and liver and in the feces). ADME, the absorption, distribution, metabolism, and elimination of drugs, are the processes of *pharmacokinetics* (Figure 2–1). Understanding these processes and their interplay and employing pharmacokinetic principles increase the probability of therapeutic success and reduce the occurrence of adverse drug events and drug-drug interactions.

The absorption, distribution, metabolism, and excretion of a drug involve its passage across numerous cell membranes. Mechanisms by which drugs cross membranes and the physicochemical properties of molecules and membranes that influence this transfer are critical to understanding the disposition of drugs in the human body. The characteristics of a drug that predict its movement and availability at sites of action are its molecular size (i.e., molecular weight) and structural features, degree of ionization, the relative lipid solubility of its ionized and nonionized forms, and its binding to serum and tissue proteins. Although physical barriers to drug movement may be a single layer of cells (e.g., intestinal epithelium) or several layers of cells and associated extracellular protein (e.g., skin), the plasma membrane is the basic barrier.

Passage of Drugs Across Membrane Barriers

The Plasma Membrane Is Selectively Permeable

The plasma membrane consists of a bilayer of amphipathic lipids with their hydrocarbon chains oriented inward to the center of the bilayer to form a continuous hydrophobic phase, with their hydrophilic heads oriented outward. Individual lipid molecules in the bilayer vary according to the particular membrane and can move laterally and organize themselves into microdomains (e.g., regions with sphingolipids and cholesterol, forming lipid rafts), endowing the membrane with fluidity, flexibility, functional organization, high electrical resistance, and relative impermeability to highly polar molecules. Membrane proteins embedded in the bilayer serve as structural anchors, receptors, ion channels, or transporters to transduce electrical or chemical signaling pathways and provide selective targets for drug actions. Far from being a sea of lipids with proteins floating randomly about, membranes are ordered and compartmented (Banani et al., 2017; Kitamata et al., 2020) with structural scaffolding elements linking to the cell interior. Membrane proteins may be associated with caveolin and sequestered within caveolae, excluded from caveolae, or organized in signaling domains rich in cholesterol and sphingolipids not containing caveolin or other scaffolding proteins.

Modes of Permeation and Transport

Passive diffusion dominates transmembrane movement of most drugs. However, carrier-mediated mechanisms (*active transport* and *facilitated diffusion*) play important roles (Figure 2–2; Figure 4–4).

Passive Diffusion

In passive transport, the drug molecule usually penetrates by diffusion along a concentration gradient by virtue of its solubility in the lipid bilayer. Such transfer is directly proportional to the magnitude of the concentration gradient across the membrane, to the lipid:water partition coefficient of the drug, and to the membrane surface area exposed to the drug. At steady state, the concentration of the unbound drug is the same on both sides of the membrane if the drug is a nonelectrolyte. For ionic compounds, the steady-state concentrations depend on the electrochemical gradient for the ion and on differences in pH across the membrane, which will influence the state of ionization of the molecule disparately on either side of

Abbreviations

ABC: ATP-binding cassette
ACE: angiotensin-converting enzyme
AUC: area under the concentration-time curve of drug absorption and elimination
BBB: blood-brain barrier
***CL*:** clearance
CNS: central nervous system
CNT1: concentrative nucleoside transporter 1
C_p: plasma concentration
CSF: cerebrospinal fluid
C_{ss}: steady-state concentration
CYP: cytochrome P450
***F*:** bioavailability
FDA: Food and Drug Administration
GI: gastrointestinal
h: hours
k: a rate constant
MDR1: multidrug resistance protein
MEC: minimum effective concentration
min: minutes
PLLR: Pregnancy and Lactation Labeling Rule
SLC: solute carrier
T, t: time
$t_{1/2}$: half-life
***V*:** volume of distribution
V_{ss}: volume of distribution at steady state

the membrane and can effectively trap ionized drug on one side of the membrane.

Influence of pH on Ionizable Drugs

Many drugs are weak acids or bases that are present in solution as both the lipid-soluble, diffusible nonionized form and the ionized species that is relatively lipid insoluble and poorly diffusible across a membrane. Common ionizable groups are carboxylic acids and amino groups (primary, secondary, and tertiary; quaternary amines hold a permanent positive charge). The transmembrane distribution of a weak electrolyte is influenced by its pK_a and the pH gradient across the membrane. The pK_a is the pH at which half the drug (weak acid or base electrolyte) is in its ionized form. The ratio of nonionized to ionized drug at any pH may be calculated from the Henderson-Hasselbalch equation:

$$\log \frac{[\text{protonated form}]}{[\text{unprotonated form}]} = pK_a - \text{pH} \qquad \text{(Equation 2–1)}$$

Equation 2–1 relates the pH of the medium around the drug and the drug's acid dissociation constant (pK_a) to the ratio of the protonated (HA or BH^+) and unprotonated (A^- or B) forms, where

$$HA \leftrightarrow A^- + H^+, \text{ where } K_a = \frac{[A^-][H^+]}{[HA]}$$

describes the dissociation of an acid, and

$$BH^+ \leftrightarrow B + H^+, \text{ where } K_a = \frac{[B][H^+]}{[BH^+]}$$

describes the dissociation of the protonated form of a base.

At steady state, an acidic drug will accumulate on the more basic side of the membrane and a basic drug on the more acidic side. This phenomenon, known as *ion trapping*, is an important process in drug distribution with potential therapeutic benefit and in management of the poisoned patient (Ornillo and Harbord, 2020). Figure 2–3 illustrates this effect and shows the calculated values for the distribution of a weak acid between the plasma and gastric compartments.

The effects of pH on transmembrane partitioning can be utilized to alter drug excretion. In the kidney tubules, urine pH can vary over a wide range, from 4.5 to 8. As urine pH drops (as $[H^+]$ increases), weak acids (A^-) and weak bases (B) will exist to a greater extent in their protonated forms (HA and BH^+); the reverse is true as pH rises, where A^- and B will be favored. Thus, alkaline urine favors excretion of weak acids; acidic urine favors excretion of weak bases. Elevation of urine pH (by giving sodium bicarbonate) will promote urinary excretion of weak acids such as aspirin (pK_a ~3.5) and urate (pK_a ~5.8). Another useful consequence of a drug being ionized at physiological pH is illustrated by the relative lack of sedative effects of second-generation histamine H_1 antagonists (e.g., *loratadine*): Second-generation antihistamines are ionized molecules (less lipophilic, more hydrophilic) that poorly cross the BBB compared to first-generation agents such as diphenhydramine, which are now used as sleep aids. Also of note, most bacterial urinary tract infections cause the urine to become alkaline, potentially altering therapy (Huang et al., 2020).

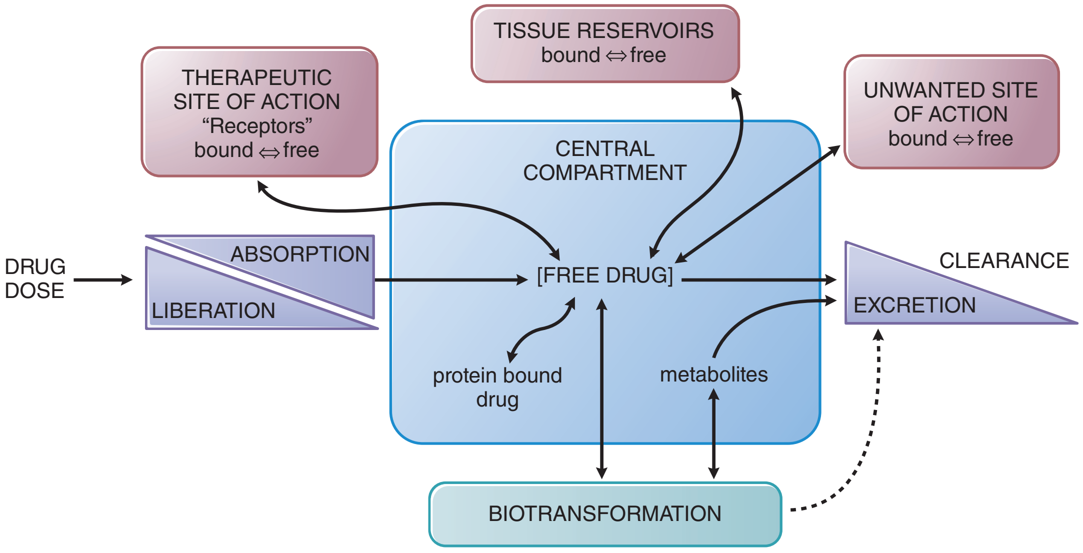

Figure 2–1 *The interrelationship of the absorption, distribution, binding, metabolism, and excretion of a drug and its concentration at its sites of action.* Possible distribution and binding of metabolites in relation to their potential actions at receptors are not depicted.

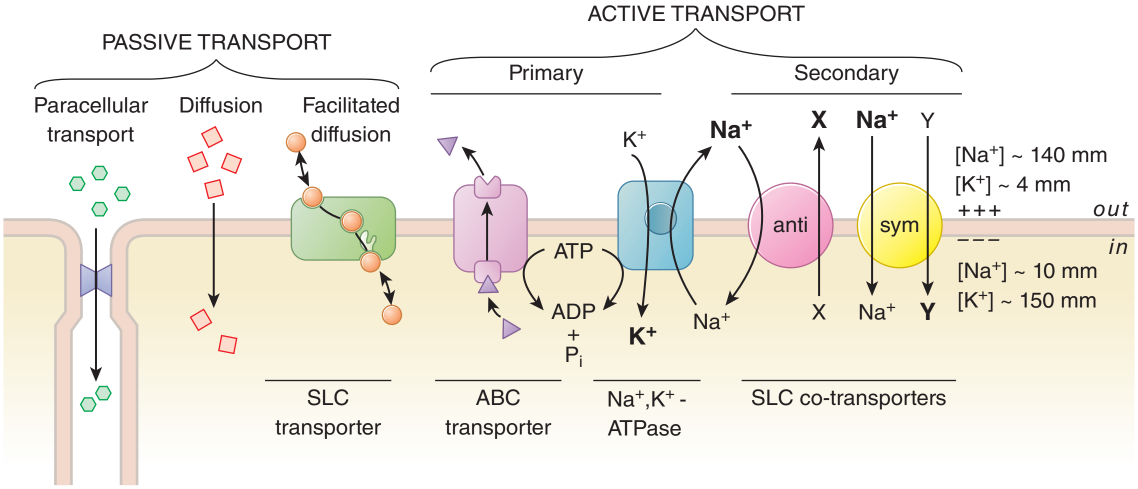

Figure 2–2 *Drugs move across membrane and cellular barriers in a variety of ways.* See details in Figures 4–1 through 4–4.

Carrier-Mediated Membrane Transport

Proteins in the plasma membrane mediate transmembrane movements of many physiological solutes; these proteins also mediate transmembrane movements of drugs and can be targets of drug action. Mediated transport is broadly characterized as *facilitated diffusion* or *active transport* (see Figure 2–2; Figure 4–4). Membrane transporters and their roles in drug response are presented in detail in Chapter 4.

Facilitated Diffusion. *Facilitated diffusion* is a carrier-mediated transport process in which the driving force is simply the electrochemical gradient of the transported solute; thus, these carriers can facilitate solute movement either in or out of cells, depending on the direction of the electrochemical gradient. The carrier protein may be highly selective for a specific conformational structure of an endogenous solute or a drug whose rate of transport by passive diffusion through the membrane would otherwise be quite slow. For instance, the organic cation transporter OCT1 (SLC22A1) facilitates the movement of a physiologic solute, thiamine (Jensen et al., 2020), and drugs, including *metformin*, which is used in treating type 2 diabetes. Chapter 4 describes OCT1 and other members of the human SLC superfamily of transporters.

Active Transport. *Active transport* is characterized by a direct requirement for energy, capacity to move solute against an electrochemical gradient, saturability, selectivity, and competitive inhibition by cotransported compounds. Na^+/K^+-ATPase is an important example of an active transport mechanism, which simultaneously exports three sodium ions in exchange for two potassium ions using ATP as the energy substrate. *Digoxin* is an important Na^+/K^+-ATPase inhibitor used in the treatment of heart failure (see Chapter 29). A group of primary active transporters, the ABC family, hydrolyze ATP to export substrates across membranes. For example, the P-glycoprotein, also called ABCB1 or MDR1, exports bulky neutral or cationic compounds from cells; its physiologic substrates include steroid hormones such as testosterone and progesterone. MDR1 exports many drugs as well, including *digoxin*, and a great variety of other agents (see Table 4–4). P-glycoprotein in the enterocyte limits the absorption of some orally administered drugs by exporting compounds into the lumen of the GI tract subsequent to their absorption (Gessner et al., 2019). ABC transporters perform a similar function in the cells of the BBB, effectively reducing net accumulation of some compounds in the brain (see Chapters 4 and 17). By the same mechanism, P-glycoprotein also can confer resistance to some cancer chemotherapeutic agents (see Chapters 69–73).

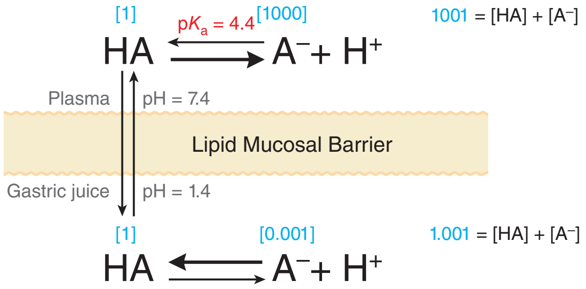

Figure 2–3 *Influence of pH on the distribution of a weak acid (pK_a = 4.4) between plasma and gastric juice separated by a lipid barrier.* **A weak acid dissociates to different extents** in plasma (pH 7.4) and gastric acid (pH 1.4): The higher pH facilitates dissociation; the lower pH reduces dissociation. The uncharged form, HA, equilibrates across the membrane. Blue numbers in brackets show relative equilibrium concentrations of HA and A^-, as calculated from Equation 2–1.

Members of the SLC superfamily can mediate secondary active transport using the electrochemical energy stored in a gradient (usually Na^+) to translocate both biological solutes and drugs across membranes. For instance, the Na^+/Ca^{2+} exchange protein (SLC8 or NCX) uses the energy stored in the Na^+ gradient established by Na^+/K^+-ATPase to export cytosolic Ca^{2+} and maintain it at a low basal level, about 100 nM in most cells. SLC8 is thus an *antiporter*, using the inward flow of Na^+ to drive an outward flow of Ca^{2+}. SLC8 also helps to mediate the positive inotropic effects of digoxin and other cardiac glycosides that inhibit the activity of Na^+/K^+-ATPase and thereby reduce the driving force for the extrusion of Ca^{2+} from the ventricular cardiac myocyte. Other SLC cotransporters are *symporters*, in which the driving force ion and solute move in the same direction. The CNT1 (SLC28A1), driven by the Na^+ gradient, moves pyrimidine nucleosides and the cancer chemotherapeutic agents *gemcitabine* and *cytarabine* into cells. DAT, NET, and SERT, transporters for the neurotransmitters dopamine, norepinephrine, and serotonin, respectively, are secondary active transporters that also rely on the energy stored in the transmembrane Na^+ gradient. These symporters coordinate movement of Na^+ and neurotransmitter in the same direction (into the neuron). DAT, NET, and SERT are also the targets of CNS-active agents used to treat depression and/or anxiety. Members of the SLC superfamily are active in drug transport in the GI tract, liver, and kidney, among other sites, and play a significant role in drug disposition (Liu, 2019).

Paracellular Transport

In the vascular compartment, paracellular passage of solutes and fluid through intercellular gaps is sufficiently large that passive transfer across the endothelium of capillaries and postcapillary venules is generally limited by blood flow. Evidence of this phenomenon can readily be seen in the dependent edema that forms in the ankles of heart failure patients. Capillaries of the CNS and a variety of epithelial tissues have tight junctions that limit paracellular movement of drugs (Spector et al., 2015).

Drug Absorption, Bioavailability, and Routes of Administration

Absorption and Bioavailability

Absorption is the movement of a drug from its site of administration into the central compartment (e.g., bloodstream; see Figure 2–1). For solid dosage forms, absorption first requires dissolution of the tablet or capsule, thus liberating the drug. Except in cases of malabsorption syndromes, the clinician is concerned primarily with bioavailability rather than absorption (Tran et al., 2013).

Bioavailability describes the fractional extent to which an administered dose of drug reaches its site of action or a biological fluid (usually the systemic circulation) from which the drug has access to its site of action. A drug given orally must be absorbed first from the GI tract, but net absorption may be limited by the characteristics of the dosage form, by the drug's physicochemical properties, by metabolic attack in the intestine, and by transport across the intestinal epithelium and into the portal circulation. The absorbed drug then passes through the liver, where metabolism and biliary excretion may occur before the drug enters the systemic circulation. Accordingly, less than all of the administered dose may reach the systemic circulation and be distributed to the drug's sites of action. If the metabolic or excretory capacity of the liver and the intestine for the drug is large, bioavailability will be reduced substantially (*first-pass effect*). This decrease in availability is a function of the anatomical site from which absorption takes place; for instance, intravenous administration generally permits all of a drug to enter the systemic circulation. Other anatomical, physiological, and pathological factors can influence bioavailability (described further in this chapter), and the route of drug administration should be chosen based on an understanding of these conditions. We can define bioavailability *F* as:

$$F = \frac{\text{Quantity of drug reaching systemic circulation}}{\text{Quantity of drug administered}} \qquad \text{(Equation 2–2)}$$

where $0 < F \leq 1$.

Factors modifying bioavailability also apply to prodrugs, in which case availability results from metabolic processes that produce the active form of the drug.

Routes of Administration

Some characteristics of the major administration routes employed for systemic drug effect are compared in Table 2–1.

Oral Administration

Oral ingestion is the most common method of drug administration. It also is the safest, most convenient, and most economical. Its disadvantages include limited absorption of some drugs because of their physical characteristics (e.g., low water solubility or poor membrane permeability), emesis as a result of irritation to the GI mucosa, destruction of some drugs by digestive enzymes or low gastric pH, irregularities in absorption or propulsion in the presence of food or other drugs, and the need for cooperation on the part of the patient. In addition, drugs in the GI tract may be metabolized by the enzymes of the intestinal microbiome, mucosa, or liver before they gain access to the general circulation. The gut microbiome comprises over 1000 species; its alteration may impact disease progression and therapeutic outcomes (see Chapter 6 and Ding et al., 2020).

Absorption from the GI tract is governed by factors such as surface area for absorption; blood flow to the site of absorption; the physical state of the drug (solution, suspension, or solid dosage form); its aqueous solubility; and the drug's concentration at the site of absorption. For drugs given in solid form, the rate of dissolution may limit their absorption. Because most drug absorption from the GI tract occurs by passive diffusion, absorption is favored when the drug is in the nonionized, more lipophilic form. Based on the pH-partition concept (see Figure 2–3), one would predict that drugs that are weak acids would be better absorbed from the stomach (pH 1–2) than from the upper intestine (pH 3–6), and vice versa for weak bases. However, the surface area of the stomach is relatively small, and a mucus layer covers the gastric epithelium. By contrast, the villi of the upper intestine provide an extremely large surface area (~200 m^2). Accordingly, the rate of absorption of a drug from the intestine will be greater than that from the stomach even if the drug is predominantly ionized in the intestine and largely nonionized in the stomach.

TABLE 2–1 ■ SOME CHARACTERISTICS OF COMMON ROUTES OF DRUG ADMINISTRATION[a]

ROUTE AND BIOAVAILABILTY (*F*)	ABSORPTION PATTERN	SPECIAL UTILITY	LIMITATIONS AND PRECAUTIONS
Intravenous *F* = 1 by definition	Absorption circumvented	Valuable for emergency use	Increased risk of adverse effects
	Potentially immediate effects	Permits titration of dosage	Must inject solutions *slowly* as a rule
	Suitable for large volumes and for irritating substances, or complex mixtures, when diluted	Usually required for high-molecular-weight protein and peptide drugs	Not suitable for oily solutions or poorly soluble substances
Subcutaneous $0.75 < F < 1$	Prompt from aqueous solution	Suitable for some poorly soluble suspensions and for instillation of slow-release implants	Not suitable for large volumes
	Slow and sustained from repository preparations		Possible pain or necrosis from irritating substances
Intramuscular $0.75 < F < 1$	Prompt from aqueous solution	Suitable for moderate volumes, oily vehicles, and some irritating substances	Precluded during anticoagulant therapy
	Slow and sustained from repository preparations	Appropriate for self-administration (e.g., insulin)	May interfere with interpretation of certain diagnostic tests (e.g., creatine kinase)
Oral ingestion $0.05 < F < 1$	Variable, depends on many factors (see text)	Most convenient and economical; usually safer	Requires patient compliance
			Bioavailability potentially erratic and incomplete

[a] See text for more complete discussion and for other routes.

Thus, any factor that accelerates gastric emptying (recumbent position right side) will generally increase the rate of drug absorption, whereas any factor that delays gastric emptying will have the opposite effect. The gastric emptying rate is influenced by numerous factors, including the caloric content of food; volume, osmolality, temperature, and pH of ingested fluid; diurnal and interindividual variation; metabolic state (rest or exercise); and the ambient temperature. Gastric emptying is influenced in women by the effects of estrogen (i.e., compared to men, emptying is slower for premenopausal women and those taking estrogen replacement therapy).

Drugs that are destroyed by gastric secretions and low pH or that cause gastric irritation sometimes are administered in dosage forms with an enteric coating that prevents dissolution in the acidic gastric contents. Enteric coatings are useful for drugs that can cause gastric irritation and for presenting a drug such as mesalamine to sites of action in the ileum and colon (see Figure 55–4).

Controlled-Release Preparations. The rate of absorption of a drug administered as a tablet or other solid oral dosage form is partly dependent on its rate of dissolution in GI fluids. This is the basis for *controlled-release, extended-release, sustained-release,* and *prolonged-action* pharmaceutical preparations that are designed to produce slow, uniform absorption of the drug for 8 h or longer. Potential advantages of such preparations are reduction in the frequency of administration compared with conventional dosage forms (often with improved compliance by the patient), maintenance of a therapeutic effect overnight, and decreased incidence and intensity of undesired effects (by dampening of the peaks in drug concentration) and nontherapeutic blood levels of the drug (by elimination of troughs in concentration) that often occur after administration of immediate-release dosage forms. Controlled-release dosage forms are most appropriate for drugs with short half-lives ($t_{1/2}$ <4 h) or in select patient groups, such as those receiving antiepileptic or antipsychotic agents (Bera, 2014). Women have also benefited from long-acting hormonal contraception produced by implanted (Friend, 2016) or oral (Conley et al., 2006) controlled-release devices.

Sublingual Administration. Absorption from the oral mucosa has special significance for certain drugs despite the fact that the surface area available is small. Venous drainage from the mouth directly enters the superior vena cava, thus bypassing the portal circulation. As a consequence, a drug held sublingually and absorbed from that site is protected from rapid intestinal and hepatic first-pass metabolism. For example, sublingual nitroglycerin (see Chapter 31) is rapidly effective because it is nonionic, has high lipid solubility, and is not subject to the first-pass effect prior to reaching the heart and regions of the circulatory system where nitroglycerin acts to relieve myocardial ischemia.

Parenteral Injection

Parenteral (i.e., not via the GI tract) injection of drugs has distinct advantages over oral administration. In some instances, parenteral administration is essential for delivery of a drug in its active form, as in the case of monoclonal antibodies and vaccine formulations. Availability is usually more rapid, extensive, and predictable when a drug is given by injection; the effective dose can be delivered more accurately to a precise dose; this route is suitable for the loading dose of medications prior to initiation of oral maintenance dosing (e.g., *digoxin*). In emergency therapy and when a patient is unconscious, uncooperative, or unable to retain anything given by mouth, parenteral therapy may be necessary. Parenteral administration also has disadvantages: Asepsis must be maintained, especially when drugs are given over time (e.g., intravenous or intrathecal administration); pain may accompany the site of injection; and it is sometimes difficult for patients to perform the injections themselves if self-medication is necessary.

The major routes of parenteral administration are intravenous, subcutaneous, and intramuscular. Absorption from subcutaneous and intramuscular sites occurs by simple diffusion along the gradient from drug depot to plasma. The rate is limited by the area of the absorbing capillary membranes and by the solubility of the substance in the interstitial fluid. Relatively large aqueous channels in the endothelial layer account for the indiscriminate diffusion of molecules regardless of their lipid solubility. Larger molecules, such as proteins, slowly gain access to the circulation by way of lymphatic channels. Drugs administered into the systemic circulation by any route, excluding the intra-arterial route, are subject to possible first-pass elimination in the lung prior to distribution to the rest of the body. The lungs also serve as a filter for particulate matter that may be given intravenously and provide a route of elimination for volatile substances.

Intravenous. Factors limiting absorption are circumvented by intravenous injection of drugs in aqueous solution because bioavailability is complete ($F = 1.0$) and distribution is rapid. Also, drug delivery is controlled and achieved with an accuracy and immediacy not possible by any other procedures. Certain irritating solutions can be given only in this manner because the drug, when injected slowly, is greatly diluted by the blood.

There are advantages and disadvantages to intravenous administration. Unfavorable reactions can occur because high concentrations of drug may be attained rapidly in plasma and tissues. There are therapeutic circumstances for which it is advisable to administer a drug by bolus injection (e.g., tissue plasminogen activator) and other circumstances where slower or prolonged administration of drug is advisable (e.g., antibiotics). Intravenous administration of drugs warrants careful determination of dose and close monitoring of the patient's response; once the drug is injected, there is often no retreat. Repeated intravenous injections depend on the ability to maintain a patent vein. Drugs in an oily vehicle, those that precipitate blood constituents or hemolyze erythrocytes, and drug combinations that cause precipitates to form *must not* be given intravenously.

Subcutaneous. Injection into a subcutaneous site can be done only with drugs that are not irritating to tissue; otherwise, severe pain, necrosis, and tissue sloughing may occur. The rate of absorption following subcutaneous injection of a drug is generally constant and slow, providing a sustained effect. Moreover, altering the period over which a drug is absorbed may be varied intentionally, as is accomplished with insulin for injection using particle size, protein complexation, and pH. The incorporation of a vasoconstrictor agent in a solution of a drug to be injected subcutaneously also retards absorption. Absorption of drugs implanted under the skin in a solid pellet form occurs slowly over a period of weeks or months; some hormones (e.g., contraceptives) are administered effectively in this manner.

Intramuscular. Absorption of drugs in aqueous solution after intramuscular injection depends on the rate of blood flow to the injection site and can be relatively rapid. Absorption may be modulated to some extent by local heating, massage, or exercise. Generally, the rate of absorption following injection of an aqueous preparation into the deltoid or vastus lateralis is faster than when the injection is made into the gluteus maximus. The rate is particularly slower for females after injection into the gluteus maximus, a feature attributed to the different distribution of subcutaneous fat in males and females and because fat is relatively poorly perfused. Slow, constant absorption from the intramuscular site results if the drug is injected in solution in oil or suspended in various other repository (depot) vehicles.

Intra-arterial. Occasionally, a drug is injected directly into an artery to localize its effect in a particular tissue or organ, such as in the treatment of liver tumors and head and neck cancers. Diagnostic agents sometimes are administered by this route (e.g., technetium-labeled human serum albumin). Inadvertent intra-arterial administration can cause serious complications and requires careful management (Ellis et al., 2015).

Intrathecal. The BBB and the blood-CSF barrier often preclude or slow the entrance of drugs into the CNS, reflecting the activity of P-glycoprotein (MDR1) and other transporters to export xenobiotics from the CNS. Therefore, when local and rapid effects of drugs on the meninges or cerebrospinal axis are desired, as in spinal anesthesia, drugs sometimes are injected directly into the spinal subarachnoid space. Brain tumors or serious CNS infections also may be treated by direct intraventricular drug administration, increasingly through the use of specialized long-term indwelling reservoir devices (De Andrés et al., 2020). Injections into the CSF and epidural space are covered in chapters on analgesia and local anesthesia (Chapters 24 and 25, respectively).

Pulmonary Absorption

Gaseous and volatile drugs may be inhaled and absorbed through the pulmonary epithelium and mucous membranes of the respiratory tract. Access to the circulation is rapid by this route because the lung's surface area is large. In addition, solutions of drugs can be atomized and the fine droplets in air (aerosol) inhaled. Advantages are the almost instantaneous absorption of a drug into the blood, avoidance of hepatic first-pass loss, and in the case of pulmonary disease, local application of the drug at the desired site of action (see Chapters 24 and 44), as in the use of inhaled nitric oxide for pulmonary hypertension in term and near-term infants and adults (see Chapter 35).

Topical Application

Mucous Membranes. Drugs are applied to the mucous membranes of the conjunctiva, nasopharynx, oropharynx, vagina, colon, urethra, and urinary bladder primarily for their local effects. Absorption from these sites is generally excellent and may provide advantages for immunotherapy because vaccination of mucosal surfaces using mucosal vaccines provides the basis for generating protective immunity in both the mucosal and systemic immune compartments due to the presence of antigen-presenting cells in the tissues underlying mucosa such as stratum corneum (Li et al., 2020).

Eye. Topically applied ophthalmic drugs are used primarily for their local effects (see Chapter 69). The use of drug-loaded contact lenses and ocular inserts allows drugs to be better placed where they are needed for direct delivery.

Skin: Transdermal Absorption. Absorption of drugs able to penetrate the intact skin is dependent on the surface area over which they are applied and their lipid solubility (see Chapter 70). Systemic absorption of drugs occurs much more readily through abraded, burned, or denuded skin. Toxic effects result from absorption through the skin of highly lipid-soluble substances (e.g., a lipid-soluble insecticide in an organic solvent). Absorption through the skin can be enhanced by suspending the drug in an oily vehicle and rubbing the resulting preparation into the skin. Hydration of the skin with an occlusive dressing may be used to facilitate absorption. Controlled-release topical patches are increasingly available, with nicotine for tobacco-smoking withdrawal, *scopolamine* for motion sickness, *nitroglycerin* for angina pectoris, testosterone and estrogen for replacement therapy, various estrogens and progestins for birth control, and *fentanyl* for analgesia.

Rectal Administration

Approximately 50% of the drug that is absorbed from the rectum will bypass the liver, thereby reducing hepatic first-pass metabolism. However, rectal absorption can be irregular and incomplete, and certain drugs can cause irritation of the rectal mucosa. In certain clinical situations, rectal administration may be desirable, as in the use of opioids in hospice care.

Novel Methods of Drug Delivery

Drug-eluting stents and other devices such as microneedle drug delivery patches (Waghule et al., 2019) are being used to target drugs locally to maximize efficacy and minimize systemic exposure. Recent advances in drug delivery include the use of biocompatible polymers and nanoparticles for drug delivery (Lee et al., 2018; Santos et al., 2018).

Bioequivalence

Drug products are considered to be pharmaceutical equivalents if they contain the same active ingredients and are identical in strength or concentration, dosage form, and route of administration. Two pharmaceutically equivalent drug products are considered to be *bioequivalent* when the rates and extents of bioavailability of the active ingredient in the two products are not significantly different under suitable and identical test conditions. The availability of generic formulations of brand name drugs has increased access and affordability of drugs. Bioequivalence has made it possible for pharmacists to provide these alternatives when not restricted by physician choice. However, courts have not always found generic and brand name drugs to be legally equivalent or permitted the pharmacist to choose generic substitution (Sacks et al., 2021).

Distribution of Drugs

Not All Tissues Are Equal

Following absorption or systemic administration into the bloodstream, a drug distributes into interstitial and intracellular fluids as functions of the physicochemical properties of the drug, the rate of drug delivery to individual organs and compartments, and the differing capacities of those regions to interact with the drug. Cardiac output, regional blood flow, capillary permeability, and tissue volume affect the rate of delivery and amount of drug distributed into tissues (Table 2–2 and Figure 2–4). Initially, liver, kidney, brain, and other well-perfused organs receive most of the drug; delivery to muscle, most viscera, skin, and fat is slower. This second distribution phase may require minutes to several hours before the concentration of drug in tissue is in equilibrium with that in blood. The second phase also involves a far larger fraction of body mass (e.g., muscle) than does the initial phase and generally accounts for most of the extravascular distribution. With exceptions such as the brain, diffusion of drug into the interstitial fluid occurs rapidly because of the highly permeable nature of the capillary endothelium. Thus, tissue distribution is determined by the partitioning of drug between blood and the particular tissue.

Binding to Plasma Proteins

Many drugs circulate in the bloodstream bound to plasma proteins, and therapies based on drug:protein formulations have been advanced (Van de Sande et al., 2020). Albumin is a major carrier for acidic drugs; α_1-acid glycoprotein binds basic drugs. Nonspecific binding to other plasma proteins generally occurs to a much smaller extent. The binding is usually reversible. In addition, certain drugs may bind to proteins that function as specific hormone carrier proteins, such as the binding of estrogen or testosterone to sex hormone–binding globulin or the binding of thyroid hormone to thyroxin-binding globulin. Drug-protein binding can also be influenced by foods, beverages, herbal medicines, and dietary supplements (López-Yerena et al., 2020).

The fraction of total drug in plasma that is bound is determined by the drug concentration, the affinity of binding sites for the drug, and the

TABLE 2–2 ■ DISTRIBUTION OF BLOOD FLOW IN 70-KG MALE AT REST

	KIDNEYS	HEART	LIVER	BRAIN	SKELETAL MUSCLE	FAT	REMAINDER	Σ
Blood flow (mL/min)	1100	250	1500	800	900	250	500	5500
Mass (kg)	0.3	0.3	2.6	1.3	34	10	21.5	70
Flow/mass (mL/min/kg)	3667	833	654	615	26	25	23	
% Cardiac output	20	4.5	31	14.5	16.4	4.5	9.1	100

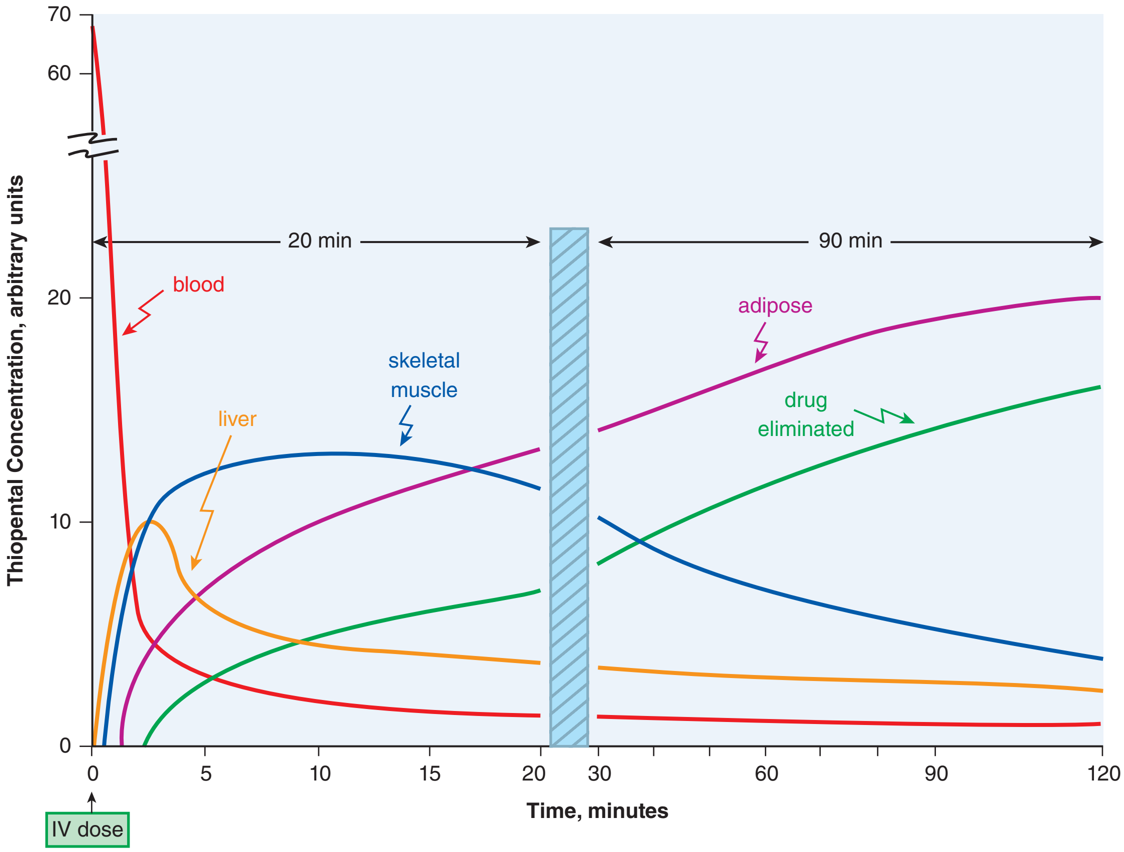

Figure 2–4 *Redistribution.* Curves depict the distribution of the barbiturate anesthetic *thiopental* into different body compartments following a single rapid intravenous dose. Note breaks and changes of scale on both axes. The drug level at *thiopental*'s site of action in the brain closely mirrors the plasma level of the drug. The rate of accumulation in the various body compartments depends on regional blood flow; the extent of accumulation reflects the differing capacities of the compartments and the steady but slow effect of elimination to reduce the amount of drug available. Emergence from the anesthetic influence of this single dose of *thiopental* relies on redistribution, not on metabolism. The drug will partition out of tissue depots as metabolism and elimination take their course. Depletion of compartments will follow the same order as accumulation, as a function of their perfusion.

concentration of available binding sites. For most drugs, the therapeutic range of plasma concentrations is limited; thus, the extent of binding and the unbound fraction are relatively constant. The extent of plasma protein binding also may be affected by disease-related factors (e.g., hypoalbuminemia). Conditions resulting in the acute-phase reaction response (e.g., cancer, arthritis, myocardial infarction, Crohn's disease) lead to elevated levels of α_1-acid glycoprotein and enhanced binding of basic drugs. Changes in protein binding caused by disease states and drug-drug interactions are clinically relevant mainly for a small subset of so-called high-clearance drugs of narrow therapeutic index that are administered intravenously, such as *lidocaine*. When changes in plasma protein binding occur in patients, unbound drug rapidly equilibrates throughout the body, and only a transient significant change in unbound plasma concentration will occur. Only drugs that show an almost-instantaneous relationship between free plasma concentration and effect (e.g., antiarrhythmics) will show a measurable effect. Thus, unbound plasma drug concentrations will exhibit significant changes only when either drug input or clearance of unbound drug occurs as a consequence of metabolism or active transport. A similar situation occurs when a new drug is added that can compete with an existing drug for plasma protein binding sites: Competition for binding sites may cause one drug to transiently elevate the concentration of another that is bound less avidly, but steady-state drug levels will be unchanged unless clearance changes. A more common problem resulting from competition of drugs for plasma protein-binding sites is misinterpretation of measured concentrations of drugs in plasma because most assays do not distinguish free drug from bound drug.

Binding of a drug to plasma proteins limits its concentration in tissues and at its site of action because only unbound drug is in equilibrium across membranes. Accordingly, after distribution equilibrium is achieved, the concentration of unbound drug in intracellular water is the same as that in plasma except when carrier-mediated active transport is involved. Binding of a drug to plasma protein limits the drug's glomerular filtration and may also limit drug transport and metabolism.

Tissue Binding

Many drugs accumulate in tissues at higher concentrations than those in the extracellular fluids and blood. Tissue binding of drugs usually occurs with cellular constituents such as proteins, phospholipids, or nuclear proteins and generally is reversible. A large fraction of drug in the body may be bound in this fashion and serve as a reservoir that prolongs drug action in that same tissue or at a distant site reached through the circulation. Such tissue binding and accumulation also can produce local toxicity (e.g., renal and ototoxicity associated with aminoglycoside antibiotics). The intracellular accumulation of antimicrobial agents has clinical implications, both therapeutic and toxicological (Pea, 2018).

CNS, the BBB, and CSF

The brain capillary endothelial cells have continuous tight junctions; therefore, drug penetration into the brain depends on transcellular rather than paracellular transport. The unique characteristics of brain capillary endothelial cells and pericapillary glial cells that constitute the BBB are described in detail in Chapter 17, and Chapter 4 describes some aspects of specific transport proteins that move drugs into and out of the CNS. At the choroid plexus, a similar blood-CSF barrier is present, formed by epithelial cells that are joined by tight junctions. The lipid solubility of the nonionized and unbound species of a drug is therefore an important determinant of its uptake by the brain; the more lipophilic a drug, the more likely it is to cross the BBB. In general, the BBB's function is well maintained; however, meningeal and encephalic inflammation increase local permeability.

Bone

The tetracycline antibiotics (and other divalent metal-ion chelating agents) and heavy metals may accumulate in bone by adsorption onto the bone crystal surface and eventual incorporation into the crystal lattice. Bone can become a reservoir for the slow release of toxic agents such as lead or radium; their effects thus can persist long after exposure has ceased. Local destruction of the bone medulla also may result in reduced blood flow and prolongation of the reservoir effect as the toxic agent becomes sealed off from the circulation; this may further enhance the direct local damage to the bone. A vicious cycle results, whereby the greater the exposure to the toxic agent, the slower is its rate of elimination. The adsorption of drug onto the bone crystal surface and incorporation into the crystal lattice have therapeutic advantages for the treatment of osteoporosis as in the use of phosphonates (Black and Rosen, 2016).

Fat as a Reservoir

Many lipid-soluble drugs are stored by physical solution in the neutral fat. In obese individuals, the fat content of the body may be as high as 50%, and even in lean individuals, fat constitutes 10% of body weight; hence, fat may serve as a reservoir for lipid-soluble drugs and toxins. Fat is a rather stable reservoir because it has a relatively low blood flow. Fat may also complicate drug treatment by serving as a reservoir for infectious agents such as HIV and by limiting the access of relatively nonlipophilic drugs (Couturier and Lewis, 2018).

Redistribution

Termination of drug effect after withdrawal of a drug usually is by metabolism and excretion, but also may result from redistribution of the drug from its site of action into other tissues or sites. Redistribution is a factor in terminating drug effect primarily when a highly lipid-soluble drug that acts on the brain or cardiovascular system is administered rapidly by intravenous injection or inhalation. Such is the case of the intravenous anesthetic *thiopental*, a lipid-soluble drug. Because blood flow to the brain is high and *thiopental* readily crosses the BBB, *thiopental* reaches its maximal concentration in brain rapidly after its intravenous injection. Subsequently, the plasma and brain concentrations decrease as *thiopental* redistributes to other tissues, such as muscle and, finally, adipose tissue. This redistribution is the mechanism by which *thiopental* anesthesia is terminated (see Figure 2–4); its actual clearance from the body is rather slow (elimination $t_{1/2}$ after a single dose is 3–8 h). The concentration of the drug in brain follows that of the plasma because there is little binding of the drug to brain constituents. Thus, both the onset and the termination of *thiopental* anesthesia are relatively rapid, and both are related directly to the concentration of drug in the brain.

Placental Transfer of Drugs

Lipid solubility, extent of plasma binding, and degree of ionization of weak acids and bases are important general determinants in drug transfer across the placenta. The placenta functions as a selective barrier to protect the fetus against the harmful effects of drugs. Members of the ABC family of transporters limit the entry of drugs and other xenobiotics into the fetal circulation via vectorial efflux from the placenta to the maternal circulation (see Figure 2–2 and Chapter 4). The fetal plasma is slightly more acidic than that of the mother (pH 7.0–7.2 vs. 7.4, respectively), so that ion trapping of basic drugs occurs. The view that the placenta is an absolute barrier to drugs is inaccurate, in part because a number of influx transporters are also present (Tetro et al., 2018). The fetus is, to some extent, exposed to all drugs taken by the mother.

The transfer of drugs across the placenta is of critical importance because drugs may cause anomalies in the developing fetus; thus, the need for evidence-based drug use in pregnancy is paramount. Drugs used during pregnancy had been categorized by the FDA in categories A–D and X, progressing from A (no evidence of fetal risk; e.g., folic acid, *levothyroxine*) to D (positive evidence of fetal risk but may be used if absolutely necessary; e.g., *alprazolam*, *losartan*) and to X (risks of use outweigh any benefits; such agents must not be used in pregnancy; e.g., statins, *methotrexate*). In 2015, the FDA established a new labeling system, the Pregnancy and Lactation Labeling Rule (PLLR). The PLLR replaces the letter codes with required labeling on (1) pregnancy, (2) lactation, and (3) issues affecting females and males of reproductive potential, with the intent of permitting better patient-specific counseling and informed decision making for pregnant women seeking medication therapies (Dinatale, 2016; Pernia and Demaagd, 2016). Physicians in the U.S. appear to be adopting the new system only slowly (Namazy et al., 2020).

Metabolism of Drugs

A Few Principles of Metabolism and Elimination

The many therapeutic agents that are lipophilic do not pass readily into the aqueous environment of the urine. The metabolism of drugs and other xenobiotics into more hydrophilic metabolites is essential for their renal elimination from the body, as well as for termination of their biological and pharmacological activity.

From the point of view of pharmacokinetics, the following are the three essential aspects of drug metabolism:

- **First-order kinetics.** For most drugs in their therapeutic concentration ranges, the amount of drug metabolized per unit time is proportional to the plasma concentration of the drug (C_p) and *the fraction of drug removed by metabolism is constant (i.e., first-order kinetics).*
- **Zero-order kinetics.** For some drugs, such as *ethanol* and *phenytoin*, metabolic capacity is saturated at the concentrations usually employed, and drug metabolism becomes *zero order; that is, a constant amount of drug is metabolized per unit time.* Zero-order kinetics can also occur at high (toxic) concentrations as drug-metabolizing capacity becomes saturated.
- **Inducible biotransforming enzymes.** The major drug-metabolizing systems are inducible, broad-spectrum enzymes with some predictable genetic variations. Drugs that are substrates in common for a metabolizing enzyme may interfere with each other's metabolism, or a drug may induce or enhance metabolism of itself or other drugs.

In general, drug-metabolizing reactions generate more polar, inactive metabolites that are readily excreted from the body. However, in some cases, metabolites with potent biological activity or toxic properties are generated. Many of the enzyme systems that transform drugs to inactive metabolites also generate biologically active metabolites of endogenous compounds, as in steroid biosynthesis. The biotransformation of drugs occurs primarily in the liver and involves *phase 1 reactions* (oxidation, reduction, or hydrolytic reactions and the activities of CYP enzymes) and *phase 2 reactions* (conjugations of the phase 1 product with a second molecule generating a polar compound) and subsequent steps that involve transporters that remove conjugates to the extracellular medium from which they are excreted. Other organs with significant drug-metabolizing capacity include the GI tract, kidneys, and lungs. Drug-metabolizing enzymes, especially CYPs, are inducible by some drugs and inhibited by drugs and competing substrates and impacted by disease (Coutant and Hall, 2018). Race, ethnicity, and sex differences play a critical role in drug response due to factors such as body composition as well as expression and activity of drug-metabolizing enzymes (Farkouh et al., 2020). Chapter 5 (Drug Metabolism) covers the basic enzymology of drug metabolism in detail. Chapters 4 (Membrane Transporters and Drug Response), 6 (GI Microbiome and Drug Response), and 7 (Pharmacogenetics) present related aspects of drug metabolism. Understanding metabolism of a given drug and how other drugs may affect that metabolism is crucial to good drug therapy and the future of personalized medicine.

Prodrugs

Prodrugs are pharmacologically inactive compounds that are converted to their active forms by metabolism. Designing prodrugs with the active form as a template can maximize the amount of the active species that reaches its site of action. Inactive prodrugs are converted rapidly to biologically active metabolites, often by the hydrolysis of an ester or amide linkage. Such is the case with a number of ACE inhibitors employed in the management of high blood pressure. *Enalapril*, for instance, is relatively inactive until converted by esterase activity to the diacid enalaprilat (see Chapter 30).

Pharmacogenetics

For a number of therapeutic areas, clinical pharmacogenetics, the study of the impact of genetic variations or genotypes of individuals on their drug response or drug metabolism, allows for improved treatment of individuals or groups (Ramamoorthy et al., 2015; see Chapter 7).

Excretion of Drugs

Drugs are eliminated from the body either unchanged or as metabolites. Excretory organs, the lung excluded, eliminate polar compounds more efficiently than substances with high lipid solubility. Thus, lipid-soluble drugs are not readily eliminated until they are metabolized to more polar compounds. The kidney is the most important organ for excreting drugs and their metabolites. Renal excretion of unchanged drug is a major route of elimination for 25% to 30% of drugs administered to humans. Substances excreted in the feces are principally unabsorbed orally ingested drugs or drug metabolites either excreted in the bile or secreted directly into the intestinal tract and not reabsorbed. Excretion from the lung is important mainly for the elimination of anesthetic gases (see Chapter 24).

Excretion of drugs in breast milk is important because the excreted drugs may affect the nursing infant (with lower body mass and poorly developed capacity to metabolize xenobiotics). Currently, there is insufficient information for the clinician to guide breastfeeding mothers in the potential dangers of excretion of medications into breast milk. An estimated 50% to 70% of breastfeeding mothers in the U.S. take some form of medication, and yet as few as 15% of recently approved drugs provide information on breastfeeding (Byrne and Spong, 2019). The most comprehensive information on drugs to which infants may be exposed from breast milk can be found on LactMed®, a database of drugs and other chemicals to which breastfeeding mothers may be exposed; the database is available on the National Library of Medicine Bookshelf. As the FDA's PLLR comes into general use, such information is becoming more widely available as a required part of the drug information on package inserts.

Renal Excretion

Excretion of drugs and metabolites in the urine involves three distinct processes: glomerular filtration, active tubular secretion, and passive tubular reabsorption (Figure 2–5). The amount of drug entering the tubular lumen by filtration depends on the glomerular filtration rate and the extent of plasma binding of the drug; only unbound drug is filtered. In the proximal renal tubule, active, carrier-mediated tubular secretion also may add drug to the tubular fluid (see Figures 4–3 and 4–4 and Chapter 29). Indeed, the majority of drugs do not enter the kidney tubule by glomerular filtration but by tubular secretion. Tubular secretion involves carriers that transport basic drugs (e.g., *amiloride*, dopamine, histamine) and carriers for acidic drugs (e.g., *furosemide, penicillin, indomethacin*) (see Figures 4–10 and 4–11). *Penicillin* is rapidly excreted from the body ($t_{1/2}$ = 30 min), largely via tubular secretion, with an injected dose largely eliminated in 2 h. Drug from the tubular lumen may be reabsorbed into the systemic circulation. In the renal tubules, especially on the distal side, the nonionized forms of weak acids and bases undergo net passive reabsorption. The tubular cells are less permeable to the ionized forms of weak electrolytes; thus, passive reabsorption of these substances depends on the pH (see Figure 2–3). When the tubular urine is made more alkaline, weak acids are largely ionized and are excreted more rapidly and to a greater extent; conversely, acidification of the urine will reduce fractional ionization and excretion of weak acids. Effects of changing urine pH are opposite for weak bases. In the treatment of drug poisoning, the excretion of some drugs can be hastened by appropriate alkalinization or acidification of the urine.

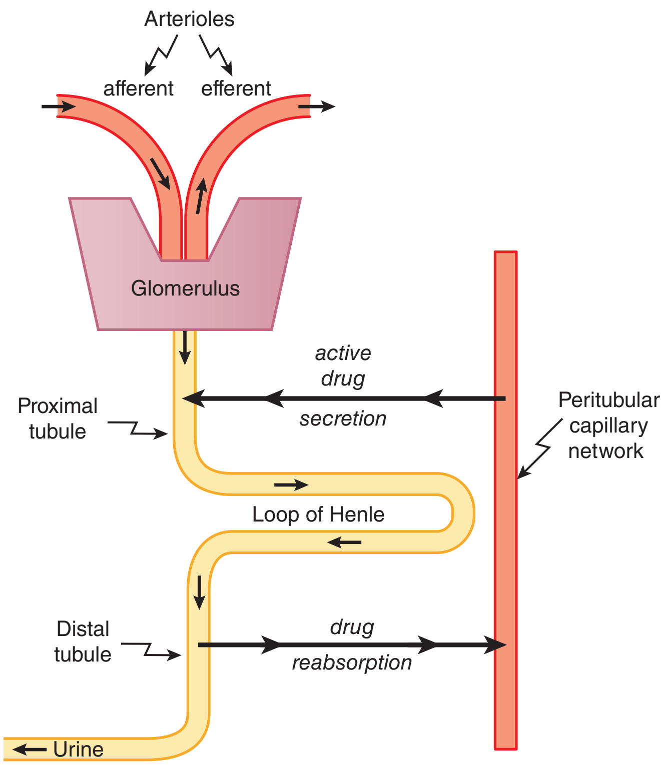

Figure 2–5 *Renal drug handling.* Drugs may be filtered from the blood in the renal glomerulus, secreted into the proximal tubule, reabsorbed from the distal tubular fluid back into the systemic circulation, and collected in the urine. Membrane transporters (OAT, OCT, MDR1, and MRP2, among others) mediate secretion into the proximal tubule (see Figures 4–12 and 4–13 for details). Reabsorption of compounds from the distal tubular fluid (generally acidic) is pH sensitive: Ionizable drugs are subject to ion trapping, and altering urinary pH to favor ionization can enhance excretion of charged species (see Figure 2–2).

The excretion and reabsorption of drugs may sometimes be usefully modified pharmacologically. For instance, co-administration of drugs that compete effectively for tubular secretion of *penicillin* can prolong the therapeutic effect of *penicillin*. *Penicillin* is secreted into the urine by the organic anion transporters OAT1 and OAT3; the acidic drug *probenecid* competes with *penicillin* at OAT1 and OAT3, thereby slowing excretion and prolonging an effective blood level of the antibiotic. *Probenecid* can also affect renal handling of uric acid. Urate is excreted into the urine by the kidney via the interplay of the processes of filtration, secretion, and reabsorption. *Probenecid* also inhibits URAT1, the urate transporter that is located on the apical surface of renal tubular cells and facilitates reabsorption of urate from the urine (Tan et al., 2016). Preventing reabsorption of uric acid (e.g., via administration of *probenecid*) promotes urate excretion, a useful effect in the treatment of hyperuricemia (i.e., gout). These actions of *probenecid* are covered in more detail in the chapters on uricosurics and β-lactam antibiotics (Chapters 42 and 58, respectively).

In older adults, renal function declines gradually at approximately 1% annually. Changes in renal excretion for drugs eliminated primarily by the kidney will be affected, resulting in increased peak drug levels and a longer duration of effect. Thus, elderly patients may require medication adjustments (O'Mahony, 2020); the FDA publishes useful guidance (https://www.fda.gov/drugs/guidance-compliance-regulatory-information/guidances-drugs).

Knowledge of the effects of pregnancy, *per se*, on drug pharmacokinetics is wanting. While enhanced drug elimination has been found in a number of cases, associated alterations in clinical responses and outcomes, or lack thereof, remain largely unknown. In neonates, renal function is low compared with body mass but matures rapidly within the first few months after birth. The PLLR initiative should bring more information to this area.

AL GRAWANY

Biliary and Fecal Excretion

Transporters present in the canalicular membrane of the hepatocyte (see Figure 4–6) actively secrete drugs and metabolites into bile. Ultimately, drugs and metabolites present in bile are released into the GI tract during the digestive process. Subsequently, the drugs and metabolites can be reabsorbed into the body from the intestine, which, in the case of conjugated metabolites such as glucuronides, may require enzymatic hydrolysis by the intestinal microflora. Such *enterohepatic recycling*, if extensive, may significantly prolong the presence of a drug (or toxin) and its effects within the body prior to elimination by other pathways. To interrupt enterohepatic cycling, substances may be given orally to bind metabolites excreted in the bile (for instance, see bile acid sequestrants and *ezetimibe*, Chapter 37). Biliary excretions and unabsorbed drug are excreted in the feces.

Excretion by Other Routes

Excretion of drugs into sweat, saliva, and tears is quantitatively unimportant. Because milk is more acidic than plasma, basic compounds may be slightly concentrated in this fluid; conversely, the concentration of acidic compounds in the milk is lower than in plasma. Nonelectrolytes (e.g., ethanol and urea) readily enter breast milk and reach the same concentration as in plasma, independent of the pH of the milk (Rowe et al., 2015). Breast milk can also contain heavy metals from environmental exposures. The administration of drugs to breastfeeding women carries the general caution that the suckling infant will be exposed to the medication or its metabolites. Insufficient data hamper a complete understanding of this problem. The PLLR initiative and the use of pharmacokinetic modeling in preclinical drug development in place of conventional animal testing for drug excretion into breast milk will improve needed guidance (Anderson, 2018). Although excretion into hair and skin is quantitatively unimportant, sensitive methods of detection of drugs in these tissues have forensic significance.

Clinical Pharmacokinetics

Clinical pharmacokinetics relate the pharmacological effects of a drug and concentration of the drug in an accessible body compartment (e.g., in blood or plasma) as these change in time. In most cases, the concentration of drug at its sites of action will be related to the concentration of drug in the systemic circulation (see Figure 2–1). The pharmacological effect that results may be the clinical effect desired or an adverse or toxic effect. Clinical pharmacokinetics attempts to provide:

- A quantitative relationship between dose and effect
- A framework within which to interpret measurements of drug concentration in biological fluids and their adjustment through changes in dosing for the benefit of the patient

The importance of pharmacokinetics in patient care is based on the improvement in therapeutic efficacy and the avoidance of unwanted effects that can be attained by application of its principles when dosage regimens are chosen and modified.

The following are the four most important parameters governing drug disposition:

1. *Bioavailability,* the fraction of drug absorbed into the systemic circulation
2. *Volume of distribution,* a measure of the apparent space in the body available to contain the drug based on how much is given versus what is found in the systemic circulation
3. *Clearance,* a measure of the body's efficiency in eliminating drug from the systemic circulation
4. *Elimination* $t_{1/2}$, a measure of the rate of removal of drug from the systemic circulation

Clearance

Clearance is the most important concept to consider when designing a rational regimen for long-term drug administration. The clinician usually wants to maintain steady-state concentrations of a drug within a *therapeutic window* or range associated with therapeutic efficacy and a minimum of toxicity for a given agent. Assuming complete bioavailability, the steady-state concentration of drug in the body will be achieved when the rate of drug elimination equals the rate of drug administration. Thus,

$$\text{Dosing rate} = CL \cdot C_{ss} \qquad \text{(Equation 2–3)}$$

where CL is clearance of drug from the systemic circulation (in units of volume/time), and C_{ss} is the steady-state concentration of drug (in units of mass/volume). When the desired steady-state concentration of drug in plasma or blood is known, the rate of clearance of drug will dictate the rate at which the drug should be administered.

Knowing the clearance of a drug is useful because its value for a particular drug usually is constant over the range of concentrations encountered clinically. This is true because metabolizing enzymes and transporters usually are not saturated; thus, the absolute rate of elimination of the drug is essentially a linear function of its concentration in plasma (first-order kinetics), where a *constant fraction* of drug in the body is eliminated per unit of time. If mechanisms for elimination of a given drug become saturated, the kinetics approach zero order (the case for *ethanol* and high doses of *phenytoin*), in which case a *constant amount* of drug is eliminated per unit of time.

With first-order kinetics, clearance CL will vary with the concentration of drug (C), often according to Equation 2–4:

$$CL = \frac{v_m}{(K_m + C)} \qquad \text{(Equation 2–4)}$$

where K_m represents the concentration at which half the maximal rate of elimination is reached (in units of mass/volume), and v_m is equal to the maximal rate of elimination (in units of mass/time). Thus, clearance is derived in units of volume cleared of drug/time. This equation is analogous to the Michaelis-Menten equation for enzyme kinetics.

Clearance of a drug is its rate of elimination by all routes normalized to the concentration of drug C in some biological fluid where measurement can be made:

$$CL = \text{Rate of elimination}/C \qquad \text{(Equation 2–5)}$$

Thus, when clearance is constant, the rate of drug elimination is directly proportional to drug concentration. Clearance indicates the volume of biological fluid such as blood or plasma from which drug would have to be completely removed to account for the clearance per unit of body weight (e.g., mL/min per kg). Clearance can be defined further as blood clearance CL_b, plasma clearance CL_p, or clearance based on the concentration of unbound drug CL_u, depending on the measurement made (C_b, C_p, or C_u). Clearance of drug by several organs is additive. Elimination of drug from the systemic circulation may occur as a result of processes that occur in the kidney, liver, and other organs. Division of the rate of elimination by each organ by a concentration of drug (e.g., plasma concentration) will yield the respective clearance by that organ. Added together, these separate clearances will equal systemic clearance:

$$CL_{renal} + CL_{hepatic} + CL_{other} = CL \qquad \text{(Equation 2–6)}$$

Any significant alteration in renal or hepatic function can result in decreased clearance for those drugs with high renal or hepatic clearance. Systemic clearance may be determined at steady state by using Equation 2–3. For a single dose of a drug with bioavailability of 1 and first-order kinetics of elimination, systemic clearance may be determined from mass balance and the integration of Equation 2–5 over time:

$$CL = \text{Dose/AUC} \qquad \text{(Equation 2–7)}$$

AUC is the total *area under the curve* that describes the measured concentration of drug in the systemic circulation as a function of time (from zero to infinity), as in Figure 2–10, panel A.

Examples of Clearance

The plasma clearance for the antibiotic *cephalexin* is 4.3 mL/min/kg, with 90% of the drug excreted unchanged in the urine. For a 70-kg man, the clearance from plasma would be 301 mL/min, with renal clearance accounting for 90% of this elimination. In other words, the kidney is able to excrete *cephalexin* at a rate such that the drug is completely removed (cleared) from about 270 mL of plasma every minute (renal clearance = 90% of total clearance). Because clearance usually is assumed to remain constant in a medically stable patient (e.g., no acute decline in kidney function), the rate of elimination of *cephalexin* will depend on the concentration of drug in the plasma (see Equation 2–5).

The β adrenergic receptor antagonist *propranolol* is cleared from the blood at a rate of 16 mL/min/kg (or 1600 mL/min in a 100-kg man), almost exclusively by the liver. Thus, the liver is able to remove the amount of *propranolol* contained in 1600 mL of blood in 1 min, roughly equal to total hepatic blood flow (see Table 2–2). In fact, the plasma clearance of some drugs exceeds the rate of blood flow to this organ. Often, this is so because the drug partitions readily into and out of red blood cells (rbc), and the rate of drug delivered to the eliminating organ is considerably higher than expected from measurement of its concentration in plasma. The relationship between plasma clearance (CL_p) and blood clearance (CL_b; all components of blood) at steady state is given by

$$\frac{CL_p}{CL_b} = \frac{C_b}{C_p} = 1 + H\left[\frac{C_{rbc}}{C_p} - 1\right] \qquad \text{(Equation 2–8)}$$

Clearance from the blood therefore may be estimated by dividing the plasma clearance by the drug's blood-to-plasma concentration ratio, obtained from knowledge of the hematocrit ($H = 0.45$) and concentration ratio of red cells to plasma. In most instances, the blood clearance will be less than liver blood flow (1.5–1.7 L/min) or, if renal excretion is involved, the sum of the blood flows to each eliminating organ. For example, the plasma clearance of the immunomodulator *tacrolimus*, about 2 L/min, is more than twice the hepatic plasma flow rate and even exceeds the organ's blood flow despite the fact that the liver is the predominant site of this drug's extensive metabolism. However, after taking into account the extensive distribution of *tacrolimus* into red cells, its clearance from the blood is only about 63 mL/min, and it is actually a drug with a rather low clearance, not a high-clearance agent as might be expected from the plasma clearance value alone. Clearance from the blood by metabolism can exceed liver blood flow, and this indicates extrahepatic metabolism. In the case of the β_1 receptor antagonist *esmolol*, the blood clearance value (11.9 L/min) is greater than cardiac output (~5.5 L/min) because the drug is metabolized efficiently by esterases present in red blood cells.

A further definition of clearance is useful for understanding the effects of pathological and physiological variables on drug elimination, particularly with respect to an individual organ. The rate of presentation of drug to the organ is the product of blood flow Q and the arterial drug concentration C_A, and the rate of exit of drug from the organ is the product of blood flow and the venous drug concentration C_V. The difference between these rates at steady state is the rate of drug elimination by that organ:

$$\begin{aligned} \text{Rate of elimination} &= Q \cdot C_A - Q \cdot C_V \\ &= Q(C_A - C_V) \end{aligned} \qquad \text{(Equation 2–9)}$$

Dividing Equation 2–8 by the concentration of drug entering the organ of elimination, C_A, yields an expression for clearance of the drug by the organ in question:

$$CL_{organ} = Q\left[\frac{C_A - C_V}{C_A}\right] = Q \times E \qquad \text{(Equation 2–10)}$$

The expression $(C_A - C_V)/C_A$ in Equation 2–10 can be referred to as the extraction ratio E of the drug. While not employed in general medical practice, calculations of a drug's extraction ratio(s) are useful for modeling the effects of disease of a given metabolizing organ on clearance and in the design of ideal therapeutic properties of drugs in development.

Hepatic Clearance (CL_H)

For a drug that is removed efficiently from the blood by hepatic processes (metabolism or excretion of drug into the bile), the concentration of drug in the blood leaving the liver will be low, the extraction ratio will approach unity, and the clearance of the drug from blood will become limited by hepatic blood flow (800–1500 mL/min). Drugs that are cleared efficiently by the liver (e.g., drugs with systemic clearances >6 mL/min/kg, such as *diltiazem*, *imipramine, lidocaine, morphine*, and *propranolol*) are restricted in their rate of elimination not by intrahepatic processes, but by the rate at which they can be transported in the blood to the liver.

Pharmacokinetic models indicate that when the capacity of the eliminating organ to metabolize the drug is large in comparison with the rate of presentation of drug to the organ, clearance will approximate the organ's blood flow. By contrast, when the drug-metabolizing capacity is small in comparison with the rate of drug presentation, clearance will be proportional to the unbound fraction of drug in blood (f_{unb}) and the drug's intrinsic clearance (CL_{int}), where intrinsic clearance represents drug binding to components of blood and tissues or the intrinsic capacity of the liver to eliminate a drug in the absence of limitations imposed by blood flow (Guner and Bowen, 2013). Thus, hepatic clearance will be:

$$CL_H = \frac{Q_H(f_{umb})(CL_{int})}{Q_H + (f_{umb})(CL_{int})} \qquad \text{(Equation 2–11)}$$

Renal Clearance

Renal clearance of a drug results in its appearance in the urine. In considering the clearance of a drug from the body by the kidney, glomerular filtration, secretion, reabsorption, and glomerular blood flow must be considered (see Figure 2–5). The rate of filtration of a drug depends on the volume of fluid that is filtered in the glomerulus and the concentration of unbound drug in plasma (because drug bound to protein is not filtered). The rate of secretion of drug into the tubular fluid is the largest factor determining renal excretion. Secretion will depend on the transporters involved in active secretion as affected by the drug's binding to plasma proteins, the degree of saturation of these transporters, the rate of delivery of the drug to the secretory site, and the presence of drugs that can compete for these transporters. In addition, one must consider processes of drug reabsorption from the tubular fluid back into the bloodstream such as occurs with uric acid. The influences of changes in protein binding, blood flow, and the functional state of nephrons will affect renal clearance.

Aspirin demonstrates the interplay among renal absorption and secretion. *Aspirin* has a bimodal effect on the renal handling of uric acid: High doses of *aspirin* (>3 g/day) are uricosuric (probably by blocking urate reabsorption), while low dosages (1–2 g/day) cause uric acid retention (probably via inhibiting urate secretion). Low-dose *aspirin*, indicated for the prophylaxis of cardiovascular events, can cause changes in renal function and uric acid handling in elderly patients.

Distribution

Volume of Distribution

The volume of distribution V relates the amount of drug in the body to the concentration of drug C in the blood or plasma, depending on the fluid measured. This volume does not necessarily refer to an identifiable physiological volume, but rather to the fluid volume that would be required to contain all of the drug in the body at the same concentration measured in the blood or plasma:

$$\text{Amount of drug in body}/V = C$$

or

$$V = \text{Amount of drug in body}/C \qquad \text{(Equation 2–12)}$$

View V as an imaginary volume because for many drugs V exceeds the known volume of any and all body compartments (Box 2–1). For example, the value of V for the highly lipophilic antimalarial *chloroquine* is

BOX 2–1 ■ *V* Values > Any Physiological Volume?

For many drugs, Equation 2–12 will give *V* values that exceed any physiological volume. For example, if 500 μg of the cardiac glycoside *digoxin* were added into the body of a 70-kg subject, a plasma concentration of about 0.75 ng/mL would be observed. Dividing the amount of drug in the body by the plasma concentration yields a volume of distribution for *digoxin* of about 667 L, or a value about 15 times greater than the total-body volume of a 70-kg man. In fact, *digoxin* distributes preferentially to muscle and adipose tissue and binds to its specific receptors, the Na^+/K^+-ATPase, leaving a very small amount of drug in the plasma to be measured. A drug's volume of distribution therefore can reflect the extent to which it is present in extravascular tissues and not in the plasma. Conversely, a small value for *V* can indicate maintenance of the drug in the bloodstream desirable in the treatment of leukemias. Many newer drug formulations encapsulate one or more drugs in liposomes or engineered nanoparticles to regulate drug distribution (Filipczak et al., 2020). Thus, *V* may vary widely depending on the relative degrees of binding to high-affinity receptor sites, plasma and tissue proteins, the partition coefficient of the drug in fat, accumulation in poorly perfused tissues, and engineered strategies such as encapsulation. The volume of distribution for a given drug can differ according to a patient's age, gender, body composition, and presence of disease. Total-body water of infants younger than 1 year of age, for example, is 75% to 80% of body weight, whereas that of adult males is 60% and that of females is 55%.

approximately 15,000 L, whereas the volume of total-body water is about 42 L in a 70-kg male. The benefit of determining *V* is to understand distribution of drug to the body away from the bloodstream as an indication of its distribution to sites of action.

For drugs that are bound extensively to plasma proteins but are not bound to tissue components, the volume of distribution will approach that of the plasma volume because drug bound to plasma protein is measurable in the assay of most drugs. In contrast, certain drugs have high volumes of distribution even though most of the drug in the circulation is bound to albumin because these drugs are sequestered elsewhere in the body.

The volume of distribution defined in Equation 2–12 considers the body as a single homogeneous compartment. In this one-compartment model, all drug administration occurs directly into the central compartment, and distribution of drug is instantaneous throughout the volume *V*. Clearance of drug from this compartment occurs in a first-order fashion, as defined in Equation 2–5; that is, the amount of drug eliminated per unit of time depends on the amount (concentration) of drug in the body compartment at that time. Figure 2–6A and Equation 2–9 describe the decline of plasma concentration with time for a drug introduced into this central compartment:

$$C = \left[\frac{\text{Dose}}{V}\right][e^{-kt}] \qquad \text{(Equation 2–13)}$$

where *k* is the rate constant for elimination that reflects the fraction of drug removed from the compartment per unit of time. This rate constant is inversely related to the $t_{1/2}$ of the drug [$kt_{1/2} = \ln 2 = 0.693$]. The idealized one-compartment model does not describe the entire time course of the plasma concentration. Certain tissue reservoirs can be distinguished from the central compartment, and the drug concentration appears to decay in a manner that can be described by multiple exponential terms (Figure 2–6B).

Rates of Distribution

In many cases, groups of tissues with similar perfusion-to-partition ratios all equilibrate at essentially the same rate such that only one apparent phase of distribution is seen (rapid initial decrease in concentration of intravenously injected drug, as in Figure 2–6B). Essentially, the drug starts in a "central" volume (see Figure 2–1), which consists of plasma and tissue reservoirs that are in rapid equilibrium, then distributes to a "final" volume, at which point concentrations in plasma decrease in a log-linear fashion with a rate constant of *k* (see Figure 2–6B). The multicompartment model of drug disposition can be viewed as though the blood and highly perfused lean organs such as heart, brain, liver, lung, and kidneys cluster as a single central compartment, whereas more slowly perfused tissues such as muscle, skin, fat, and bone behave as the final compartment (the tissue compartment).

If blood flow to certain tissues changes within an individual, rates of drug distribution to these tissues also will change. Changes in blood flow

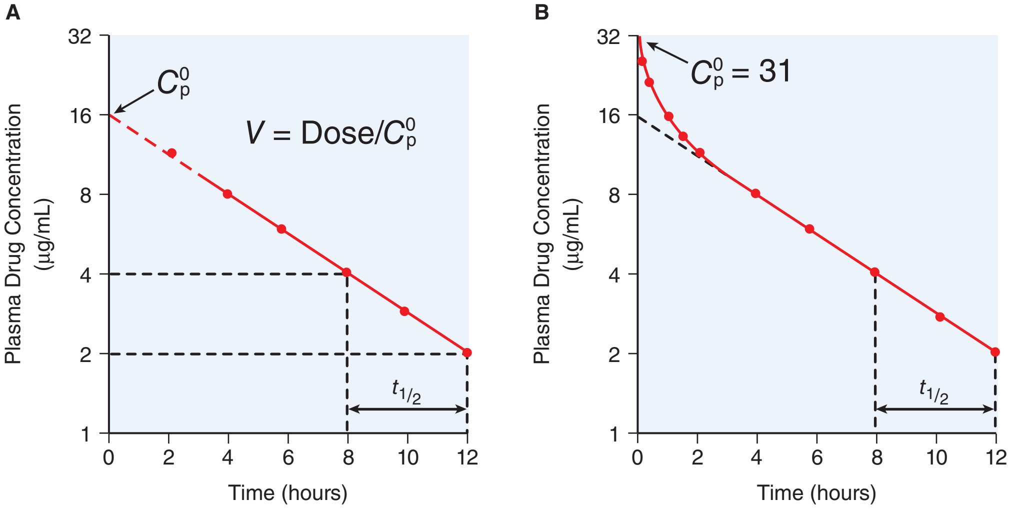

Figure 2–6 *Plasma concentration-time curves following intravenous administration of a drug (500 mg) to a 70-kg patient.* **A.** Drug concentrations are measured in plasma at 2-h intervals following drug administration. The semilogarithmic plot of plasma concentration C_p versus time suggests that the drug is eliminated from a single compartment by a first-order process (see Equation 2–13) with a $t_{1/2}$ of 4 h ($k = 0.693/t_{1/2} = 0.173\ \text{h}^{1}$). The volume of distribution *V* may be determined from the value of C_p obtained by extrapolation to zero-time. Volume of distribution (see Equation 2–12) for the one-compartment model is 31.3 L, or 0.45 L/kg ($V = \text{dose}/C_p^0$). The clearance for this drug is 90 mL/min; for a one-compartment model, $CL = kV$. **B.** Sampling before 2 h indicates that the drug follows multiexponential kinetics. The terminal disposition $t_{1/2}$ is 4 h, clearance is 84 mL/min (see Equation 2–7), and V_{ss} is 26.8 L (see Equation 2–14). The initial or "central" distribution volume for the drug ($V = \text{dose}/C_p^0$) is 16.1 L. The example indicates that multicompartment kinetics may be overlooked when sampling at early times is neglected. In this particular case, there is only a 10% error in the estimate of clearance when the multicompartment characteristics are ignored. For many drugs, multicompartment kinetics may be observed for significant periods of time, and failure to consider the distribution phase can lead to significant errors in estimates of clearance and in predictions of appropriate dosage.

may cause some tissues that were originally in the "central" volume to equilibrate sufficiently more slowly so they appear only in the "final" volume. This means that central volumes will appear to vary with disease states that cause altered regional blood flow (such as would be seen in cirrhosis of the liver). After an intravenous bolus dose, drug concentrations in plasma may be higher in individuals with poor perfusion (e.g., shock) than they would be if perfusion were better. These higher systemic concentrations may in turn cause higher concentrations (and greater effects) in tissues such as brain and heart, whose usually high perfusion has not been reduced. Thus, the effect of a drug at various sites of action can vary depending on perfusion of these sites.

Multicompartment Volumes

In multicompartment kinetics, a volume of distribution term is useful especially when the effect of disease states on pharmacokinetics is to be determined. The volume of distribution at steady-state V_{ss} represents the volume in which a drug would appear to be distributed during steady state if the drug existed throughout that volume at the same concentration as that in the measured fluid (plasma or blood). V_{ss} also may be appreciated as shown in Equation 2–14, where V_C is the volume of distribution of drug in the central compartment and V_T is the volume term for drug in the tissue compartment:

$$V_{ss} = V_C + V_T \qquad \text{(Equation 2–14)}$$

Steady-State Concentration

Equation 2–3 (Dosing rate = $CL \cdot C_{ss}$) indicates that a steady-state concentration eventually will be achieved when a drug is administered at a constant rate. At this point, drug elimination (the product of clearance and concentration; Equation 2–5) will equal the rate of drug availability. This concept also extends to regular intermittent dosage (e.g., 250 mg of drug every 8 h). During each interdose interval, the concentration of drug rises with absorption and falls by elimination. At steady state, the entire cycle is repeated identically in each interval (Figure 2–7). Equation 2–3 still applies for intermittent dosing, but it now describes the average steady-state drug concentration during an interdose interval. Note the extension of this idea to derive $\bar{C}_{ss}$ during continuous intravenous drug infusion, as explained in the legend to Figure 2–7.

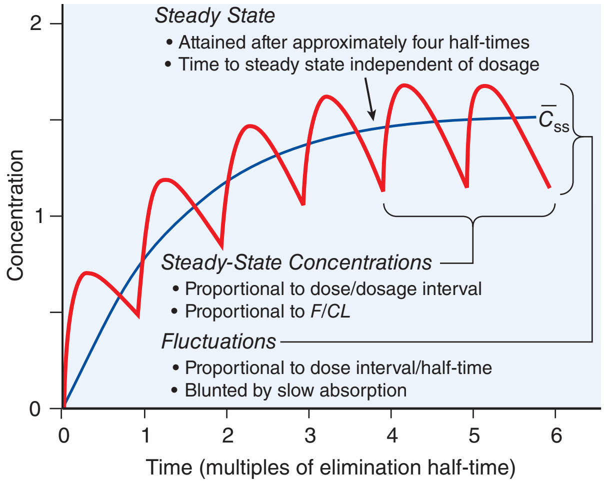

Figure 2–7 *Fundamental pharmacokinetic relationships for repeated administration of drugs.* The red line is the pattern of drug accumulation during repeated administration of a drug at intervals equal to its elimination half-time. With instantaneous absorption, each dose would add 1 concentration unit to C_p at the time of administration, and then half of that would be eliminated prior to administration of the next dose, resulting in the oscillation of C_p between 1 and 2 after four or five elimination half-times. However, this more realistic simulation uses a rate of drug absorption that is not instantaneous but is 10 times as rapid as elimination; drug is eliminated throughout the absorption process, blunting the maximal blood level achieved after each dose. With repeated administration, C_p achieves steady state, oscillating around the blue line at 1.5 units. The blue line depicts the pattern during administration of equivalent dosage by continuous intravenous infusion. Curves are based on the one-compartment model. Average drug concentration at steady state $\bar{C}_{ss}$ is:

$$C_{ss} = \frac{F \cdot \text{dose}}{CL \cdot T} = \frac{F \cdot \text{dosing rate}}{CL}$$

where the dosing rate is the dose per time interval and is dose/T, F is the fractional bioavailability, and CL is clearance. Note that substitution of infusion rate for [F · dose/T] provides the concentration maintained at steady state during continuous intravenous infusion (F = 1 with intravenous administration).

Half-Life

The $t_{1/2}$ is the time it takes for the plasma concentration of drug to be reduced by 50%. For the one-compartment model of Figure 2–6A, $t_{1/2}$ may be determined readily by inspection of the data and used to make decisions about drug dosage. However, as indicated in Figure 2–6B, drug concentrations in plasma often follow a multicomponent pattern of decline.

Half-Life, Volume of Distribution, and Clearance

When using pharmacokinetics to calculate drug dosing in disease, note that $t_{1/2}$ changes as a function of both clearance and volume of distribution:

$$t_{1/2} \cong 0.693 \cdot V_{ss}/CL \qquad \text{(Equation 2–15)}$$

This $t_{1/2}$ reflects the decline of systemic drug concentrations during a dosing interval at steady state as depicted in Figure 2–7.

Terminal Half-Life

With prolonged dosing (or with high drug concentrations), a drug may penetrate beyond the central compartment into "deep" or secondary body compartments that equilibrate only slowly with the plasma. When the infusion or dosing stops, the drug will be initially cleared from plasma as expected. Then the concentration will drop to a point at which net diffusion from the secondary compartments begins, and this slow equilibration will produce a prolongation of the half-life of the drug, referred to as the terminal half-life.

Steady-State $t_{1/2}$ and Terminal $t_{1/2}$ Compared

Examples of drugs with marked differences in terminal $t_{1/2}$ versus steady-state $t_{1/2}$ are *gentamicin* and *indomethacin*. *Gentamicin* has a $t_{1/2}$ of 2 to 3 h following a single administration, but a terminal $t_{1/2}$ of 53 h because drug accumulates in spaces such as kidney parenchyma, where this accumulation can result in toxicity). Biliary cycling probably is responsible for the 120-h terminal value for *indomethacin* (compared to the steady-state value of 2.4 h). Intravenous anesthetics provide a good example; many have *context-sensitive* half-times; these agents, with short half-times after single intravenous doses, exhibit longer half-times in proportion to the duration of exposure when used in maintenance anesthesia (see Figure 21–2).

Clearance is the measure of the body's capacity to eliminate a drug. Thus, as clearance decreases, owing to a disease process for example, $t_{1/2}$ will increase as long as the volume of distribution remains unchanged. Alternately, the volume of distribution may change, but CL remains constant or both can change. For example, the $t_{1/2}$ of *diazepam* increases with increasing age; however, this does not reflect a change in clearance, but rather a change in the volume of distribution. Similarly, changes in protein binding of a drug (e.g., hypoalbuminemia) may affect its clearance as well as its volume of distribution, leading to unpredictable changes in $t_{1/2}$ as a function of disease. The $t_{1/2}$ defined in Equation 2–15 provides an approximation of the time required to reach steady state after a dosage regimen is initiated or changed (e.g., four half-lives to reach ~94% of a new steady state).

Extent and Rate of Absorption

Bioavailability

It is important to distinguish between the amount of drug that is administered and the quantity of drug that ultimately reaches the systemic circulation. Dissolution and absorption of drug may be incomplete; some drug may be destroyed prior to entering the systemic circulation, especially by hepatic first-pass metabolism. The first-pass effect is extensive for many oral medications that enter the portal vein and pass directly to the liver. The fraction of a dose F that is absorbed and escapes first-pass elimination measures the drug's *bioavailability*; thus, $0 < F \leq 1$ (see Equation 2–2).

For some drugs, extensive first-pass metabolism precludes their use as oral agents (e.g., *lidocaine, naloxone*), while other agents, though administered orally, must be given to avoid hepatic metabolism (e.g., *glyceryl trinitrate*) or can be dosed to account for the large first-pass effect (e.g., *propranolol*). For other agents, the extent of absorption may be very low, thereby reducing bioavailability. When drugs are administered by a route that is subject to significant first-pass loss or incomplete absorption, the equations presented previously that contain the terms *dose* or *dosing rate* (see Equations 2–3, 2–7, and 2–13) also must include the bioavailability term F such that the available dose or dosing rate is used (Box 2–2). For example, Equation 2–2 is modified to

$$F \cdot \text{Dosing rate} = CL \cdot C_{ss} \qquad \text{(Equation 2–16)}$$

where the value of F is between 0 and 0.85.

Rate of Absorption

The rate of absorption can be important with a drug given as a single dose, such as a sleep-inducing medication that must act in a reasonable time frame and achieve an effective blood level that is maintained for an appropriate duration. However, with periodic and repeated dosing, the rate of drug absorption does not, in general, influence the average steady-state concentration of the drug in plasma, provided the drug is stable before it is absorbed; the rate of absorption may, however, still influence drug therapy. If a drug is absorbed rapidly (e.g., a dose given as an intravenous bolus) and has a small "central" volume, the concentration of drug initially will be high. It will then fall as the drug is distributed to its "final" (larger) volume (see Figure 2–6B). If the same drug is absorbed more slowly (e.g., by slow infusion), a significant amount of the drug will be distributed while it is being administered, and peak concentrations will be lower and will occur later. Controlled-release oral preparations are designed to provide a slow and sustained rate of absorption to produce smaller fluctuations in the plasma concentration-time profile during the dosage interval compared with more immediate-release formulations. Because the beneficial, nontoxic effects of drugs are based on knowledge of an ideal or desired plasma concentration range, maintaining that range while avoiding large swings between peak and trough concentrations can improve therapeutic outcome.

Nonlinear Pharmacokinetics

Nonlinearity in pharmacokinetics (i.e., changes in such parameters as clearance, volume of distribution, and $t_{1/2}$ as a function of dose or concentration of drug) is usually caused by saturation of protein binding, hepatic metabolism, or active renal transport of the drug.

BOX 2–2 ■ Notwithstanding Poor Absorption, Some Agents with Low Bioavailability are Effective Orally

The value of F varies widely for drugs administered by mouth, and successful therapy can still be achieved for some drugs with F values as low as 0.03 (e.g., *etidronate* and *aliskiren*). *Aliskiren* is the first orally applicable direct renin inhibitor approved for treatment of hypertension; its bioavailability is 2.6%. *Etidronate*, a bisphosphonate used to stabilize bone matrix in the treatment of Paget's disease and osteoporosis, has a similarly low bioavailability of 0.03, meaning that only 3% of the drug appears in the bloodstream following oral dosing. In these cases, therapy using oral administration is still useful, although the administered dose of the drug per kilogram is larger than would be given by injection.

Saturable Protein Binding

As the molar concentration of small drug molecules increases, the unbound fraction eventually also must increase (as all binding sites become saturated when drug concentrations in plasma are in the range of tens to hundreds of micrograms per milliliter). For a drug that is metabolized by the liver with a low intrinsic clearance-extraction ratio, saturation of plasma-protein binding will cause both V and CL to increase as drug concentrations increase; $t_{1/2}$ thus may remain constant (see Equation 2–15). For such a drug, C_{ss} will not increase linearly as the rate of drug administration is increased. For drugs that are cleared with high intrinsic clearance-extraction ratios, C_{ss} can remain linearly proportional to the rate of drug administration. In this case, hepatic clearance will not change, and the increase in V will increase the half-time of disappearance by reducing the fraction of the total drug in the body that is delivered to the liver per unit of time. Most drugs fall between these two extremes.

Saturable Elimination

In the case of saturable elimination, the Michaelis-Menten equation (see Equation 2–4) usually describes the nonlinearity. All active processes are undoubtedly saturable, but they will appear to be linear if values of drug concentrations encountered in practice are at or less than K_m for that process (Box 2–3). When drug concentrations exceed K_m, nonlinear kinetics are observed. Saturable metabolism causes oral first-pass metabolism to be less than expected (higher *fractional bioavailability*),

BOX 2–3 ■ Saturable Metabolism: Phenytoin

The antiseizure medication *phenytoin* is a drug for which metabolism can become saturated by levels of the drug in the therapeutic range. Factors contributing to this are *phenytoin*'s variable half-life and clearance and an effective concentration that varies and can saturate clearance mechanisms, such that the C_{ss} may be saturating clearance mechanisms or be well above or below that value. The $t_{1/2}$ of *phenytoin* is 6 to 24 h. For clearance, K_m (5–10 mg/L) is typically near the lower end of the therapeutic range (10–20 mg/L). For some individuals, especially young children and newborns being treated for emergent seizures, K_m may be as low as 1 mg/L. Consider an extreme case of a 70-kg adult in whom the target concentration (C_{ss}) is 15 mg/L, K_m is 1 mg/L, and the maximal elimination rate, v_m (from Appendix I), is 5.9 mg/kg per day, or 413 mg/day per 70 kg. Substituting into Equation 2–17:

$$15 \text{ mg/L} = (\text{dosing rate})(1 \text{ mg/L})/(413 \text{ mg/day} - \text{dosing rate})$$
$$\text{dosing rate} = 387 \text{ mg/day}$$

In this case, the dosing rate is just below the elimination capacity. If the dosing rate were to vary upward by 10% (to 387 + 38.7 or ~426 mg/day), the dosing rate would exceed the elimination capacity by 13 mg/day and the C_p of *phenytoin* would begin a slow climb to toxic levels due to accumulation. Conversely, if the dosing rate were to vary downward by 10% (to 387 – 38.7 or ~348 mg/day), the C_{ss} achieved would be 5.4 mg/L, a drastic reduction to a level below the therapeutic range.

Consider a more common K_m, 8 mg/L, such that the desired C_{ss} of 15 mg/L is farther from saturating the elimination capacity. In a 70-kg subject (v_m = 413 mg/day), these data require a dosing rate of only 269 mg/day. An increase in this rate by 10% (to 296 mg/day) would not saturate the elimination capacity but would lead to a C_{ss} = 20.2 mg/L. A 10% downward variance in the dosing rate (to 242 mg/day) will produce a C_{ss} = 11.3 mg/L, a much less drastic decrease than above and still in the therapeutic range.

Factoring in all the variables, predicting and controlling dosage so precisely (<10% error) can be difficult. Therefore, for patients in whom the target concentration for *phenytoin* is ≥10 times the K_m, alternating between inefficacious therapy and toxicity is common, careful monitoring is essential, and a pharmacokinetic consult to establish or revise dosing may be appropriate.

Other agents exhibiting saturated metabolism at or near the commonly employed concentrations include *aspirin*, *fluoxetine*, *verapamil*, and *ethanol*.

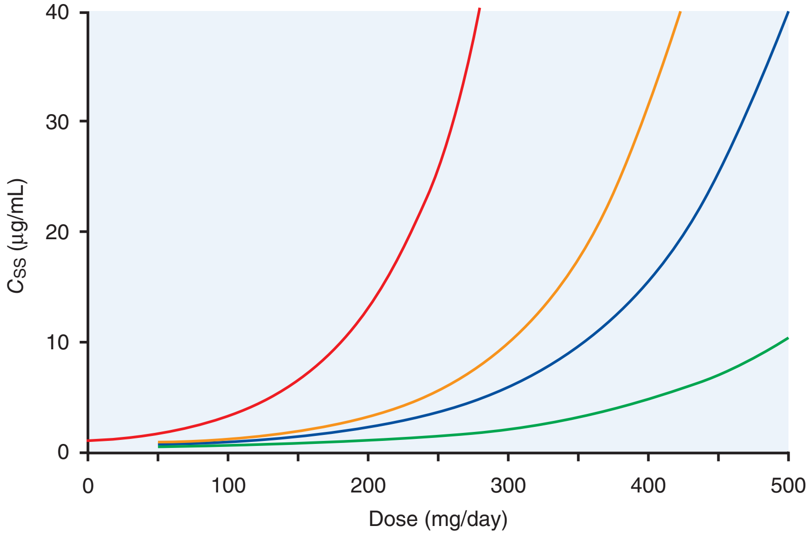

Figure 2–8 *Effect of altered elimination on the steady-state levels of a drug in the central compartment.* Blue line represents the effect of dose on the therapeutic drug level of a drug dosed once daily and eliminated by both hepatic metabolism and renal excretion. The effect of an increase in the rate of elimination, v_m, as might be seen by concurrent administration of drugs that induce metabolism (e.g., *phenytoin*, *rifampin*), is shown in green. The effect of an increase in K_m for elimination, as might be seen with concurrent administration of inhibitors of hepatic CYPs (e.g., *fluconazole*, *fluoxetine*), is shown in orange. The red line depicts the effect of hepatic cirrhosis and/or renal insufficiency, as might be found in an elderly patient. A significantly prolonged $t_{1/2}$ would cause rapid development of toxicity.

resulting in a greater fractional increase in C_{ss} than the corresponding fractional increase in the rate of drug administration; basically, the rate of drug entry into the systemic circulation exceeds the maximum possible rate of drug metabolism, and elimination becomes zero order. The major consequences of saturation of metabolism or transport are the opposite of those for saturation of protein binding. Saturation of protein binding will lead to increased *CL* because *CL* increases as drug concentration increases, whereas saturation of metabolism or transport may decrease *CL*.

Figure 2–8 presents hypothetical curves that show the effects of altered metabolism/elimination on plasma drug levels as functions of drug dosage.

Phenytoin offers a good clinical example of how the altered metabolism can alter the clinical outcome of drug therapy. *Phenytoin* is an antiepileptic drug widely used in the treatment of focal epilepsy and status epilepticus and effective in controlling focal seizures with and without tonic-clonic generalization and status epilepticus (see Chapter 20). Ninety percent of the metabolism of *phenytoin* is carried out by CYP2C9. Genetic polymorphisms in CYP2C9 may reduce the metabolism of *phenytoin* by 25% to 50% in patients. The frequency distribution of CYP2C9 polymorphism alleles in patients with epilepsy around the world ranges from 4.5% to 13.6%, being less frequent in African Americans and Asians. *Phenytoin* has a narrow therapeutic range and a nonlinear pharmacokinetic profile. Alterations in *phenytoin* metabolism can have significant clinical implications causing frequent and more serious adverse effects requiring discontinuation of treatment, despite clinical effectiveness. Table 2–3 summarizes some of these alterations, and Box 2–3 uses the example of *phenytoin* to present issues to be considered when a drug's metabolism can be saturated by plasma drug levels within a narrow therapeutic range and monitoring and adjustment of dosage become critical.

C_{ss} can be computed by substituting Equation 2–4 (with $C = C_{ss}$) into Equation 2–3 and solving for the steady-state concentration:

$$C_{ss} = \frac{\text{Dosing rate} \cdot K_m}{v_m - \text{dosing rate}} \qquad \text{(Equation 2–17)}$$

As the dosing rate approaches the maximal elimination rate v_m, the denominator of Equation 2–17 approaches zero, and C_{ss} increases disproportionately. Because saturation of metabolism should have no effect on the volume of distribution, clearance and the relative rate of drug elimination decrease as the concentration increases; therefore, the log C_p time curve is concave-downward until metabolism becomes sufficiently desaturated such that first-order elimination is observed (Figure 2–9).

Thus, during saturation of metabolism, the concept of a constant $t_{1/2}$ is not applicable. Consequently, changing the dosing rate for a drug with nonlinear metabolism is difficult and unpredictable because the resulting steady state is reached more slowly, and importantly, the effect is disproportionate to the alteration in the dosing rate.

Figure 2–9 compares the effects of first-order and zero-order elimination kinetics on important pharmacokinetic parameters.

Design and Optimization of Dosage Regimens

The Therapeutic Window

The intensity of a drug's effect is related to its concentration (usually C_p) above a minimum effective concentration, whereas the duration of the drug's effect reflects the length of time the drug level is above this value (Figure 2–10). These considerations, in general, apply to both desired and

TABLE 2–3 ■ CONDITIONS THAT CHANGE METABOLISM OF PHENYTOIN

CYP2C9	DISEASE OR CONDITION	EXAMPLE
V_{max} is increased	Enzyme induction	Concurrent administration of phenobarbital, rifampicin, or carbamazepine
V_{max} is decreased	Hepatic cirrhosis	Decreased enzyme activity: presence of either of 2 clinically significant CYP2C9 polymorphisms
K_m is increased	Competitive inhibition	Concurrent administration of antidepressants (e.g., fluoxetine), antimicrobials (e.g., chloramphenicol), or others including cimetidine
K_m is decreased	Decreased plasma protein binding	Hypoalbuminemia, competition for binding (e.g., valproic acid or salicylates)

K_m, [drug] at which rate of metabolism = 50% of V*max*; V_{max}, maximal rate of metabolism.

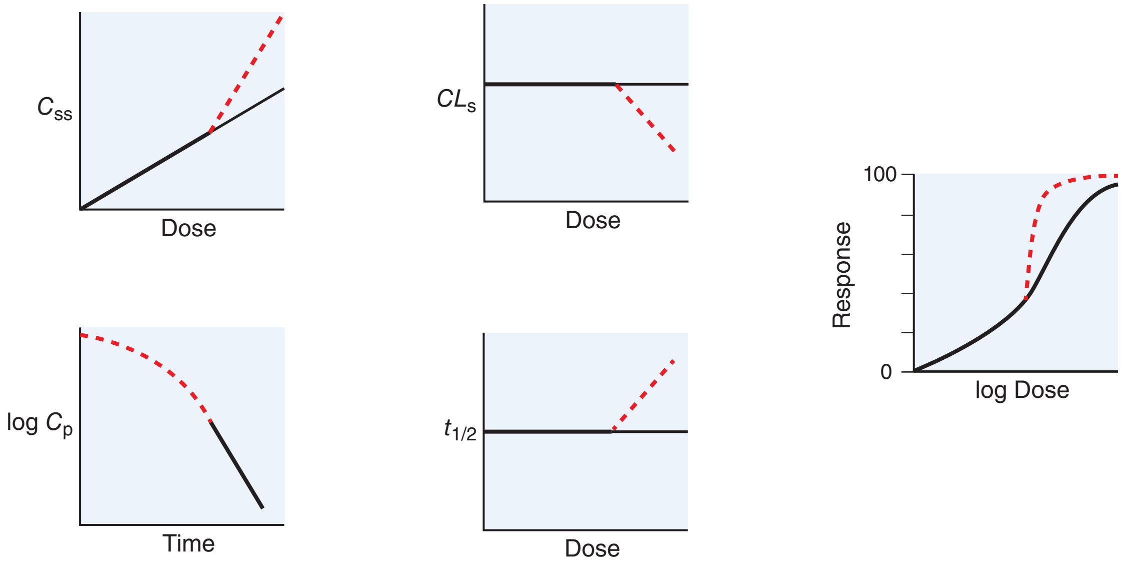

Figure 2–9 *Comparative pharmacokinetic parameters with first-order and zero-order elimination.* Black lines represent the relationships under first-order kinetics of elimination. Dashed red lines indicate the effects of transitioning to a region of saturated elimination (zero-order kinetics).

undesired (adverse) drug effects; as a result, a *therapeutic window* exists that reflects a concentration range that provides efficacy without unacceptable toxicity. Following administration of a single dose, a lag period precedes the onset of the drug effect, after which the magnitude of the effect increases to a maximum and then declines; if a subsequent dose is not administered, the effect eventually disappears as the drug is eliminated. This time course reflects changes in the drug's concentration as determined by the pharmacokinetics of its absorption, distribution, and elimination.

Similar considerations apply after multiple dosing associated with long-term therapy, and they determine the amount and frequency of drug administration to achieve an optimal therapeutic effect. *In general, the lower limit of a drug's therapeutic range is approximately equal to the drug concentration that produces about half the greatest possible therapeutic effect, and the upper limit of the therapeutic range is such that no more*

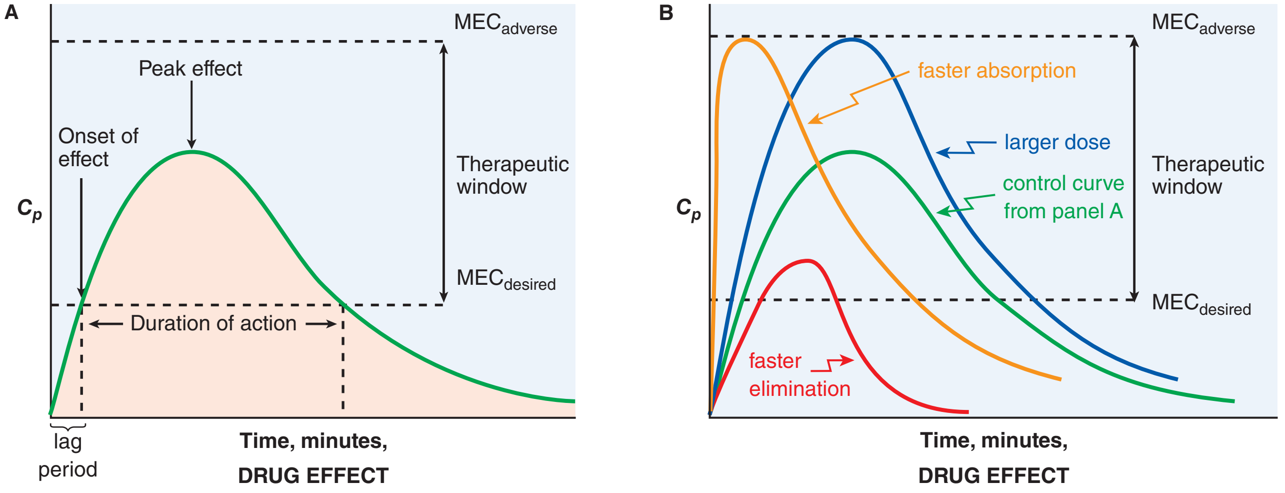

Figure 2–10 **A.** *Temporal characteristics of drug effect and relationship to the therapeutic window (e.g., single dose, oral administration).* A lag period is present before the plasma drug concentration C_p exceeds the minimum effective concentration (MEC) for the desired effect, MEC$_{desired}$. Following onset of the response, the intensity of the effect increases as the drug continues to be absorbed and distributed. This reaches a peak, after which drug elimination results in a decline in C_p and in the effect's intensity. Effect disappears when the drug concentration falls below the MEC$_{desired}$. The duration of a drug's action is determined by the time period over which concentrations exceed the MEC$_{desired}$. An MEC also exists for each adverse response (MEC$_{adverse}$), and if the drug concentration exceeds this, toxicity will result. The therapeutic goal is to obtain and maintain concentrations within the therapeutic window for the desired response with a minimum of toxicity. Drug response *below* the MEC$_{desired}$ will be subtherapeutic; *above* the MEC$_{adverse}$, the probability of toxicity will increase. The AUC (pale red) can be used to calculate the clearance (see Equation 2–7) for first-order elimination. The AUC is also used as a measure of bioavailability (defined as 100% for an intravenously administered drug). Bioavailability is less than 100% for orally administered drugs, due mainly to incomplete absorption and first-pass metabolism and elimination. Changing drug dosage shifts the curve up or down the C_p scale and is used to modulate the drug's effect, as shown in panel B.

B. *Effects of altered absorption, elimination, and dosage and the temporal profile of a single dose administered orally.* The bold green curve is the same as that shown in panel A. Increasing the dose (blue line) decreases the lag period and prolongs the drug's duration of effectiveness but at the risk of increasing the likelihood of adverse effects. Unless the drug is nontoxic (e.g., penicillins), increasing the dose is not a useful strategy for extending the duration of action if the increase puts the drug level near MEC$_{adverse}$. Instead, another dose of drug should be given, timed to maintain concentrations within the therapeutic window (see Figure 2–7). An increased rate of absorption of the dose (orange line) reduces the lag period, leads to a higher maximum C_p at an earlier time, but results in a shorter duration of action (time above MEC$_{desired}$). Increasing the rate of elimination of the dose decreases the maximum C_p and reduces the time of C_p > MEC$_{desired}$.

than 5% to 10% of patients will experience a toxic effect. For some drugs, this may mean that the upper limit of the range is no more than twice the lower limit. Of course, these figures can be highly variable, and some patients may benefit greatly from drug concentrations that exceed the therapeutic range, whereas others may suffer significant toxicity at much lower values (e.g., *digoxin*).

For a limited number of drugs, some effect of the drug is easily measured (e.g., blood pressure, blood glucose) and can be used to optimize dosage using a trial-and-error approach. Even in an ideal case, certain quantitative issues arise, such as how often to change dosage and by how much. These usually can be settled with simple rules of thumb based on the principles presented (e.g., change dosage by no more than 50% and no more often than every three or four half-lives to assure that a new near-steady-state drug concentration has been achieved). Alternatively, some drugs have little dose-related toxicity, and maximum efficacy usually is desired. In such cases, doses well in excess of the average required will ensure efficacy (if this is possible) and prolong drug action. Such a "maximal dose" strategy typically is used for penicillins. For many drugs, therapeutic effects are difficult to measure (or the drug is given for prophylaxis); hence, drug toxicity and lack of efficacy are both potential dangers, especially if the therapeutic index is narrow. In these circumstances, doses must be titrated carefully, and drug dosage is limited by toxicity rather than efficacy.

Thus, the therapeutic goal is to maintain steady-state drug levels within the therapeutic window. When the concentrations associated with this desired range are not known, it is sufficient to understand that efficacy and toxicity depend on concentration and how drug dosage and frequency of administration affect the blood level of the drug. However, for a small number of drugs for which there is a small (2- to 3-fold) difference between concentrations resulting in efficacy and toxicity (e.g., *digoxin, theophylline, lidocaine*, aminoglycosides, *cyclosporine, tacrolimus, sirolimus, warfarin*, and some anticonvulsants), a plasma concentration range associated with effective therapy has been defined. In these cases, a desired (target) steady-state concentration of the drug (usually in plasma) associated with efficacy and minimal toxicity is chosen, and a dosage is computed that is expected to achieve this value. Drug concentrations are subsequently measured, and dosage is adjusted if necessary (described further in the chapter).

Maintenance Dose

In most clinical situations, drugs are administered in a series of repetitive doses or as a continuous infusion to maintain a steady-state concentration of drug associated with the therapeutic window. Calculation of the appropriate maintenance dosage is a primary goal. To maintain the chosen steady-state or target concentration, the rate of drug administration is adjusted such that the rate of input equals the rate of loss. This relationship is expressed here in terms of the desired target concentration:

$$\text{Dosing rate} = \text{Target } C_p \cdot CL/F \qquad \text{(Equation 2–18)}$$

If the clinician chooses the desired concentration of drug in plasma and knows the clearance and bioavailability for that drug in a particular patient, the appropriate dose and dosing interval can be calculated (Box 2–4).

Dosing Interval for Intermittent Dosage

In general, marked fluctuations in drug concentrations between doses are not desirable. If absorption and distribution were instantaneous, fluctuations in drug concentrations between doses would be governed entirely by the drug's elimination $t_{1/2}$. If the dosing interval T were chosen to be equal to the $t_{1/2}$, then the total fluctuation would be 2-fold; this is often a tolerable variation. Pharmacodynamic considerations modify this. If a drug is relatively nontoxic such that a concentration many times that necessary for therapy can be tolerated easily, the maximal dose strategy can be used, and the dosing interval can be much longer than the elimination $t_{1/2}$ (for patient convenience). The $t_{1/2}$ of *amoxicillin* is about 2 h, but dosing every 2 h would be impractical. Instead, *amoxicillin* often is given in large doses every 8 or 12 h.

BOX 2–4 ■ Calculating Dosage of Digoxin in Heart Failure

Oral *digoxin* is to be used as a maintenance dose to gradually "digitalize" a 63-year-old, 84-kg patient with congestive heart failure. A steady-state plasma concentration of 0.7 to 0.9 ng/mL is selected as a conservative target based on prior knowledge of the action of the drug in patients with heart failure to maintain levels at or below the 0.5- to 1.0-ng/mL range (Bauman et al., 2006). This patient's creatinine clearance CL_{Cr} is given as 56 mL/min/84 kg; knowing that *digoxin*'s clearance may be estimated by consulting the entry for *digoxin* in Appendix I: $CL = 0.88\ CL_{Cr} + 0.33$ mL/min/kg. Thus,

$$\begin{aligned} CL &= 0.88\ CL_{Cr} + 0.33 \text{ mL/min/kg} \\ &= 0.88 \times 56/84 + 0.33 \text{ mL/min/kg} \\ &= 0.92 \text{ mL/min/kg} \end{aligned}$$

For this 84-kg patient:

$$CL = (84 \text{ kg})(0.92 \text{ mL/min/kg}) = 77 \text{ mL/min} = 4.6 \text{ L/h}$$

Knowing that the oral bioavailability of *digoxin* is 70% ($F = 0.7$) and with a target C_p of 0.75 ng/mL, one can use Equation 2–18 to calculate an appropriate dose rate for this 84-kg patient:

$$\begin{aligned} &\text{Dosing rate} = \text{Target } C_p \cdot CL/F \\ &= [0.75 \text{ ng/mL} \times 77 \text{ mL/min}] \div [0.7] = 82.5 \text{ ng/min} \\ &\text{or } 82.5 \text{ ng/min} \times 60 \text{ min/h} \times 24 \text{ h/day} = 119\ \mu\text{g/day} \end{aligned}$$

In practice, the dosing rate is rounded to the closest oral dosage size, 0.125 mg/day, which would result in a C_{ss} of 0.79 ng/mL (0.75 × 125/119 or using Equation 2–16). *Digoxin* is a well-characterized example of a drug that is difficult to dose, has a low therapeutic index (~2–3), and has a large coefficient of variation for the clearance equation in patients with heart failure (52%); the effective blood level in one patient may be toxic or ineffective in another. Thus, monitoring the clinical status of patients (new or increased ankle edema, inability to sleep in a recumbent position, decreased exercise tolerance), whether accomplished by home health follow-up or regular visits to the clinician, is essential to avoid untoward results (see Chapter 33).

For some drugs with a narrow therapeutic range, it may be important to estimate the maximal and minimal concentrations that will occur for a particular dosing interval. The minimal steady-state concentration $C_{ss,\min}$ may be reasonably determined by:

$$C_{ss,\,\min} = \frac{F \cdot \text{dose}/V_{ss}}{1 - e^{-kT}} \cdot e^{-kT} \qquad \text{(Equation 2–19)}$$

where k equals 0.693 divided by the clinically relevant plasma $t_{1/2}$, and T is the dosing interval. The term e^{-kT} is the fraction of the last dose (corrected for bioavailability) that remains in the body at the end of a dosing interval.

For drugs that follow multiexponential kinetics (administered orally), estimation of the maximal steady-state concentration $C_{ss,\max}$ involves a set of parameters for distribution and absorption (Box 2–5). If these terms are ignored for multiple oral dosing, one easily may estimate a maximal steady-state concentration by omitting the e^{-kT} term in the numerator of Equation 2–19 (see Equation 2–20 in Box 2–5). Because of the approximation, the predicted maximal concentration from Equation 2–20 will be greater than that actually observed.

Loading Dose

As noted, repeated administration of a drug more frequently than its complete elimination will result in accumulation of the drug to or around a steady-state level (see Figure 2–7). When a constant dosage is given, reaching a steady-state drug level (the desired therapeutic concentration) will take four to five elimination half-times. This period can be too long when treatment demands a more immediate therapeutic response. In such a case, one can employ a *loading dose*, one or a series of doses given at the onset of therapy with the aim of achieving the target concentration rapidly. The loading dose is calculated as

$$\text{Loading dose} = \text{Target } C_p \cdot V_{ss}/F \qquad \text{(Equation 2–22)}$$

Consider the case for treatment of arrhythmias with *lidocaine*, for example. The $t_{1/2}$ of *lidocaine* is usually 1 to 2 h. Arrhythmias encountered after myocardial infarction may be life threatening, and one cannot wait four half-lives (4–8 h) to achieve a therapeutic concentration of *lidocaine* by infusion of the drug at the rate required to attain this concentration. Hence, use of a loading dose of *lidocaine* in the coronary care unit is standard.

The use of a loading dose also has significant disadvantages. First, the particularly sensitive individual may be exposed abruptly to a toxic concentration of a drug that may take a long time to decrease (i.e., long $t_{1/2}$). Loading doses tend to be large, and they are often given parenterally and rapidly. This can be particularly dangerous if toxic effects occur because of drug actions at sites in rapid equilibrium with plasma. This occurs because the loading dose calculated on the basis of V_{ss} subsequent to drug distribution is at first constrained within the initial and smaller "central" volume of distribution. It is therefore usually advisable to divide the loading dose into a number of smaller fractional doses that are administered over a period of time (Box 2–6). Alternatively, the loading dose should be administered as a continuous intravenous infusion over a period of time using computerized infusion pumps.

BOX 2–5 ■ Estimating Maximal and Minimal Blood Levels of Digoxin

In the 84-kg patient with congestive heart failure discussed in Box 2–4, an oral maintenance dose of 0.125 mg *digoxin* per 24 h was calculated to achieve an average plasma concentration of 0.79 ng/mL during the dosage interval. *Digoxin* has a narrow therapeutic index, and plasma levels ≤1.0 ng/mL usually are associated with efficacy and minimal toxicity. What are the maximum and minimum plasma concentrations associated with this regimen? This first requires estimation of *digoxin*'s volume of distribution based on pharmacokinetic data (Appendix I).

$$\begin{aligned} V_{ss} &= 3.12\, CL_{Cr} + 3.84 \text{ L} \cdot \text{kg}^{-1} \\ &= 3.12 \times (56/84) + 3.84 \text{ L} \cdot \text{kg}^{-1} \\ &= 5.92 \text{ L/kg} \end{aligned}$$

or 497 L in this 84-kg patient.

Combining this value with that of *digoxin*'s clearance provides an estimate of *digoxin*'s elimination $t_{1/2}$ in the patient (Equation 2–15).

$$\begin{aligned} t_{1/2} &= 0.693\, V_{ss}/CL \\ &= \frac{0.693 \times 497 \text{ L}}{4.6 \text{ L/h}} = 75 \text{ h} = 3.1 \text{ days} \end{aligned}$$

Accordingly, the fractional rate constant of elimination k is equal to 0.22 day^{-1} (0.693/3.1 days). Maximum and minimum *digoxin* plasma concentrations then may be predicted depending on the dosage interval. With T = 1 day (i.e., 0.125 mg given every day),

$$\begin{aligned} C_{ss,max} &= \frac{F \cdot \text{dose}/V_{ss}}{1 - e^{-kT}} \\ &= \frac{0.7 \times 0.125 \text{ mg}/497 \text{ L}}{0.2} \qquad \text{(Equation 2–20)} \\ &= 0.88 \text{ ng/mL } (\sim 0.9 \text{ ng/mL}) \end{aligned}$$

$$\begin{aligned} C_{ss,\,min} &= C_{ss,\,max} \cdot e^{-kt} \\ &= (0.88 \text{ ng/mL})(0.8) = 0.7 \text{ ng/mL} \qquad \text{(Equation 2–21)} \end{aligned}$$

Thus, the plasma concentrations would fluctuate minimally about the steady-state concentration of 0.79 ng/mL, well within the recommended therapeutic range of 0.5 to 1.0 ng/mL.

BOX 2–6 ■ A Loading Dose of Digoxin

In the 84-kg patient described previously, accumulation of *digoxin* to an effective steady-state level was gradual when a daily maintenance dose of 0.125 mg was administered (for at least 12.4 days, based on $t_{1/2}$ = 3.1 days). A more rapid response could be obtained (if deemed necessary) by using a loading dose strategy and Equation 2–22. Choosing a target C_p of 0.9 ng/mL (the $C_{ss,max}$ calculated in Box 2–5 and below the recommended maximum of 1.0 ng/mL):

$$\text{Loading dose} = 0.9 \text{ ng} \cdot \text{mL}^{-1} \times 497 \text{ L}/0.7 = 639\ \mu\text{g}$$

Using standard dosage sizes, one would use a loading dose of 0.625 mg given in divided doses. To avoid toxicity, this oral loading dose would be given as an initial 0.25-mg dose followed by a 0.25-mg dose 6 to 8 h later, with careful monitoring of the patient, and the final 0.125-mg dose given another 6 to 8 h later. Equivalent loading strategy can be accomplished by administering *digoxin* by intravenous injection.

Therapeutic Drug Monitoring

The major use of measured concentrations of drugs (at steady state) is to refine the estimate of CL/F for the patient being treated, using Equation 2–16 as rearranged:

$$CL/F_{\text{patient}} = \text{Dosing rate}/C_{ss} \text{ (measured)} \qquad \text{(Equation 2–23)}$$

The new estimate of CL/F for an individual patient can be used in Equation 2–18 to adjust the maintenance dose to achieve the desired target concentration (Box 2–7).

Practical details associated with therapeutic drug monitoring should be kept in mind. The first of these relates to the time of sampling for measurement of the drug concentration.

The purpose of sampling during supposed steady state is to modify the estimate of CL/F and thus the choice of dosage. Early postabsorptive concentrations do not reflect clearance. They are determined primarily by the rate of absorption, the "central" (rather than the steady-state) volume of distribution, and the rate of distribution, all of which are pharmaco-kinetic features of virtually no relevance in choosing the long-term maintenance dosage. When the goal of measurement is adjustment of dosage, the sample should be taken just before the next planned dose, when the concentration is at its minimum.

If it is unclear whether efficacious concentrations of drug are being achieved, a sample taken shortly after a dose may be helpful. On the other hand, if a concern is whether low clearance (as in renal failure) may cause accumulation of drug, concentrations measured just before the next dose will reveal such accumulation and are considerably more useful than the maximal concentration.

BOX 2–7 ■ Adjusting the Dose at Steady State

If a drug follows first-order kinetics, the average, minimum, and maximum concentrations at steady state are linearly related to dose and dosing rate (see Equations 2–16, 2–19, and 2–20). Therefore, the ratio between the measured and desired concentrations can be used to adjust the dose, consistent with available dosage sizes:

$$\frac{C_{ss} \text{ (measured)}}{C_{ss} \text{ (predicted)}} = \frac{\text{Dose (previous)}}{\text{Dose (new)}} \qquad \text{(Equation 2–24)}$$

Consider the previously described patient given 0.125 mg *digoxin* every 24 h, for example. If the measured minimum (trough) steady-state concentration were found to be 0.35 ng/mL rather than the predicted level of 0.7 ng/mL, an appropriate, practical change in the dosage regimen would be to increase the daily dose by 0.125 mg to 0.25 mg *digoxin* daily.

Determination of both maximal and minimal concentrations is recommended. These two values can offer a more complete picture of the behavior of the drug in a specific patient (particularly if obtained over more than one dosing period) and can better support pharmacokinetic modeling to adjust treatment.

Recall that when constant dosage is given, steady state is reached after four to five elimination half-times. If a sample is obtained too soon after dosage is begun, it will not reflect steady state and clearance accurately. In such cases, the first sample should be taken after two $t_{1/2}$ (75% of expected C_{ss}) assuming that no loading dose has been given. If the concentration already exceeds 90% of the eventual expected mean steady-state concentration, the dosage rate should be halved, another sample obtained in another two (supposed) $t_{1/2}$, and the dosage halved again if this sample exceeds the target. If the first concentration is not too high, the initial rate of dosage is continued. Even if the concentration is lower than expected, it is usually reasonable to await the attainment of steady state after two additional half-lives and then adjust the dose as described in Box 2–7.

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Chapter 3

Pharmacodynamics: Molecular Mechanisms of Drug Action

David R. Manning and Donald K. Blumenthal

PHARMACODYNAMIC CONCEPTS

- Drug Targets
- Specificity of Drug Responses
- Additivity and Synergism: Isobolograms
- Attenuation of Drug Responses
- Pharmacodynamic Drug Interactions
- Precision Medicine: A Pharmacodynamic Perspective
- Concentration- and Dose-Response Relationships
- Pharmacodynamic Variability: Individual and Population Pharmacodynamics
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RECEPTOR-MEDIATED MECHANISMS OF DRUG ACTION

- Quantitative Aspects of Drug Interactions With Receptors
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- Diseases Resulting From Receptor and Pathway Dysfunction

INTRINSIC PATHWAYS REGULATED BY NUTRIENTS, ENERGY, AND CELL DAMAGE

- AMPK and TOR Pathways
- Autophagy
- Apoptosis

PHYSIOLOGICAL SYSTEMS MUST INTEGRATE MULTIPLE SIGNALS

SIGNALING PATHWAYS AND DRUG ACTION

Pharmacodynamic Concepts

Pharmacodynamics is the study of the biochemical, cellular, and physiological actions of drugs, including the molecular mechanisms by which these actions are achieved. Most drugs are small molecules that interact with macromolecular entities, or *drug targets*, intrinsic to the body or to pathogens. Drug targets include receptors for endocrine and paracrine factors, enzymes, voltage-gated ion channels, membrane transporters, and, for pathogens chiefly, structures relevant to cell viability and replication. As such, targets can be located anywhere on or within a cell, including the cell-surface membrane, cytosol, and nucleus, or entirely in the extracellular compartment. In keeping with the nature of these targets, drugs almost always alter the rate or magnitude of intrinsic cellular or physiological processes rather than create biologically novel phenomena.

Of the new drugs approved by the FDA (U.S. Food and Drug Administration), a growing percentage, averaging approximately 25% over the past 5 years (Figure 1–7), are *therapeutic biological products*. These are defined by the FDA (in the Therapeutic Biologics Applications) as:

- Monoclonal antibodies for *in vivo* use
- Cytokines, growth factors, enzymes, immunomodulators, and thrombolytics
- Proteins intended for therapeutic use that are extracted from animals or microorganisms, including recombinant versions of these products and other nonvaccine therapeutic immunotherapies

A distinction is commonly made between these products, which are often proteins, and the much larger number of small-molecule drugs. Targets in the case of monoclonal antibodies and certain recombinant proteins include inflammatory mediators, immunological checkpoint inhibitors, and cell-surface molecules (Chapters 39, 42, 44, and 72). Genetically modified viruses, for example oncolytic viruses, and microbes, too, are considered biological products and are actively investigated as recombinant vectors for candidate vaccines (Chapter 40).

Another important dimension to pharmacodynamics is gene therapy. Gene therapy uses viruses as vectors to replace defective genes that cause debilitating or lethal diseases, or it can introduce other genes altogether. Recently approved examples of gene therapy include the treatments of a congenital form of retinoblastoma and of spinal muscular atrophy with proteins (RPE65 [retinal epithelium-specific 65-kDa protein] and SMN1 [survival motor neuron 1], respectively) introduced by means of adeno-associated virus (AAV) vectors. The insertion of an anti-CD19 chimeric antigen receptor into T cells for the treatment of B-cell acute lymphoblastic leukemia makes use of a lentiviral vector (Chapter 72). Gene therapy also has the capacity for gene silencing, for example, in the treatment of hereditary transthyretin-mediated amyloidosis with *patisiran* and *inotersen*, two transthyretin-directed short interfering RNAs (siRNAs). Exon skipping represents another facet of gene therapy, as exemplified in the treatment of certain forms of Duchenne muscular dystrophy with the antisense oligonucleotide *eteplirsen*. The CRISPR-Cas9 (clustered regularly interspersed short palindromic repeats/CRISPR-associated protein 9) genome-editing system holds considerable potential in providing highly targeted forms of editing. Drugs can also act by influencing epigenetic regulation. For example, *tazemetostat*, used in the treatment of epithelioid carcinoma, is a recently approved inhibitor of histone methyltransferase EZH2. Clearly, the boundaries among pharmacology, immunology, and genetics overlap.

Quarterly updated eChapters in Section X of the online version of this textbook can provide the reader with reviews of new and noteworthy FDA approvals, including first-in-class drugs and breakthrough therapies (see *AccessMedicine.com or AccessPharmacy.com*; select *Goodman & Gilman*).

Drug Targets

The actions of the vast majority of drugs can be ascribed to their interactions with a relatively small number of protein classes. These classes in humans are receptors for endocrine and paracrine factors; enzymes; voltage-gated ion channels and other ion channels apart from receptors; and membrane transporters (Figure 3–1). Of these, receptors and enzymes are the targets for the majority of drugs in current therapeutic use and are a focus of this chapter. Voltage-gated ion channels are addressed here, as well as in Chapters 16 and 25. Membrane transporters are the subject of Chapter 4.

Abbreviations

AC: adenylyl cyclase
ACE: angiotensin-converting enzyme
ACh: acetylcholine
AKAP: A-kinase anchoring protein
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AMPK: AMP-activated protein kinase
AngII: angiotensin II
ANP: atrial natriuretic peptide
AP-1: activator protein-1 (transcription factor)
Apaf-1: apoptotic activating protease factor 1
***ATG*:** autophagy gene
AT_1R: angiotensin subtype 1 receptor
BNP: brain natriuretic peptide
CaM: calmodulin
CAR: constitutive androstane receptor
CNP: C-type natriuretic peptide
CYP: cytochrome P450
DAG: diacylglycerol
DHFR: dihydrofolate reductase
EC_{50}: half-maximally effective concentration
ED_{50}: half-maximally effective dose
EGF: epidermal growth factor
eNOS: endothelial NOS (NOS3)
EPAC: exchange protein activated by cyclic AMP
ER: endoplasmic reticulum
FBP: fructose 1,6-bisphosphate
FXR: farnesoid X receptor
GABA: γ-aminobutyric acid
GC: guanylyl cyclase
GEF: guanine nucleotide exchange factor
GnRH: gonadotropin-releasing hormone
GPCR: G protein-coupled receptor
GRK: GPCR kinase
HMG-CoA: hydroxymethylglutaryl–coenzyme A
HRE: hormone response element
5HT: 5-hydroxytryptamine (serotonin)
IFN: interferon
IL: interleukin
iNOS: inducible NOS (NOS2)
IP_3: inositol 1,4,5-trisphosphate
JAK: Janus kinase
JNK: c-Jun N-terminal kinase
K_i: affinity of a competitive antagonist
K_{ir}: inward rectifying K^+ channel
LD_{50}: half-maximal lethal dose
LKB1: liver kinase B1
LXR: liver X receptor
MAPK: mitogen-activated protein kinase
MLCK: myosin light chain kinase
mTOR: mechanistic or mammalian target of rapamycin, a protein kinase
mTORC: mechanistic or mammalian target of rapamycin complex
NAM: negative allosteric modulator
NE: norepinephrine
NF-κB: nuclear factor-κB
NMDA: *N*-methyl-D-aspartate
nNOS: neuronal NOS
NO: nitric oxide
NOS: NO synthase
NPR: natriuretic peptide receptor
NSAID: nonsteroidal anti-inflammatory drug
PAMP: pathogen-associated molecular pattern
PDE: cyclic nucleotide phosphodiesterase
PAM: positive allosteric modulator
PDGF: platelet-derived growth factor
PI3K: phosphatidylinositol 3-kinase
PIP_2: phosphatidylinositol 4,5-bisphosphate
PK: protein kinase, e.g., PKA, PKB (also known as Akt), PKC
PLC: phospholipase C
PPAR: peroxisome proliferator-activated receptor
PTB: phosphotyrosine-binding
PXR: pregnane X receptor
RAR: retinoic acid receptor
RGS: regulator of G protein signaling
RhoGEF: RhoA guanine nucleotide exchange factor
RIP1: receptor interacting protein 1
ROCK: Rho-associated protein kinase
RXR: retinoid X receptor
SERM: selective estrogen receptor modulator
sGC: soluble guanylyl cyclase
SH2: Src homology 2
SMAC: second mitochondria-derived activator of caspase
SMN1: survival motor neuron protein
STAT: signal transducer and activator of transcription
TGF-β: transforming growth factor β
TLR: toll-like receptor
TNF-α: tumor necrosis factor α
TNFR: TNF-α receptor
TOR: target of rapamycin
TRAIL: TNF-related apoptosis-inducing ligand
TRP: transient receptor potential
VEGF: vascular endothelial growth factor
VSMC: vascular smooth muscle cell

More than a few drugs operate through multiple mechanisms, and in this respect the picture regarding their actions continues to evolve. *Amphetamines*, for example, have long been known to be competitive inhibitors of the dopamine transporter but are now recognized to operate additionally through displacement of *dopamine* from vesicular stores and engagement of the trace amine-associated receptor TAAR1 (Chapters 15 and 16). *Metformin* exerts its hypoglycemic actions in large part through the AMP-dependent protein kinase (AMPK) and via inhibition of mitochondrial glycerol phosphate dehydrogenase, but almost certainly through additional mechanisms (Chapter 51). The hypolipidemic actions of *niacin* are achieved through one or more G protein-coupled receptors (GPCRs) at the level of adipocytes, but also through inhibition, in hepatocytes, of diacylglycerol acyltransferase, again among other actions (Chapter 37). The mechanism by which estrogen acts in certain situations extends beyond nuclear receptors to GPCRs at the cell surface (Chapter 48). One should note that the multiple mechanisms by which a drug may act are also quite important elements of unintended adverse drug responses.

The actions of some drugs do not require targets *per se*. *Aluminum* and *magnesium hydroxides* reduce gastric acid chemically, neutralizing H^+ and raising gastric pH. *Methenamine* achieves a formaldehyde-dependent antibacterial action at urinary pH (Chapter 57). *Mannitol* acts osmotically to cause changes in the distribution of water to promote diuresis, catharsis, expansion of circulating volume in the vascular compartment, or reduction of cerebral edema. Chelators are used in situations of acute metal intoxication (Chapters 9 and 76). However, some distinctions of biological targets are a matter of perspective. Bile acid sequestrants lower plasma cholesterol by inhibiting the recycling of bile acids within the

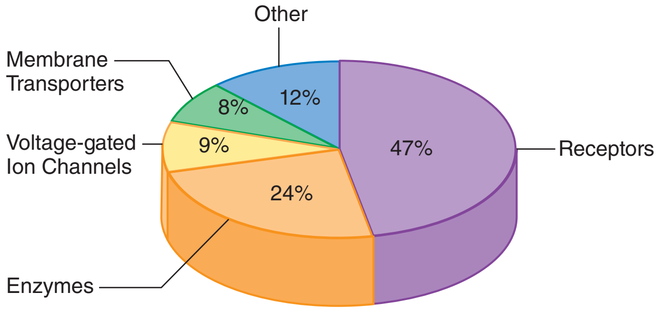


Figure 3–1 *Drug targets in humans.* Depicted are categories of drug targets expressed in humans as a percentage of total number of targets. The principal categories are receptors for endocrine and paracrine factors, enzymes, voltage-gated ion channels (plus a few other ion channels apart from those classified as receptors), and membrane transporters. "Other" includes DNA, RNA, and ribosomes, signaling molecules themselves, enzyme-interacting proteins, and structural proteins. The source for these data is Rask-Andersen et al. (2011), with adjustment according to distinctions in this chapter between ion channels and membrane transporters. The reader may wish to consult as well Imming et al. (2006) and Santos et al. (2017). The figure does not take into account microbial targets. Drugs from the top 100 prescribed in the U.S. for 2018 (source: CliniCalc) for ***receptors*** include: *for GPCRs*, AT$_1$ receptor antagonists, *albuterol*, *carvedilol*, *tramadol* and other opioids, *tamsulosin* and other α adrenergic receptor antagonists, *atenolol*, *clopidogrel*, *propranolol*, *cyclobenzaprine*, *ranitidine*, *loratadine* and other histamine H$_1$ receptor blockers, *clonidine* and other α$_2$ receptor agonists, *buspirone*, *latanoprost*, and *sumatriptan*; for *ligand-gated ion channels*, *alprazolam* and other benzodiazepines and positive GABA$_A$ receptor modulators and *ondansetron*; for *receptor tyrosine kinases*, various forms of *insulin*; for *nuclear hormone receptors*, *levothyroxine*, *fluticasone* and other glucocorticoid agonists, *estradiol* and other estrogen receptor agonists, *norethindrone* and other progesterone receptor agonists, *ergocalciferol*, *spironolactone*, and *fenofibrate*. Those drugs for ***enzymes*** include *lisinopril* and other ACE inhibitors, *atorvastatin* and other HMG-CoA reductase inhibitors, *metformin*, *ibuprofen* and other NSAIDs, *warfarin*, *allopurinol*, *finasteride*, *apixaban*, and *sitagliptin*. Those drugs for ***voltage-gated ion channels*** and other ion channels include *amlodipine*, *gabapentin* and other anticonvulsants that inhibit neuronal voltage-gated calcium channels, *glipizide* and other activators of the ATP-regulated K$^+$ channel, *lamotrigine* and other anticonvulsants that inhibit voltage-gated sodium channels, and *diltiazem*. Those drugs for ***membrane transporters*** include *omeprazole* and other proton pump inhibitors, *hydrochlorothiazide*, *sertraline* and other monoamine reuptake inhibitors, *digoxin*, and *furosemide*.

lumen of the intestine; bile acids are not "targets" as defined in this chapter, but a key to the mechanism by which bile acid sequestrants work is in fact consequent de-repression of 7α-hydroxylase, which may well be considered a target.

A large number of drugs bind to serum albumin, a protein important to oncotic pressure and the transport of free fatty acids within the bloodstream. The drugs that bind to albumin are generally organic acids. Yet, albumin cannot be considered a drug target in any pharmacodynamic sense, as it does not translate the binding of the drug into a therapeutic action. Nor for the same reason can α$_1$-acid glycoprotein, a serum protein that binds organic bases, be considered a target. Interactions of drugs with these proteins are more appropriately considered in the context of pharmacokinetics, as the binding impedes the distribution of drugs into tissues (Chapter 2). Likewise, the redistribution of lipophilic drugs such as thiopental into adipose tissue (see Figure 2–4), so important to how the body handles general anesthetics, is the province of pharmacokinetics, since the partitioning of the drug is without a pharmacodynamic effect on a target.

Receptors

Pharmacologists often define a *receptor* as a protein that recognizes a signaling molecule endogenous to the organism, i.e., an endocrine, paracrine, autocrine, or juxtacrine factor (Box 3–1), and that translates that recognition into a meaningful cellular event. Receptors include GPCRs, ligand-gated ion channels, enzyme-linked (i.e., catalytic) receptors, other membrane-associated receptors, and nuclear receptors. Many receptors recognize signals at the cell surface; others, for example the nuclear receptors, recognize those that pass through the cell membrane. The term *ligand* is used for any molecule that binds to a receptor, whether endogenous or not.

BOX 3–1 ■ Types of Intercellular Signaling Molecules

Endocrine factor: A chemical messenger that is released into the circulation to produce effects distant from the point of release. Also referred to as a hormone. Originally, hormones were considered a product of a ductless gland; however, many organs are now considered "endocrine."

Paracrine factor: A chemical messenger that is released from one cell to produce effects on a neighboring cell. Neurotransmitters, cytokines, morphogens, and many growth factors exert paracrine effects.

Autocrine factor: A chemical messenger that exerts actions on the same cell from which it is released. Many endocrine and paracrine factors also play roles in autocrine signaling, exerting negative or positive feedback on their own release, roles that are especially important in neuronal and cytokine signaling.

Juxtacrine factor: A chemical messenger that remains affixed to the cell in which it is produced and exerts actions on a physically juxtaposed cell. The mechanism by which a T cell and an antigen-presenting cell establish an immunological "synapse" is an example of juxtacrine signaling.

Agonism refers to the capacity of a ligand to activate a receptor. If a ligand binds to the same site as the endogenous signaling molecule to achieve activation, it—like the endogenous molecule—is said to be an *orthosteric agonist*. If, instead, the ligand binds to a different site to achieve activation, it is identified as an *allosteric agonist*. Ligands that produce a maximal response for a given population of receptor are *full agonists*, whereas those that produce a response but fall short of producing a maximal response despite fully occupying that population are *partial agonists*. The use of "response" in place of "receptor activation" in defining full and partial agonism here is pragmatic. What constitutes an activated conformation or set of conformations of a receptor is difficult to assess; downstream responses are more easily measured. An important qualification exists in both cases: Any ranking of ligands according to agonism for a given receptor may change depending on the tissue and specific response being measured, i.e., ranking according to agonism, which is later described in terms of *efficacy*, can be a function of context. Context is discussed more fully in the section Biased Agonism.

Antagonism at the receptor level relates to the property of a ligand, almost always an exogenous ligand (i.e., a drug) or toxin, to block the action of an agonist. Numerous *antagonists* are orthosteric and competitive in nature, that is, they bind essentially to the same receptor site as that used by the endogenous agonist and they do so reversibly. A few antagonists bind irreversibly. *Clopidogrel* and *prasugrel*, which inhibit platelet activation as antagonists of the P2Y$_{12}$ subtype of ADP receptor (Chapter 36), are examples of irreversible inhibitors. Many orthosteric antagonists are relatively *neutral* in the sense of having little if any impact *per se* on basal receptor activity, their effect becoming noticeable only in the presence of an agonist. Other antagonists are *inverse agonists*, as discussed below.

Forms of antagonism exist that operate apart from the receptor altogether. *Chemical antagonism* involves neutralization of an agonist through physical complexation. *Adalimumab* and *infliximab*, used in the treatment of several autoimmune disorders (Chapters 39 and 55), bind tightly to tumor necrosis factor α (TNF-α) and antagonize its actions. *Bevacizumab*, used in the treatment of certain cancers and ocular disorders, antagonizes the action of vascular endothelial growth factor (VEGF) by similar complexation (Chapter 72).

Functional antagonism refers to the inhibition of an agonist's action downstream of the receptor. The use of *epinephrine* in countering anaphylaxis represents functional antagonism. Epinephrine increases cyclic AMP levels within bronchiolar smooth muscle cells, which in turn inhibits myosin light chain kinase and thereby diminishes the contractile response engendered by histamine, thromboxane A_2, and other substances released by mast cells (Chapter 43).

Pharmacokinetic antagonism relates to the impediment of the absorption or distribution, or the enhancement of elimination, of a therapeutic drug, as discussed in Chapter 2.

Many receptors exhibit at least a modicum of constitutive activity, that is, activity in the absence of a ligand, which is the product of normal thermodynamic equilibria between active and inactive conformations. *Inverse agonism* refers to the suppression of this activity by ligands, and such ligands are termed *inverse agonists*, acting through stabilization of receptors in an inactive conformation. Depending on the level of constitutive activity and degree of suppression, it can be difficult to distinguish inverse agonists from neutral antagonists. At an orthosteric level, both can be viewed as antagonists of partial or full agonist action.

The actions of full agonists, partial agonists, neutral antagonists, and inverse agonists are easily accommodated by a *two-state model* of receptor activity wherein an equilibrium between inactive and active states for a population of any given receptor is pulled one way or another (or not, in the case of neutral antagonists) by ligands (Figure 3–2). More sophisticated models exist to account for what appears to be a large number of functional states for GPCRs, either intrinsic or dependent on proteins with which the receptors interact (Weiss and Kobilka, 2018).

As implied above for inverse agonists, what defines a ligand as an antagonist at an operational level can sometimes be a matter of semantics. Focusing on orthosteric ligands for the moment, inverse agonists can antagonize the actions of partial and full agonists. A partial agonist will antagonize the actions of a full agonist, or of an inverse agonist for that matter, similarly by occluding the binding site. The duality of agonism and antagonism for partial agonists is therapeutically relevant. *Varenicline*, used in the treatment of nicotine addiction, is a partial agonist at the α4β2 nicotinic receptor; it is posited to at least partly block the actions of nicotine derived from nicotine-containing products but to have sufficient activity as a partial nicotinic agonist to blunt the craving for nicotine (Chapter 13). *Buprenorphine* likely operates by a similar principle in the treatment of opioid abuse and withdrawal (Chapter 23). *Aripiprazole* is a partial agonist used in the treatment of psychosis to inhibit activation of the D_2 receptor by endogenous dopamine but also provides an important, low-level degree of activation (Chapter 19). Certain β_1 receptor–selective antagonists (e.g., *pindolol* and *acebutolol*) are in fact partial agonists whose "intrinsic sympathomimetic activity" may prevent profound bradycardia or negative inotropy (Chapter 14).

Receptor ligands also include allosteric modulators, both positive and negative. These are discussed in the section Allosteric Modulation of Receptor Function.

Enzymes

Enzymes are important targets for drugs for essentially two reasons. First, enzymes have especially varied and selective roles in essential life processes. Second, the mechanisms of catalysis and allosteric regulation are well defined and therefore amenable to pharmacological manipulation. Enzymes that are drug targets are almost always those that are rate-limiting or otherwise necessary in the metabolic process of interest, for example, hydroxymethylglutaryl–coenzyme A (HMG-CoA) reductase in the synthesis of cholesterol, which is inhibited by *statins* (Chapter 37); angiotensin-converting enzyme (ACE) in the synthesis of angiotensin II,

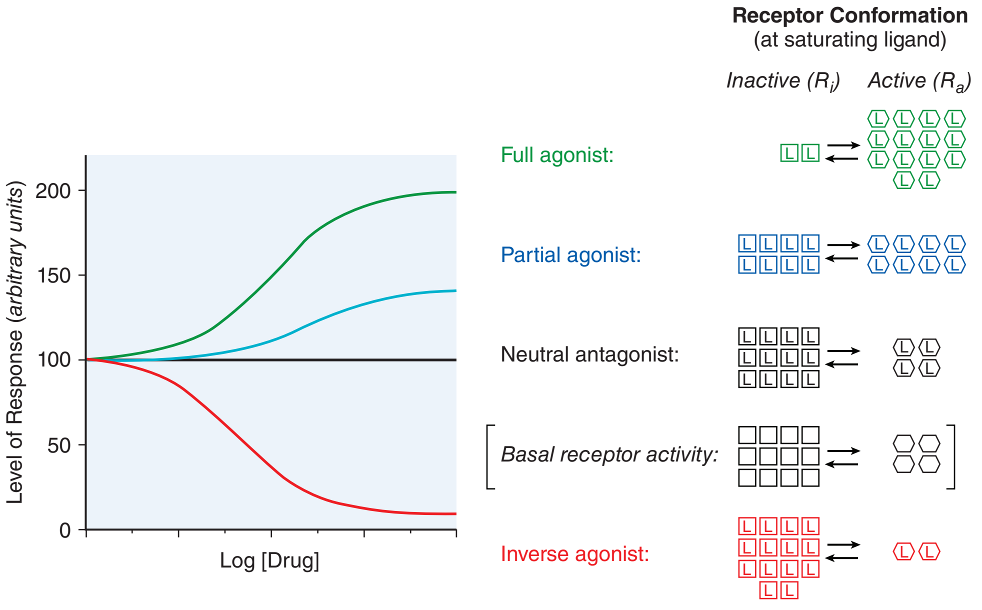


Figure 3–2 *Two-state model of receptor activity.* In this model, receptor *R* can exist in active (R_a, depicted as a hexagon) and inactive (R_i, depicted as a square) conformations in thermodynamic equilibrium. The equilibrium in the absence of drug or endogenous ligands (a "response" set arbitrarily to "100" units in the graph to the left and noted in brackets in the depiction of receptor states to the right) underlies what is commonly referred to as "basal" or "constitutive" activity, terms connoting a certain—usually small—level of active receptor. Drugs binding individual receptor states or both states simultaneously can influence the balance of the two forms of *R* and the net effect of receptor-controlled events. The ordinate of the graph on the left is the response of the receptor produced by R_a, the active receptor conformation (e.g., stimulation of AC by an active β adrenergic receptor conformation). If a drug *L* (*L*, for ligand) binds to R_a with a certain degree of selectivity relative to R_i, it will produce a positive response; the greater the selectivity for R_a, the greater the response. The difference in selectivity differentiates *full* from *partial* agonists; no "full" agonist, however, is likely able to pull the equilibrium entirely to the right. If *L* has an equal affinity for R_i and R_a, it will not perturb the basal equilibrium between them and will therefore have no effect on basal activity; this is the case for a *neutral antagonist*. If the drug selectively binds to R_i, then the net influence and amount of R_a will be diminished. If there is sufficient R_a to produce an observable basal response, and *L* binds selectively to R_i, then that basal response will be inhibited; *L* will then be an *inverse agonist*. In systems in which basal activity is not observable, inverse agonists seem to behave like neutral antagonists, which helps explain that the properties of inverse agonists and the number of such agents previously described as competitive antagonists were only recently appreciated. The panel on the right represents equilibria at saturating concentrations of *L*; whether the ligands bind through induced fit or conformational selection is not depicted.

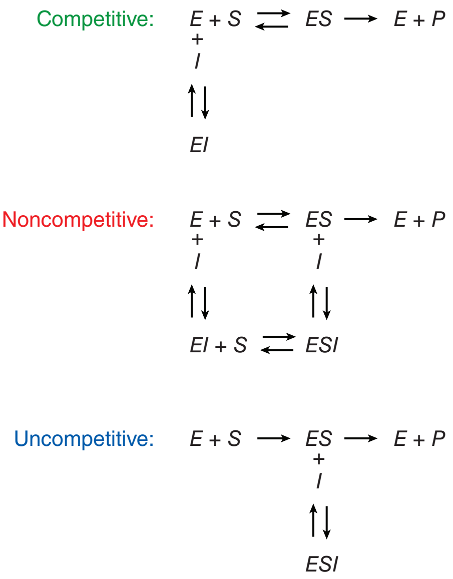


Figure 3–3 *Reversible enzyme inhibition.* The three different forms of reversible inhibition for enzymes are depicted. *E*, enzyme; *S*, substrate; *I*, inhibitor; *P*, product. The reader is directed to an excellent monograph covering enzyme inhibition, both reversible and irreversible, in a pharmacological context by Copeland (2013).

which is inhibited by *ACE inhibitors* (Chapter 30); cyclooxygenase in the synthesis of prostaglandins, which is inhibited by *aspirin* (acetylsalicylate) and other nonsteroidal anti-inflammatory drugs (NSAIDs; Chapter 42); and dihydrofolate reductase in the production of the nucleotide thymidylate, which is inhibited by *methotrexate* (Chapter 70).

Most drugs that utilize enzymes as targets can be divided broadly into *reversible* and *irreversible inhibitors* of the catalytic process. Reversible inhibitors bind to the enzyme in a fashion that can be described reasonably by on- and off-rate constants serving to establish a dynamic chemical equilibrium (Figure 3–3). Most of these inhibitors are *competitive*, wherein the binding of the inhibitor and substrate to the enzyme is mutually exclusive. Others are *noncompetitive* or *uncompetitive*. Noncompetitive inhibition refers to a mechanism in which the inhibitor binds to the enzyme regardless of bound substrate, whereas uncompetitive inhibition is that in which the binding of inhibitor requires the conjoint binding of substrate. In both noncompetitive and uncompetitive inhibition, the enzyme to which substrate and inhibitor are bound is less able to effect catalysis than with substrate alone.

Irreversible enzyme inhibitors form stable covalent linkages with the enzyme, thereby disrupting catalytic activity generally for the enzyme's lifetime. *Aspirin*, which acetylates cyclooxygenases near the catalytic site, is an irreversible inhibitor of arachidonic acid conversion to prostaglandins G_2 and H_2, the first intermediates in the synthesis of a variety of prostanoids (Chapter 41). Irreversible inhibitors also include those that are mechanism-based, commonly referred to as *suicide substrates*. These inhibitors are recognized by the enzyme as a substrate, but during the process of catalysis, generally at the transition state, they are converted to intermediates capable of attacking the enzyme. *Organophosphates*, which irreversibly inhibit acetylcholinesterase through phosphorylation, provide an example of such a mechanism-based irreversible inhibition (Chapter 12). *Omeprazole*, an inhibitor of the gastric H^+/K^+ ATPase, is another example (Chapter 53). *Heparin*, an anticoagulant, potentiates the ability of an endogenous suicide substrate, antithrombin, to irreversibly inhibit thrombin and factor Xa (Chapter 36).

Not all drugs that target enzymes are inhibitors. *Organic nitrates*, for example, activate soluble guanylyl cyclase. In this case, nitric oxide (NO), a product of enzymatic transformation of the organic nitrates, combines with the heme of the enzyme to achieve activation. The subsequent increase in intracellular cyclic GMP is the basis for NO-induced vasodilation (Chapter 31).

Ion Channels

Changes in the flux of ions across the plasma membrane achieved through ion channels are critical regulatory events in both excitable and nonexcitable cells. These changes are superimposed on electrochemical gradients, prominently those underlying cell-surface membrane electrical potential, which are established by ion transporters for Na^+, K^+, Ca^{2+}, and Cl^-. For instance, the Na^+/K^+-ATPase expends cellular ATP to pump Na^+ out of the cell and K^+ into the cell. The electrochemical gradients thus established are used by excitable tissues such as nerve and muscle through ion channels to generate and transmit electrical impulses, by nonexcitable cells to trigger biochemical and secretory events, and by all cells to support a variety of secondary symport and antiport processes (see Figures 2–2 and 4–4).

Humans express about 230 ion channels (Jegla et al., 2009). These are divided into families based principally on the ions they conduct, molecular architecture, and method of gating. They are subdivided according to properties of conduction, including the kinetics of activation and deactivation. Those relevant to pharmacology are primarily voltage-, G protein-, and ligand-gated ion channels. Voltage-gated ion channels are discussed here; G protein- and ligand-gated ion channels are discussed in the section Receptor-Mediated Mechanisms of Drug Action.

Voltage-gated ion channels are sensitive to changes in the electrochemical potential across a membrane, undergoing activation upon membrane depolarization. Any introduction to pharmacodynamics must pay attention to them: They are exceedingly important drug targets; a considerable number of them are downstream of receptors engaged by endocrine and paracrine factors, for example neurotransmitters; and they provide a context for understanding all other types of channels. Moreover, mutations in voltage-gated ion channels can lead to diseases (referred to as *channelopathies*) including various seizure disorders (Chapter 20), other neurological diseases, and cardiac arrhythmias (Chapter 34).

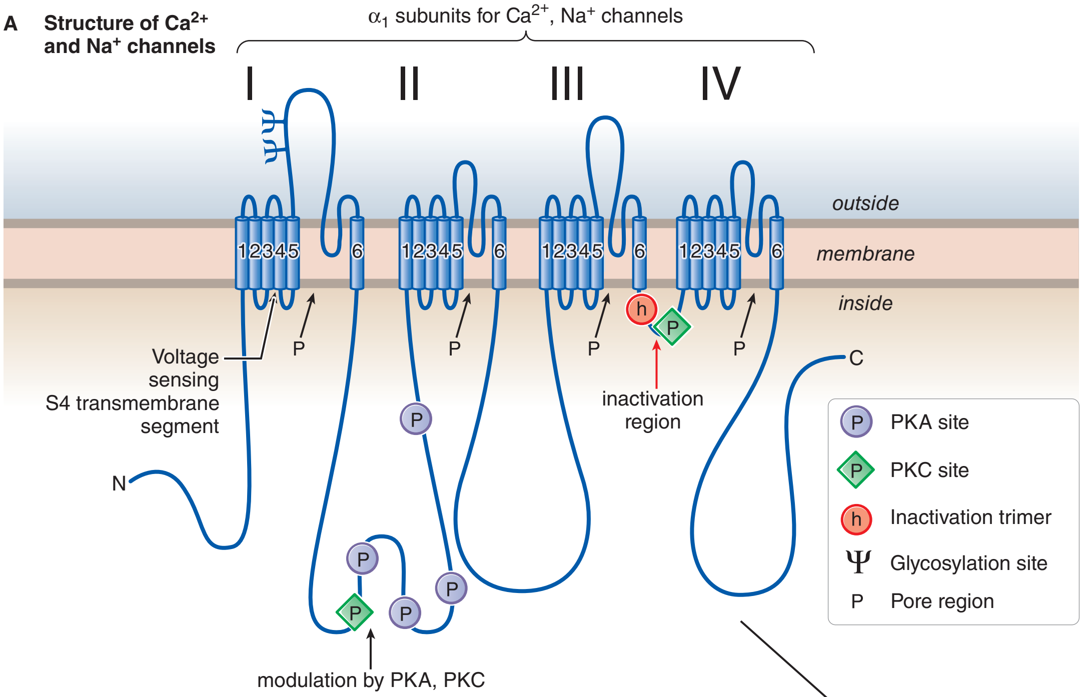
Voltage-Gated Na^+ Channels. Voltage-gated Na^+ channels are responsible for the propagation in nerve and muscle cells of robust action potentials that depolarize the membrane from its resting potential of about −70 mV, depending on the cell, to a potential of +20 mV within a few milliseconds. The activation of these channels that sets into motion the action potential generally occurs in response to a localized depolarization achieved by ligand-gated cation channels in neurons and skeletal muscle and by activated L-type Ca^{2+} channels in the sinoatrial node of the heart; the propagation of the action potential is achieved through sequential activation of, and by, the Na^+ channels themselves.

Voltage-gated Na^+ channels comprise three subunits, a pore-forming α subunit and two regulatory β subunits (Catterall et al., 2020). The α subunit is a single 260-kDa protein containing four domains, each consisting of six membrane-spanning helices (S1–S6), that arrange into a pseudotetrameric shape to form a Na^+ ion-selective pore (Figures 3–4A and 25–2). The β subunits are 36-kDa proteins that span the membrane once. An extracellular loop between S5 and S6 of the α subunit, termed the pore-forming or P loop, dips back into the pore and, combined with residues from the corresponding P loops from the other domains, provides a selectivity filter for the Na^+ ion. Four other helices surrounding the pore (one S4 helix from each of the domains) each contain a set of charged amino acids that form the voltage sensor and cause the pore to open at more positive membrane voltages, those that initiate activation.

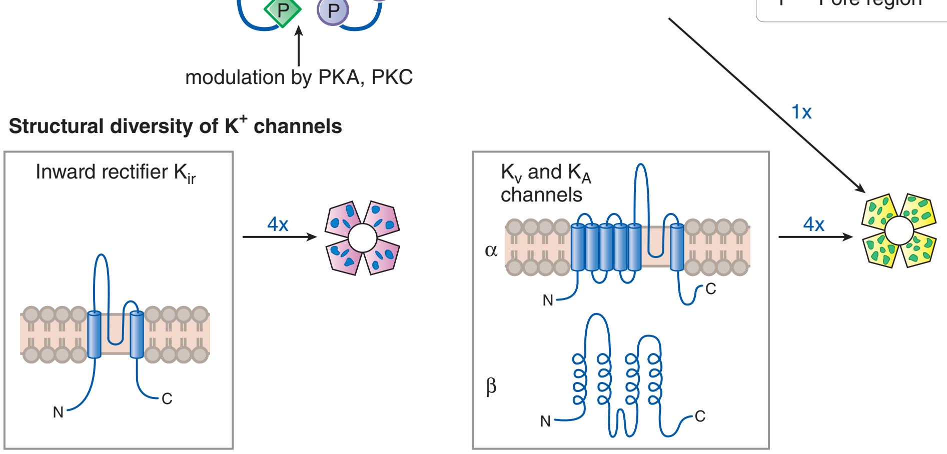
The voltage-gated Na^+ channels in neurons that sense pain (*nociception*) are targets for local anesthetics, such as *lidocaine* and *tetracaine*, which block the pore to inhibit depolarization and thus block the transmission relevant to the sensation of pain (see Figure 25–3). They are also targets for certain *antiepileptics* (Chapter 20) and *antiarrhythmics* (Chapter 34). Catterall et al. (2020) provide an extensive discussion of the state-dependent blockade by these drugs (i.e., the dependence of these drugs on resting membrane potential and frequency of the action potential). The channels are also the targets of the naturally occurring marine toxins, including *tetrodotoxin*, *saxitoxin*, and a number of *conotoxins*.

Voltage-Gated Ca^{2+} Channels. Voltage-gated Ca^{2+} channels are architecturally quite similar to Na^+ channels, with a large α subunit (four

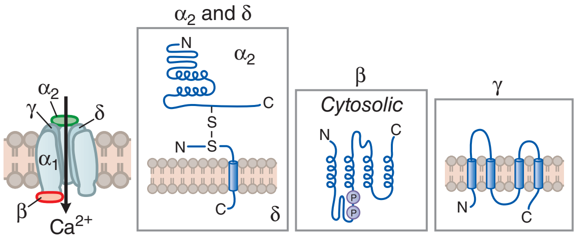
A Structure of Ca^{2+} and Na^+ channels


α_1 subunits for Ca^{2+}, Na^+ channels
I
II
III
IV
outside
membrane
inside
12345
6
12345
6
12345
6
12345
6
P
P
P
P
h
P
Voltage sensing S4 transmembrane segment
inactivation region
N
C
P
P
P
P
P
modulation by PKA, PKC
P PKA site
P PKC site
h Inactivation trimer
Ψ Glycosylation site
P Pore region

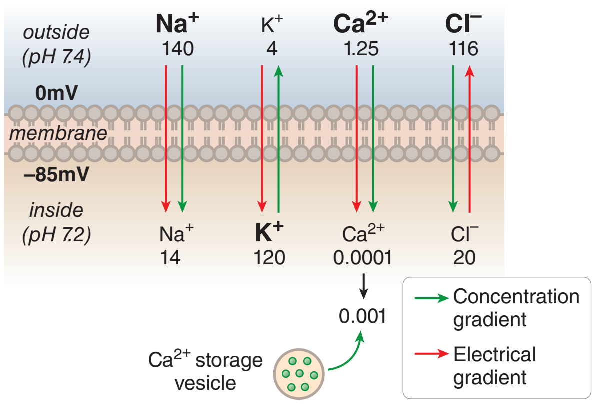
B Structural diversity of K^+ channels


Inward rectifier K_{ir}
N
C
4x
K_v and K_A channels
α
N
C
β
N
C
4x
1x

C Multisubunit assembly of Ca^{2+} channels


α_2
γ
δ
α_1
β
Ca^{2+}
α_2 and δ
N
α_2
C
S
S
N
C
δ
β
Cytosolic
N
C
P
P
γ
N
C

D Ionic gradients across a mammalian nerve cell membrane


outside (pH 7.4)
0mV
membrane
–85mV
inside (pH 7.2)
Na^+ 140
K^+ 4
Ca^{2+} 1.25
Cl^- 116
Na^+ 14
K^+ 120
Ca^{2+} 0.0001
Cl^- 20
0.001
Ca^{2+} storage vesicle
Concentration gradient
Electrical gradient

domains of six membrane-spanning helices) and auxiliary regulatory subunits (e.g., β and $\alpha_2\delta$ subunits; Figures 3–4C and 16–2) (Catterall et al., 2020). Ca^{2+} channels can be major components of an action potential, as in pacemaker cells of the heart, but are more commonly responsible for translating, via Ca^{2+} influx, action potentials into intracellular responses. There are three general types of Ca^{2+} channels. L-type Ca^{2+} channels are expressed in cardiac, smooth muscle, and skeletal muscle cells and in cells of endocrine tissues, where they are involved in excitation-contraction coupling and secretion. Currents conducted by L-type Ca^{2+} channels may require strong depolarization, and they are long-lasting (hence, "L" as the type identifier). These channels are blocked by *dihydropyridines*, such as *nifedipine*, and by *diltiazem* and *verapamil*, drugs widely used to treat hypertension, angina, and certain cardiac arrhythmias (see Chapters 31, 32, and 34). P/Q-, N-, and R-type Ca^{2+} channels are expressed mostly in neurons and are involved in neurotransmitter release. They also require strong depolarization for activation. The T-type channels are expressed in neurons and in cardiac and smooth muscle cells. The currents generated by ion flux through these channels are activated by weak depolarizations and are transient. These channels facilitate cardiac pacemaker depolarization. They are also found in thalamic neurons, where the current amplifies oscillations in membrane potential, one oscillation being that observed in absence seizure (Chapter 20); *ethosuximide* and *valproate* are antiepileptics used to inhibit the T-type channel. L-type Ca^{2+} channels are subject to modulation via phosphorylation by protein kinase A (PKA), while the P/Q, N-, and R-type channels are more proximately modulated by GPCRs, as discussed later (Receptor-Mediated Mechanisms of Drug Action).

Voltage-Gated K$^+$ Channels. Voltage-gated K$^+$ channels are the most numerous of the voltage-gated ion channel family (Gutmann et al., 2005; Wulff et al., 2009). These channels are tetramers of four similar or identical six-transmembrane $K_V\alpha$ subunits that together form a conductance pore with voltage-sensing capability (Figure 3–4B). There are 40 human $K_V\alpha$ genes that sort into 12 subfamilies. The channels regulate the waveform and pattern of action potentials (Attali et al., 2019). Several antiarrhythmic drugs, for example *amiodarone* and *sotalol*, modulate these channels directly or indirectly and typically together with other voltage-gated ion channels (Chapter 34). A common mechanism by which drugs prolong action potentials in heart and provoke arrhythmias is inhibition of a specific delayed rectifier current generated by hERG, a channel more often referred to as K_V11.1 (the product of *KCNH2*; Chapter 34).

Specificity of Drug Responses

The degree to which a drug achieves an intended action relative to other actions is referred to as *specificity* or *selectivity* of action. The affinity of a drug for its molecular target is of obvious importance in this regard, as a high-affinity interaction minimizes the chance of off-target effects at therapeutic concentrations. Specificity depends on other factors as well. One is the extent to which the site at which a drug interacts is unique to that target. The conservation of orthosteric sites among subtypes of muscarinic receptors, for example, makes the pharmacological manipulation of any single subtype difficult (Chapter 11). Moreover, certain seemingly disparate receptors or enzymes have orthosteric binding sites of common ontological origin. This is the case for muscarinic receptors and the H_1-histamine receptor, as manifest in the atropine-like effects of classical antihistamines (Chapter 43). That said, an example of an orthosteric site where there has been some success in achieving relatively high target specificity is the ATP-binding site of protein kinases, for which there are approximately 800 different genes in the human genome, many of which are involved in the pathogenesis of cancer (Chapter 71). *Imatinib* targets the ATP-binding site of the Bcr-Abl protein kinase and is used to treat chronic myeloid leukemia; *imatinib* was the first protein kinase inhibitor targeting a specific protein kinase to be FDA-approved (Cohen et al., 2021).

Specificity of action can also depend on the extent to which the target is distributed among tissues. A target expressed in one or only a few tissues will almost certainly support a higher degree of therapeutic specificity than one expressed throughout the body, as drug-elicited effects in the latter case would occur in tissues likely irrelevant to the pathological event. The manner in which the drug itself distributes among tissues, an important concept in pharmacokinetics, will also have an impact on specificity of action. Peripherally restricted μ-opioid receptor antagonists such as *methylnaltrexone*, unlike *naloxone* or *naltrexone*, have little impact on the (central) analgesic actions of opioids (Chapter 23).

No drug is entirely specific in terms of action. The departure from a high degree of specificity in certain circumstances can be advantageous. The sedation exerted by classical antihistamines, for example, can be a welcome action secondary to the peripheral blockade of histamine in the treatment of pruritis. However, more often, the unintended actions of a drug are a nuisance, or worse if the unintended actions rise to the level of physical or psychological harm (i.e., to the point of becoming adverse drug events).

On occasion, the promiscuity is not that of a drug, but of the target. The K_V11.1 (hERG) channel contains a site that can interact with a variety of structurally dissimilar molecules. The prolongation of the QT interval with consequent *torsades de pointes* (a form of ventricular tachycardia) is a serious off-target effect of many drugs. The FDA now requires all new drugs to be tested for their capacity to bind the K_V11.1 channel and prolong the QT interval.

Complicating the picture of specificity is the fact that some drugs are administered as racemic mixtures of stereoisomers, each of which can

Figure 3–4 *Voltage-dependent Na$^+$, Ca^{2+}, and K$^+$ channels.* Voltage-dependent channels provide for rapid changes in ion permeability along axons and within dendrites and for excitation-secretion coupling that causes neurotransmitter release from presynaptic sites. **A.** *Structure of Ca^{2+} and Na$^+$ channels.* The α subunit in both Ca^{2+} and Na$^+$ channels consists of four sub-subunits or segments (labeled **I** through **IV**), each with six transmembrane (TM) hydrophobic domains (blue cylinders). The hydrophobic regions that connect TM5 and TM6 in each segment associate to form the pore of the channel. Segment 4 in each domain includes the voltage sensor. (Adapted with permission from Catterall W. *Neuron* **2000**, *26*:13–25. © Elsevier). **B.** *Structural diversity of K$^+$ channels.* Inward rectifier, K_{ir}: The basic subunit of the inwardly rectifying K$^+$ channel protein K_{ir} has the general configuration of TM5 and TM6 of a segment of the α subunit shown in panel **A**. Four of these subunits assemble to create the pore. Voltage-sensitive K$^+$ channel, K_v: The α subunits of the voltage-sensitive K$^+$ channel K_v and the rapidly activating K$^+$ channel K_A share a hexaspanning structure resembling in overall configuration a single segment of the Na$^+$ and Ca^{2+} channel structure, with six TM domains. Four of these assemble to form the pore. Regulatory β subunits (cytosolic) can alter K_v channel functions. **C.** *Multisubunit assembly of Ca^{2+} channels.* Ca^{2+} channels variably require several auxiliary small proteins (α_2, β, γ, and δ); α_2 and δ subunits are linked by a disulfide bond. Likewise, regulatory subunits also exist for Na$^+$ channels. **D**. *Ionic gradients across a mammalian nerve cell membrane.* The kidney is the primary regulator of the extracellular ionic environment. Active transport of cations and the relatively selective permeabilities of ion channels maintain the intracellular milieu. In this figure, the numbers below the various ions are resting state concentrations in mM; the large bold lettering of the elements indicates the location of the higher concentration of the ion; the red and green arrows indicate the direction of the electrical and concentration gradients. In the resting state, Na$^+$ channels are closed, K$^+$ channels are open, and the membrane potential approaches the Nernst potential for K$^+$. The opening of Na$^+$ channels results in depolarization. In contrast, the K$^+$ gradient is such that increased permeability to K$^+$ results in *hyperpolarization*. Changes in the concentration of intracellular Ca^{2+} (entry via Ca^{2+} channels and mobilization of Ca^{2+} sequestered in the cell) affect multiple cellular processes and are critical for the release of neurotransmitters. Cl$^-$ flows through membrane channels, a large fraction of which are gated by GABA or glycine. Activation of neuronal $GABA_A$ receptors generally leads to a net influx of Cl$^-$, resulting in membrane hyperpolarization and inhibition of depolarization. The equilibrium potential for Cl$^-$ is relatively close to the membrane resting potential, and small changes in cellular Cl$^-$, the membrane potential, and the activities of Cl$^-$ transporters (e.g., KCC2 and NKCC1) can influence transmembrane Cl$^-$ movements.

exhibit different pharmacodynamic as well as pharmacokinetic properties. The antiarrhythmic drug *sotalol* is a good example. While the *D*- and *L*-enantiomers of *sotalol* are equipotent as K^+ channel blockers, the *L*-enantiomer is a much more potent β adrenergic antagonist. A variety of drugs initially available as racemic mixtures are now available in single-stereoisomeric forms, often underscoring differences in the properties of resolved forms.

The metabolic conversion of a drug to one or more metabolites that retain activity is also a confounding factor in specificity. Active metabolites typically differ from the parent drug in efficacy, but some differ qualitatively as well in their actions. Aside from prodrugs, these are commonly recognized as toxic metabolites and include, for example, normeperidine, a metabolite of *meperidine* likely responsible for CNS excitation (Chapter 23) and *N*-acetyl-*p*-benzoquinone imine, a metabolite of *acetaminophen* responsible for hepatic and renal necrosis (Chapter 5).

Additivity and Synergism: Isobolograms

Drugs with different mechanisms of action are often used in combination to achieve *additive* and *positive synergistic* effects. Such interactions of two agents may permit use of reduced concentrations of each drug, thereby reducing concentration-dependent adverse effects. Positive synergism refers to the *superadditive* effects of drugs used in combination, in which the effect of the two drugs together is larger than the sum of their individual effects. Drugs used in combination can also demonstrate *negative synergism* or *subadditive* effects, where the efficacy of the drug combination is less than would be expected if the effects were additive. Isobolograms, such as that depicted in Figure 3–5, provide a quantitative depiction of positive and negative synergism. The basis for the use of isobolograms has been developed and reviewed by Tallarida (2012) and explained in the legend to Figure 3–5. Using isobolograms to characterize the possible synergistic pharmacodynamic interactions of several different antiseizure drugs in a mouse model is demonstrated by Metcalf et al. (2018).

Attenuation of Drug Responses

Feedback regulation is a defining feature of biological systems. A cell or an organism may perceive the actions of a drug as a perturbation of homeostasis and attempt to counter the actions of that drug in order to restore the *status quo* through molecular and physiological mechanisms. Mechanisms include those accomplished through the sympathetic nervous system and the renin-angiotensin-aldosterone system (Chapters 29 and 32), among many other cellular and system-wide feedback loops. Reflex tachycardia mediated by the sympathetic nervous system is a common response to vasodilators used to lower blood pressure, such as *dihydropyridines*, *hydralazine*, and *organic nitrates*, sometimes requiring administration of a β blocker to blunt the increase in heart rate (Chapters 31–33).

Tachyphylaxis and *fade* are overlapping terms for a decline in response to a drug with repeated application or time, respectively. The two terms generally have a physiological connotation. While no mechanism needs be implied (Neubig et al., 2003), underlying processes include modulation of target expression and function, responses of superimposed tissue homeostatic mechanisms, and, in the limit for therapies directed toward cancer or pathogens, mechanisms of frank resistance. *Desensitization* also refers to attenuation of response, but typically at the level of the drug target itself. Desensitization is often distinguished from *downregulation*, the loss of a target with prolonged exposure to the ligand, although the two can be intimately related mechanistically.

Desensitization and downregulation are particularly well understood for GPCRs. *Homologous desensitization* refers to a relatively rapid decline

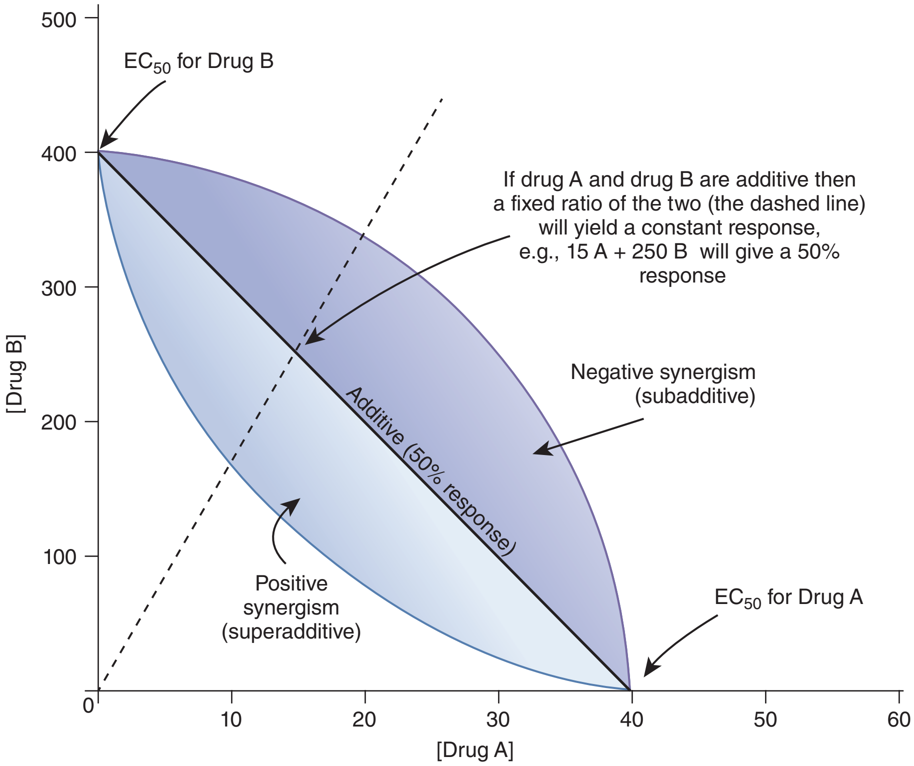


Figure 3–5 *Isobologram showing additivity and synergism of a drug combination.* The isobologram shows the line of additivity for a 50% effect obtained with a combination of two drugs (concentrations of drug A are on the *x* axis, concentrations of drug B are on the *y* axis) that have similar effects but different mechanisms of action. The intercept of the line of additivity (50% effect) with the *x* axis is the EC_{50} for A, while the intercept on the *y* axis is the EC_{50} for B. If the combination of A and B exhibits positive synergism (superadditivity), then the 50% effect with a combination of the two drugs will fall somewhere below the line of additivity, whereas negative synergism (subadditivity) will fall above the line of additivity. Lines of additivity for different percentage effects (e.g., 90% effect) are parallel to the 50% line of additivity. The isobologram can be used to estimate the concentrations of two drugs needed to obtain a given effect when used in combination. For a full explanation of the concept and utility of isoboles, consult Tallarida (2012).

in response of a GPCR to an agonist as a consequence of an interaction between the two. One form of homologous desensitization entails phosphorylation of the GPCRs by G protein-coupled receptor kinases (GRKs) with subsequent engagement of arrestins and internalization of receptors, as discussed in the section GRK- and Arrestin-Mediated Desensitization. Other forms of homologous desensitization exist, including those mediated by protein kinases, with or without arrestins, and involving other forms of internalization. *Heterologous desensitization* refers to a decline in response of a receptor to an agonist as a result of the activation of a different receptor-signaling pathway, typically by a different agonist. Phosphorylation of receptors by PKA and PKC figure prominently in heterologous desensitization, as do other posttranslational modifications, such as ubiquitination and palmitoylation (Patwardhan et al., 2021). Desensitization of GPCRs, while permitting a variable degree of recycling from endosomes, predisposes the receptors to downregulation through lysosomal degradation.

Desensitization and downregulation, while generally perceived to blunt the efficacy of agonists, are in some instances therapeutically valuable. The mechanisms by which selective serotonin reuptake inhibitors (SSRIs) attenuate depression are thought to involve downregulation of autoinhibitory $5HT_{1A}$, $5HT_{1D}$, and $5HT_7$ receptors and postsynaptic $5HT_{2A}$ receptors, while other serotonergic receptors remain responsive to the now increased levels of serotonin (Chapter 18). The downregulation of the gonadotropin-releasing hormone (GnRH) receptor by the sustained signaling achieved with GnRH congeners is the basis for the utility of these drugs in the treatment of GnRH-dependent precocious puberty (Chapter 46).

The phenomenon of *supersensitivity* reflects an adaptive process as well, often an adaptation to prolonged suppression of receptor signaling and usually manifested as enhanced receptor expression. It is most noticeable when an inhibitor or antagonist is abruptly withdrawn. Supersensitivity related to the dopaminergic D2 receptor is proposed to account for tolerance to antipsychotics (Chapter 19) and the enhanced β adrenergic responsiveness in physically active patients who, after long-term treatment with a β antagonist, abruptly stop the medication (Chapter 14). Supersensitivity is also easily observed for certain postsynaptic neurotransmitter receptors in instances of chronic denervation.

Attenuation of response to drugs that inhibit enzymes can be related to increases in target expression. The inhibition by *statins* of HMG-CoA reductase is opposed, albeit incompletely, by a substantive elevation in levels of the enzyme through autoregulatory forms of gene transcription and reduced protein degradation.

Attenuation of a drug's response, regardless of its target, can result from *auto*-induced expression of the enzymes responsible for the drug's degradation, a pharmacokinetic phenomenon. The induction of CYP3A4 (cytochrome P450 isoform 3A4) by *carbamazepine* is a classic example: After a single dose, the half-life ($t_{1/2}$) of *carbamazepine* is approximately 36 h; after repeated dosing, the $t_{1/2}$ drops by half.

The term *drug resistance* is used almost solely in relation to antimicrobial and cancer therapy and refers to the capacity of the pathogen or cell to avoid destruction by the drug. The overuse of antimicrobials leads to the selection of drug-resistant strains of microbes, a critical problem in human medicine and in animal husbandry for food production. Common mechanisms of antimicrobial resistance, discussed in detail in Chapter 56, are:

- Reduced entry of the drug into the pathogen or cell
- Enhanced export of the drug out of the pathogen or cell
- Alteration of target proteins
- Development of alternative pathways to circumvent those inhibited by the drug
- Release of enzymes that alter or destroy the drug

In cancer cells, one of several mechanisms of resistance to *methotrexate*, a competitive inhibitor of dihydrofolate reductase (DHFR), is upregulation of the enzyme through gene amplification or altered gene regulation (Chapter 70). Other mechanisms of resistance to *methotrexate* include impaired drug transport into cells due to low expression of the reduced folate carrier, diminished intracellular retention of the drug due to decreased polyglutamylation and/or an increased expression of an efflux transporter, and production of altered forms of DHFR that have a decreased affinity for *methotrexate*.

Pharmacodynamic Drug Interactions

While drugs can be used in combination to achieve an additive or synergistic therapeutic effect, combinations are also used simply to treat multiple concurrent conditions. In either case, *drug interactions* must be considered. The term *interactions*, as generally used, is not related to therapeutic interactions, but rather to interactions of the drugs that have the potential to cause adverse effects. Understanding the basis of such interactions provides the backdrop for preventing them.

Drug interactions are most often framed from a pharmacokinetic perspective in which one drug alters the absorption, distribution, metabolism, or elimination (ADME) of another drug. Pharmacokinetic drug interactions are discussed in Chapter 2. Those of a pharmacodynamic nature are less well categorized. Perhaps the most commonly encountered type of pharmacodynamic drug interaction relates to sedation. A large number of drugs exert sedative actions, and a combination of such drugs, through additive or synergistic forms of sedation, can cause excessive sedation, loss of consciousness, and even death. Another type of pharmacodynamic drug interaction pertains to prolongation of the QTc interval, wherein coadministration of two drugs that both cause prolongation of the interval has a greater chance of causing polymorphic ventricular tachycardia than either agent alone.

Other pharmacodynamic drug interactions are more selective or idiosyncratic in nature. For example, nitrovasodilators produce vasodilation via NO-dependent elevation of cyclic GMP in vascular smooth muscle, while *sildenafil*, *tadalafil*, and *vardenafil* achieve their pharmacological effects in the vasculature from inhibition of phosphodiesterase (PDE) 5 that hydrolyzes cyclic GMP to 5′GMP. Thus, coadministration of an NO donor (e.g., *nitroglycerin*) with a PDE5 inhibitor can cause profound vasodilation and potentially catastrophic hypotension. *Warfarin* has a narrow margin between therapeutic inhibition of clot formation and bleeding complications and is subject to numerous important pharmacokinetic and pharmacodynamic drug interactions. Alterations in dietary vitamin K intake may significantly affect the pharmacodynamics of *warfarin* and mandate altered dosing; antibiotics that alter the intestinal flora reduce the bacterial synthesis of vitamin K, thereby enhancing the effect of *warfarin*; and concurrent administration of NSAIDs with *warfarin* increases the risk of gastrointestinal bleeding almost 4-fold compared with *warfarin* alone.

What About Pharmacokinetics and Pharmacodynamics at the Extremes of Age?

Most drugs are evaluated in young and middle-aged adults. Data for children and the elderly are relatively sparse. Yet, pharmacokinetic and pharmacodynamic parameters at the extremes of age can differ radically from those of the prototypic adult, requiring avoidance of certain drugs or substantial alteration in the dose or dosing regimen to safely produce the desired clinical effect. A variety of resources cover neonatal and pediatric pharmacology. With regard to the elderly, the American Geriatrics Society publishes the Beers Criteria for Potentially Inappropriate Medication Use in Older Adults, which is an explicit list of drugs that should be avoided in older adults, drugs that should be avoided or be used at lower doses in older patients with reduced kidney function, and specific drug-disease and drug-drug interactions that are known to be harmful in this population.

Precision Medicine: A Pharmacodynamic Perspective

The intent of precision medicine is to understand an individual's genetic makeup, environment, and lifestyle in order to optimize approaches to the prevention and treatment of disease. From a pharmacodynamic perspective, the key to this pursuit is the identification of *biomarkers* or other surrogates that anticipate a response, whether therapeutic or adverse, to a

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given drug. The overwhelming emphasis to date has been on biomarkers at a genetic level, in part due to the relative ease in sequencing DNA. The classification of such biomarkers is the province of *pharmacogenetics* and *pharmacogenomics*, which are discussed in Chapter 7. *Epigenetics*, the study of modifications and changes in chromatin structure superimposed on the DNA sequence, will almost certainly prove as important, especially given its connection to environment and lifestyle.

Examples of genetic biomarkers at this point in time relate primarily to singular changes in DNA structure through somatic mutations and through single-nucleotide or structural polymorphisms. Good examples of biomarkers relating to therapeutic effectiveness are the V600E mutation in Raf-B required for the treatment of melanoma with *vemurafenib*; the overexpression of the HER2/neu receptor required for the treatment of breast cancer with *trastuzumab*; and the Philadelphia chromosome required for the treatment of chronic myelogenous leukemia with *imatinib* (Chapter 71). Landscapes of multiple polymorphisms and mutations, whose functional impact individually are not necessarily known, are proving important as well. For example, a high "mutational load" and tumor-specific neoantigens relating to T-cell recognition are associated with the degree of clinical benefit afforded by CTLA4 (cytotoxic T lymphocyte–associated protein 4) blockade in melanoma (Snyder et al., 2014).

Requirements for genetic testing that relate to minimization of especially harmful adverse effects include those for the human leukocyte antigen allele B*1502 polymorphism prior to administration of *carbamazepine* in patients of Asian ancestry and for glucose-6-phosphate dehydrogenase deficiency prior to administration of *rasburicase* in patients of African or Mediterranean ancestry. Testing for the AA polymorphic variant of the vitamin K epoxide reductase complex-1 (together with polymorphic variants of CYP2C9) can provide guidance in the initial dosing of *warfarin*. Why? Because the optimal dose can vary by greater than 10-fold among patients, with significant consequences for dosing that is too high (bleeding complications) or too low (clot formation).

Pharmacokinetic perspectives relating to precision medicine, and to pharmacogenetics/genomics in general, are provided in Chapter 7. The Pharmacogenomics Knowledge Base (PharmGKB) and FDA/European Medicines Agency drug labels provide guidelines at both pharmacodynamic and pharmacokinetic levels.

Concentration- and Dose-Response Relationships

The terms most often employed to describe the capacity of a drug to achieve an effect are *efficacy* and *potency*. These terms can be approached through evaluating the relationship between the concentration or dose of a drug and the elicited response(s). *Concentration-* or *dose-response curves* are typically modeled, as we do here, for the effects of agonists through receptors, but they are extensible to all targets. The focus of these curves tends to be placed on individuals, as opposed to populations, or on cells, tissues, or experimental samples in such a manner as to minimize variability. Populations are more easily evaluated by means of quantal concentration- or dose-effect curves (see the next section).

The concentration- or dose-response curve as it relates to receptors depicts the observed effect of an agonist as a function of its concentration in the receptor compartment or of the dose administered to an individual. Figure 3–6 shows a concentration-response curve, usually plotted as in Figure 3–6B. The exact placement and shape of the curve will depend on the affinity of the drug for the receptor, the extent to which the population of receptors might be in excess of the number required for a maximal response (*spare receptors*), and cooperative effects. Responses conforming to the depicted relationship share the property of being mathematically continuous, as opposed to quantal, in nature, a difference discussed later.

Some agonists elicit a response at one range of concentrations and suppress the same response at a higher range. The basis for this biphasic relationship, sometimes referred to as *hormesis*, is generally unknown; however, it may be at the root of some adverse drug responses (see Figure 9–2).

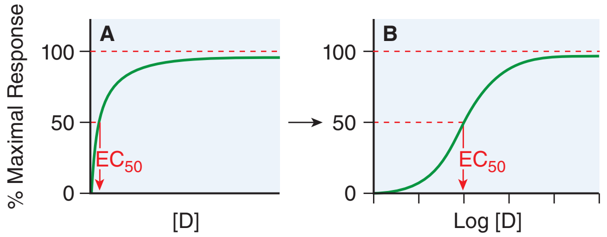


Figure 3–6 *Concentration-response curve.* **A.** *A selected response to an agonist "D" as a function of the agonist's concentration is plotted as a percentage of the maximum response to the agonist.* The concentration of agonist that produces 50% of the maximal response is referred to as EC$_{50}$ (effective concentration, indexed to 50% maximal response). **B.** *The hyperbolic shape of the curve in panel* ***A*** *becomes sigmoid when plotted semi-logarithmically.* The range of concentrations needed to fully depict the dose-response relationship (~3 log$_{10}$ [10] units) is too wide to be useful in the linear format of Figure 3–6A; thus, most dose-response curves use *log [Drug]* on the *x* axis, as in Figure 3–6B. The sigmoidal shape has three noteworthy features: threshold, slope, and maximal asymptote.

At a molecular level, efficacy relates to the ability of a ligand to bind to the receptor and promote a change in receptor conformation that evokes the measured downstream response. Differences in efficacy among ligands are most easily evaluated at saturating concentrations of the ligands, when the targeted receptors are fully occupied and the maximal response for each ligand is attained. This is depicted in Figure 3–7A, where the asymptotes relating to maximal effects, and hence efficacies, of two drugs differ. Full and partial agonists differ in efficacy; a neutral antagonist, having no effect on receptor conformation, has zero efficacy.

Potency is an expression of the activity of a drug in terms of the concentration or amount required to produce a defined effect (Neubig et al., 2003). The *median effective concentration* (EC$_{50}$) or dose (ED$_{50}$) is commonly used. In this case, a drug whose concentration-response curve lies to the left of another, as in Figure 3–7B, is said to be the more potent, regardless of differences in maximal responses. That said, the notion of a "defined effect" in the definition above lends itself to other interpretations of potency, for example, those not based on EC$_{50}$ or ED$_{50}$ but on

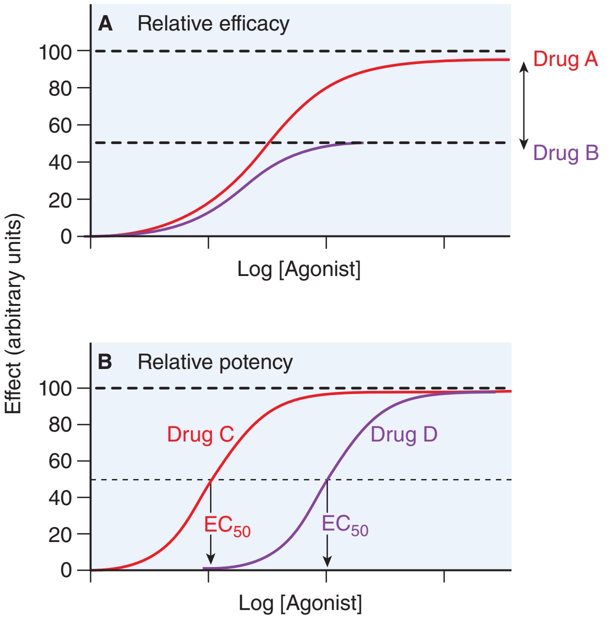


Figure 3–7 *Two ways of quantifying agonism.* **1.** The relative efficacy of two agonists (*Drug A*, ——; *Drug B*, ——) for a given type of receptor in the same cell or tissue is evaluated based on a comparison of responses. The asymptotic response of Drug A is two times that of Drug B; hence, Drug A is twice as efficacious as Drug B. **2.** The EC$_{50}$ of Drug C is one-tenth that of Drug D; hence, Drug C is 10-fold more potent than Drug D.

concentrations or doses required to achieve an absolute or given therapeutic effect. In either case, potency does not refer to the maximum effect attainable.

Differences in efficacies and potencies between two or more drugs operating through a given receptor can change depending on the response measured and the cellular context. Context can refer to the relative proximity of downstream effectors, receptor conformations differentially recognized in different cells, whether receptors are limiting for the measured response, and adaptive responses. Moreover, the translation of efficacy from a molecular to a physiological or clinical level, where the desired therapeutic effect is the most important concern, can be a fraught process. The therapeutic effect is often far downstream of changes in receptor conformation and represents an integration of actions and reactions at the level of cells, tissues, and superimposed homeostatic mechanisms. Furthermore, the variability among humans in terms of response is confounding (see below). Moreover, an antagonist, while having no effect on a receptor in its own right, cannot be said to have no effect *in vivo* if it blocks the actions of an endogenous agonist, which could rightly be considered a measurable, therapeutic response.

Pharmacodynamic Variability: Individual and Population Pharmacodynamics

Individuals vary among themselves in the magnitude of their response to the same concentration of a single drug, and a given individual may not always respond in the same way to the same drug. Factors underlying this variability are poorly understood but are both pharmacokinetic and pharmacodynamic in nature (Figure 3–8). As such, these factors can change in relation to physiological and pathophysiological status, for example, pregnancy, age, and cardiovascular, hepatic, or renal function. Further complicating issues of variability is the fact that molecular targets for drugs are often up- or downregulated by endogenous or exogenous factors, including previous or concurrent drug administration.

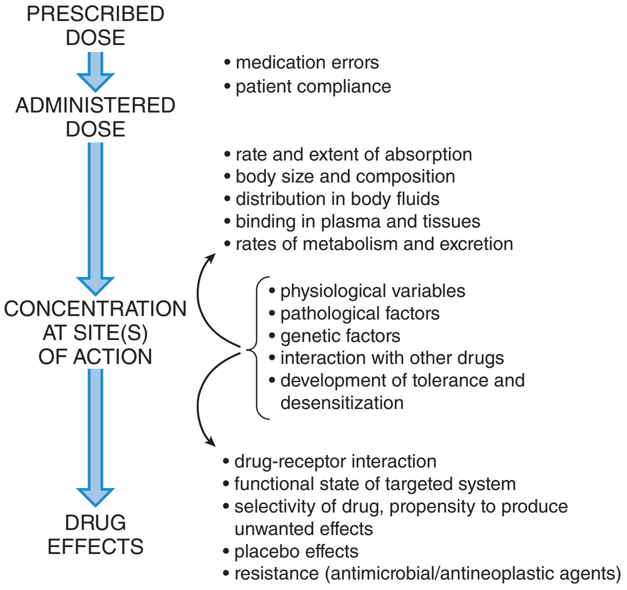

PRESCRIBED DOSE
ADMINISTERED DOSE
CONCENTRATION AT SITE(S) OF ACTION
DRUG EFFECTS
• medication errors
• patient compliance
• rate and extent of absorption
• body size and composition
• distribution in body fluids
• binding in plasma and tissues
• rates of metabolism and excretion
• physiological variables
• pathological factors
• genetic factors
• interaction with other drugs
• development of tolerance and desensitization
• drug-receptor interaction
• functional state of targeted system
• selectivity of drug, propensity to produce unwanted effects
• placebo effects
• resistance (antimicrobial/antineoplastic agents)

Figure 3–8 *Factors influencing the response to a prescribed drug dose.*

Data on the correlation of drug levels with therapeutic response and toxicity for a population must be interpreted within this context of variability. The concentration- and dose-response curves discussed previously, which attempt to minimize variability, will not generally suffice. Potency and efficacy as they relate to a population must instead be handled through *quantal* concentration- or dose-effect curves (Figure 3–9). Quantal refers to whether an individual responds, or not, to a given concentration or dose of drug; the concentration of the drug, if used instead of the dose, is usually that in the plasma. In the case of biological events that are themselves quantal (e.g., consciousness, convulsion, or death), the response is simply the occurrence of the event. In the case of endpoints that are mathematically continuous (e.g., blood pressure, level of pain, plasma glucose concentration, P_{CO_2}), a specified increment of change constitutes the quantal response. In either case, the number or percentage of individuals responding in this quantal fashion is plotted as a function of concentration or dose of drug. The concentration or dose required to produce a specified response in 50% of the population is the EC_{50} or ED_{50}.

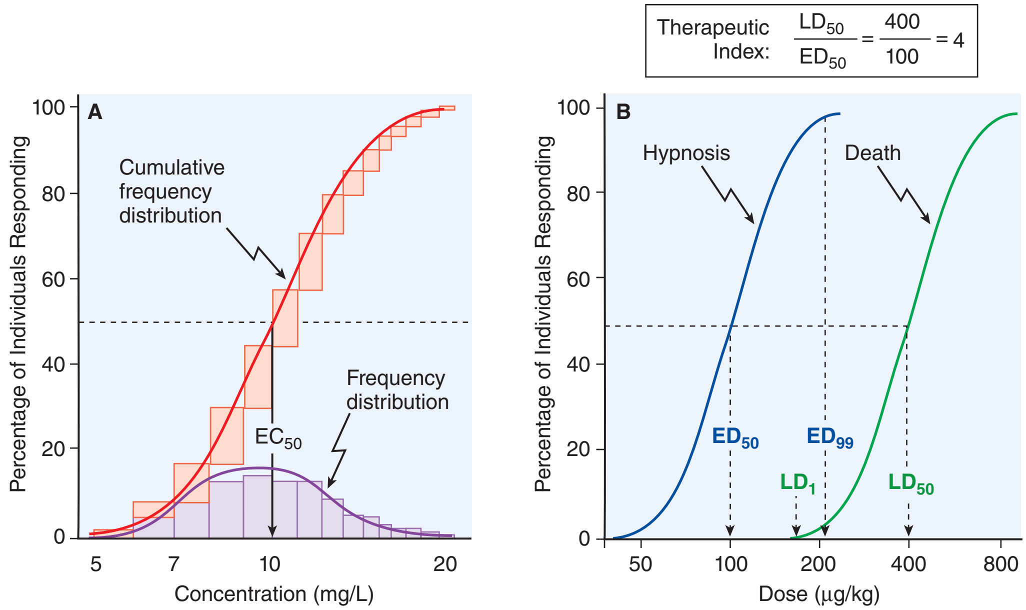


Figure 3–9 *Quantal concentration- and dose-response curves.* **A.** *Frequency distribution and concentration-response curve.* An experiment was performed on 100 subjects, in which the plasma concentration of drug that produced a quantal response, in this case hypnosis, was determined for each individual. The number of subjects responding to each concentration was plotted, giving a log-normal frequency distribution (purple bars). The normal frequency distribution, when summated, yields the cumulative frequency distribution—a sigmoidal curve that is a quantal concentration-response curve (red bars, red line). **B.** *Quantal dose-response curves.* Animals were injected with varying doses of a drug, and the responses were determined and plotted. The therapeutic index, the ratio of the LD_{50} to the ED_{50}, is an indication of how selective a drug is in producing its desired effects relative to its lethality. See text for additional explanation.

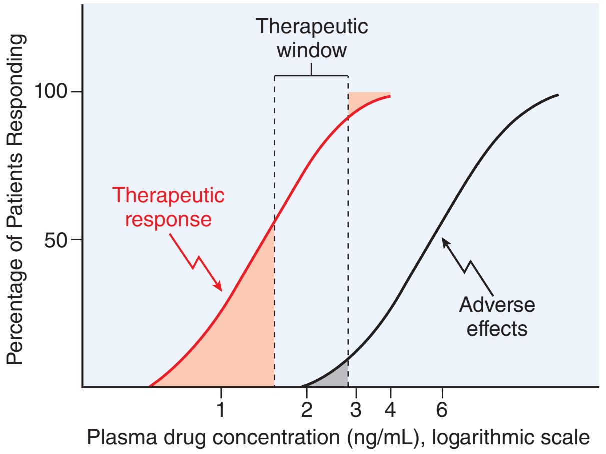


Figure 3–10 *Relation of the therapeutic window of drug concentrations to therapeutic and adverse effects in the population.* The ordinate is linear; the abscissa is logarithmic. This particular therapeutic window represents the difference in drug concentrations eliciting a therapeutic response in 50% of the patients and adverse effects in 10%.

In preclinical studies of drugs, the *median lethal dose* (LD_{50}) is determined in experimental animals (Figure 3–9B). The LD_{50}/ED_{50} ratio in animals is an indication of the *therapeutic index* in humans, a term that reflects how selective the drug is in producing desired versus serious adverse effects. Another term, the *therapeutic window*, is the range of concentrations or doses of drug that provide therapeutic efficacy with minimal toxicity (Figures 2–11 and 3–10). The concentration or dose of a drug required to produce a therapeutic effect in most of the population will usually overlap to some extent with that required to produce toxicity in some of the population. Thus, a therapeutic window defined for a population expresses a range of concentrations or doses for which the likelihood of efficacy is high and the probability of adverse effects is low but does not guarantee efficacy or safety for any single individual. Use of the therapeutic window to optimize the dosage of a drug in a given patient should therefore be complemented by monitoring appropriate clinical and surrogate markers of the drug's effects.

Antimicrobial Pharmacodynamics

In antimicrobial pharmacology, macromolecules that serve metabolic, replicative, and structural needs *distinct from the host* come to the fore as targets. Such targets allow for selective toxicity (microbe as opposed to human host). A good example is the inhibition of folic acid synthesis by a combination of a sulfonamide with *trimethoprim* (Chapter 57). A very large number of microorganisms must synthesize folic acid, while mammalian cells require preformed folic acid from the diet. *Sulfonamides* block the synthesis of dihydropteroic acid, a precursor to dihydrofolic acid, through competitive inhibition of the enzyme dihydropteroate synthase, for which *para*-aminobenzoic acid is the normal substrate. *Trimethoprim* blocks the subsequent conversion of dihydrofolic acid to tetrahydrofolic acid through competitive inhibition of DHFR. While this latter conversion is required in mammalian cells, *trimethoprim* has a several thousand–fold greater affinity for the microbial reductase.

Pharmacologically exploitable differences between microbes and the host are extensive. Among the many additional examples are the production of heme cofactor by plasmodia responsible for malaria, which provides the basis for the therapeutic actions of *artemisinin*, *quinolines*, and *chloroquine* (Chapter 66); electron transport components in certain protozoa and bacteria that have a uniquely negative redox potential, leading to activation of *metronidazole* (Chapters 57 and 67); a glutamate-gated Cl^- channel found only in invertebrates, the target for *avermectins* (Chapter 68); the bacterial cell wall, whose synthesis is the target of *β-lactams*, *glycopeptides*, and *lipopeptides* (Chapter 58); the 30S ribosomal subunit unique to bacteria, the target of *aminoglycosides* and *tetracyclines* (Chapters 59 and 60); the 50S ribosomal subunit also unique to bacteria, the target of *chloramphenicol*, *macrolides*, *lincosamides*, and *oxazolidinones* (Chapter 60); unique topological rearrangements of DNA coiling represented by requirements for DNA gyrase and topoisomerase IV, the targets of *quinolone antibiotics* (Chapter 57); unique composition of surface membranes of protozoa, fungi, and bacteria, whose constituents are the target of *amphotericin B*, *polymyxin*, *daptomycin*, and *isoniazid* (Chapters 59, 61, and 65); viral forms of thymidylate kinase and DNA polymerase, relevant to the actions of *acyclovir* and *ganciclovir* (Chapter 62); and enzymes and structures unique to retroviral replication, targets for inhibitors of fusion, uncoating, reverse transcription, and viral release and maturation (Chapters 62–64).

Receptor-Mediated Mechanisms of Drug Action

The investigation of receptors in terms of their identities and properties has played a pivotal role in the development of pharmacology as a discipline. The majority of drugs used therapeutically, moreover, are directed toward receptors or toward ligands that receptors recognize. Given these facts, and that voltage-gated ion channels, transporters, and enzymes are devoted substantial attention in other chapters of this textbook or elsewhere, the remainder of the chapter will focus primarily on receptors and, specifically, the mechanisms by which receptors mediate drug action.

Quantitative Aspects of Drug Interactions With Receptors

Binding and Fractional Occupancy

The reactions describing the interaction of a drug, or more generally ligand *L*, with a receptor are illustrated in Equation 3–1. The first reaction is the reversible formation of the ligand-receptor complex, *LR*. The second, depending on the nature of the ligand, is conversion of the complex to LR^*, in which the receptor attains a conformation capable of engendering a biological response.

$$L+R \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} LR \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} LR^* \qquad \text{(Equation 3–1)}$$

The affinity of the ligand for the receptor, *R*, ignoring for the moment conversion to LR^*, depends on the forward or *association rate constant*, k_{+1}, and the reverse or *dissociation rate constant*, k_{-1}. The concentration of *LR* at any given instant is equal to the rate of its formation, $k_{+1}[L][R]$, minus the rate of its dissociation, $k_{-1}[LR]$. At equilibrium, where $[LR]$ is unchanging:

$$k_{+1}[L][R]=k_{-1}[LR] \qquad \text{(Equation 3–2)}$$

The ratio of the dissociation and association rate constants, k_{-1}/k_{+1}, defines what is referred to as the *equilibrium dissociation constant*, K_D. Thus, with a small amount of rearrangement of terms, and at equilibrium:

$$\frac{[L][R]}{[LR]}=\frac{k_{-1}}{k_{+1}}=K_D \qquad \text{(Equation 3–3)}$$

A numerically low K_D signifies a high affinity of the ligand for receptor, while a high K_D signifies a low affinity. As a practical matter, differences in affinities of similar compounds most often reflect differences in dissociation rate constants. Also of note, the *affinity constant* or *equilibrium association constant*, K_A, is the reciprocal of the equilibrium dissociation constant (i.e., $K_A = 1/K_D$).

Given that the concentration of free receptor is equal to that of total receptor, R_t, minus receptor bound to ligand, and assuming that the concentration of drug is unaffected by receptor binding, Equation 3–3 can be rearranged to describe *fractional occupancy*, or *f*, which is the ratio of $[LR]$ to $[R_t]$, as a function of ligand concentration:

$$\textit{Fractional occupancy}=f=\frac{[LR]}{[R_t]}=\frac{[L]}{K_D+[L]} \qquad \text{(Equation 3–4)}$$

Equation 3–4 describes a hyperbolic relationship (Figure 3–11). Here, K_D is revealed as a "positioning constant"; namely, K_D is the concentration

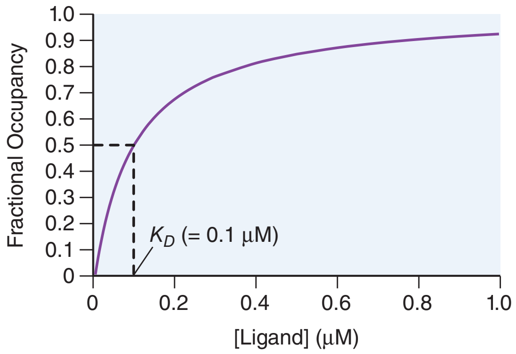


Figure 3–11 *Receptor fractional occupancy.* Portrayed is the binding of a ligand, expressed as fractional occupancy, to a population of homogeneous receptors as a function of ligand concentration. In this example, the equilibrium dissociation constant, K_D, is 0.1 µM. The K_D, defined as the ratio of k_{-1}/k_{+1}, is equal to the concentration of ligand that supports half-maximal occupancy; it can be viewed graphically as a positioning constant. Were the ligand to have a higher affinity (i.e., lower K_D), the curve would be shifted leftward. Were the ligand to have a lower affinity (i.e., higher K_D), the curve would be shifted rightward.

of ligand that yields half-maximal occupancy of the receptor. This follows from setting fractional occupancy in Equation 3–4 to 0.5 (i.e., 50% occupancy).

While the derivation of fractional occupancy is correct for complexation of *L* with *R*, and remains appropriate for the behavior of an antagonist, for which no transition to LR^* occurs, the conversion to LR^* in the case of an agonist results in a higher fractional occupancy than would be predicted by K_D alone. This is because more receptor-bound ligand exists than can be accounted for by *LR*, or said another way, the conversion to LR^* pulls the equilibrium rightward. With GPCRs, the binding of LR^* to a G protein to form the so-called ternary complex further distorts the equilibrium.

If ligand-bound receptors are limiting with regard to the ultimate measured response throughout the entire range of occupancy, then K_D and the concentration of the drug producing a half-maximal effect (EC_{50}) will be nearly equal, the effects of LR^* on binding notwithstanding. Owing to downstream amplification, however, many signaling systems can reach a full biological response with only a fraction of receptors occupied. In this case, the EC_{50} is shifted leftward from K_D; the receptors are said to be in excess, or that *spare receptors* exist.

Quantifying Antagonism

Characteristic patterns of antagonism are associated with certain mechanisms of receptor blockade. One is straightforward *competitive antagonism*, in which a drug with an affinity for a receptor but lacking intrinsic efficacy competes with the agonist for the orthosteric site. The characteristic pattern of such antagonism is a parallel, rightward shift of the agonist concentration- or dose-response curve with no change in maximal response (Figure 3–12A). The magnitude of the rightward shift depends on the concentration of the antagonist and its affinity for the receptor (Schild, 1957). A competitive antagonist at sufficiently high concentrations relative to the agonist can reduce the response to near zero; however, the antagonism remains *surmountable*.

Other mechanisms of receptor blockade are *pseudoirreversible* and *irreversible antagonism*, in which the antagonist once bound to receptor, whether through noncovalent or covalent mechanisms, respectively, dissociates quite slowly or not at all. Both types of antagonists will produce the pattern of antagonism shown in Figure 3–12B. Pseudoirreversible and irreversible forms of antagonism are not surmountable.

The majority of orthosteric receptor antagonists are competitive. One may write mathematical expressions of fractional occupancy of the receptor by an agonist, *f*, and by the agonist in the presence of a competitive antagonist, f_{+I}.

For the agonist alone, the fractional occupancy is provided by the previously developed Equation 3–4:

$$\textit{Fractional occupancy} = f = \frac{[LR]}{[R_t]} = \frac{[L]}{K_D + [L]}$$

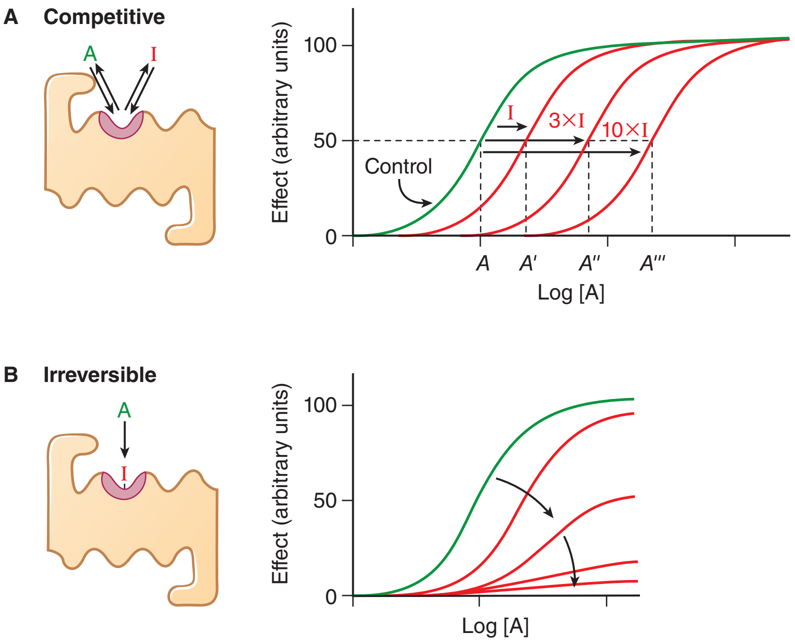


Figure 3–12 *Mechanisms of receptor antagonism.* The effect achieved by an agonist unmodulated by antagonist is depicted in each panel by the green curve. **A.** Competitive antagonism occurs when the agonist **A** and antagonist **I** compete for the same binding site on the receptor. Response curves for the agonist are shifted to the right in a manner dependent on antagonist concentration such that the EC_{50} for the agonist increases (e.g., *L* vs. *L′*, *L″*, and *L‴*). **B.** Irreversible antagonism occurs when the antagonist forms a covalent bond with the receptor, in this example, at the same site as agonist, causing a progressive depression of the maximal response as the concentration of antagonist increases; the rightward shift in EC_{50} initially would suggest some degree of spare receptors. Irreversible antagonism need not occur at the orthosteric site, and sometimes, the antagonism is in fact pseudoirreversible, in which case, the antagonist does not form a covalent bond but instead dissociates quite slowly.

For the agonist *plus* antagonist, one must first consider two equilibria. The first is the equilibrium already described between the agonist, L, and receptor, R, and summarized here as Equation 3–5.

$$L+R \rightleftharpoons LR;\ K_D=\frac{[L][R]}{[LR]},\ \text{or}\ [LR]=\frac{[L][R]}{K_D} \qquad \text{(Equation 3–5)}$$

The second is the equilibrium between the antagonist, defined as the inhibitor I, and R.

$$I+R \rightleftharpoons IR;\ K_i=\frac{[I][R]}{[IR]},\ \text{or}\ [IR]=\frac{[I][R]}{K_i} \qquad \text{(Equation 3–6)}$$

Fractional occupancy by the agonist L in the presence of I is defined as:

$$f_{+I}=\frac{[LR]}{[LR]+[IR]+[R]} \qquad \text{(Equation 3–7)}$$

The concentration of agonist needed to achieve a designated fractional occupancy in the presence of antagonist, $[L']$, will be greater than the concentration of agonist needed to achieve the same fractional occupancy in the absence of inhibitor, $[L]$. Using Equations 3–5 and 3–6, and applying some algebraic tinkering to the right-hand side of Equation 3–7, the fractional occupancy in the presence of the competitive inhibitor, f_{+I}, can be expressed in terms of L', K_D, K_i, and I:

$$f_{+I}=\frac{[L']}{[L']+K_D\left(1+\frac{[I]}{K_i}\right)} \qquad \text{(Equation 3–8)}$$

Assuming that equal responses result from equal fractional receptor occupancies in both the absence and presence of antagonist, one can set the fractional occupancies equal at experimentally determined agonist concentrations ($[L]$ and $[L']$) that generate equivalent responses, as depicted in Figure 3–12A. Thus,

$$f=f_{+I} \qquad \text{(Equation 3–9)}$$

$$\frac{[L]}{[L]+K_D}=\frac{[L']}{[L']+K_D\left(1+\frac{[I]}{K_i}\right)} \qquad \text{(Equation 3–10)}$$

Simplifying, one obtains

$$\frac{[L']}{[L]}-1=\frac{[I]}{K_i} \qquad \text{(Equation 3–11)}$$

where all values are known except K_i. Thus, one can determine the K_i for a reversible, competitive antagonist without knowing the K_D for the agonist and without needing to define the precise relationship between receptor and response.

Allosteric Modulation of Receptor Function

Some drugs interact with receptors through allosteric mechanisms, wherein drug binding at a site distinct from the orthosteric site can alter the receptor's affinity for the orthosteric agonist, the capacity of the orthosteric agonist to induce or stabilize conformational changes equated with activation, or both. These drugs are termed *allosteric modulators*. Some patterns of allosteric modulation are shown in Figure 3–13. *Cinacalcet* is a *positive allosteric modulator* (PAM), enhancing the actions of Ca^{2+} on the calcium-sensing receptor (Chapter 52). Benzodiazepines and barbiturates are also PAMs; they *potentiate* the actions of γ-aminobutyric acid (GABA) on the $GABA_A$ receptor (see Figure 16–11 and Chapter 22). *Maraviroc* is a *negative allosteric modulator* (NAM), blocking the binding of the HIV outer envelope protein gp120 to the CCR5 chemokine receptor and hence fusion and entry of HIV into macrophages and $CD4^+$ T cells (see Figure 64–7). *Ticagrelor* and *cangrelor* are similarly NAMs, blocking the actions of ADP binding to the orthosteric site on platelet $P2Y_{12}$ receptors (Chapter 36). Allosteric modulation is not confined to receptors. Allosteric modulators also act on voltage-gated ion channels, including L-type and T-type Ca^{2+} channels. For example, the dihydropyridine Ca^{2+} channel blocker *amlodipine* allosterically modulates binding of *diltiazem* and Ca^{2+} to the pore of the L-type Ca^{2+} channel by causing the channel to mimic the conformation of the inactivation state. Of note, *diltiazem* can both directly block and allosterically modulate Ca^{2+} binding in the channel's pore (Tang et al., 2019).

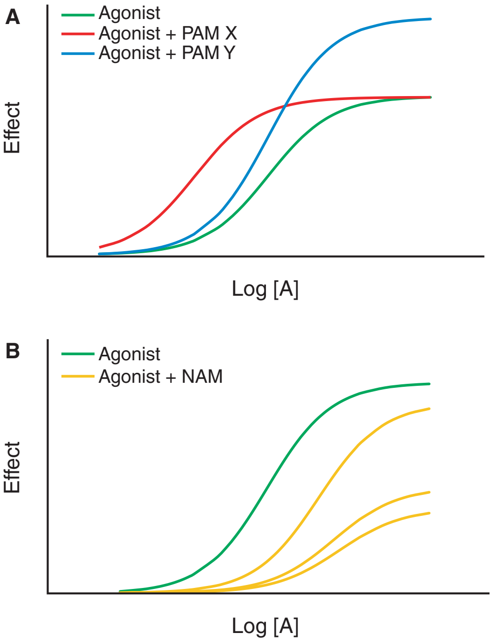


Figure 3–13 *Allosteric modulation.* **A.** Concentration-response curves for an orthosteric agonist in the absence and presence of two positive allosteric modulators (PAMs). For clarity, assume maximal occupancy of the receptor population by the PAMs. PAM X shifts the EC_{50} for the agonist leftward (i.e., increases the potency of the agonist), whereas PAM Y increases the E_{max} for the agonist (i.e., increases efficacy). Note that neither modulator has any effect in the absence of the agonist, which is to say that they are not allosteric agonists. **B.** Concentration-response curves for an orthosteric agonist in the absence and presence of *increasing* concentrations of a negative allosteric modulator (NAM). In this instance, the NAM has a negative impact on both EC_{50} and E_{max}, reducing potency and efficacy.

Despite the small number of allosteric modulators recognized at present, considerable effort is currently devoted to the development of those that distinguish receptor subtypes for which orthosteric sites are highly conserved, as discussed in Chapter 1 (see Protein-Drug Binding: Affinity and Allostery) and in Chapter 11 for muscarinic receptors. Because many positive allosteric modulators have no activity *per se* but rather condition the receptor to activation by the endogenous agonist, they have the advantage of preserving the spatial and temporal pattern of receptor activation when present (Foster and Conn, 2017).

Classes of Receptors Relevant to Drug Actions

Cellular receptors of relevance to therapeutics include GPCRs, ligand-gated ion channels, enzyme-linked (catalytic) receptors, still other cell-surface membrane receptors, and nuclear receptors (Table 3–1).

The activation of receptors by agonists sets into motion a number of proximal subcellular events. One such set of events, achieved primarily by GPCRs through G proteins as transducers, is the generation or mobilization of *second messengers*. Second messengers are small, intracellular, and sometimes interdependent molecules that include the cyclic nucleotides cyclic AMP and cyclic GMP, NO, inositol trisphosphate (IP_3), diacylglycerol (DAG), and Ca^{2+}. Also achieved by GPCRs, and more directly by ligand-activated ion channels, are alterations in membrane permeability to one or more ions, notably Na^+, K^+, Ca^{2+}, and Cl^-. Changes in permeability result in changes in electrical potential across the cell membrane and hence the degree of cell excitability. Other actions are attributable to receptors that have intrinsic enzymic activity,

TABLE 3–1 ■ CELLULAR RECEPTORS OF RELEVANCE TO THERAPEUTICS

CLASSES	SUBCLASSES OR FAMILIES	PHYSIOLOGICAL LIGANDS	TRANSDUCERS, PRINCIPAL EFFECTS, OR IONS
G protein-coupled receptors (GPCRs)[a]	Adhesion Frizzled Glutamate Rhodopsin Secretin	Very large number of endocrine and paracrine factors, but other types of ligands as well	G proteins G_s: Adenylyl cyclases (stimulation) G_i: Adenylyl cyclases (inhibition), delayed rectifier K^+ channel (stimulation), N-type voltage-gated Ca^{2+} channel (inhibition) G_q: Phospholipase C-β (stimulation) $G_{12/13}$: Rho GEFs (stimulation) Arrestins: MAP kinases, nonreceptor tyrosine kinases, transcription factors
Ligand-gated ion channels[b]	Glutaminergic	Glutamate	Na^+ and K^+ principally, but Ca^{2+} as well in certain circumstances
	Nicotinic cholinergic	ACh	Na^+, K^+, Ca^{2+}
	P2X	ATP	Na^+, K^+, Ca^{2+}
	5-HT_3	5-HT_3	Na^+, K^+, Ca^{2+}
	TRP	Many ligands	Na^+, Ca^{2+}, Mg^{2+}
	$GABA_A$	GABA	Cl^-
	Glycine	Glycine	Cl^-
Enzyme-linked (catalytic) receptors	Receptor tyrosine kinases	Insulin, PDGF, EGF, VEGF, growth factors[d]	Proteins containing SH2 and PTB domains
	Receptor serine kinases	TGF-β family	SMADs
	Membrane bound GC	Natriuretic peptides	Cyclic GMP
Other cell-surface membrane receptors	Cytokine receptors	Interleukins and other cytokines, growth hormone, prolactin	JAK/STATs, soluble tyrosine kinases
	Toll-like receptors	PAMPs	TIRAP, TRAM
	TNFα receptors	TNF-α	TRADD, RIP-1, TRAF2
Nuclear receptors[c]	Steroid receptors (subfamily 3)	Corticosteroids, sex hormones	Coactivators
	Nonsteroid receptors (subfamilies 1, 2, 4–6)	Thyroxine, retinoic acid, hydroxycholesterols, bile acids, Vitamin D	Coactivators, corepressors

SMAD, a concatenation of SMA (small worm phenotype in *C. elegans*) and MAD (Mothers Against Decapentaplegic in *Drosophila*); TIRAP, toll-interleukin 1 receptor domain-containing adaptor protein; TRADD, TNF receptor–associated death domain; TRAF2, TNF receptor–associated factor 2; TRAM, TRIF-related adapter molecule, wherein TRIF represents TIR domain-containing adapter-inducing interferon-β and TIR is toll-interleukin 1 receptor.

[a] The GPCR families are listed according to the GRAFS system (Fredriksson et al., 2003), which is based on phylogenetic analyses of the human genome; other systems of classification exist. It is important to note that each family of GPCRs contains a large number of receptors that respond to agonists and communicate with transducers distinct from the family's namesake. See Figure 3–14 and legend.

[b] Glutaminergic receptors comprise the AMPA, kainate, and NMDA receptors. Many permutations of each of these subtypes and of other subclasses of ligand-gated ion channels exist owing to combinatorial diversity in subunit composition. Ion channels that are gated by internal ligands are not listed for the purposes of brevity but are discussed in the text.

[c] Nuclear receptors are generally grouped into six subfamilies. The table groups the subfamilies into two major classes, steroid receptors and nonsteroid receptors, as does the IUPHAR/BPS Guide to Pharmacology, available at: https://www.guidetopharmacology.org/.

[d] The list comprises only a selection from among many different agonists.

for example, receptor tyrosine kinases. Tyrosine phosphorylation promotes the stable interaction of the phosphorylated proteins, including the receptors themselves through autophosphorylation, with proteins containing Src homology 2 (SH2) and phosphotyrosine-binding (PTB) domains; changes in the conformations of the interacting proteins and the process of scaffolding itself propagates the internal signal. Stable interactions with proteins can also be achieved by receptors without intrinsic enzymic activity, through conformational changes alone or abetted by phosphorylation by protein kinases distinct from the receptor. This is the case for cytokine and toll-like receptors in their interaction with adaptor proteins, nuclear receptors with coactivators and corepressors, and GPCRs with arrestins. Still another important setting for receptor activity is transcription. While all of the above phenomena can affect transcription, nuclear hormone receptors interact directly with transcriptional regulatory elements.

One tends to think of an activated receptor as eliciting a singular, linear chain of events. That is true in some instances, but viewing events in the context of multiple and branching pathways is usually more realistic. Many GPCRs interact with two or more G proteins that have distinct downstream actions and with transducers beyond G proteins, such as arrestins. Signals from multiple pathways are frequently integrated within the responsive cell, and there is ample cross talk among multiple signaling pathways. For example, pathways employing cyclic AMP and Ca^{2+} are integrated in most excitable tissues, where levels of cyclic AMP are controlled by GPCRs and those of intracellular Ca^{2+} by ligand- or voltage-gated ion channels or by other GPCRs. In cardiac myocytes, activation of the β_1 receptor–G_s–adenylyl cyclase (AC)–cyclic AMP–PKA pathway enhances cardiac contractility by augmenting Ca^{2+} entry through voltage-gated Ca^{2+} channels and Ca^{2+} mobilization from intracellular stores via the ryanodine receptor; thus, both cyclic AMP and Ca^{2+} are positive contractile signals in cardiac myocytes. By contrast, smooth muscle cells integrate these signals differently: In smooth muscle, Ca^{2+} is a contractile signal, but an elevation of cyclic AMP leads to relaxation via the phosphorylation of proteins that mediate Ca^{2+} signaling, such as myosin light chain kinase (MLCK).

BOX 3–2 ■ Fertile Ground

Research on cell signaling involving GPCRs, G proteins, and cyclic nucleotides has garnered a number of Nobel Prizes in Physiology or Medicine and in Chemistry. Early work in cell signaling involved the regulation of glycogen metabolism, building on the accomplishments of Gerty and Carl Cori, who shared the 1947 Nobel Prize in Physiology or Medicine "for their discovery of the course of the catalytic conversion of glycogen." Earl Sutherland, who discovered cyclic AMP, won the 1972 Nobel Prize in Physiology or Medicine "for his discoveries concerning the mechanisms of the action of hormones." In 1992, Edmond Fischer and Edwin Krebs won "for their discoveries concerning reversible protein phosphorylation as a biological regulatory mechanism." Two years later, Alfred Goodman Gilman and Martin Rodbell won "for their discovery of G-proteins and the role of these proteins in signal transduction in cells." Three pharmacologists, Robert Furchgott, Louis Ignarro, and Ferid Murad, shared the 1998 Nobel Prize in Physiology or Medicine "for their discoveries concerning nitric oxide as a signaling molecule in the cardiovascular system," and in 2000, Arvid Carlsson, Paul Greengard, and Eric Kandel shared the prize "for their discoveries concerning signal transduction in the nervous system." In 2012, Robert Lefkowitz and Brian Kobilka won the Nobel Prize in Chemistry "for studies of G-protein-coupled receptors." Fertile ground indeed.

Transmembrane Signaling via GPCRs and G Proteins

In general terms, agonists at the cell surface interact with GPCRs that couple to G proteins at the inner membrane leaflet, which in turn interact with effectors similarly situated or present within the cytosol. We consider these individual elements of transmembrane signaling below. The study of signaling through GPCRs and G proteins has been recognized at the level of several Nobel Prizes (Box 3–2).

G Protein-Coupled Receptors. GPCRs are a large family of receptors (Figure 3–14) that exhibit a seven-transmembrane α-helical motif. Of the over 800 GPCRs expressed by the human genome, some 130 are current targets for therapeutic drugs, either agonists or antagonists (Sriram and Insel, 2018). The remainder include "orphan" receptors (~130), for which endogenous ligands and functions have yet to be ascribed, sensory receptors (~420), most of which are olfactory but may also have other roles, and receptors not yet targeted. GPCRs are typically, though not always, situated in the cell-surface membrane. They recognize a very large number of endocrine and paracrine factors, as well as visual, olfactory, and gustatory stimuli. GPCRs engage G proteins and/or arrestins as transducers on the cytoplasmic aspect of the membrane. Many cells will contain several dozen different GPCRs and hundreds to thousands of copies each. The variety, accessibility, specificity, distribution, and relevance of GPCRs to disease make them important targets for drugs.

GPCR Subtypes. Quite commonly an endogenous agonist (e.g., an endocrine or paracrine factor) is recognized by two or more GPCRs. These receptors are defined as *receptor subtypes*. Acetylcholine, for example, is recognized by five subtypes of muscarinic (M) receptor, M_1 to M_5; histamine by four subtypes of receptor, H_1 to H_4; 5-hydroxytryptamine (5HT), or serotonin, by at least 10 subtypes; and so on. In fact, the existence of receptor subtypes is more the rule than the exception. Initially identified through binding studies with endogenous and synthetic ligands, the number has climbed through genomic analyses.

Receptor subtypes, while recognizing a given endogenous agonist, can differ among themselves in several important respects. First, subtypes often exhibit differences in affinities for endogenous or synthetic ligands (i.e., subtypes can be distinguished pharmacologically). Second, subtypes may be differentially distributed among cells or tissues, and the distribution of a single subtype may be relatively restricted. Pharmacological targeting of a given subtype can therefore minimize the extent to which generalized adverse effects occur. Subtypes of the β adrenergic receptor are a good example. β_2 Adrenergic receptor agonists such as *terbutaline* are used for bronchodilation in the treatment of asthma in the hope of minimizing cardiac side effects caused by stimulation of the cardiac β_1 adrenergic receptor (see Chapter 14). Conversely, the use of β_1 receptor–selective antagonists in patients being treated for hypertension or angina (see Chapters 14, 31, and 32) minimizes the likelihood of bronchoconstriction.

Receptor subtypes can also differ with respect to the G proteins (or other transducers) with which they interact. For instance, M_1, M_3, and M_5 muscarinic receptors couple to the G protein G_q with consequent increases in intracellular Ca^{2+}; M_2 and M_4 receptors couple to G_i to decrease the activity of AC (thus reducing intracellular cyclic AMP accumulation) and, in a variety of excitable cells, to activate inwardly rectifying K^+ channels and inhibit voltage-gated Ca^{2+} channels.

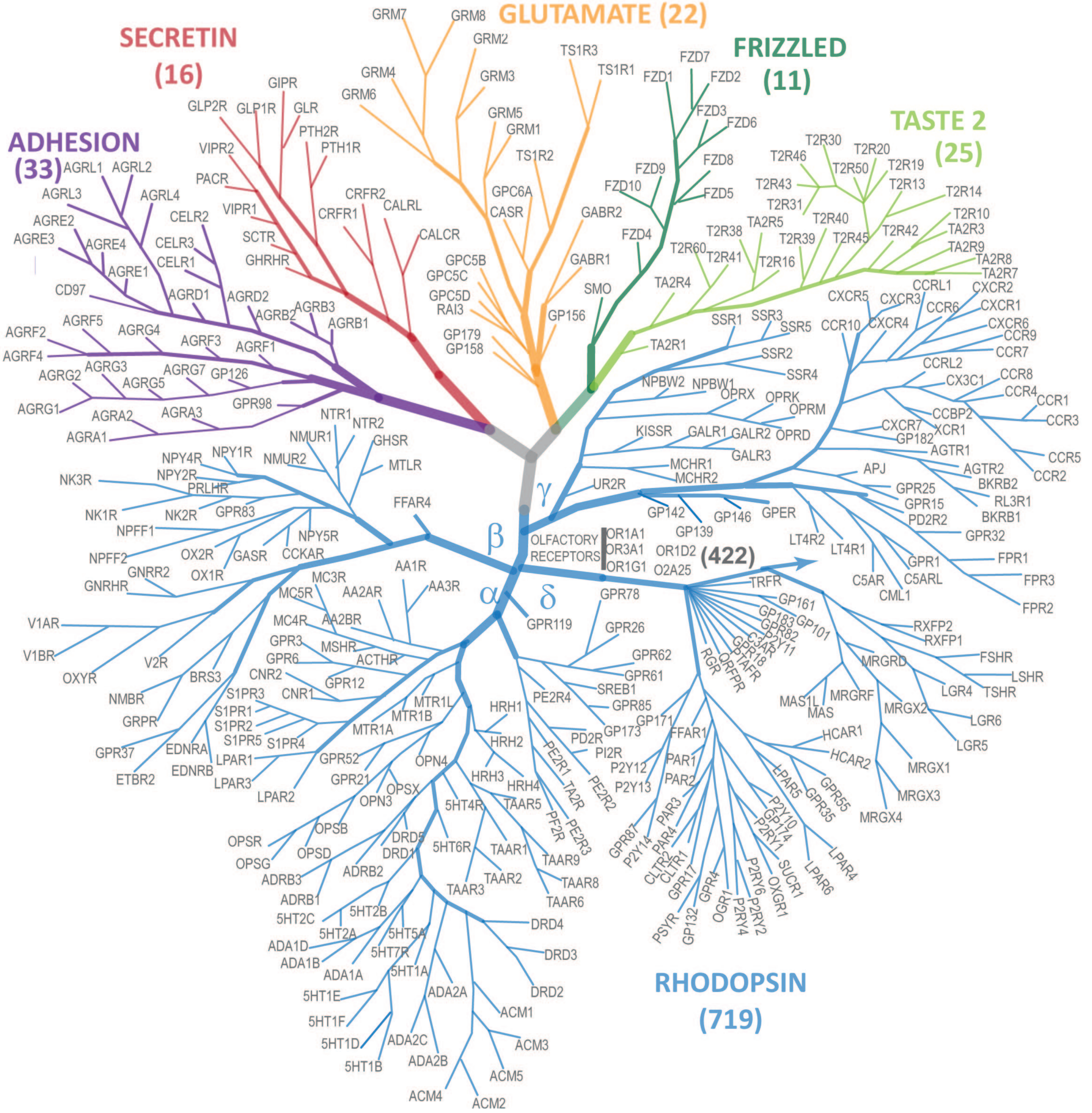
G Proteins. GPCRs couple to heterotrimeric GTP-binding regulatory proteins, or G proteins. G proteins, upon activation by agonist-occupied GPCRs, activate or inhibit any number of target enzymes and ion channels; G proteins are thus the transducers in the process of *transmembrane signal transduction*. The structure of a G protein conforms to that of an α, β, and γ subunit heterotrimer (Figure 3–15). Based on primary structural similarities among the α subunits (Strathmann and Simon, 1991), G proteins sort into four families (Table 3–2)—the G_s, G_i (sometimes $G_{i/o}$), G_q, and G_{12} (sometimes $G_{12/13}$) families—each with 2 to 10 members that have more or less common activities depending on the differentiated properties of responsive cell types. Virtually every cell in the body contains one or more members of each family. A given GPCR can couple to G proteins of one or two families and, in some instances, more.

With regard to the transduction event, an agonist-activated GPCR promotes exchange of GDP for GTP on the α subunit of the G protein heterotrimer, resulting in dissociation of the α subunit from the βγ heterodimer. The actions of the G protein on a target enzyme or ion channel are achieved through the monomeric α subunit alone, the βγ heterodimer alone, and sometimes both working coordinately. Reversion of the activated G protein to a heterotrimeric configuration occurs upon hydrolysis of GTP by the α subunit and recombination of the subunit with the βγ heterodimer.

Activation of members of the G_s family most often equates with the *activation* of ACs and an increase in cellular cyclic AMP. Activation of members of the G_i family links to the *inhibition* of ACs and to the activation of inwardly rectifying K^+ channels and inhibition of voltage-gated Ca^{2+} channels. *Transducin*, a member of the G_i family, couples to rhodopsin in retinal outer segments and cones and activates a cyclic GMP-selective phosphodiesterase (see Figure 74–9). Activation of members of the G_q family results in the activation of phospholipase C-β and the consequent release of DAG and IP_3. DAG and IP_3 initiate many downstream effects: IP_3 mobilizes Ca^{2+} from intracellular stores, thereby activating myriad Ca^{2+}-dependent events; Ca^{2+} and DAG are essential cofactors in the activation of PKC. The two members of the G_{12} family, G_{12} and G_{13}, activate the monomeric G protein RhoA through RhoA-selective guanine nucleotide exchange factors (GEFs). However, the G proteins of the G_{12} family, and those of every other G protein family, have an array of targets well beyond those highlighted here.

GRK- and Arrestin-Mediated Desensitization. GPCRs are subject to various forms of desensitization following exposure to agonists. One of the best characterized is a homologous desensitization initiated by phosphorylation of the activated receptor by one or more GPCR-specific kinases (GRKs) (Figure 3–16) (DeWire et al., 2007). The phosphorylation occurs at specific serine or threonine residues on the cytosolic aspect of the receptor, often within the C-terminal tail. Specificity for phosphorylation of the activated receptor is keyed to the receptor's conformation and, for some GRKs, recruitment of the GRK to the inner cell surface membrane by heterodimeric βγ subunits released upon activation of G proteins.

The phosphorylation of the receptor results in recruitment of arrestins. Of the several arrestins, β-arrestins-1 and -2 are widely expressed. The recruited arrestin binds conjointly to the phosphorylated C-terminal tail and exposed portions of the transmembrane core of the activated GPCR. This interaction of arrestin with the GPCR disrupts the interaction of the receptor with the G protein, thus terminating G protein signaling.


GLUTAMATE (22)
SECRETIN (16)
FRIZZLED (11)
TASTE 2 (25)
ADHESION (33)
RHODOPSIN (719)
α
β
γ
δ
OLFACTORY RECEPTORS
(422)
GRM7
GRM8
GRM2
GRM4
GRM3
GRM6
GRM5
GRM1
TS1R3
TS1R1
TS1R2
GPC6A
CASR
GABR2
GABR1
GPC5B
GPC5C
GPC5D
RAI3
GP179
GP158
GP156
SMO
FZD7
FZD1
FZD2
FZD3
FZD6
FZD9
FZD8
FZD10
FZD5
FZD4
GIPR
GLP2R
GLP1R
GLR
PTH2R
PTH1R
VIPR2
PACR
VIPR1
SCTR
GHRHR
CRFR2
CRFR1
CALRL
CALCR
AGRL1
AGRL2
AGRL3
AGRL4
AGRE2
AGRE3
CELR2
CELR3
AGRE4
CELR1
AGRE1
CD97
AGRD1
AGRD2
AGRB2
AGRB3
AGRB1
AGRF5
AGRF2
AGRG4
AGRF4
AGRF3
AGRF1
AGRG3
AGRG2
AGRG7
GP126
AGRG5
AGRG1
AGRA2
AGRA3
GPR98
AGRA1
T2R30
T2R46
T2R20
T2R50
T2R19
T2R43
T2R13
T2R14
T2R31
T2R10
T2R5
T2R40
T2R38
TA2R5
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TA2R3
TA2R9
T2R60
T2R41
T2R16
TA2R8
TA2R7
TA2R4
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CCRL1
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CCR10
CXCR4
CXCR6
CCR9
CCR7
SSR2
SSR4
CCRL2
CX3C1
CCR8
CCR4
NPBW2
NPBW1
OPRX
OPRK
OPRM
CCR1
CCBP2
CCR3
KISSR
GALR1
GALR2
OPRD
CXCR7
XCR1
GP182
GALR3
AGTR1
CCR5
CCR2
MCHR1
MCHR2
APJ
AGTR2
BKRB2
UR2R
GPR25
GPR15
RL3R1
PD2R2
BKRB1
GP142
GP146
GPER
GP139
GPR32
OR1A1
OR3A1
OR1G1
OR1D2
O2A25
LT4R2
LT4R1
GPR1
FPR1
C5AR
C5ARL
FPR3
CML1
FPR2
TRFR
GP161
GP183
GP101
GPR82
P2Y11
GPR18
C3AR
GP174
FPR
ORFR
RGR
RXFP2
RXFP1
FSHR
LSHR
MRGRD
LGR4
TSHR
MAS1L
MAS
MRGRF
MRGX2
LGR6
LGR5
HCAR1
HCAR2
MRGX1
MRGX3
MRGX4
GPR55
GPR35
LPAR5
P2Y10
GP34
P2RY1
SUCR1
LPAR6
LPAR4
OXGR1
P2RY2
P2RY6
P2RY4
OGR1
GPR4
GP132
PSYR
GPR17
CLTR1
CLTR2
PAR4
P2Y14
GPR87
PAR3
PAR2
PAR1
FFAR1
GP171
GPR173
P2Y12
P2Y13
PD2R
PI2R
GPR78
GPR26
GPR62
GPR61
SREB1
GPR85
GPR119
NTR1
NTR2
NMUR1
GHSR
NMUR2
MTLR
NPY4R
NPY1R
NPY2R
PRLHR
NK3R
FFAR4
NK1R
NK2R
GPR83
NPFF1
NPY5R
NPFF2
OX2R
GASR
CCKAR
GNRR2
OX1R
GNRHR
MC3R
MC5R
AA2AR
AA1R
AA3R
MC4R
AA2BR
V1AR
V1BR
GPR3
MSHR
GPR6
ACTHR
OXYR
V2R
CNR2
BRS3
GPR12
CNR1
NMBR
S1PR3
S1PR1
S1PR2
S1PR5
S1PR4
MTR1L
MTR1B
MTR1A
GRPR
HRH1
PE2R4
EDNRA
EDNRB
GPR37
ETBR2
LPAR1
LPAR3
LPAR2
GPR52
GPR21
OPN4
HRH2
HRH3
HRH4
PE2R1
TA2R
PE2R2
OPSX
OPN3
5HT4R
TAAR5
PF2R
PE2R3
OPSR
OPSB
OPSG
OPSD
DRD5
DRD1
5HT6R
TAAR1
TAAR9
ADRB3
ADRB2
TAAR2
TAAR3
TAAR8
ADRB1
TAAR6
5HT2C
5HT2B
5HT2A
5HT5A
DRD4
ADA1D
ADA1B
5HT7R
DRD3
ADA1A
5HT1A
5HT1E
ADA2A
DRD2
5HT1F
ACM1
5HT1D
ADA2C
ACM3
5HT1B
ADA2B
ACM5
ACM4
ACM2

Figure 3–14 *The human GPCR superfamily.* Human GPCRs are targeted by about 30% of marketed drugs. This dendrogram, constructed using sequence similarities within the seven-transmembrane region, identifies GPCRs by their names in the UniProt database. There are over 825 human GPCRs, which can be subdivided into the color-coded groups named by the capitalized words on the outer edge of the dendrogram (number of group members in parentheses). These groups can be further subdivided on the basis of sequence similarity. The large Rhodopsin class is subdivided into four broad groups: α, β, δ, and γ. Olfactory receptors constitute the largest fraction of the Rhodopsin class of GPCRs, with 422 members. Receptors on the dendrogram that readers will frequently encounter include AA2AR, A_{2A} adenosine receptor; ACM3, M_3 muscarinic acetylcholine receptor; ADRB1, β_1 adrenergic receptor; AGTR1, AT_1 angiotensin receptor; CNR1, CB_1 cannabinoid receptor; CXCR4, CXC_4 chemokine receptor; DRD2, D_2 dopamine receptor; EDNRA, ET_A endothelin receptor; FPR1, f-Met-Leu-Phe receptor; GCGR, glucagon receptor; GRM1, $mGluR_1$ metabotropic glutamate receptor; HRH1, H_1 histamine receptor; 5HT2B, the $5HT_{2B}$ serotonin receptor; OPRM, μ opioid receptor; RHO, rhodopsin; SMO, smoothened homolog; S1PR1, S1P1 sphingosine-1-phosphate receptor, also known as EDG_1; TSHR, thyrotropin (TSH) receptor; and VIPR1, V_1 vasoactive intestinal peptide receptor. Details of entries on the dendrogram are available from the GPCR Network (http://gpcr.usc.edu). Additional information on GPCRs is available from the IUPHAR/BPS Guide to Pharmacology (http://www.guidetopharmacology.org). (Reproduced with permission from Angela Walker, Vsevolod Katrich, and Raymond Stevens of the GPCR Network at the University of Southern California, as created in the Stevens lab by Yekaterina Kadyshevskaya.)

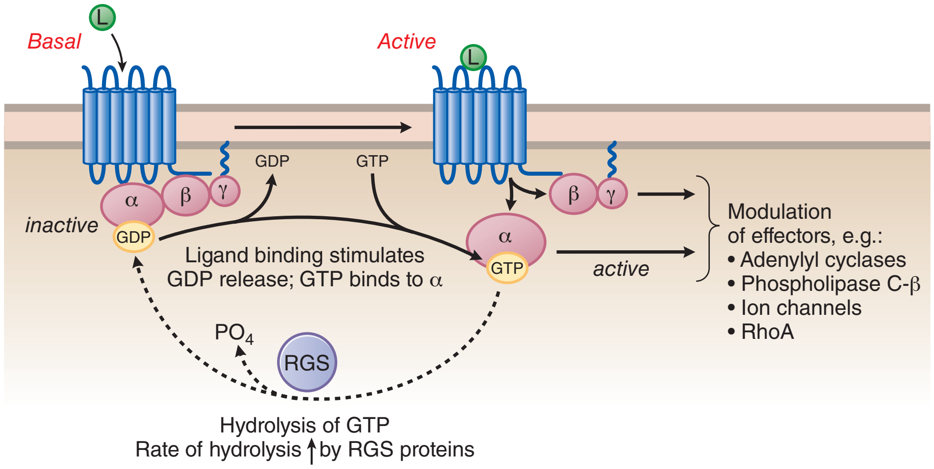


Figure 3–15 *The basic GPCR–G protein–effector pathway.* The GPCR and G protein heterotrimer, absent an activating ligand ("Basal"), are generally thought to form a complex in the cell surface membrane, in which GDP is bound to the Gα subunit. Following the binding of an activating ligand "L" to the receptor, the receptor and G protein α subunit undergo a conformational change leading to exchange of GDP for GTP and dissociation of both the complex and the G protein into monomeric Gα and heterodimeric Gβγ subunits. The activated GTP-bound Gα subunit and the Gβγ dimer bind to and regulate effectors individually or in coordination. The system returns to the basal state upon hydrolysis of the GTP by the α subunit, a reaction that can be markedly enhanced by regulator of G-protein signaling (RGS) proteins. Detailed descriptions of these signaling pathways are given throughout the text in relation to the therapeutic actions of drugs affecting them. The physical interaction between the inactive GPCR and G protein has been posited in the ternary complex model but has not been explicitly demonstrated for any but several GPCRs and G proteins. The βγ dimer is tethered to the membrane by a geranylgeranyl modification. Not shown are the lipid modifications for most α subunits, notably palmitoylation and myrisotylation.

The recruited arrestin also links to cytoskeletal elements, promoting internalization of the receptor for recycling to the membrane or lysosomal destruction. Some GPCRs, designated class A receptors, interact only transiently with arrestins (DeWire et al., 2007). Others, designated class B receptors, interact stably. A stable interaction is associated with a decreased rate of recycling to the cell surface.

Arrestins as Transducers. Quite importantly, while arrestins have the ability to displace G proteins from GPCRs, they serve as transducers in their own right (DeWire et al., 2007). The binding of an arrestin to an activated, phosphorylated GPCR induces a change in conformation of the arrestin. The "activated" arrestin can serve as a scaffold, an essential step in the activation of certain mitogen-activated protein kinases (MAPKs). Effectors for arrestins include the MAPKs (ERK1/2, JNK3, and p38), nonreceptor tyrosine kinases such as Src, certain members of the Ras superfamily of GTP-binding proteins (e.g., ARF6 and RhoA), and nuclear factor-κB (NF-κB). Arrestins that are stably bound to class B GPCRs can signal deep within the cytoplasm from endocytotic vesicles. Moreover, arrestins may signal within the nucleus, as both β-arrestin-1 and -2 contain nuclear localization signals.

TABLE 3–2 ■ FAMILIES OF HETEROTRIMERIC G PROTEINS

FAMILY	A SUBUNITS
G_s	α_s (short and long forms) α_{olf}
G_i (or $G_{i/o}$)	$\alpha_{i1}, \alpha_{i2}, \alpha_{i3}$ α_{oA}, α_{oB} α_{t1}, α_{t2} α_g α_z
G_q	α_q $\alpha_{11}, \alpha_{14}, \alpha_{15}, \alpha_{16}$
G_{12} (or $G_{12/13}$)	α_{12}, α_{13}

G proteins that serve as transducers for GPCRs are αβγ heterotrimers. Many subtypes of α, β, and γ subunits exist; however, a G protein is typically defined by its α subunit. The G protein containing the α_{11} subunit, for example, is G_{11}. Based on primary structural homology among α subunits, G proteins sort into four families.

The activation of G proteins and arrestins is often posited to occur sequentially, with G proteins preceding arrestins. Interactions of G proteins with effectors in this scenario would be constrained to the inner surface of the plasma membrane, and those of arrestins could extend more deeply into the cell, depending on the receptor. Yet, the signaling through G proteins by some GPCRs is sustained, and a variety of biophysical data for G_s-mediated signaling indicate that class B GPCRs, G proteins, and arrestins can exist as megacomplexes that persist at the level of endocytotic vesicles (Cahill et al., 2017; Thomsen et al., 2016). The link of arrestin to the GPCR in such a complex is through the C-terminal tail of the receptor alone, not the transmembrane core. The temporal and spatial import of GPCR signal transduction within subcellular compartments will almost certainly prove significant.

Biased Agonism. The two-state model of receptor activity is a convenient and useful simplification, but GPCRs can exist in a variety of active conformations, some of which may have the capacity to communicate differentially with downstream elements of transduction. *Biased agonism* refers to the property of an agonist to stabilize one conformation relative to another of a receptor and thus to set into motion a qualitatively distinct set of cellular events. The concept of biased agonism emerged first in the differential activation of G proteins, for example, G_i versus G_q and G_i versus G_{12}, depending on the agonist. Subsequently, differences between G protein and arrestin signaling were recognized (Smith et al., 2018), where certain agonists were found to stabilize conformations that signal through G proteins predominantly, while others stabilize conformations that signal through arrestins instead (Figure 3–17).

Carvedilol, for example, has long been classified as a β adrenergic receptor antagonist. However, in addition to antagonizing β receptor activation of G_s, the carvedilol-receptor complex also engages arrestin (Wisler et al., 2007). Thus, from the viewpoint of arrestin signaling, carvedilol is an agonist. Can the two pathways be manipulated separately? Can therapeutic and adverse effects be distinguished according to the pathway engaged? Drug discovery efforts are seeking to answer these questions, synthesizing putative *biased agonists*, especially targeting the GPCRs for *opioids* (Chapter 23), *dopamine* (Chapter 15), and *angiotensin* (Chapter 30).

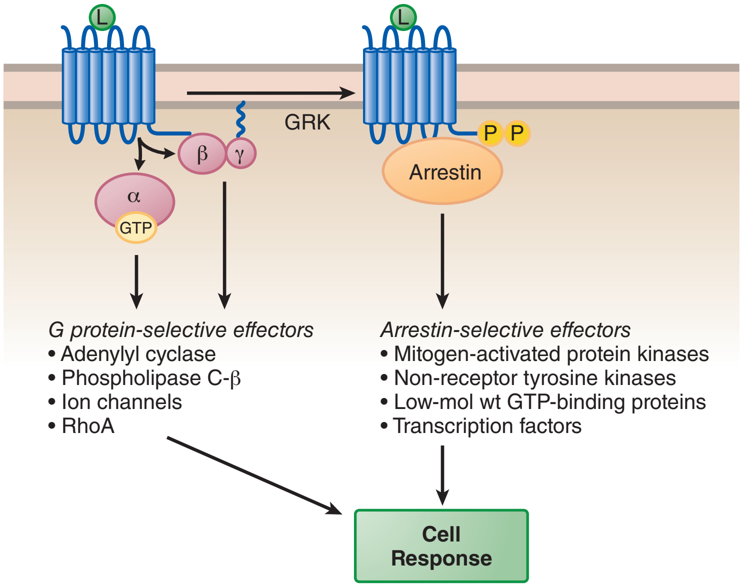

L
L
GRK
β
γ
α
GTP
P P
Arrestin
G protein-selective effectors
• Adenylyl cyclase
• Phospholipase C-β
• Ion channels
• RhoA
Arrestin-selective effectors
• Mitogen-activated protein kinases
• Non-receptor tyrosine kinases
• Low-mol wt GTP-binding proteins
• Transcription factors
Cell Response

Figure 3–16 *Dual actions of arrestin.* Arrestins were initially characterized in the context of receptor desensitization related to G protein signaling. The recruitment of an arrestin requires phosphorylation of the GPCR by a GRK. The recruitment, and specifically the binding of arrestin to the GPCR "core," sterically hinders subsequent G protein activation. The recruitment also effects a change in the conformation of arrestin that is equated with an adaptor functionality and/or frank activation of signaling intermediates and thus downstream signaling in its own right. At an overt level, the signaling achieved by activation of G proteins at the outset integrates with the signaling achieved by arrestin subsequently. The temporal and subcellular (e.g., endosomal) aspects of signaling are not shown in this figure but are discussed in the text. Adapted from Lefkowitz and Shenoy (2005).

Consider the analgesic actions of opioids. One hypothesis being tested is that the analgesic actions of opioids through the μ opioid receptor are exerted through G proteins and that signaling via β-arrestin-2 mediates many of the adverse responses to opiates (e.g., respiratory depression, tolerance, dependence). A study of opiate action in β-arrestin-2 gene knockout [*βarr2(–/–)*] animals reported potentiation of morphine analgesia and reduction of side effects (Raehal et al., 2005). Development of the recently approved *oliceridine*, designed as a biased μ receptor agonist, is based on this premise (Markham, 2020). Some initial findings were promising, but subsequent data on several putatively biased agonists have not supported such a clean separation of antinociceptive and adverse effects (Gillis et al., 2020a) and have challenged the proposition that *oliceridine* provides increased separation of desired and adverse effects (see Chapter 23). In addition, other explanations of apparent bias (e.g., low intrinsic agonism and differential signal amplification in the G protein and arrestin pathways) have been offered and refuted (Azevedo Neto et al., 2020; Gillis et al., 2020b; Stahl and Bohn, 2021). Stay tuned. Biased agonists could offer advantages for a higher level of specificity in drug-induced signaling through GPCRs.

A distinction must be made between biased agonism, or *ligand bias*, as discussed here and *receptor bias* and *system bias* (Smith et al., 2018). Receptor bias is the predisposition of a receptor toward signaling through one G protein or another or through a G protein versus arrestin. Certain receptors signal through arrestin alone, and others can be made (through mutation) to signal only through G proteins. System bias is the difference among cells in expression of transducers, effectors, and downstream proteins, brought into sharp focus for systems in which the expression of a G protein relative to arrestin differs substantially. As always, but especially with the emergence of bias as a concept, it is important to remember that efficacy depends on the context in which it is evaluated.

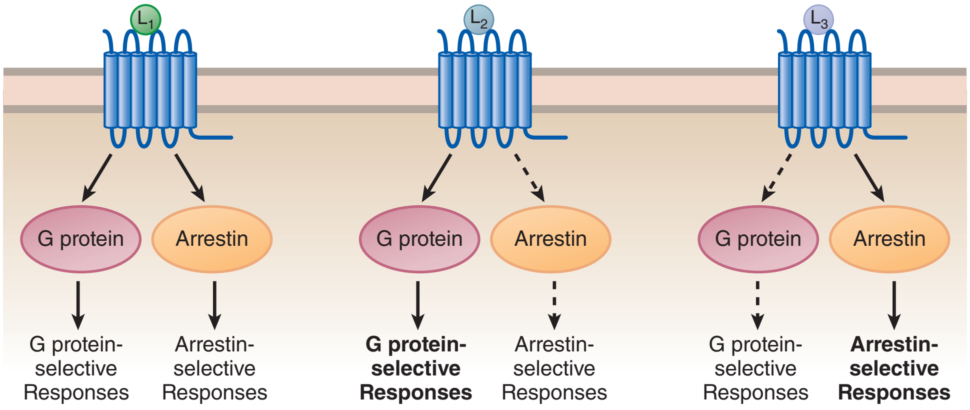

L1
L2
L3
G protein
Arrestin
G protein
Arrestin
G protein
Arrestin
G protein-selective Responses
Arrestin-selective Responses
G protein-selective Responses
Arrestin-selective Responses
G protein-selective Responses
Arrestin-selective Responses

Figure 3–17 *Biased agonism.* One form of biased agonism is that relating to differential engagement of G proteins and arrestins. A single GPCR can assume any number of conformations (not expressly depicted) that are differentially stabilized by activating ligands. One or more of these conformations can translate into the engagement of both G protein and arrestin (*left*), the G protein selectively (*middle*), or arrestin selectively (*right*). In this sense, the ligand *biases*, or predisposes, the GPCR toward a particular scheme of signaling. See the text for other forms of bias.

AL GRAWANY

Proximal Forms of Signaling by GPCRs Through G Proteins. The second messengers regulated by GPCRs through G proteins include cyclic nucleotides, DAG, IP_3, and (through IP_3) Ca^{2+}. Also engaged as important forms of signaling are G protein-gated ion channels and the monomeric G protein RhoA.

Cyclic AMP. Cyclic AMP is synthesized by the enzyme AC, for which nine membrane-bound isoforms and one soluble isoform exist in mammals (Dessauer et al., 2017; Hanoune and Defer, 2001). All membrane-bound isoforms are activated by the α subunit of G_s. Inhibition of the activated enzyme by G_i is usually attributed to release of the βγ heterodimer and consequent sequestration of α_s; however, the heterodimer and α_i can have direct and idiosyncratic actions, depending on the isozyme of AC (Taussig et al., 1994). Cyclic AMP generated by ACs has two major targets in most cells: the cyclic AMP-dependent protein kinase (PKA) and the cyclic AMP-regulated GEFs termed EPACs (exchange proteins activated by cyclic AMP) (Cheng et al., 2008; Roscioni et al., 2008). The transcription factor CREB (cyclic AMP response element–binding protein) is activated via PKA phosphorylation and provides a link of cellular cyclic AMP transients to transcriptional regulation (Mayr and Montminy, 2001; Sands and Palmer, 2008). In cells with specialized functions, cyclic AMP can have additional targets, such as the cyclic nucleotide-gated (CNG) ion channels and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (Wahl-Schott and Biel, 2009) and cyclic nucleotide-regulated PDEs. For an overview of cyclic nucleotide action and a historical perspective, see Beavo and Brunton (2002). A discussion of cyclic GMP is presented later (Guanylyl Cyclases).

PKA. The PKA holoenzyme consists of two catalytic (C) subunits reversibly bound to an inhibitory regulatory (R) subunit dimer to form a heterotetrameric complex (R_2C_2). In response to an increase in cellular cyclic AMP, four cyclic AMP molecules bind to the R_2C_2 complex, two to each R subunit, causing a conformational change in the R subunits that removes the inhibitory domain from the C subunit catalytic domain, resulting in their activation. The active C subunits phosphorylate serine and threonine residues on specific protein substrates. There are multiple isoforms of PKA; molecular cloning has revealed α and β isoforms of both the regulatory subunits (RI and RII), as well as three C subunit isoforms, Cα, Cβ, and Cγ. The R subunits exhibit different subcellular localization and binding affinities for cyclic AMP, giving rise to PKA holoenzymes with different thresholds for activation (Taylor et al., 2008). PKA function and specificity are also modulated by subcellular localization mediated by A-kinase anchoring proteins (AKAPs). Indeed, compartmentation of cyclic AMP signaling components (including components described below as well as GPCRs, ACs, phosphodiesterases, and protein phosphatases) within multiprotein nanometer scale *signalosomes* is now recognized as being essential for normal cell responses to cyclic AMP (Brunton et al., 1981; reviewed by Zaccolo et al., 2020).

EPAC. EPAC, also known as cyclic AMP-GEF, is a novel cyclic AMP–dependent signaling protein (Schmidt et al., 2013). EPAC serves as a cyclic AMP–regulated GEF for the family of small Ras GTPases (especially the Rap small GTPases), catalyzing the exchange of GTP for GDP, thereby activating the small GTPase. The two isoforms of EPAC, EPAC1 and EPAC2, differ in their architecture and tissue expression. Both EPAC isoforms are multidomain proteins that contain a regulatory cyclic AMP–binding domain, a catalytic domain, and domains that determine their intracellular localization. Compared to EPAC2, EPAC1 contains an additional N-terminal low-affinity cyclic AMP–binding domain. The expressions of EPAC1 and EPAC2 are differentially regulated during development and in a variety of disease states. EPAC2 can promote incretin-stimulated insulin secretion from pancreatic β cells through activation of Rap1 (see Figure 51–3). *Sulfonylureas*, oral drugs used to treat type 2 diabetes mellitus, may act in part by activating EPAC2 in β cells and increasing insulin release.

PDEs. Cyclic nucleotide phosphodiesterases (PDEs) hydrolyze the cyclic 3′,5′-phosphodiester bond in cyclic AMP and cyclic GMP, thereby terminating action of the cyclic nucleotide. The PDEs comprise a superfamily with more than 50 different proteins (Conti and Beavo, 2007). The substrate specificities of the different PDEs include those specific for cyclic AMP hydrolysis and for cyclic GMP hydrolysis and some that hydrolyze both cyclic nucleotides. The activities of PDEs are regulated via gene transcription as well as by cyclic nucleotides, Ca^{2+}-calmodulin, and interactions with other signaling proteins such as arrestins and protein kinases. Some PDEs are localized to specific signaling complexes via AKAPs and other scaffolding proteins. PDEs (mainly PDE3 isoforms) are drug targets for treatment of diseases such as asthma (Chapter 44), a variety of cardiovascular diseases (Chapters 31–33), and atopic dermatitis (Chapter 75), among others. PDE5 inhibitors (e.g., *sildenafil*) are used in treating chronic obstructive pulmonary disease (see Figures 44–4 and 44–5) and erectile dysfunction (see Figure 49–6).

DAG/IP_3/Ca^{2+}. The activation of GPCRs that are coupled to G_q (and occasionally to G_i) results in recruitment of an isoform of phospholipase C (PLC), PLCβ-2, to the plasma membrane. Activated PLC hydrolyzes a minor membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP_2), to generate two intracellular signals, DAG and IP_3. DAG directly activates some members of the PKC family. IP_3 diffuses to the endoplasmic reticulum (ER), where it activates the IP_3 receptor in the ER membrane, causing release of stored Ca^{2+}. This raises Ca^{2+} levels in the cytoplasm manyfold within seconds and activates Ca^{2+}-dependent enzymes such as some of the PKCs and Ca^{2+}/calmodulin-sensitive enzymes such as PDE1 and a family of Ca^{2+}/calmodulin-sensitive PKs (e.g., phosphorylase kinase, MLCK, and CaM [calmodulin] kinases II and IV) (Hudmon and Schulman, 2002). The second messenger functions of Ca^{2+} cannot be understated. Ca^{2+} is integral to the regulation of diverse metabolic processes, secretion, contraction, gene expression, and electrical activity across the membrane.

G Protein-Gated Ion Channels. Of the several types of ion channels regulated directly by G proteins, the inwardly rectifying K^+ (K_{ir}) channels have risen to prominence. The subset of K_{ir} channels activated through GPCRs are homo- and heterotetrameric complexes of two-transmembrane helical subunits from the $K_{ir}3$ subfamily (Hibino et al., 2010) (Figure 3B). These subunits are expressed to varying extents in neurons, atrial myocytes, and endocrine cells, among other cells. The channels are activated by the βγ heterodimer released from G_i, perhaps through βγ-enhanced PIP_2 binding. The selectivity for G_i as opposed to other G proteins that likewise contain βγ is probably related to preformed complexes between the channels and the G_i heterotrimer.

K_{ir} channels help stabilize the resting potential of the cell-surface membrane. Because the resting membrane potential is generally positive to the K^+ equilibrium potential, the activated channel conducts net outward current and thereby hyperpolarizes the membrane, making cells less responsive to depolarizing stimuli. In neurons, the $K_{ir}3$ subunit–containing channels can be activated by acetylcholine, adenosine, dopamine, cannabinoids, GABA (through the $GABA_B$ receptor), serotonin, somatostatin, and opioids. Considerable attention has been devoted to the postsynaptic inhibition achieved by opioids through G protein-regulated K_{ir} channels in both central and peripheral neurons as a basis for the analgesic actions of these compounds. G protein-regulated K_{ir} channel signaling is associated with behavioral responses of various drugs of abuse, psychostimulants, and ethanol (Luján et al., 2014).

The decrease in heart rate by acetylcholine via the M_2 muscarinic cholinergic receptor is achieved in part through $K_{ir}3$ subunit–containing channels as well. These channels are an important component of the heart's response to parasympathetic tone. The $K_{ir}3$ subunit–containing channels in heart respond also to adenosine through adenosine A_1 receptors; adenosine is used therapeutically to rapidly halt supraventicular tachyarrhythmias (Chapter 34).

Members of the G_i family also regulate voltage-gated Ca^{2+} channels. The channels best studied in this context are those formed from Ca_V2 subunits, that is, the P/Q-, N-, and R-type channels, in contrast to L- and T-type channels (Proft and Weiss, 2015). The regulation is again achieved by the βγ heterodimer, which *inhibits* channel activity. The Ca_V2 subunit–containing neuronal channels are located presynaptically, and their inhibition is manifest as an inhibition of neurotransmitter release. Opioids, for instance, not only repress neuronal activation through activation of inwardly rectifying K^+ channels but inhibit the release of neurotransmitters through inhibition of voltage-gated Ca^{2+} channels at the presynaptic terminal. Virtually all endogenous agonists and drugs operating through $G_{i/o}$-linked GPCRs within the CNS can achieve these molecular actions, with the precise neurological impact depending on the type and location of the neuron.

RhoA Activation. The activation of members of the $G_{12/13}$ family invariably links to the activation of RhoA, an important member of the Ras superfamily of low-molecular-weight (~21 kDa) monomeric G proteins. RhoA controls phenomena related to cell shape, migration, and contraction, accomplished largely through the Rho-associated protein kinase (ROCK). RhoA action through ROCK and other protein kinases also regulates the expression of numerous genes (Yu and Brown, 2015). The importance of RhoA to events of pharmacological interest is illustrated in the actions of angiotensin II (Ang II) on vascular smooth muscle cells in relation to hypertension, as discussed in Physiological Systems Must Integrate Multiple Signals. The activation of RhoA, together with events engaged by G_q and arrestin, is also implicated in the growth and proliferation of cardiomyocytes and vascular smooth muscle cells and in abnormalities that are associated with the progression of heart failure (Balakumar and Jagadeesh, 2014; Chapters 30 and 33). The activation of RhoA by thrombin is the basis for the shape change and activation of platelets in hemostasis. Inhibitors of ROCK are of interest in the treatment of pulmonary hypertension (Chapter 35), pathologies involving bronchoconstriction (Chapter 44), erectile dysfunction (Chapter 49), and elevated ocular pressure in patients with open-angle glaucoma or ocular hypertension (Chapter 74). *Netarsudil*, FDA approved for the treatment of these ocular disorders, is a first-in-kind inhibitor of ROCK for therapeutic use.

The $G_{12/13}$ family activates Rho via the interaction of the α subunit of G_{12} or G_{13} with one of several RhoA guanine nucleotide exchange factors (RhoGEFs), each containing a regulator of G protein signaling (RGS) domain. GEFs facilitate the exchange of GDP for GTP and, hence, activation of low-molecular-weight G proteins such as RhoA; RGS domains interact with activated heterotrimeric G protein α subunits to hasten GTP hydrolysis yet are often contained within proteins that have downstream signaling functionalities (Ross and Wilkie, 2000). In certain cells, the activation of RhoA can be achieved by G_i and G_q, also through RGS-containing RhoGEFs.

Ligand-Gated Ion Channels

Channels Gated by Excitatory and Inhibitory Neurotransmitters.

The principal ligand-gated ion channels in the nervous system are those activated by *excitatory* or *inhibitory neurotransmitters*. Those activated by excitatory neurotransmitters conduct Na^+ and K^+ nonspecifically, and sometimes Ca^{2+}, and include the cholinergic nicotinic receptors, glutaminergic receptors (AMPA [α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid], kainate, and NMDA [*N*-methyl-D-aspartate] subtypes), and certain purinergic and serotonergic receptors. Channels activated by inhibitory neurotransmitters conduct Cl^- and are the $GABA_A$ and glycine receptors. Activation of channels by excitatory and inhibitory neurotransmitters is responsible for the majority of events relevant to synaptic transmission by neurons both in the CNS and in the periphery. These ligand-gated ion channels are pentamers of distinct subunits, each a large protein with four transmembrane spans (Figure 3–18).

The nicotinic acetylcholine (ACh) receptor is an instructive example of an excitatory ligand-gated ion channel. Isoforms of this channel are expressed in the CNS, in autonomic ganglia, and at the neuromuscular junction. The channel is a pentamer that, in neurons, consists of two to five α subunits (drawn from among the α_2–α_{10} subtypes) and up to three β subunits (drawn from among the β_2–β_4 subtypes) and, at the neuromuscular junction, consists of two α_1, one β_1, one δ, and one γ (embryo) or one ε (adult) subunit. Each subunit of the receptor contains a large, extracellular N-terminal domain, four membrane-spanning helices (one of which helps to line the pore in the assembled complex), and an internal loop between helices 3 and 4 that forms the intracellular domain of the channel (see Figure 3–18). ACh binds at interfaces involving the α subunits (e.g., at α/α or α/β interfaces). The different compositions of the subunits account for the ability of competitive antagonists such as *rocuronium* to inhibit the receptor in the neuromuscular junction without effect on the ganglionic or CNS receptor. This property is exploited to provide muscle relaxation during surgery with minimal autonomic side effects (Chapter 13). The pore opening in the channel measures about 3 nm, whereas the diameter of a Na^+, K^+, or Ca^{2+} ion is only 0.3 nm or less, and for this reason, the channel does not possess the exquisite ion selectivity found in most voltage-activated channels. The passage of Na^+, K^+, and Ca^{2+} ions has the net effect of depolarization.

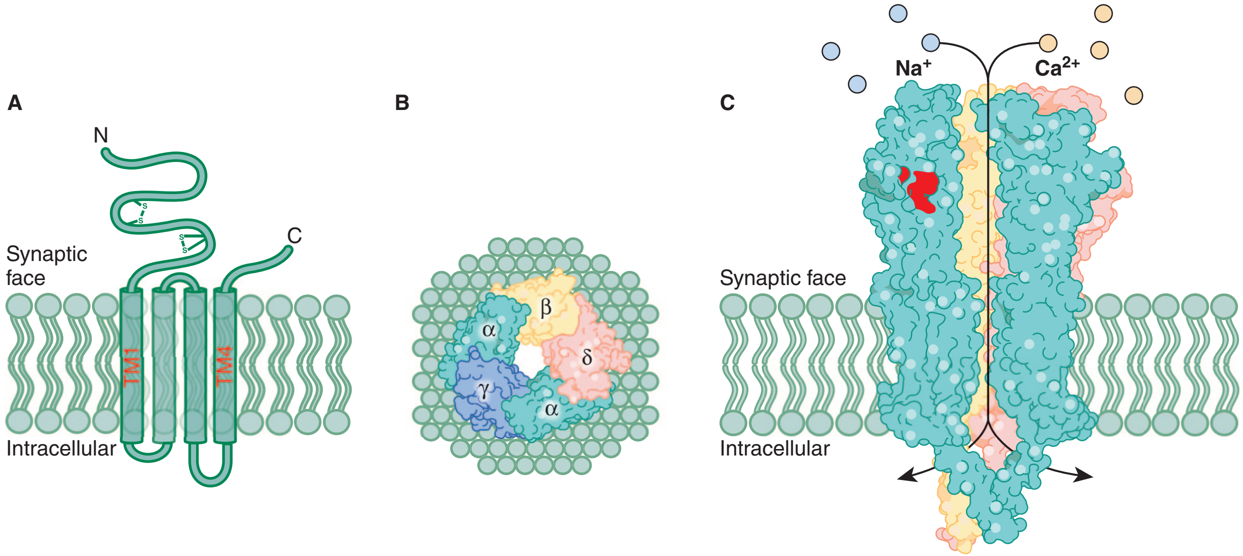


Figure 3–18 *Structure of the nicotinic acetylcholine receptor.* **A.** A schematic representation of a nicotinic ACh receptor subunit, one of the five that constitute the receptor pentamer. It depicts the four-transmembrane helical motif with the extracellular N- and C-terminal domains. **B.** The nicotinic receptor structure viewed from the perspective of the neuromuscular junction. Conductance is achieved through the central axis pore, defined by the pseudosymmetric arrangement of receptor subunits. **C.** Longitudinal view of the same receptor as recently determined by electron microscopy, with the γ subunit removed; the depolarizing ions alone (Na^+ and Ca^{2+}) are shown. See Chapter 13 for further details.

The pentameric $GABA_A$ receptor containing α, β, and γ subunits conducts Cl^- when activated by GABA. With an equilibrium potential for Cl^- around −65 mV, the activation of the receptor does not generally cause membrane hyperpolarization but does impede generation and propagation of action potentials. The $GABA_A$ receptor is a target for *benzodiazepines* and *barbiturates*, among many other drugs. The multiplicity of isoforms of each of the constituent subunits of these pentameric ion channels accounts for the distinct pharmacology of these receptors.

Channels Gated by Intracellular Ligands. There is a category of ion channels activated by *intracellular* ligands: the IP_3-sensitive Ca^{2+} channel responsible for release of Ca^{2+} from the ER; cyclic nucleotide-gated ion channels responsible for nonselective cation conductance; and the sulfonylurea "receptor" (SUR1) that associates with the $K_{ir}6.2$ channel to regulate the ATP-gated K^+ current in pancreatic β cells. This latter channel, or K_{ATP} channel, is the target of oral hypoglycemic drugs such as *sulfonylureas* and *meglitinides* that stimulate insulin release from pancreatic β cells and are used to treat type 2 diabetes (see Chapter 51).

Transient Receptor Potential Channels. Transient receptor potential (TRP) cation channels are involved in a variety of sensory processes, including nociception, heat and cold sensation, mechanosensation, and perception of chemicals such as capsaicin and menthol. The superfamily consists of 28 channels in six families (Moran, 2018). Most TRP channels are homotetramers, with each monomer consisting of six transmembrane helices (S1–S6) with a pore-forming loop between S5 and S6 and large intracellular regions at the intracellular amino and carboxyl termini. TRP channels are relatively nonselective with regard to the conductance of cations, generally conducting Na^+ and Ca^{2+} and sometimes Mg^{2+}. Two scientists who have done seminal work on TRP channels, David Julius and Ardem Patapoutian, shared the 2021 Nobel Prize in Physiology/Medicine "for their discoveries of receptors for temperature and touch" (Latorre and Diaz-Franulic, 2021).

Pharmacological agents are under development to treat heritable diseases associated with TRP channel mutations and to treat pain, itching, and skin and respiratory disorders (Bamps et al., 2021). Formulations of *capsaicin*, an agonist for the TRPV1 receptor, are available for certain forms of pain relief, including that associated with postherpetic neuralgia.

Enzyme-Linked (Catalytic) Receptors

Receptor Tyrosine Kinases. The receptor tyrosine kinases include receptors for hormones such as insulin; growth factors such epidermal growth factor (EGF), platelet-derived growth factor (PDGF), nerve growth factor (NGF), fibroblast growth factor (FGF), and VEGF; and ephrins. With the exception of the insulin receptor, consisting of α and β polypeptide chains (see Chapter 51), these receptors are single polypeptide chains. Each has a large, cysteine-rich extracellular domain, a short transmembrane segment, and an intracellular region containing one or two protein tyrosine kinase domains. Activation of growth factor receptors generally supports cell survival, cell proliferation, and differentiation. Activation of the ephrin receptors supports neuronal angiogenesis, axonal migration, and guidance.

Ligand binding induces dimerization of the receptor and cross-phosphorylation of tyrosine residues within the now proximal intracellular regions, notably in the kinase domains themselves to enhance activity but also in stretches beyond these domains. Phosphotyrosine residues constitute docking sites on the receptor for proteins containing SH2 and PTB domains. Over 100 such proteins, namely enzymes and adaptors, are encoded in the human genome.

Recruited enzymes are often phosphorylated and activated in turn. These include PLCγ, the activity of which raises intracellular levels of Ca^{2+} and activates PKC, and the α and β isoforms of phosphatidylinositol 3-kinase (PI3K). PI3K binds directly to the phosphorylated receptor by means of SH2 domains or indirectly via insulin receptor substrate-1 (IRS-1), is activated, and increases the level of phosphatidylinositol 3,4,5-trisphosphate (PIP_3) with consequent activation of PKB (also known as Akt). PI3K can also be activated directly by the monomeric GTP-binding protein Ras. PKB can regulate mTOR (mechanistic/mammalian target of rapamycin), which is upstream of various signaling pathways (Figure 3–19A, also see Autophagy below) and the *Bad* protein that is important in apoptosis (Figure 3–25; also see section on Apoptosis below).

Adaptors are proteins without enzymic activity that serve accessory functions, often those of placing potentially interacting proteins in close proximity to each other. Grb2, for example, is an adaptor prebound to Sos, a GEF that can activate Ras (Figure 3–19B). Activation of Ras leads in turn to activation of a protein kinase cascade termed the Ras-MAPK pathway. Activation of the MAPK pathway is one of the major routes used by growth factor receptors to signal to the nucleus and stimulate cell growth. Oncogenic mutations that result in constitutively activated growth factor receptors and Ras can also activate the MAPK pathway and drive tumor proliferation. Anticancer agents that target the MAPK pathway and the protein tyrosine kinase activity of oncogenic growth factors are now important agents in treating several forms of cancer (see Chapter 69 and 71).

Receptor Serine-Threonine Kinases. The transforming growth factor β (TGF-β) family of ligands, which include different forms of TGF-β and bone morphogenic proteins (BMPs), activate receptors that are analogous to receptor tyrosine kinases but have a serine-threonine kinase functionality. The ligands bind and stabilize a heteromeric complex of two type I and two type II cell-surface receptors. Humans express seven type I and five type II receptors, which are engaged differentially by TGF-β family members (Derynck and Budi, 2019). Ligand-induced changes in conformation and/or proximity allow the type II receptors to phosphorylate the type I receptors at serine and threonine residues and activate downstream signaling via SMADs (Figure 3–20). Drugs that inhibit TGF-β ligand signaling are under development and are of particular interest in the therapy of cancer and fibrosis.

Guanylyl Cyclases. The synthesis of intracellular cyclic GMP in cells is achieved either through ligand-activated cell-surface receptors with intrinsic guanylyl cyclase (GC) activity or through soluble GC (sGC) (Figure 3–21). The cell-surface receptors are those for natriuretic peptides; sGC is responsive to NO. The downstream effects of cyclic GMP are carried out by multiple isoforms of PKG, cyclic GMP–gated ion channels, and cyclic GMP–modulated PDEs that degrade cyclic AMP.

Transmembrane Receptors With Intrinsic GC Activity. Natriuretic peptides are small peptide ligands released from cells in cardiac tissues, the vascular system, and certain other tissues. The peptides are: (1) atrial natriuretic peptide (ANP), released from atrial storage granules following expansion of intravascular volume or stimulation with pressor hormones; (2) brain natriuretic peptide (BNP), synthesized and released in large amounts from ventricular tissue in response to volume overload; and (3) C-type natriuretic peptide (CNP), synthesized in the brain, endothelial cells, and chondrocytes and released in response to growth factors and sheer stress on vascular endothelial cells (Potter et al., 2009). The major physiological effects of these hormones are to decrease blood pressure (ANP, BNP), to reduce cardiac hypertrophy and fibrosis (BNP), and to stimulate long-bone growth (CNP). The receptors for the natriuretic peptides are natriuretic peptide receptor (NPR)-A, which responds to ANP and BNP, and NPR-B, which responds to CNP. NPR-C is thought to function as a clearance receptor, removing excess natriuretic peptide from the circulation. Chapter 29 has an extensive discussion of the effects of the natriuretic peptides and of the pathways involved. *Nesiritide*, a synthetic BNP agonist, and *sacubitril*, an inhibitor of an enzyme (neprilysin) that degrades ANP and BNP, are used in the treatment of heart failure.

sGC, a Cytosolic Receptor/Enzyme That Responds to a Membrane-Permeable Paracrine Factor, NO. NO is produced locally in cells by *nitric oxide synthase* (NOS). There are three forms of NOS: neuronal NOS (nNOS or NOS1), endothelial NOS (eNOS or NOS3), and inducible NOS (iNOS or NOS2). All three forms are widely expressed but are especially important in the cardiovascular system, where they are found in myocytes, vascular smooth muscle cells, endothelial cells, hematopoietic cells, and platelets. Elevated cell Ca^{2+}, acting via CaM, markedly activates nNOS and eNOS; the inducible form (iNOS) is less sensitive to Ca^{2+}, but its synthesis can be induced many fold by inflammatory stimuli such as endotoxin, TNF-α, interleukin (IL)-1β, and interferon (IFN) γ.

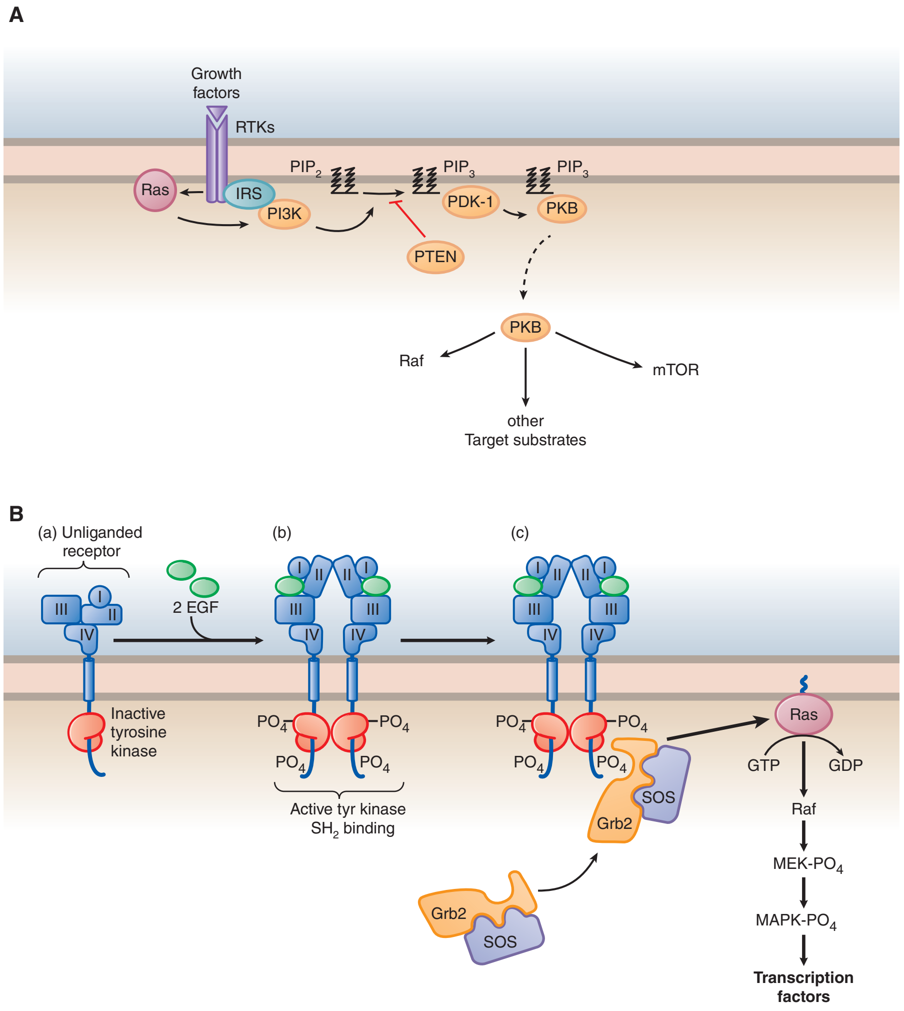


Figure 3–19 *Two events downstream of receptor tyrosine kinases.* **A.** *Activation of the mTOR pathway.* Signaling via this pathway promotes growth, proliferation, and survival of cells via a complex web of signaling pathways (see Guri and Hall, 2016). mTOR signaling is emerging as a major consideration in immunosuppression and cancer pharmacotherapy, and inhibitors of mTOR signaling are sometimes included as adjunct therapy. **B.** *Activation of the EGF receptor.* The extracellular structure of the unliganded receptor (a) contains four domains (I–IV), which rearrange significantly on binding two EGF molecules. In (b), the conformational changes lead to activation of the cytoplasmic tyrosine kinase domains and tyrosine phosphorylation of intracellular regions to form SH2-binding sites. (c) The adaptor molecule Grb2 bound to Sos via two so-called SH3 domains binds to the phosphorylated tyrosine residues and activates the Ras-MAPK cascade. Ras is attached to the inner surface of the plasma membrane normally by a farnesyl moiety. Translocation of Sos to the inner surface of the membrane occurs by the binding of Grb2 to an activated receptor through an SH2 domain. The translocation places Sos in proximity to the membrane-bound Ras and thus supports activation of Ras.

NOS produces NO by catalyzing the oxidation of the guanido nitrogen of L-arginine, producing L-citrulline and NO. NO activates sGC, which is an αβ heterodimer that contains a protoporphyrin-IX heme domain. NO binds to the heme domain at low nanomolar concentrations and produces a 200- to 400-fold increase in the V_{max} of the enzyme, leading to an elevation of cellular cyclic GMP (Murad, 2006).

In vascular smooth muscle, activation of PKG leads to vasodilation by inhibiting IP_3-mediated Ca^{2+} release from intracellular stores; phosphorylating voltage-gated Ca^{2+} channels to inhibit Ca^{2+} influx; phosphorylating phospholamban, a modulator of the sarcoplasmic Ca^{2+} pump, leading to a more rapid reuptake of Ca^{2+} into intracellular stores; phosphorylating and opening the Ca^{2+}-activated K^+ channel, leading to hyperpolarization of the cell membrane, which closes L-type Ca^{2+} channels and reduces the flux of Ca^{2+} into the cell; phosphorylating and thereby inhibiting myosin light chain kinase; and phosphorylating and thereby activating myosin light chain phosphatase.

Drugs that activate sGC are the organic nitrates (*nitroglycerin*, *isosorbide dinitrate*, and *isosorbide-5-monitrate*), which produce NO, and inhaled NO gas, all of which are used in the treatment of stable angina (Chapter 31). NO is also used as a tocolytic agent (Chapter 48) and in the treatment of term and near-term neonates with persistent pulmonary hypertension and acute hypoxemic respiratory failure (Chapter 35). The recently approved drugs *riociguat* and *vericiguat* are used as well in the treatment of pulmonary hypertension (Chapter 35); *riociguat* sensitizes sGC to endogenous NO and also stimulates the enzyme directly.

The counterpart to activation of sGC is the inhibition of cyclic GMP–selective PDE (i.e., PDE5). The inhibition is accomplished by the drugs *sildenafil*, *vardenafil*, *tadalafil*, and *avanafil*. PDE5 inhibitors are used in the treatment of erectile dysfunction (Chapter 49) and pulmonary hypertension (Chapter 35).

Other Cell-Surface Membrane Receptors

JAK-STAT Receptor Pathway. Cytokines (interleukins, interferons, erythropoietin, and colony-stimulating factors) and certain hormones (e.g., growth hormone and prolactin) signal to transcriptional elements via *signal transducers and activators of transcription*, or STATs. Most of the receptors, although not all, are multi-subunit complexes consisting of separate ligand-binding and signal-transducing subunits. The receptors have no intrinsic enzymatic activity; rather, each is associated with a distinct intracellular tyrosine kinase termed a *Janus kinase*, or JAK (Figure 3–22A). Receptor dimerization or oligomerization induced by a

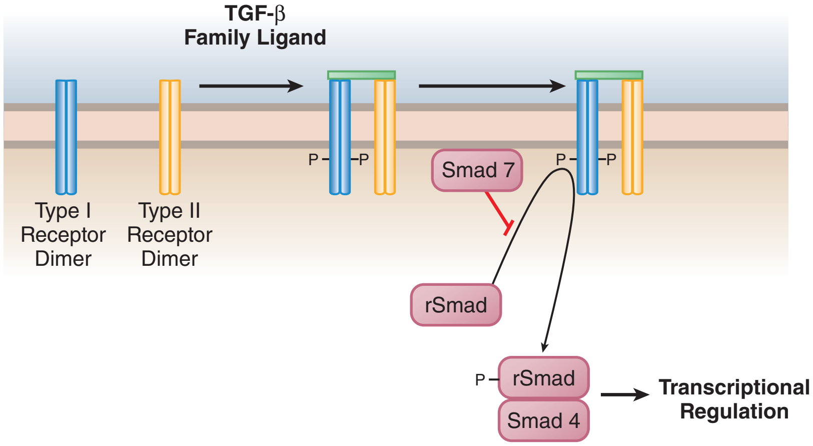

TGF-β
Family Ligand
Type I
Receptor
Dimer
Type II
Receptor
Dimer
P
P
Smad 7
P
P
rSmad
P
rSmad
Smad 4
Transcriptional
Regulation

Figure 3–20 *Signaling through type I and type II heteromeric receptors by TGF-β family members.* TGF-β ligands stabilize a heteromeric receptor structure consisting of two type I and two type II receptors. The type II receptors then catalyze phosphorylation of the type I receptors on serine residues, permitting them to interact with and phosphorylate, again on serine residues, any number of "receptor-activated" Smads (rSmads). Phosphorylated rSmads bind Smad4, an effector Smad, to subsequently translocate to the nucleus where they regulate transcriptional events. Smad7 (and Smad6) are inhibitory Smads. Variations and dynamics of type I and type II receptor association, posttranslational modifications relevant to their stability, non-Smad signaling through these receptors, and the use by TGF-β–like ligands of alternate receptors are reviewed by Derynck and Budi (2019).

ligand brings at least two JAKs into close proximity, resulting in their transphosphorylation and the phosphorylation of the cytoplasmic tails of the receptors. STATs are recruited to the receptors via their SH2 domains and phosphorylated in turn by the JAKs. Phosphorylated STATs translocate as dimers to the nucleus to directly regulate transcription. The entire pathway is termed the JAK-STAT pathway. There are four JAKs and seven STATs in mammals that, depending on the cell type and signal, combine differentially to regulate gene transcription.

A variety of cytokines employing JAK-STAT pathways are used clinically, including *aldesleukin* (a recombinant IL-2) in metastatic renal cell cancer and metastatic melanoma (Chapter 72), *pegylated IFN-α* in viral hepatitis (Chapter 63), *sargramostim* (a recombinant granulocyte-macrophage colony-stimulating factor) to stimulate myelopoiesis, *oprelvekin* (a recombinant IL-11) to stimulate megakaryocyte maturation, and recombinant forms of *erythropoietin* to stimulate red blood cell production (Chapter 45). Receptor antagonists include *dupilumab* (IL-4

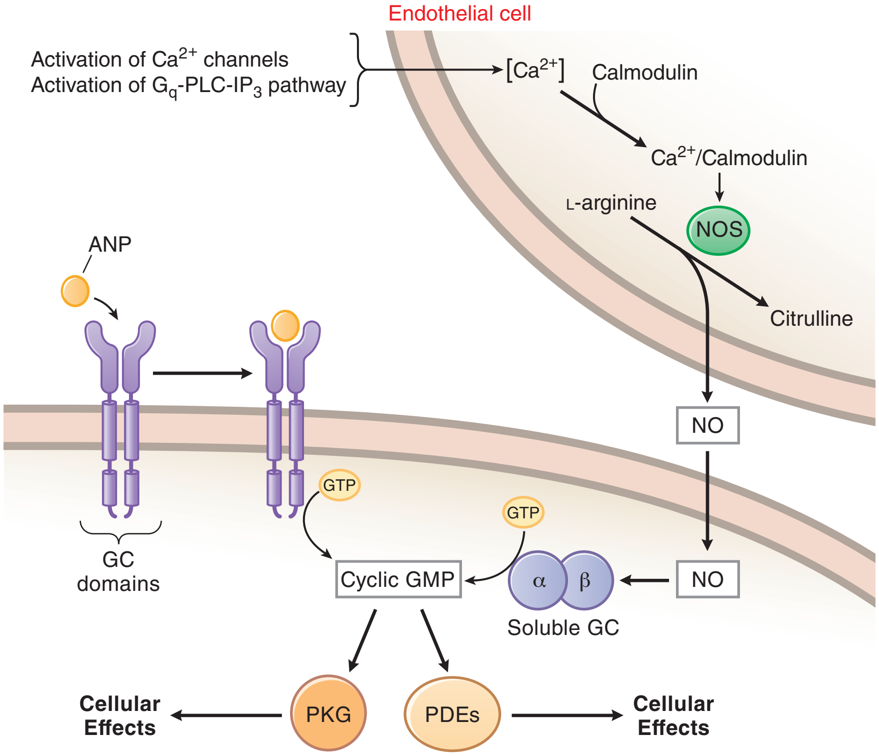

Endothelial cell
Activation of Ca2+ channels
Activation of Gq-PLC-IP3 pathway
[Ca2+]
Calmodulin
Ca2+/Calmodulin
L-arginine
NOS
Citrulline
ANP
NO
GTP
GTP
GC
domains
Cyclic GMP
α
β
NO
Soluble GC
Cellular
Effects
PKG
PDEs
Cellular
Effects

Figure 3–21 *Cyclic GMP signaling pathways.* Formation of cyclic GMP is regulated by cell-surface receptors with intrinsic GC activity and by soluble forms of GC. The cell-surface receptors respond to natriuretic peptides such as ANP with an increase in cyclic GMP. sGC responds to NO generated from L-arginine by NOS. Cellular effects of cyclic GMP are carried out by PKG and cyclic GMP–regulated PDEs. In this diagram, NO is produced by a Ca^{2+}/calmodulin–dependent NOS in an adjacent endothelial cell. Detailed descriptions of these signaling pathways are given throughout the text in relation to the therapeutic actions of drugs affecting these pathways.

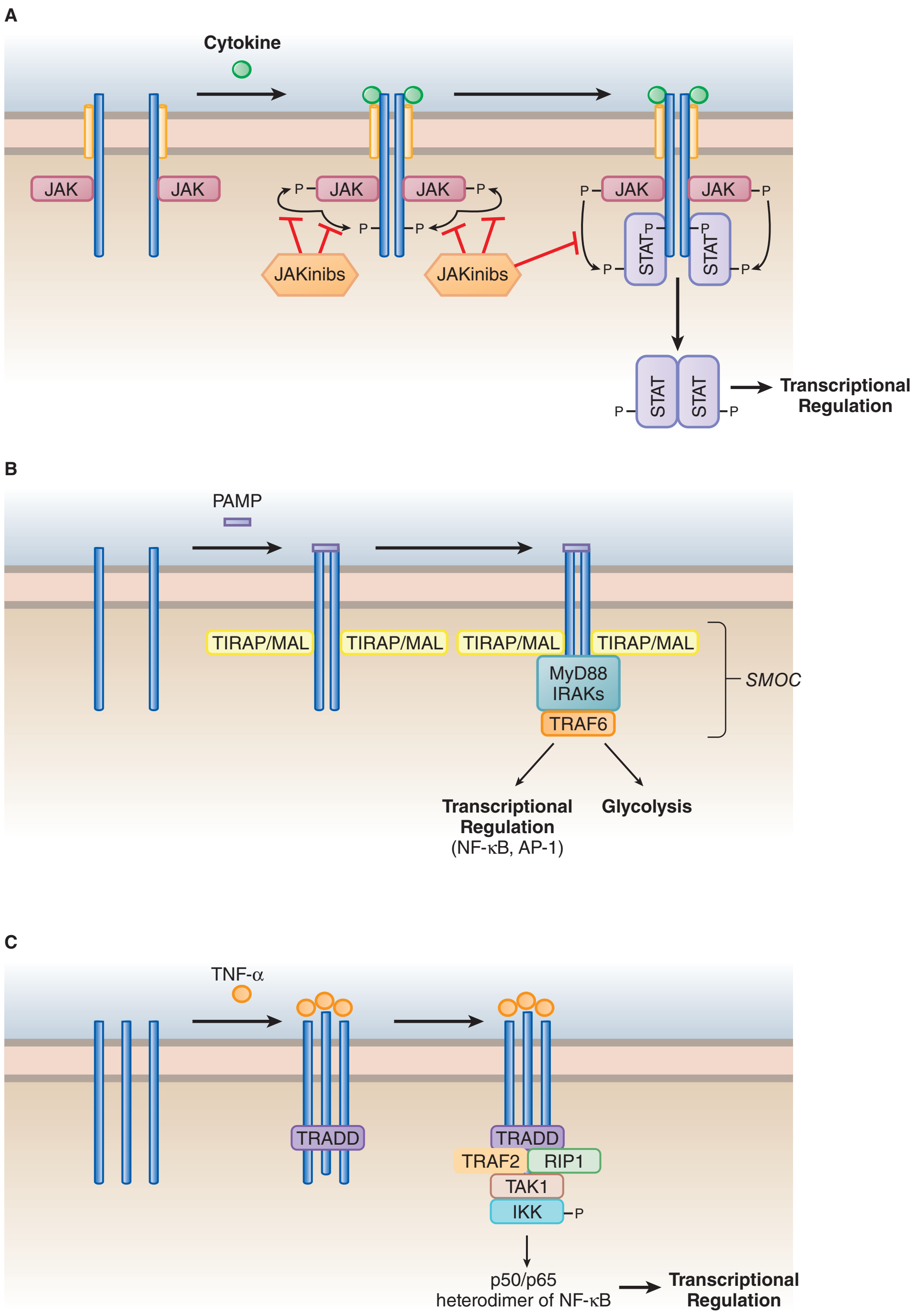

A
Cytokine
JAK
JAK
P
JAK
JAK
P
P
P
JAKinibs
JAKinibs
P
JAK
JAK
P
STAT
STAT
P
P
P
P
P
STAT
STAT
P
Transcriptional Regulation
B
PAMP
TIRAP/MAL
TIRAP/MAL
TIRAP/MAL
TIRAP/MAL
MyD88
IRAKs
TRAF6
SMOC
Transcriptional Regulation (NF-κB, AP-1)
Glycolysis
C
TNF-α
TRADD
TRADD
TRAF2
RIP1
TAK1
IKK
P
p50/p65 heterodimer of NF-κB
Transcriptional Regulation

Figure 3–22 *Signaling by several other cell-surface membrane receptors.* **A.** *Cytokine signaling through JAK/STAT.* Cytokine-induced dimerization of cytokine receptors (highly schematized) results in cross-phosphorylation and activation of associated JAKs. These tyrosine kinases in turn phosphorylate the receptor, leading to recruitment of STATs via their SH2 domains and phosphorylation of them as well. The phosphorylation promotes STAT dimerization. The phosphorylated, dimerized STATs translocate to the nucleus to act directly as transcription factors. Orally effective JAK inhibitors, JAKinibs, are now available as immunomodulators (see text). **B.** *Signaling by toll-like receptors.* PAMP-induced dimerization of a TLR, here at the cell-surface membrane, attracts TIRAP/MAL to the dimerized cytosolic TIR domains of the receptor. TIRAP/MAL engages multiple copies of MyD88 (myeloid differentiation protein 88) and the serine kinases of the IRAK (interleukin-1 receptor–associated kinase) family, which recruit the E3 ubiquitin lyase TNF receptor–associated factor (TRAF) 6. TRAF6 sets into motion events resulting in activation of NF-κB and AP-1 and glycolysis, among other events depending on the cell. SMOC refers to the aggregate of these proteins as the supramolecular organizing center. Other SMOCs exist depending on the identity and subcellular location of the TLR. **C.** *Signaling by TNF-α through TNFR1.* The signaling shown is that relevant to activation of NF-κB; other activities exist. The signaling begins with the formation of TNFR1 homotrimers stabilized by TNF-α (here, the soluble form). Activated TNFR1 recruits the TNFR type 1 death-associated protein (TRADD), which in turn recruits the receptor interacting protein-1 (RIP1) kinase and TRAF2. RIP1 and TRAF2 achieve activation of NF-κB by promoting phosphorylation via TGF-β–activated kinase-1 (TAK1), causing ubiquitin-dependent degradation of the inhibitor of NF-κB, IKK (IκB kinase), allowing the p50/p65 heterodimer to translocate to the nucleus and activate the transcription of inflammatory genes. Activated TNFR2 (not shown) recruits TRAF2 directly, among other proteins. TRAF2 also results in activation of the kinase JNK, relevant to the activation of the transcription factors c-Jun, AP-1, and ATF-2. Signaling by TNF-α through the two receptors is reviewed by Wajant and Siegmund (2019).

receptor) for atopic dermatitis, *ustekinumab* (IL-12 and IL-13 receptors) for plaque psoriasis and psoriatic arthritis (Chapter 75), and *pegvisomant* (pegylated recombinant growth hormone receptor) in the treatment of growth hormone excess (Chapter 46). A number of inhibitors of JAKs, referred to as *JAKinibs*, inhibit ATP binding to the JAK catalytic domain and are employed as immunomodulators (Chapter 39), especially in the treatment of ulcerative colitis (Chapter 55), psoriasis (Chapter 75), and myelofibrosis, polycythemia vera, and graft-versus-host disease (Chapter 71).

Toll-Like Receptors. Signaling related to the innate immune system is carried out in part by a family of 10 single membrane-spanning *toll-like receptors* (TLRs). These receptors are highly expressed in macrophages, monocytes, dendritic cells, and natural killer cells (Chapter 38). TLRs contain a large extracellular ligand-binding domain and a cytoplasmic region devoid of intrinsic enzymatic activity but containing a "TIR" domain that mediates protein/protein interactions.

Ligands for TLRs represent conserved microbial products often referred to as *pathogen-associated molecular patterns* (PAMPs), which include lipopolysaccharide, bacterial lipoproteins, single- and double-stranded RNA, unmethylated CpG-containing single-stranded DNA, and bacterial ribosomal DNA. TLRs that detect nucleic acids are expressed in endosomes; the others are present at the cell surface. Activation of TLRs produces an inflammatory response to the pathogens consisting principally of the production of inflammatory cytokines via enhanced gene transcription and a variety of cell-specific changes.

The first step in the activation of TLRs by ligands is dimerization of the receptors as either homo- or heterodimers (Figure 3–22B). The dimerization recruits adaptor proteins that, in turn, instigate assembly of a supramolecular organizing center. Scaffolded proteins include protein kinases relevant to the activation of the transcription factors NF-κB, activator protein-1 (AP-1), and IRF-3, and of glycolysis (Fitzgerald and Kagan, 2020).

Agonists for TLRs, especially for TLR3, TLR7, TLR8, and TLR9, have generated interest as immunomodifiers in cancer chemotherapy, as adjuvants in vaccines utilizing specific tumor agonists, and, tentatively, as viral prophylaxis (Vanpouille-Box et al., 2019). *Imiquimod* is a TLR7 agonist approved for the treatment of actinic keratosis, superficial basal cell carcinoma, and genital and anal warts. Recombinant herpes zoster virus and hepatitis B virus vaccines are formulated with TLR4 and TLR9 agonists, respectively. Antagonists for the TLRs are being investigated for the treatment of autoimmune diseases.

TNF-α Receptors. There are two primary TNF-α receptors (TNFRs), TNFR1 and TNFR2, both single-spanning membrane proteins. TNFR1 is the more widely expressed and is responsible for TNF-induced apoptosis (see Apoptosis below); however, signaling is more relevant to the production of inflammatory cytokines, principally through activation of the transcription factor NF-κB. Figure 3–22C summarizes signaling through TNFR1 to NF-κB. Humanized monoclonal antibodies to TNF-α itself, such as *infliximab* and *adalimumab*, are important in the treatment of rheumatoid arthritis and Crohn's disease.

Nuclear Hormone Receptors

Nuclear hormone receptors in humans constitute a superfamily of 48 receptors that respond to ligands capable of traversing the cell-surface membrane. The *steroid receptors* (family 3; Table 3–3) are a subset that comprise those for androgens, estrogens, glucocorticoids, mineralocorticoids, and progesterone. Other subsets, in which receptors show the capacity to form heterodimers with retinoid X receptors (RXRs) (families 1 and 2), are receptors for lipid metabolites, xenobiotics, vitamin D, and thyroid hormones. Included in these families are the receptors that mediate induction of drug-metabolizing enzymes such as CYP3A4 (see Figure 5–13). Receptors from yet other subsets serve uncertain functions, some apparently in a ligand-independent manner (Evans and Mangelsdorf, 2014). Isoforms of virtually all the receptors are generated by means of alternate transcriptional or translational start sites and differential RNA splicing.

Nuclear hormone receptors consist of five or six domains, denoted A through F, based on regions of conserved sequence and function (Figure 3–23A). The N-terminal A/B domain contains a *transcriptional activation region* (AF-1). This region is poorly conserved among receptors and is often portrayed to be autonomous. However, it can be controlled by ligands, depending on the receptor, and is a target for posttranslational modification. Domain C is the *DNA-binding domain*. This domain is highly conserved among receptors apart from elements that contribute to *hormone response element* (HRE) specificity (see below). Domain D is the hinge region between the DNA-binding domain, and domain E, the *ligand-binding domain*, has many functions. Beyond presenting a binding site for the ligand, domain E plays a role in receptor dimerization and provides surfaces for the binding of coactivators and corepressors. It also contains a ligand-dependent transcriptional activation region (AF-2). Domain F, if present, can serve as a site for posttranslational modification.

Steroid receptors exist as monomers in the absence of agonist. The monomers exist in the cytosol, complexed with chaperone proteins, although some may exist as well in the nucleus (Chapters 48 and 50). Upon binding agonist, the receptors form homodimers, with those in the cytosol translocating to the nucleus. The activated homodimers bind HREs, which are in this instance two inverted six-nucleotide repeats separated by three nucleotides and are specific for the identity of the homodimer. Activated homodimers associate at the same time with *coactivators*, a diverse group of proteins responsible for chromatin remodeling and posttranslational modifications of the general transcriptional machinery, in order to facilitate transcription. Mechanisms of action may exist beyond the genomic level for several of the steroid receptors. In the case of the estrogen receptor, a small fraction of the receptor associates with the plasma membrane and, together with a GPCR (GPER/GPR30), may mediate the relatively rapid actions of estrogen.

Receptors that can bind RXR exist in the nucleus as homo- or heterodimers (with RXR) regardless of agonist. Those that can function either as homodimers or as heterodimers with RXR include the RXRs themselves, thyroid receptors, vitamin D receptors, and retinoic acid receptors (RARs). Those that require heterodimerization with RXR are peroxisome proliferator-activated receptors (PPARs), liver X receptor (LXR), farnesoid X receptor (FXR), pregnane X receptor (PXR), and constitutive androstane receptor (CAR). The dimeric receptors bind two six-nucleotide "half-sites" in tandem, the sequence and spacing depending on the dimer. Some of the receptors in the absence of agonist exist in association with corepressors to keep the DNA tightly packed and otherwise inhibit transcription (Figure 3–23B). Agonists for the heterodimers promote exchange of bound corepressors for coactivators. Some RXR heterodimers are *permissive*, that is, they can be activated by ligands for either RXR or the partner nuclear receptor; others are *nonpermissive*, being activated only by the ligand for the partner nuclear receptor (Evans and Mangelsdorf, 2014).

An agonist-bound nuclear hormone receptor often activates a large number of genes to carry out a program of cellular differentiation or metabolic regulation. The activity of a receptor in a given cell depends not only on the ligand but also on the ratio of coactivators and corepressors recruited to the complex. *Selective estrogen receptor modulators* (SERMs) such as *tamoxifen* and *raloxifene* (Chapter 48) are thought to recruit either coactivators or corepressors depending on the cell.

The means by which agonists for nuclear receptors, particularly those for glucocorticoids, exert anti-inflammatory actions involves considerable complexity beyond the traditional gene activation paradigm (Hardy et al., 2020). Featuring prominently are mechanisms of *transrepression* that undercut the actions of NF-κB and AP-1. Transrepression can include competition between dimeric nuclear receptors and NF-κB and AP-1 for coactivators, enhanced expression of proteins by the dimeric receptors that interfere with the activation of such transcription factors, and binding of the dimeric receptors to *negative response elements* (i.e., inhibiting in some fashion gene expression directly).

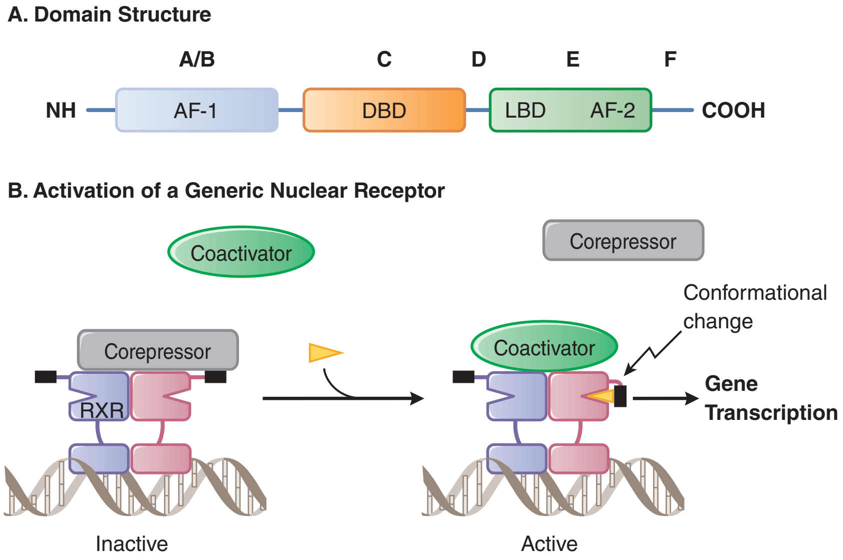
TABLE 3–3 ■ NUCLEAR HORMONE RECEPTORS[a]

FAMILY[b]	RECEPTORS	PHYSIOLOGICAL LIGANDS	EXAMPLES OF THERAPEUTIC AGENTS[c]
1A	Thyroid hormone receptors: THR-α and THR-β	Thyroxine (T_4) and thyroid hormone	Levothyroxine, liothyronine
1B	Retinoic acid receptors: $RAR_α$, $RAR_β$, and $RAR_γ$	Vitamin A–derived tretinoin and alitretinoin	Tretinoin, tazarotene, adapalene ($RAR_{β, γ}$), alitretinoin, etretinate, acitretin
1C	Peroxisome proliferator-activated receptors: $PPAR_α$, $PPAR_{β/δ}$, and $PPAR_γ$	Leukotriene B ($PPAR_α$), 15-Deoxy-$Δ^{12,14}$-PGJ_2 ($PPAR_γ$), many fatty acids and eicosanoids, among others	Fibrates ($PPAR_α$), thiazolidinediones ($PPAR_γ$)
1H	Liver X receptor-like receptors: liver X receptor (LXR) and farnesoid X receptor (FXR)	Hydroxycholesterols (LXR), bile acids (FXR)	Obeticholic acid (FXR)
1I	Vitamin D receptors (VDR), pregnane X receptor (PXR), and constitutive androstane receptor (CAR)	Vitamin D (VDR), 17β-estradiol (PXR), and a wide range of exogenous compounds (PXR and CAR)	Calcitriol (VDR), calcipotriene (VDR) Note: PXR and CAR are activated by a wide variety of drugs in the context of inducing CYPs, phase 2 enzymes, and drug transporters.
2B	Retinoid X receptors: $RXR_α$, $RXR_β$, and $RXR_γ$	Vitamin A–derived alitretinoin	Bexarotene, alitretinoin
3A	Estrogen receptors: $ER_α$ and $ER_β$	Estriol (ERα), estrone (ERα)	Estrogens; selective estrogen receptor modulators (SERMs) tamoxifen, raloxifene, and toremifene
3C	3-Ketosteroid receptors: androgen receptor (AR), glucocorticoid receptor (GR), mineralocorticoid receptor (MR), and progesterone receptor (PR)	Dihydrotestosterone and testosterone (AR), cortisol and corticosterone (GR), aldosterone (MR), progesterone (PR)	Testosterone esters (AR), and the antagonists flutamide, bicalutamide, nilutamide, and enzalutamide (AR); hydrocortisone, prednisone, and dexamethasone (GR); the antagonists spironolactone and eplerenone (MR); progesterone, progesterone esters, and 19-nor steroids (PR)

[a] Listed are receptors known to be activated by physiological ligands, with the exception of CAR, which is included by virtue of its importance (together with that of PXR) to the handling of xenobiotics.

[b] The nomenclature regarding families and physiological ligands is taken from Alexander et al., 2019.

[c] Listed are examples of agents whose therapeutic actions are mediated by one or more members of the noted family, unless otherwise specified. If specific for a specific member of the family, this is noted parenthetically.


A. Domain Structure

B. Activation of a Generic Nuclear Receptor

Figure 3–23 *Structure and activation of a nuclear hormone receptor.* **A.** The domain structure of a nuclear hormone receptor (after Moore et al., 2006). The functions of domains A through F are described in the text. **B.** A generic nuclear hormone receptor is shown as a dimer with an RXR. When an agonist (yellow triangle) and coactivator bind, a conformational change occurs in helix 12 (black bar), and gene transcription is stimulated.

Diseases Resulting From Receptor and Pathway Dysfunction

Alteration in receptors and their downstream signaling pathways can be the cause of disease. The loss of a receptor in a highly specialized signaling system may cause a phenotypic disorder (e.g., deficiency of the androgen receptor and testicular feminization syndrome; see Chapter 49). Deficiencies in widely employed signaling pathways have broad effects, as are seen in myasthenia gravis (due to autoimmune disruption of nicotinic cholinergic receptor function; Chapter 13) and in some forms of insulin-resistant diabetes mellitus (as a result of autoimmune depletion of insulin-producing cells and interference with insulin receptor function; Chapter 51). Mutations in GPCRs cause a variety of monogenic diseases (Schöneberg and Liebscher, 2021). The same is true for mutations in TRP channels (Moran, 2018). Many forms of cancer are now known to arise from mutations that result in constitutive activity of growth factor receptors and downstream signaling enzymes in the Ras-MAPK pathway, or loss of tumor suppressors and other proteins that regulate cell proliferation (see Chapter 72).

Common polymorphisms in receptors and proteins downstream of the receptor can also lead to variability in therapeutic responses in patient populations from different geographic and ethnic origins (Johnson, 2019).

Intrinsic Pathways Regulated by Nutrients, Energy, and Cell Damage

In addition to cell-extrinsic regulation of cell growth mediated by growth factors and cytokines, cell-intrinsic pathways that arose during the early evolution of eukaryotes regulate cell growth and survival by sensing the availability of nutrients and cellular energy status. These ancient nutrient-sensing pathways consist of the *AMP-activated protein kinase* (AMPK) pathway and the *target of rapamycin* (TOR) pathway, which work in opposition to control cell growth and the process of *autophagy* (González et al., 2020). Autophagy is an intracellular degradation pathway that is important for cell survival during conditions of stress or cell damage. Programmed cell death (*apoptosis*) is also dually regulated by extrinsic factors, including TNF-α, Fas ligand, and TNF-related apoptosis-inducing ligand (TRAIL), and by intrinsic pathways that sense cell damage. Organ development and renewal require a balance between cell population growth and survival versus cell death and removal, and both autophagy and apoptosis play important roles in normal tissue and cell function. However, aberrant autophagy or apoptosis pathways play roles in many disease processes, and pharmacological perturbation of these pathways may be important therapeutically in such diseases, including neurodegenerative diseases, and drug- and radiation-resistant cancers.

AMPK and TOR Pathways

AMPK is a serine/threonine protein kinase that occurs as a heterotrimeric complex consisting of a catalytic α subunit and regulatory β and γ subunits. Canonical activation of AMPK occurs in the cytoplasm and is triggered by energy stress (cellular increases in AMP:ATP or ADP:ATP ratios) mediated by binding of AMP or ADP to allosteric sites on the AMPK γ-subunit (Figure 3–24). AMP/ADP binding to these sites is antagonized by ATP. Canonical activation of AMPK also requires phosphorylation by LKB1 (liver kinase B1) on Thr172 in the activation loop of the kinase domain of AMPK.

Noncanonical AMPK activation results from glucose deprivation, release of Ca^{2+} from the ER, or increased Ca^{2+} in the nucleus. The activation of AMPK in response to glucose starvation (the *lysosomal pathway*) occurs independently of changes in adenine nucleotide ratios and is mediated by aldolase, the glycolytic enzyme that binds fructose 1,6-bisphosphate (FBP) and converts it to triose phosphates. Glucose starvation leads to FBP depletion, which alters aldolase's interactions with the v-ATPase complex on the surface of the lysosome, promoting interactions of LKB1 with the lysosome that lead to phosphorylation and activation of lysosome-associated AMPK. Noncanonical Ca^{2+} activation of AMPK in the cytoplasm and nucleus is mediated by CaMKK2 (Ca^{2+}/calmodulin-dependent kinase kinase-2) in response to IP_3-dependent release of Ca^{2+} from the ER (cytoplasmic activation) or increases in nuclear Ca^{2+} caused by DNA damage (activation in the nucleus).

Activated AMPK phosphorylates more than 60 downstream targets that activate catabolic pathways and inactivate anabolic (biosynthetic) pathways, with acute overall effects of increasing ATP synthesis, reducing ATP consumption, and inhibiting cell growth (González et al., 2020). An important target for AMPK is the *mechanistic or mammalian target of rapamycin complex 1* (mTORC1), which is inactivated by AMPK phosphorylation.

TOR is a serine/threonine protein kinase that was first identified in yeast mutants that were resistant to the growth-inhibiting effects of *rapamycin*. Rapamycin (*sirolimus*) and its several structurally and mechanistically related compounds (*everolimus, temsirolimus*) are described in more detail in Chapter 39. In humans, there are two structurally and functionally different forms of mTOR, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2); only mTORC1 is inhibited by rapamycin. mTORC1 is regulated intrinsically by amino acids that cause translocation of mTORC1 from the cytosol to lysosomes and extrinsically by growth factors that activate the small G protein RHEB (Ras homolog enriched in brain) on the surface of lysosomes. Figure 3–24 summarizes some of the details of the regulation of mTORC1 and AMPK and the anabolic and catabolic processes of the cell.

Autophagy

Autophagy is a highly conserved, tightly regulated, multistep catabolic pathway in which cellular contents (including aggregation-prone proteins, organelles such as mitochondria and peroxisomes, and infectious agents) are sequestered within double-membrane vesicles known as *autophagosomes* and then delivered to lysosomes, where fusion occurs and autophagosome contents are degraded by lysosomal proteases (Bento et al., 2016). The functions of autophagy are to remove cell contents that are damaged and to provide cells with substrates for energy and biosynthesis under conditions of stress and starvation. Autophagy plays an important protective role in a number of diseases, including neurodegenerative diseases (e.g., Alzheimer's, Parkinson's, and Huntington's diseases) caused by aggregation-prone proteins and certain infectious diseases (*Salmonella typhi* and *Mycobacterium tuberculosis*). Autophagy-related genes may also play a role in tumor suppression, and decreased autophagic capacity is correlated with poor prognosis in brain tumors. However, in breast, ovarian, and prostate cancers, autophagy can function as a tumor promoter and may enhance the survival of metastatic cells at sites where nutrients are limited.

Autophagy is directly controlled by autophagy-related genes (known as *ATG*s, AuTophaGy genes). More than 30 *ATG*s have been identified in eukaryotes, and the ATG proteins function at various steps in autophagy, including induction of cargo packaging, vesicle formation, vesicle fusion with lysosomes, and degradation of vesicular contents. Autophagy is primarily regulated at the cellular level by stress-mediated and growth factor signaling pathways that integrate signaling output via mTORC1 and AMPK as described above (see Figure 3–24). Activated mTORC1 inhibits autophagy; AMPK can promote autophagy.

Apoptosis

Apoptosis is a highly regulated program of biochemical reactions that leads to cell rounding, shrinkage of the cytoplasm, condensation of the nucleus and its contents, and changes in the cell membrane that eventually lead to presentation of phosphatidylserine on the outer surface of the cell. Phosphatidylserine is recognized as a sign of apoptosis by macrophages, which engulf and phagocytize the dying cell. During this process, the membrane of the apoptotic cell remains intact, and the cell does not release its cytoplasm or nuclear material. Thus, unlike necrotic cell death, the apoptotic process does not initiate an inflammatory response. Alterations in apoptotic pathways are implicated in cancer, neurodegenerative diseases, and autoimmune diseases. Thus, maintaining or restoring normal apoptotic

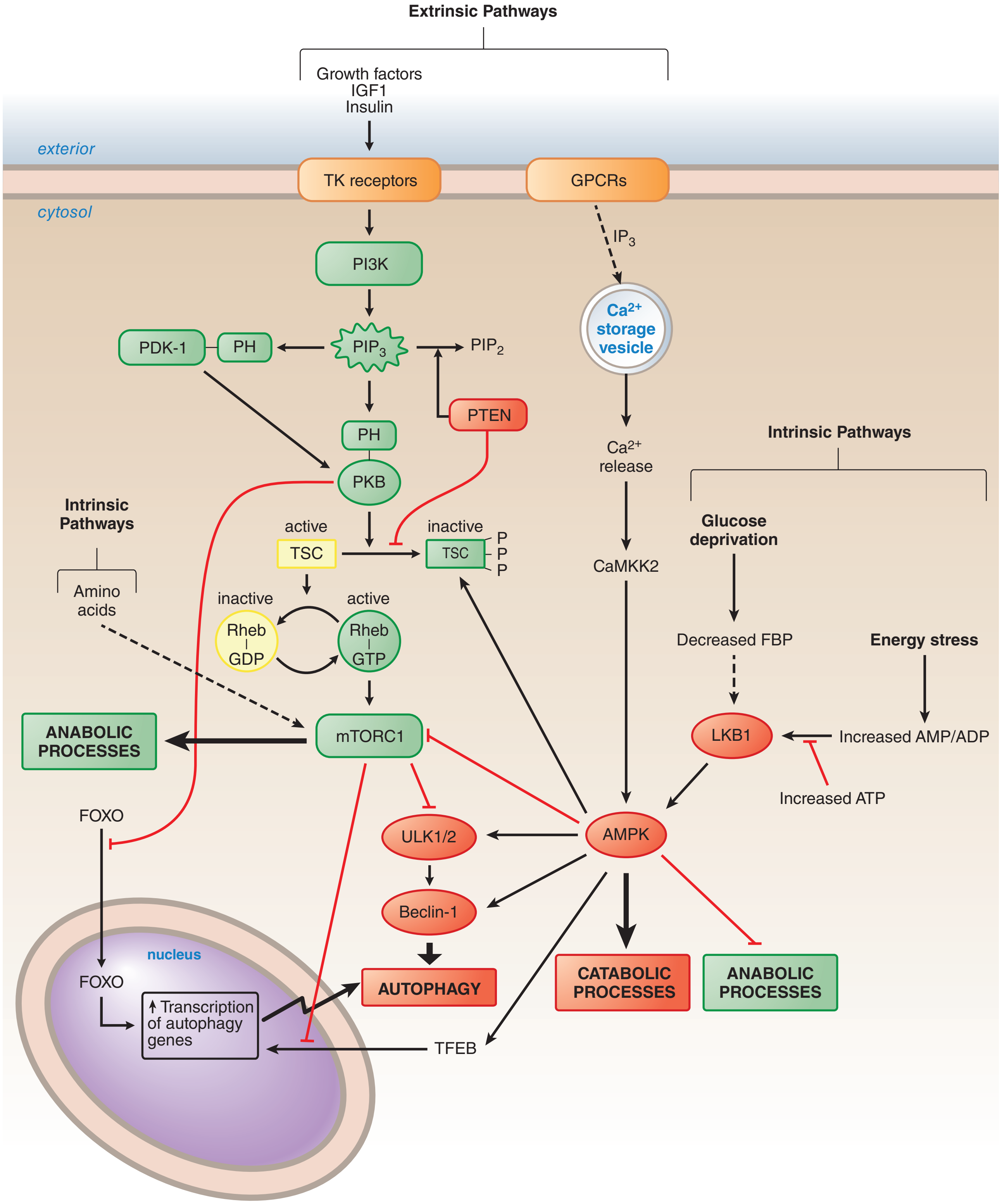

Extrinsic Pathways
Growth factors
IGF1
Insulin
exterior
TK receptors
GPCRs
cytosol
PI3K
IP_3
Ca^{2+} storage vesicle
PDK-1
PH
PIP_3
PIP_2
PTEN
PH
PKB
Ca^{2+} release
Intrinsic Pathways
Intrinsic Pathways
active
TSC
inactive
TSC
P
P
P
Glucose deprivation
Amino acids
CaMKK2
inactive
active
Rheb
GDP
Rheb
GTP
Decreased FBP
Energy stress
ANABOLIC PROCESSES
mTORC1
LKB1
Increased AMP/ADP
Increased ATP
FOXO
ULK1/2
AMPK
Beclin-1
nucleus
FOXO
AUTOPHAGY
CATABOLIC PROCESSES
ANABOLIC PROCESSES
↑ Transcription of autophagy genes
TFEB

Figure 3–24 *Pathways regulating cell proliferation, cell survival, and autophagy.* The primary regulators of cell proliferation and growth (*anabolic processes*) and opposing cell survival (*catabolic processes*) and autophagy are growth factor signaling (*extrinsic pathways*) and *intrinsic pathways* regulated by amino acids, glucose, and cellular stress. Growth factor signaling pathways that lead to activation of mTORC1 (green boxes) promote anabolic processes and inhibit autophagy (red boxes), whereas cellular stress caused by nutrient starvation enhance catabolic processes and autophagy through activation of AMPK (red boxes). These pathways interact not only with one another but also with other pathways including apoptosis pathways, as described in the text. See Figure 39–2 for the effect of mTOR inhibitors as immunosuppressants. Mammalian FOXOs (FOXO 1, 3, 4, and 6) are a subclass of Forkhead transcription factors that function mainly as transcriptional activators, with effects on myriad cell functions including apoptosis and drug resistance; insulin and growth factor signaling can inhibit FOXO activity. Additional signaling pathways not depicted here regulate transcription factor EB (TFEB).

AL GRAWANY

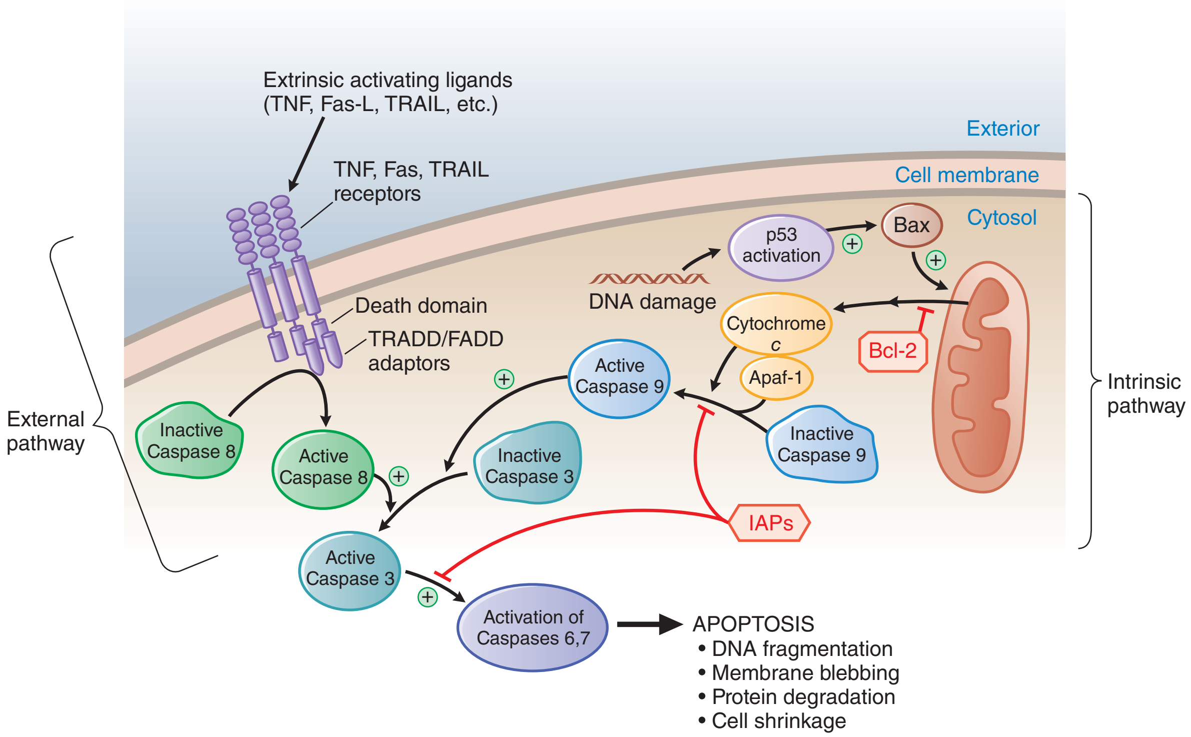


Figure 3–25 *Two pathways leading to apoptosis.* Apoptosis can be initiated by external ligands such as TNF, Fas ligand, or TRAIL, binding to specific transmembrane receptors (left half of figure: the *external pathway*). Activation leads to trimerization of the receptor, and binding of adaptor molecules such as TRADD, to the intracellular death domain. The adaptors recruit caspase 8 and activate it, leading to cleavage and activation of the effector caspase, caspase 3, which activates the caspase pathway leading to apoptosis. Apoptosis can also be initiated by an *intrinsic pathway* regulated by Bcl-2 family members such as Bax and Bcl-2. Bax is activated by DNA damage or malformed proteins via p53 (right half of figure). Activation of this pathway leads to release of cytochrome *c* from the mitochondria and formation of a complex with Apaf-1 and caspase 9. Caspase 9 is activated in the complex and initiates apoptosis through activation of caspase 3. Either the external or the intrinsic pathway can overwhelm the inhibitors of apoptosis proteins (IAPs), which otherwise keep apoptosis in check.

pathways is the goal of major drug development efforts to treat diseases that involve dysregulated apoptotic pathways. Resistance to many cancer chemotherapies is associated with reduced function of apoptotic pathways.

Two major signaling pathways induce apoptosis. Apoptosis can be initiated by external signals that have features in common with those used by ligands such as TNF-α or by an internal pathway activated by DNA damage, improperly folded proteins, or withdrawal of cell survival factors (Figure 3–25). The apoptotic program is carried out by a large family of cysteine proteases termed *caspases*. The caspases are highly specific cytoplasmic proteases that are inactive in normal cells but become activated by apoptotic signals.

The external or extrinsic apoptosis signaling pathway, also known as the death receptor pathway, can be activated by ligands such as TNF, Fas ligand, or TRAIL. The receptors for TNF (TNF receptor), Fas ligand (Fas or Apo-1), and TRAIL (TRAIL receptor) are transmembrane receptors with no enzymatic activity, similar to the organization of the TNF receptor described previously. On binding TNF, Fas ligand, or TRAIL, the cognizant receptors form a homotrimer, undergo a conformational change, and recruit adaptor proteins to the receptor's death domain. The adaptor proteins then recruit RIP1 (receptor-interacting protein 1) and caspase 8 to form a complex that results in the activation of caspase 8. Activation of caspase 8 leads to the activation of caspase 3, which initiates the apoptotic program. The final steps of apoptosis are carried out by caspases 6 and 7, leading to degradation of enzymes, structural proteins, and DNA fragmentation characteristic of cell death (see Figure 3–25).

The internal apoptosis pathway can be activated by signals such as DNA damage, leading to increased transcription of the *p53* gene, and involves damage to the mitochondria by proapoptotic members of the Bcl-2 family of proteins. This family includes proapoptotic members such as Bax, Bak, and Bad, which induce damage at the mitochondrial membrane. There are also antiapoptotic Bcl-2 members, such as Bcl-2, Bcl-X, and Bcl-W, which serve to inhibit mitochondrial damage and are negative regulators of the system. When DNA damage occurs, p53 transcription is activated and the cell is held at a cell cycle checkpoint until the damage is repaired. If the damage cannot be repaired, apoptosis is initiated through the proapoptotic Bcl-2 members, such as Bax. Bax is activated, translocates to mitochondria, overcomes the antiapoptotic proteins, and induces the release of cytochrome *c* and a protein termed SMAC (second mitochondria-derived activator of caspase). SMAC binds to and inactivates the inhibitor of apoptosis proteins (IAPs) that normally prevent caspase activation. Cytochrome *c* combines in the cytosol with another protein, Apaf-1 (apoptotic activating protease factor 1), and with caspase 9. This complex leads to activation of caspase 9 and ultimately to the activation of caspase 3. Once activated, caspase 3 activates the same downstream pathways as the external pathway described previously, leading to the cleavage of proteins, cytoskeletal elements, and DNA repair proteins, with subsequent DNA condensation and membrane blebbing that eventually lead to cell death and engulfment by macrophages.

Physiological Systems Must Integrate Multiple Signals

Consider the vascular wall of an arteriole (Figure 3–26). Several types of cells interact at this site, including vascular smooth muscle cells (VSMCs), endothelial cells, platelets, and postganglionic sympathetic neurons. The contractility of VSMCs in the arteriole is the focal point for local and systemic forms of regulation related to vascular resistance. Local regulation by substances such as adenosine, CO_2, and lactic acid can decrease VSMC contractility and hence promote local blood flow. Systemic regulation of blood pressure homeostasis occurs via endocrine, paracrine, and neuronal signaling.

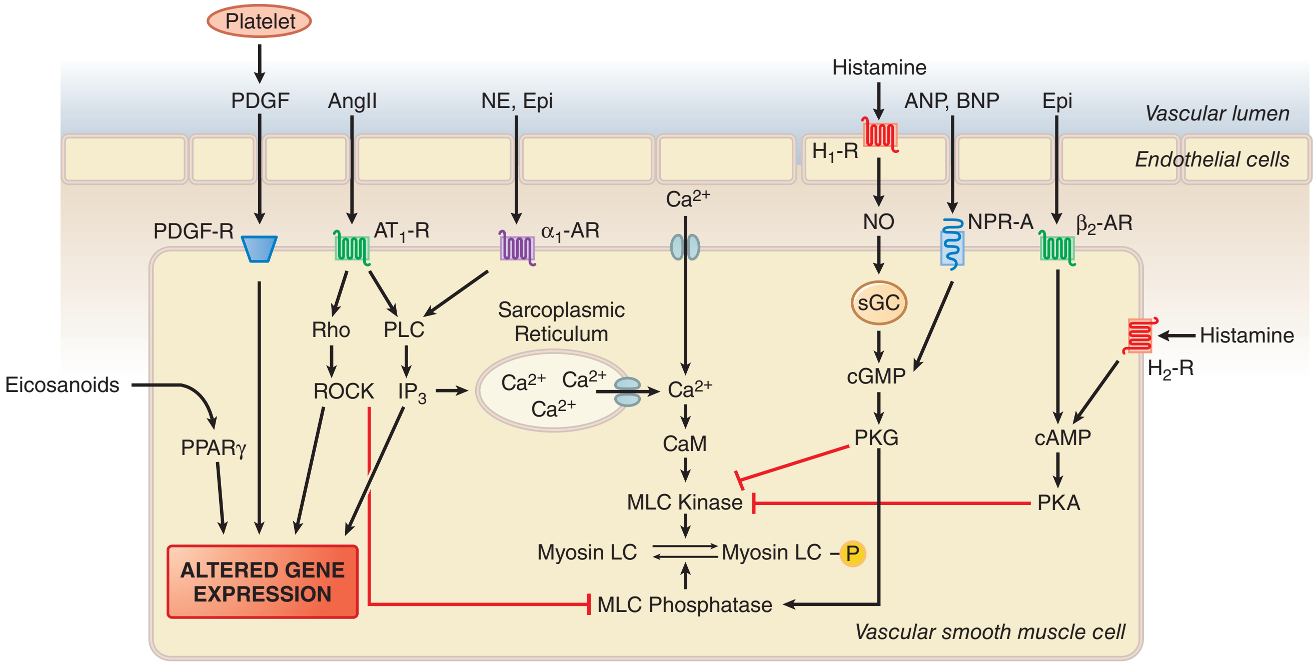


Figure 3–26 *Interaction of multiple signaling systems regulating VSMCs.* It is essential to know that the contractile activity of VSMCs is based on the degree to which myosin, and specifically the light chain of myosin (MLC), is phosphorylated. It is myosin with a phosphorylated light chain that is able to interact with actin filaments to generate force. Note that the degree of phosphorylation is a balance between MLC kinase, which phosphorylates the MLC, and MLC phosphatase, which dephosphorylates MLC. Depicted is a composite VSMC; the relaxation in response to epinephrine is best fit to VSMCs in the skeletal muscle vasculature. See text for a detailed explanation of signaling.

Activation of the sympathetic nervous system regulates VSMC tone in part through release of norepinephrine (NE) from postganglionic sympathetic neurons. NE binds α_1 adrenergic receptors on VSMCs, which activates the G_q-PLC-IP_3-Ca^{2+} pathway. Ca^{2+} binds and activates CaM, which in turn activates MLCK. MLCK phosphorylates light chains of myosin, leading to an increase in VSMC contractile activity. Epinephrine released from the adrenal medulla activates the same pathway in the vasculature of most tissues; however, for the vasculature of skeletal muscle, epinephrine binds β_2 adrenergic receptors and thus engages the G_s-AC-cyclic AMP pathway, as does histamine via the H_2 receptor. The increase in cyclic AMP inhibits MLCK through PKA-mediated phosphorylation and therefore causes a *decrease* in VSMC contraction. The differential engagement of α_1 versus β_2 adrenergic receptors by epinephrine is the basis for the shunt of blood flow from viscera and skin to skeletal muscle in the fight-or-flight response.

Activation of the renin-angiotensin-aldosterone system, which occurs in response to a drop in blood pressure and/or loss in fluid volume, produces angiotensin II (AngII). AngII binding to the AT_1 receptor in VSMCs mobilizes stored Ca^{2+} via the G_q-PLC-IP_3-Ca^{2+} pathway and therefore increases VSMC contractility in part through the same means as NE. But AngII-activated AT_1 receptor also engages $G_{12/13}$ and as a consequence activates the RhoA monomeric G protein. RhoA activates ROCK, which phosphorylates and inhibits myosin light chain phosphatase. The increase in phosphorylated myosin light chain, and hence contractile activity, is thus the product of two pathways. As noted, the activation of RhoA through $G_{12/13}$ and events engaged by G_q and arrestin are also implicated in the growth and proliferation of vascular smooth muscle cells.

The contraction of VSMCs is opposed by mediators that promote relaxation, including NO, atrial natriuretic peptides, and, in the skeletal muscle vasculature, epinephrine. NO is formed in endothelial cells by eNOS when the G_q-PLC-IP_3-Ca^{2+} pathway is activated, for example by histamine via H_1 histaminergic receptors, and by iNOS when that isoform is induced (e.g., by proinflammatory cytokines). The NO formed in the endothelium diffuses into VSMCs and activates sGC, which catalyzes the formation of cyclic GMP and leads to activation of PKG and phosphorylation of both MLCK and myosin light chain phosphatase to inhibit and stimulate, respectively, their activities and to promote relaxation. PKG also inhibits IP_3-mediated Ca^{2+} release from intracellular stores and activates a K^+ channel to inhibit, through hyperpolarization, the activity of the L-type Ca^{2+} channel, thereby reducing Ca^{2+} influx (not shown). Intracellular concentrations of cyclic GMP are also increased by activation of transmembrane natriuretic peptide receptors by ANP and BNP, which are released from cardiac atrial cells and cardiomyocytes, respectively, in response to volume overload.

As a consequence of the variety of pathways that affect arteriolar tone, a patient with hypertension may be treated with one or several drugs that alter signaling through these pathways. Drugs commonly used to treat hypertension include β_1 adrenergic receptor antagonists to reduce secretion of renin, the first step in AngII synthesis; a direct inhibitor of renin (*aliskiren*); ACE inhibitors (e.g., *enalapril*) to reduce conversion of AngI to AngII; angiotensin subtype 1 receptor (AT_1R) blockers (e.g., *losartan*) to block AngII binding to AT_1Rs on VSMCs; α_1 adrenergic blockers to block NE binding to VSMCs; *sodium nitroprusside* to increase quantities of NO produced; and Ca^{2+} channel blockers (e.g., *nifedipine*) to block Ca^{2+} entry into VSMCs. β_1 Adrenergic receptor antagonists would also block the baroreceptor reflex-mediated increase in heart rate and blood pressure that would otherwise attempt to counter the drop in blood pressure induced by the therapy. ACE inhibitors also inhibit the degradation of a vasodilating peptide bradykinin (see Chapter 43). Thus, the choices and mechanisms are complex, and the appropriate therapy in a given patient depends on many considerations, including the diagnosed causes of hypertension in the patient, possible side effects of the drug, efficacy in a given patient, and cost.

Signaling Pathways and Drug Action

Throughout this text, cellular signaling pathways figure prominently in explaining the actions of therapeutic agents. Not all pathways have been mentioned or fully explored in this chapter. To aid readers in finding more information on signaling and important sites of action, Table 3–4 lists relevant figures that appear *in other chapters*.

TABLE 3–4 ■ SUMMARY: IMPORTANT SITES OF DRUG ACTION

RECEPTOR/PATHWAY	FIGURE TITLE	FIGURE NUMBER
Drug transport proteins	Major mechanisms by which transporters mediate adverse drug responses	**Figure 4–3**
CYPs, drug metabolism	Location of CYPs in the cell	**Figure 5–2**
Nuclear receptors	Induction of drug metabolism by nuclear receptor–mediated signal transduction	**Figure 5–14**
GI Microbiome	Pathway of an orally administered drug	**Figure 6–1**
CYP variant	Effects of Splice Variant in CYP3A5 on PK	**Figure 7–3**
General neurotransmission	Steps involved in excitatory and inhibitory neurotransmission	**Figure 10–3**
Exocytosis	Molecular basis of exocytosis: docking and fusion of synaptic vesicles with neuronal membranes	**Figure 10–4**
Cholinergic neurotransmission	A typical cholinergic neuroeffector junction	**Figure 10–6**
Adrenergic neurotransmission	A typical adrenergic neuroeffector junction	**Figure 10–8**
AChE and its inhibition	Steps involved in the hydrolysis of ACh by AChE and in the inhibition and reactivation of the enzyme	**Figure 12–2**
Transmission at the NMJ	A pharmacologist's view of the motor end plate	**Figure 13–4**
α and β effects/CV system	Comparative effects of intravenous infusion of NE, EPI, and INE.	**Figure 14–2**
β Blockers and vasodilation	Mechanisms underlying actions of vasodilating β blockers in blood vessels	**Figure 14–4**
Serotonergic neurotransmission	A serotonergic synapse	**Figure 15–4**
Dopaminergic neurotransmission	A dopaminergic synapse	**Figure 15–9**
Voltage-sensitive cation channels	Voltage-sensitive Na$^+$, Ca^{2+}, and K$^+$ channels	**Figure 16–2**
Neurotransmission	Transmitter release, action, and inactivation	**Figure 16–4**
Ligand-gated ion channels	Pentameric ligand-gated ion channels	**Figure 16–6**
NMDA receptor	Pharmacologic binding sites on the NMDA receptor	**Figure 16–9**
GABA$_A$ receptor	Pharmacologic binding sites on the GABA$_A$ receptor	**Figure 16–11**
Histamine signaling	Signal transduction pathways for histamine receptors	**Figure 16–12**
Actions of antidepressants	Sites of action of antidepressants at noradrenergic and serotonergic nerve terminals	**Figure 18–1**
Ketamine action	Proposed mechanisms for the antidepressant effects of ketamine.	**Figure 18–2**
Na$^+$ channel	Antiseizure drug–enhanced Na$^+$ channel inactivation	**Figure 20–2**
GABA$_A$ receptor/channel	Some antiseizure drugs enhance GABA synaptic transmission	**Figure 20–3**
T-type Ca^{2+} channel	Antiseizure drug–induced reduction of current through T-type Ca^{2+} channels	**Figure 20–4**
Opioid signaling	Simplified scheme of opioid receptor signaling	**Figure 23–2**
Cation signaling	Structure and function of voltage-gated Na$^+$ channels	**Figure 25–2**
Local anesthetic action on Na$^+$ channels	A structural view of the interaction of a local anesthetic with a voltage-gated Na$^+$ channel	**Figure 25–3**
Cannabinoids	Cannabinoid synthesis and signaling	**Figures 26–2, 26–3, 26–4**
Diuretics	Sites and mechanisms of action of diuretics	**Figure 29–5**
Aldosterone signaling	Diuretic mechanism of aldosterone antagonists	**Figure 29–6**
ANP signaling	Inter medullary collecting duct Na$^+$ transport and its regulation	**Figure 29–7**
V$_1$ receptor signaling	Mechanism of V$_1$ receptor-effector coupling	**Figure 29–11**
V$_2$ receptor signaling	Mechanism of V$_2$ receptor-effector coupling	**Figure 29–12**
Signals regulating renin release	Mechanisms by which the macula densa regulates renin release	**Figure 30–3**
Renin-Angiotensin System	Schematic diagram of opposing arms in the RAS	**Figure 30–4**
Signals regulating blood pressure	Principles of blood pressure regulation and its modification by drugs	**Figure 32–3**
E-C coupling	Cardiac excitation-contraction coupling and positive inotropic drugs	**Figure 33–6**
NO/cGMP signaling in PAH	Stimulators of NO/cGMP signaling	**Figure 35–3**
cAMP signaling in PAH	Membrane receptor agonists that increase cAMP	**Figure 35–4**
PLC signaling in PAH	Membrane receptor antagonists that inhibit activation of phospholipase C	**Figure 35–5**

(Continued)

TABLE 3–4 ■ SUMMARY: IMPORTANT SITES OF DRUG ACTION (*CONTINUED*)

RECEPTOR/PATHWAY	FIGURE TITLE	FIGURE NUMBER
Endothelium–smooth muscle signaling	Interactions between endothelium and vascular smooth muscle in PAH	**Figure 35–7**
Aggregatory signaling	Platelet adhesion and aggregation	**Figure 36–1**
Coagulatory signaling	Major reactions of blood coagulation	**Figure 36–2**
Fibrinolytic signaling	Fibrinolysis	**Figure 36–3**
Blood clotting and its prevention	Sites of action of antiplatelet drugs	**Figure 36–7**
LDLR and endocytosis	Regulation of LDL-receptor life cycle	**Figure 37–5**
T cell activation	T cell activation: costimulation and coinhibitory checkpoints	**Figure 38–3**
T cell receptor (TCR) ligands	TCR signaling and its modulation by co-receptors and antibodies	**Figure 38–4**
Leukocyte inflammatory response	The leukocyte inflammatory response	**Figure 38–6**
T cell signaling	T cell activation and sites of action of immunosuppressive agents	**Figure 39–2**
Sphingosine-1-phosphate signaling	S1P formation and action at S1P$_1$R; fingolimod and its active metabolite.	**Figure 39–3**
Prostanoid receptors	Prostanoid receptors and their primary signaling pathways	**Figure 41–1**
Eicosanoid signaling	Human Eicosanoid Receptors	**Table 41–4**
Probenecid action; urate transport	A pharmacologist's view of renal urate transport and its inhibition by drugs.	**Figure 42–2**
Inflammatory signaling; asthma	Mechanism of anti-inflammatory action of corticosteroids in asthma	**Figure 44–7**
Growth hormone receptor (GHR)	Mechanisms of GH and PRL action and of GHR antagonism	**Figure 46–5**
Oxytocin receptor signaling	Sites of action of oxytocin and tocolytic drugs in the uterine myometrium	**Figure 46–8**
Estrogen receptor signaling	Molecular mechanism of action of nuclear ER	**Figure 48–4**
Soluble guanylyl cyclase and PDE5	Mechanism of action of PDE5 inhibitors in the corpus cavernosum	**Figure 49–6**
Glucocorticoid receptor (GR)	Mechanisms of transcriptional regulation by GR.	**Figure 50–8**
Insulin secretion	Regulation of insulin secretion from a pancreatic β cell	**Figure 51–3**
Insulin receptor	Pathways of insulin signaling	**Figure 51–4**
FGF receptor	FGF23-FGFR-Klotho complex	**Figure 52–4**
H$_2$ and gastrin receptors; gastric secretion	Pharmacologist's view of gastric secretion and its regulation: the basis for therapy of acid-peptic disorders	**Figure 53–1**
EP$_2$ and EP$_4$ receptors; GI ion transporters; cAMP, cGMP	Mechanism of action of drugs that alter intestinal epithelial secretion and absorption	**Figure 54–4**
Emetic signaling	Pharmacologist's view of emetic stimuli	**Figure 54–6**
EGF receptor	Targeting the EGFR in cancer	**Figure 71–1**
Growth factor receptors	Cancer cell signaling pathway and drug targets	**Figure 71–2**
B cell kinases	Inhibitors of signaling in B cells	**Figure 71–4**
mTOR, AMPK, growth factor signaling	Caveat mTOR: effect of rapamycin on growth factor signaling	**Figure 71–5**
Hypoxia-inducible genes.	Oxygen-sensing and hypoxia-inducible genes targeted	**Figure 71–6**
Apoptotic signaling	BH3 mimetics enhance apoptosis	**Figure 71–10**
IgG1 structure	IgG1 elements relevant for their use in cancer treatment.	**Figure 72–1**
EGF receptor	Antibody targeting and domains of EGFR (HER1).	**Figure 72–2**
T cell/APC signaling	Targeting of immune checkpoints	**Figure 72–3**
IL-2 receptor	A pharmacologist's view of IL-2 receptors, signaling pathways, and inhibitors	**Figure 72–4**
CAR T cells	Chimeric antigen receptor (CAR) T-cell activation by a target antigen on cancer cells.	**Figure 72–6**
Rhodopsin	Pharmacologist's view of photoreceptor signaling	**Figure 74–9**

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Chapter 4

Membrane Transporters and Drug Response

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TRANSPORTERS IN REGULATORY SCIENCES

Membrane transport proteins are present in all organisms. These proteins control the influx of essential nutrients and ions and the efflux of cellular waste, environmental toxins, drugs, and other xenobiotics (Figure 4–1). Consistent with their critical roles in cellular homeostasis, about 2000 genes in the human genome, or ~7% of the total number of genes, code for transporters or transporter-related proteins. The functions of membrane transporters may be facilitated (equilibrative, not requiring energy) or active (requiring energy). In considering the transport of drugs, pharmacologists generally focus on transporters from two major superfamilies, ABC and SLC transporters (Nigam, 2015).

Most ABC (ATP binding cassette) proteins are primary active transporters, which rely on ATP hydrolysis to actively pump substrates across membranes. Among the best-recognized transporters in the ABC superfamily are Pgp (encoded by *ABCB1*, also termed *MDR1*) and CFTR (encoded by *ABCC7*).

The SLC (solute carrier) superfamily includes genes that encode facilitated transporters and ion-coupled secondary active transporters. Sixty-five SLC families with about 460 transporters have been identified in the human genome (Pizzagalli et al., 2021). Many SLC transporters serve as drug targets or in drug absorption and disposition. Widely recognized SLC transporters include SERT and DAT, both targets for antidepressant medications.

Membrane Transporters in Therapeutic Drug Responses

Pharmacokinetics

Transporters important in pharmacokinetics generally are located in intestinal, renal, and hepatic epithelia, where they function in the selective absorption and elimination of endogenous substances and xenobiotics, including drugs. Transporters work in concert with drug-metabolizing enzymes to eliminate drugs and their metabolites (Figure 4–2). In addition, transporters in various cell types mediate tissue-specific drug distribution (drug targeting). Conversely, transporters also may serve as protective barriers to particular organs and cell types. For example, Pgp in the BBB protects the CNS from a variety of structurally diverse drugs through its efflux mechanisms.

Pharmacodynamics: Transporters as Drug Targets

Membrane transporters are the targets of many clinically used drugs. SERT (*SLC6A4*) is a target for a major class of antidepressant drugs, the SSRIs. Other neurotransmitter reuptake transporters serve as drug targets for the tricyclic antidepressants, various amphetamines (including

Abbreviations

ABC: ATP binding cassette
ABCC: ATP binding cassette family C
ACE: angiotensin-converting enzyme
AUC: area under the concentration-time curve
BBB: blood-brain barrier
BCRP: breast cancer resistance protein
BSEP: bile salt export pump
CAR: constitutive androstane receptor
CFTR: cystic fibrosis transmembrane regulator
$CL_{int,all}$: overall hepatic intrinsic clearance
CL_{met}: metabolic clearance
CPT-11: irinotecan hydrochloride
Cryo-EM: cryo-electron microscopy
CSF: cerebrospinal fluid
DA: dopamine
DAT: dopamine transporter
FDA: U.S. Food and Drug Administration
FXR: farnesoid X receptor
GABA: γ-aminobutyric acid
GAT: GABA reuptake transporter
GI: gastrointestinal
GLUT: glucose transporter
GSH, GSSG: reduced and oxidized glutathione
HCV: hepatitis C virus
HIV: human immunodeficiency virus
HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A
HNF4α: hepatic nuclear factor 4 alpha
5HT: serotonin
α-KG: α-ketoglutarate
LAT: large amino acid transporter
LeuT: leucine transporter
MAO: monoamine oxidase
MATE1: multidrug and toxin extrusion protein 1
MDMA: 3,4-methylenedioxymethamphetamine
MFS: major facilitator superfamily
MRP: multidrug resistance protein
NBDs: nucleotide-binding domains
NE: norepinephrine
NET: NE transporter
NME: new molecular entity
NTCP: Na^+-taurocholate cotransporting polypeptide
OAT1: organic anion transporter 1
OCT1: organic cation transporter 1
OCTN: novel organic cation transporter
OSTα/β: organic solute transporter α/β heterodimer
PAH: *p*-aminohippurate
PBPK: physiologically based pharmacokinetic
PGE_2: prostaglandin E_2
Pgp: P-glycoprotein
PPARα: peroxisome proliferator-activated receptor α
PXR: pregnane X receptor
RAR: retinoic acid receptor
RFC: reduced folate carrier
RXR: retinoid X receptor
SERT: serotonin transporter
SHP1: Src homology region 2 domain-containing phosphatase-1
SLC: solute carrier
SNP: single-nucleotide polymorphism
SSRI: selective serotonin reuptake inhibitor
SXR: steroid X receptor
TMD: transmembrane domain
URAT1: uric acid transporter 1
XOI: xanthine oxidase inhibitor

amphetamine-like drugs used in the treatment of attention-deficit disorder in children), and anticonvulsants.

These transporters also may be involved in the pathogenesis of neuropsychiatric disorders, including Alzheimer's and Parkinson's diseases. An inhibitor of the vesicular monoamine transporter VMAT2 (SLC18A2), *tetrabenazine*, is approved for the symptomatic treatment of Huntington's disease; the antichorea effect of *tetrabenazine* likely relates to its capacity to deplete stores of biogenic amines by inhibiting their uptake into storage vesicles by VMAT2. Transporters that are nonneuronal also may be potential drug targets (e.g., cholesterol transporters in cardiovascular disease, nucleoside transporters in cancers, glucose transporters in metabolic syndromes, and Na^+-Cl^- cotransporters in the SLC12 family in hypertension).

Recently, first-in-class drugs that inhibit Na^+-glucose transporters in the SLC5 family (SGLT1 and SGLT2) have been FDA-approved for the treatment of type 2 diabetes. These drugs, the gliflozins, which include *canagliflozin, dapagliflozin*, and *empagliflozin*, reduce renal reabsorption of glucose, thereby facilitating glucose elimination in the kidney. All three are prescribed as second-line therapy for treatment of inadequately controlled diabetes. *Dapagliflozin* has proven useful in treating declines in kidney function in patients with chronic kidney disease, irrespective of the presence or absence of diabetes (Heerspink et al., 2020). *Dapagliflozin* is also being used in treating heart failure with reduced ejection fraction (see Chapter 33).

In addition, renal transporters for uric acid such as URAT1 (SLC22A12) (Nakata et al., 2020) and GLUT9 (SLC2A9) are being targeted by drugs in clinical trials for the treatment of gout when used with an XOI. These drugs are uricosurics and act by selectively inhibiting uric acid reabsorption in the kidney.

Mutations in the transmembrane gated anion (chloride) channel CFTR (cystic fibrosis transmembrane conduction regulator or ABCC7) reduce function of that protein and cause thickened secretions in the lung and other tissues. Drugs directed toward CFTR can modulate and enhance CFTR function in patients with cystic fibrosis. A first-in-class drug, *ivacaftor*, is used for the treatment of patients who harbor the coding mutation CFTR-p.G551D. *Ivacaftor*, termed a *potentiator*, increases the probability that the mutant chloride channel, CFTR-p.G551D, remains in the open state. Other agents, termed *correctors* (e.g., *tezacaftor, elexacaftor*), enhance trafficking and insertion of mutant CFTR proteins into the plasma membrane. Combinations of potentiators and correctors of CFTR mutants are now in use for cystic fibrosis patients who are homozygous or heterozygous for the common deletion mutation, CFTR-p.F508del (Gramegna et al., 2020).

Drug Resistance

Membrane transporters play critical roles in the development of resistance to anticancer drugs, antiviral agents, and anticonvulsants. *Decreased uptake of drugs*, such as folate antagonists, nucleoside analogues, and platinum complexes, is mediated by reduced expression of influx transporters required for these drugs to access the tumor. *Enhanced efflux of hydrophobic drugs* is one mechanism of antitumor resistance in cellular assays of resistance. The overexpression of MRP4 is associated with resistance to antiviral nucleoside analogues (Aceti et al., 2015). Pgp (MDR1, ABCB1) and BCRP (ABCG2) can be overexpressed in tumor cells after exposure to cytotoxic anticancer agents and are implicated in resistance to these agents, exporting anticancer drugs, reducing their intracellular concentration, and rendering cells resistant to the drugs' cytotoxic effects. Modulation of MDR1 expression and activity to regulate drug resistance could be a useful adjunct in pharmacotherapy (Seelig, 2020).

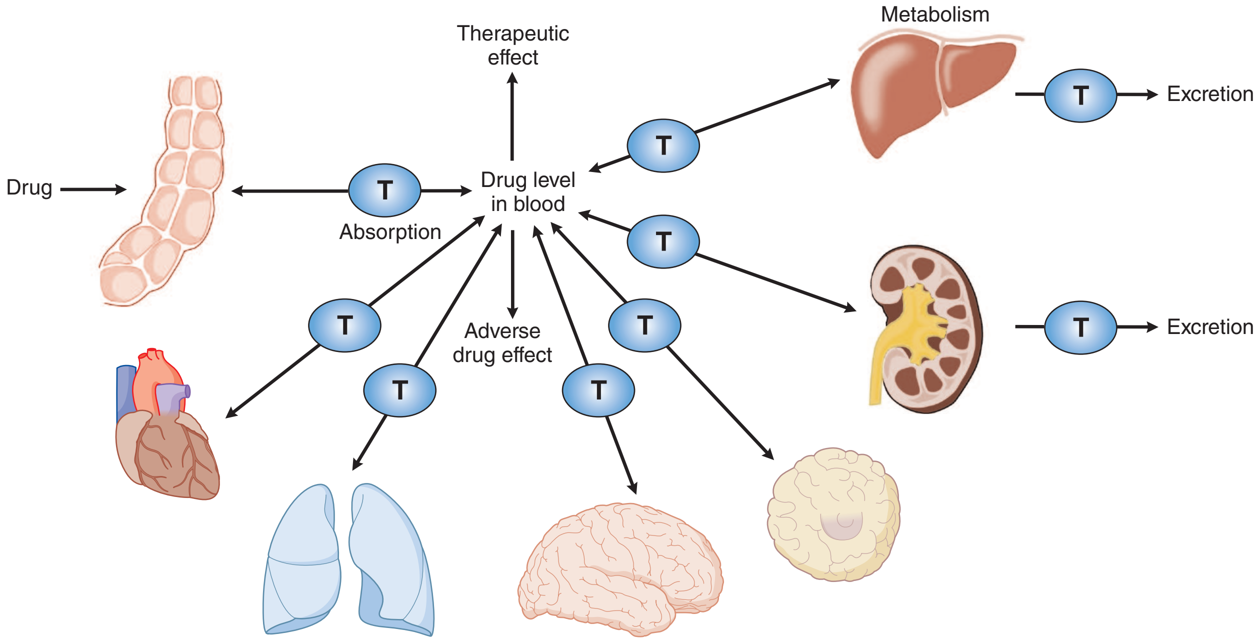

Figure 4–1 *Membrane transporters in pharmacokinetic pathways.* Membrane transporters (T) play roles in pharmacokinetic pathways (drug absorption, distribution, metabolism, and excretion), thereby setting systemic drug levels. Drug levels often drive therapeutic and adverse drug effects.

Membrane Transporters and Adverse Drug Responses

As controllers of import and export, transporters ultimately control the exposure of cells to chemical carcinogens, environmental toxins, and drugs. Thus, transporters play crucial roles in the cellular activities and toxicities of these agents. Transporter-mediated adverse drug responses generally can be classified into three categories (Figure 4–3):

- Decreased uptake or excretion at clearance organs
- Increased uptake or decreased efflux at target organs
- Altered transport of endogenous compounds at target organs

Transporters expressed in the liver and kidney, as well as metabolic enzymes, are key determinants of drug exposure in the systemic circulation, thereby affecting exposure, and hence toxicity, in all organs (Figure 4–3, top panel). For example, after oral administration of an HMG-CoA reductase inhibitor (e.g., *pravastatin*), the efficient first-pass hepatic uptake of the drug by the SLC OATP1B1 maximizes the effects of such drugs on hepatic HMG-CoA reductase. Uptake by OATP1B1 also minimizes the escape of these drugs into the systemic circulation, where they can cause adverse responses, such as skeletal muscle myopathy.

Transporters expressed in tissues that may be targets for drug toxicity (e.g., brain) or in barriers to such tissues (e.g., the BBB) can tightly control local drug concentrations and thus control the exposure of these tissues to the drug (Figure 4–3, middle panel). For example, endothelial cells in the BBB are linked by tight junctions, and some efflux transporters are expressed on the blood-facing (luminal) side, thereby restricting the penetration of compounds into the brain. The interactions of *loperamide* and *quinidine* are good examples of transporter-controlled drug exposure at this site. *Loperamide* is a peripheral opioid used in the treatment of diarrhea and is a substrate of Pgp, which prevents accumulation of

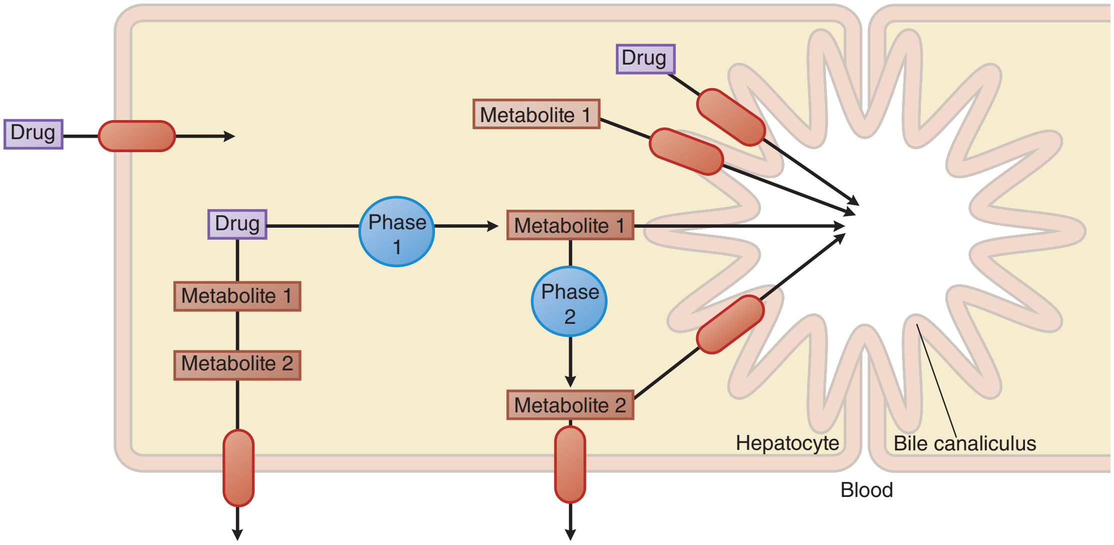

Figure 4–2 *Hepatic drug transporters.* Membrane transporters (red ovals with arrows) work in concert with phase 1 and phase 2 drug-metabolizing enzymes in the hepatocyte to mediate the uptake and efflux of drugs and their metabolites.

AL GRAWANY

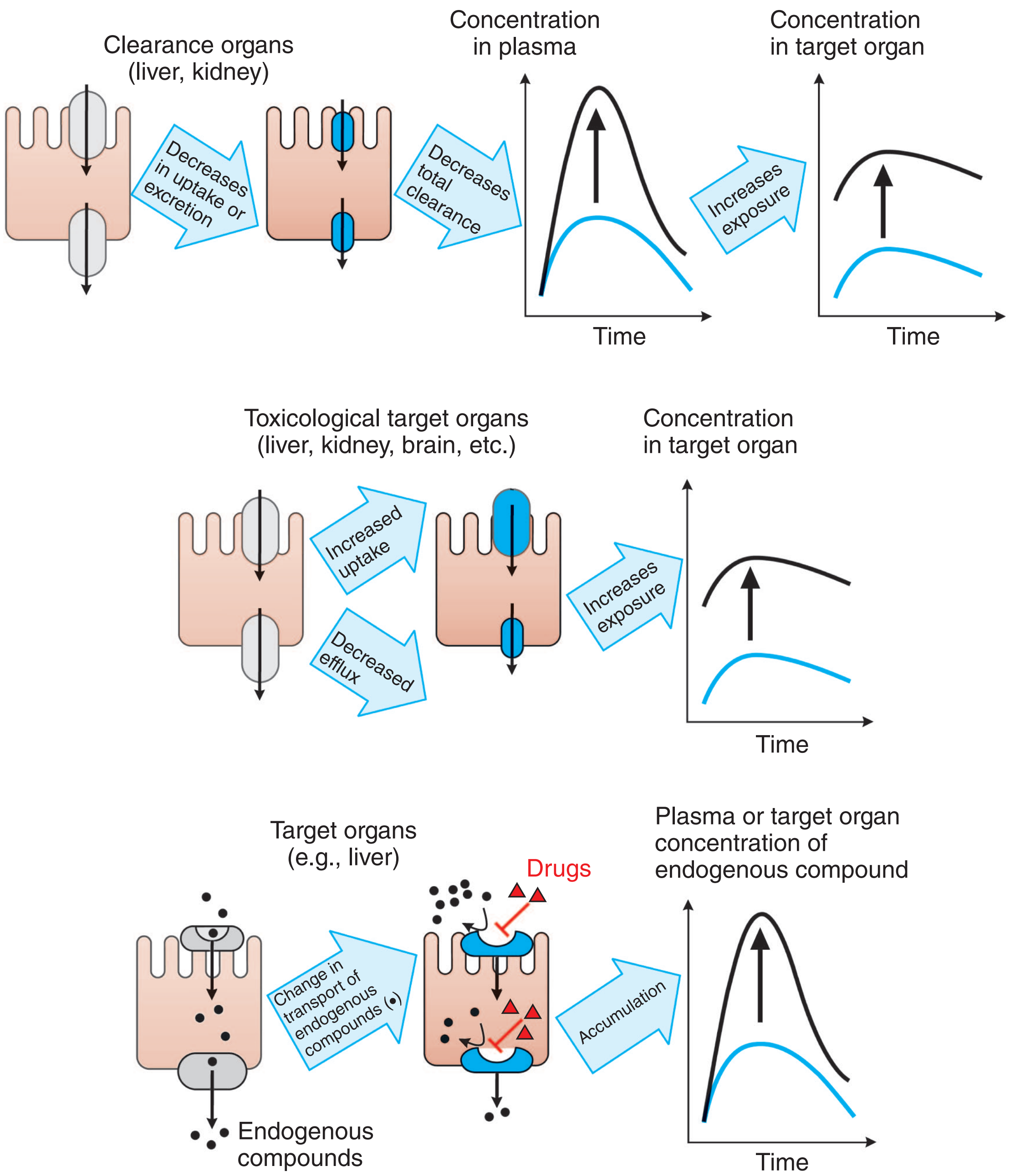
Clearance organs
(liver, kidney)
Decreases in uptake or excretion
Decreases total clearance
Concentration in plasma
Time
Increases exposure
Concentration in target organ
Time
Toxicological target organs
(liver, kidney, brain, etc.)
Increased uptake
Decreased efflux
Increases exposure
Concentration in target organ
Time
Target organs
(e.g., liver)
Change in transport of endogenous compounds (•)
Drugs
Endogenous compounds
Accumulation
Plasma or target organ concentration of endogenous compound
Time

Figure 4–3 *Major mechanisms by which transporters mediate adverse drug responses.* Three cases are given. The left panel of each case provides a representation of the mechanism; the right panel shows the resulting effect on drug levels. (*Top panel*) Increase in the plasma concentrations of drug due to a decrease in the uptake or secretion in clearance organs (e.g., liver and kidney). (*Middle panel*) Increase in the concentration of drug in toxicological target organs due to enhanced uptake or reduced efflux. (*Bottom panel*) Increase in the plasma concentration of an endogenous compound (e.g., a bile acid) due to a drug inhibiting the influx of the endogenous compound in its eliminating or target organ. The diagram also may represent an increase in the concentration of the endogenous compound in the target organ owing to drug-inhibited efflux of the endogenous compound.

loperamide in the CNS. Inhibition of Pgp-mediated efflux in the BBB would cause an increase in the concentration of *loperamide* in the CNS and potentiate adverse effects. Indeed, coadministration of *loperamide* and the potent Pgp inhibitor *quinidine* results in significant respiratory depression, an adverse response to *loperamide*. Pgp is also expressed in the intestine, where inhibition of Pgp will reduce intestinal efflux of *loperamide*, increase its systemic concentrations, and contribute to increased concentrations in the CNS.

Drug-induced toxicity sometimes is caused by the concentrative tissue distribution mediated by influx transporters. For example, biguanides (e.g., *metformin*), used for the treatment of type 2 diabetes mellitus, can produce lactic acidosis, a lethal side effect. Biguanides are substrates of OCT1 (SLC22A1), which is highly expressed in the liver; of OCT2 (SLC22A2), expressed in the kidney; and of OCT3 (SLC22A3) in adipocytes and skeletal muscle. In experimental animals lacking OCT1, hepatic uptake of biguanides and development of lactic acidosis are greatly reduced. These results indicate that OCT1-mediated hepatic uptake of biguanides and uptake into tissues such as kidney and skeletal muscle mediated by other OCTs play an important role in facilitating tissue concentrations of biguanides and thus the development of lactic acidosis (Wang et al., 2003), which may result from biguanide-induced impairment of mitochondrial function and consequent increased glycolytic flux (Dykens et al., 2008). Biguanides are exported by the MATE1 transporter, and inhibition of this efflux by a variety of drugs, including tyrosine kinase inhibitors, enhances biguanide toxicity (DeCorter et al., 2012).

OAT1 (SLC22A1), OCT1, and OCT2 provide other examples of transporter-related toxicity. OAT1 is expressed mainly in the kidney and is responsible for the renal tubular secretion of anionic compounds. Substrates of OAT1, such as *cephaloridine* (a β-lactam antibiotic) and *adefovir* and *cidofovir* (antiviral drugs), reportedly cause nephrotoxicity. Exogenous expression of OCT1 and OCT2 enhances the sensitivities of tumor cells to the cytotoxic effect of *oxaliplatin* for OCT1 and *cisplatin* and *oxaliplatin* for OCT2 (Zhang et al., 2006a). Renal toxicity of *cisplatin* is modulated by OCT2 present on the basolateral membrane of the proximal tubule as well as by transporters in the SLC47 family, MATE1 (SLC47A1) and MATE2 (SLC47A2), on the apical membrane (Harrach and Ciarimboli, 2015).

Drugs may modulate transporters for endogenous ligands and thereby exert adverse effects (Figure 4–3, bottom panel). For example, bile acids are taken up mainly by NTCP and excreted into the bile by BSEP (*ABCB11*). Bilirubin is taken up by OATP1B1 and conjugated with glucuronic acid; bilirubin glucuronide is excreted into the bile by MRP2 (*ABCC2*) and transported into the blood by MRP3. Bilirubin glucuronide in the blood undergoes reuptake into the liver by OATP1B1. Inhibition of these transporters by drugs may cause cholestasis or hyperbilirubinemia.

In addition to the simple mechanism of controlling plasma and tissue concentrations of xenobiotics, transporters may act through more complicated mechanisms to mediate adverse drug reactions. For example, Pgp is involved in the extrusion of inflammatory cytokines from T cells and dendritic cells. Notably, Pgp knockout mice may spontaneously develop inflammation associated hepatocellular carcinoma (Seelig, 2020). Pgp modulators may thus dysregulate immune responses.

Uptake and efflux transporters determine the plasma and tissue concentrations of endogenous compounds and xenobiotics, thereby influencing the systemic or site-specific toxicity of drugs.

Basic Mechanisms of Membrane Transport

Transporters Versus Channels

Both channels and transporters facilitate the membrane permeation of inorganic ions and organic compounds. Recent technological advances in X-ray crystallography and cryo-electron microscopy have resulted in a plethora of new ion channel structures leading to enhanced understanding of the mechanisms underlying their function (Thompson and Baenziger, 2020). In general, ion channels follow two archetypal classes: voltage-gated channels and ligand-gated channels. In both models, the channel forms a gated pore that controls the passage of ions as they move through the pore and across a membrane (Isacoff et al., 2013). The gate may be controlled by voltage or by ligand binding, leading to two primary states, open and closed, that are stochastic phenomena. Only in the open state do such channels act as pores that allow the selected ions to flow down their electrochemical gradients. After opening, channels stop conducting ions as a function of time by either returning to the closed state or by inactivating. As noted, drugs termed *potentiators* (e.g., *ivacaftor*) may increase the probability that a channel is in the open state. By contrast, a *transporter* forms an intermediate complex with the substrate (solute), and a subsequent conformational change in the transporter induces translocation of the substrate to the other side of the membrane. As a consequence, the kinetics of solute movement differ between transporters and channels. Typical turnover rate constants of channels are 10^6 to 10^8 sec^{-1}; those of transporters are, at most, 10^1 to 10^3 sec^{-1}. Because a particular transporter forms intermediate complexes with specific compounds (referred to as *substrates*), transporter-mediated membrane transport is characterized by saturability and inhibition by substrate analogues, as described in the section Kinetics of Transport.

The basic mechanisms involved in solute transport across biological membranes include passive diffusion, facilitated diffusion, and active transport. Active transport can be further subdivided into primary and secondary active transport. Figure 4–4 depicts these mechanisms.

Passive Diffusion

Simple diffusion of a solute across the plasma membrane consists of three processes: partition from the aqueous to the lipid phase, diffusion across the lipid bilayer, and repartition into the aqueous phase on the opposite side. Passive diffusion of any solute (including drugs) occurs down an electrochemical potential gradient of the solute.

Facilitated Diffusion

Diffusion of ions and organic compounds across the plasma membrane may be facilitated by a membrane transporter. Facilitated diffusion is a form of transporter-mediated membrane transport that does not require

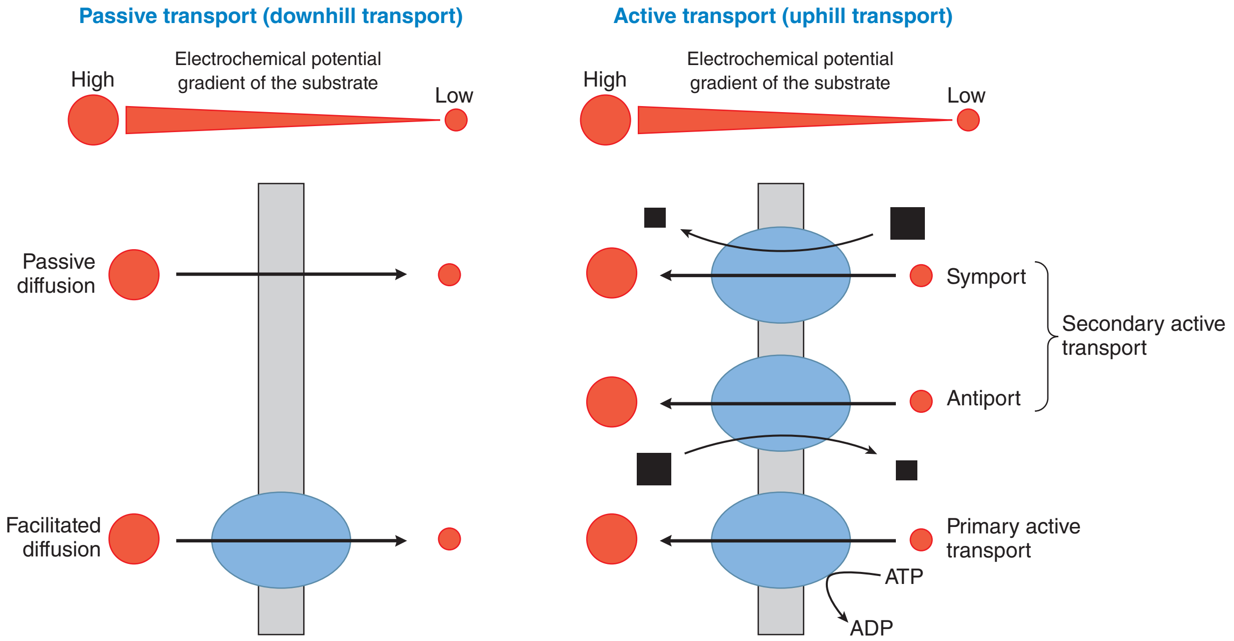
Passive transport (downhill transport)
Electrochemical potential gradient of the substrate
High
Low
Passive diffusion
Facilitated diffusion
Active transport (uphill transport)
Electrochemical potential gradient of the substrate
High
Low
Symport
Antiport
Secondary active transport
Primary active transport
ATP
ADP

Figure 4–4 *Classification of membrane transport mechanisms.* Red circles depict the substrate. Size of the circles is proportional to the concentration of the substrate. Arrows show the direction of flux. Black squares represent the ion that supplies the driving force for transport (size is proportional to the concentration of the ion). Blue ovals depict transport proteins.

energy input. Just as in passive diffusion, the transport of ionized and nonionized compounds across the plasma membrane occurs down their electrochemical potential gradients. Therefore, steady state will be achieved when the electrochemical potentials of a compound on both sides of the membrane become equal.

Active Transport

Active transport is the form of membrane transport that requires the input of energy. It is the transport of solutes against their electrochemical gradients, leading to the concentration of solutes on one side of the plasma membrane and the creation of potential energy in the electrochemical gradient formed. Active transport plays an important role in the uptake and efflux of drugs and other solutes. Depending on the driving force, active transport can be subdivided into primary active transport in which ATP hydrolysis is coupled directly to solute transport, and secondary active transport, in which transport uses the energy in an existing electrochemical gradient established by an ATP-using process to move a solute uphill against its electrochemical gradient. Secondary active transport is further subdivided into symport and antiport. Symport describes movement of driving ion and transported solute in the same direction. Antiport occurs when the driving ion and the transported solute move in opposite directions, as when the sodium/calcium exchanger (SLC8A1) transports $3Na^+$ *into* and $1Ca^{2+}$ *out of* a cardiac ventricular myocyte (see Figure 4–4).

Primary Active Transport

Membrane transport that directly couples with ATP hydrolysis is called *primary active transport*. ABC transporters are examples of primary active transporters. In mammalian cells, ABC transporters mediate the unidirectional efflux of solutes across biological membranes. Another example of primary active transport that establishes the inward Na^+ gradient and outward K^+ gradient across the plasma membrane, found in all mammalian cells, is the Na^+,K^+-ATPase.

Secondary Active Transport

In secondary active transport, the transport across a biological membrane of a solute, S_1, against its concentration gradient is energetically driven by the transport of another solute, S_2, in accordance with its electrochemical gradient. Depending on the transport direction of the solute, secondary active transporters are classified as either symporters or antiporters. For example, using the inwardly directed Na^+ concentration gradient across the plasma membrane that the Na^+,K^+-ATPase maintains, the inward movement of 3 Na^+ can drive the outward movement of 1 Ca^{++} via the Na^+/Ca^{++} exchanger, NCX. This is an example of *antiport*, or exchange transport, in which the transporter moves S_2 and S_1 in opposite directions. *Symporters*, also termed *cotransporters*, transport S_2 and S_1 in the same direction, as for glucose transport into the body from the lumen of the small intestine by the Na^+-glucose transporter SGLT1 (see Figure 4–4).

Kinetics of Transport

The flux of a substrate (rate of transport) across a biological membrane via a transporter-mediated process is characterized by saturability. The relationship between the flux v and substrate concentration S in a transporter-mediated process is given by the Michaelis-Menten equation:

$$v = \frac{V_{max}S}{K_m + S} \qquad \text{(Equation 4–1)}$$

where V_{max} is the maximum transport rate and is proportional to the density of transporters on the plasma membrane, and K_m is the Michaelis constant, which represents the substrate concentration at which the flux is half the V_{max} value. K_m is an approximation of the dissociation constant of the substrate from the intermediate complex. The K_m and V_{max} values can be determined by examining the flux at different substrate concentrations. Rearranging Equation 4–1 gives

$$v/S = -v/K_m + V_{max}/K_m \qquad \text{(Equation 4–2)}$$

Plotting v/S versus v provides a convenient graphical method for determining the V_{max} and K_m values, the Eadie-Hofstee plot (Figure 4–5): The slope is $-1/K_m$ and the x intercept is V_{max}.

Transporter-mediated membrane transport of a substrate is also characterized by inhibition by other compounds. The manner of inhibition can be categorized as one of three types: *competitive*, *noncompetitive*, and *uncompetitive*. Competitive inhibition occurs when substrates and inhibitors share a common binding site on the transporter, resulting in an increase in the apparent K_m value in the presence of inhibitor. The flux of a substrate in the presence of a competitive inhibitor is

$$\text{Competitive inhibition} \quad v = \frac{V_{max} \cdot S}{K_m \cdot \left(1 + \frac{I}{K_i}\right) + S} \qquad \text{(Equation 4–3)}$$

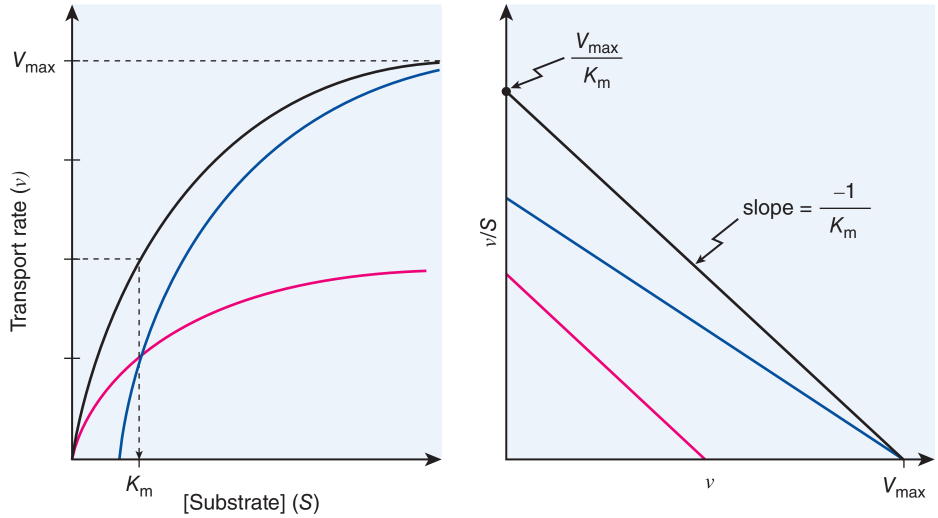

Figure 4–5 *Eadie-Hofstee plot of transport data.* The black lines show the hyperbolic concentration-dependence curve (v vs. S, left panel) and the Eadie-Hofstee transformation of the transport data (v/S vs. v, right panel) for a simple transport system. The blue lines depict transport in the presence of a competitive inhibitor (surmountable inhibition; achieves same V_{max}). The red lines depict the system in the presence of a noncompetitive inhibitor that effectively reduces the number of transporting sites but leaves the K_m of the functional sites unchanged. Involvement of multiple transporters with different K_m values gives an Eadie-Hofstee plot that is curved and can be resolved into multiple components. Algebraically, the Eadie-Hofstee plot of kinetic data is equivalent to the Scatchard plot of equilibrium binding data (see Chapter 3).

where I is the concentration of inhibitor, and K_i is the inhibition constant. Noncompetitive inhibition assumes that the inhibitor has an allosteric effect on the transporter, does not inhibit the formation of an intermediate complex of substrate and transporter, but does inhibit the subsequent translocation process.

$$\text{Noncompetitive inhibition} \quad v = \frac{V_{max} \cdot S}{\left(1 + \frac{I}{K_i}\right)\left(K_m + S\right)} \qquad \text{(Equation 4–4)}$$

Uncompetitive inhibition assumes that inhibitors can form a complex only with an intermediate complex of the substrate and transporter and inhibit subsequent translocation.

$$\text{Uncompetitive inhibition} \quad v = \frac{V_{max} \cdot S}{K_m + S\left(1 + \frac{I}{K_i}\right)} \qquad \text{(Equation 4–5)}$$

Transporter Structure and Mechanism

Predictions of secondary structure of membrane transport proteins based on hydropathy analysis indicate that membrane transporters in the SLC and ABC superfamilies are multimembrane-spanning proteins. Emerging crystal structures are adding to our ideas of the mechanisms of transport via these proteins.

ABC Transporters

The ABC superfamily includes 49 genes, each containing one or two conserved ABC regions. The core catalytic ABC regions of these proteins bind and hydrolyze ATP, using the energy for uphill transport of their substrates across the membrane. Most ABC transporters in eukaryotes move compounds from the cytoplasm to the cell exterior or into an intracellular compartment (endoplasmic reticulum, mitochondria, peroxisomes). ABC transporters also are found in prokaryotes, where they are involved predominantly in the import of essential compounds that cannot be obtained by passive diffusion (sugars, vitamins, metals, etc.).

Recent studies using cryo-electron microscopy (cryo-EM) have led to an enhanced understanding of the structure and function of ABC transporters. In general, ABC transporters are organized symmetrically with two major components, a nucleotide-binding domain (NBD) and a transmembrane domain (TMD), each associated with a function (Lusvarghi et al., 2020). The NBDs on the cytoplasmic side are considered the motor domains of ABC transporters and contain conserved motifs (e.g., Walker-A motif, ABC signature motif) that participate in binding and hydrolysis of ATP. Crystal structures of all four full ABC transporters show two NBDs, which are in contact with each other, and a conserved fold. The mechanism shared by these ABC transporters appears to involve binding of ATP to the NBDs, which subsequently triggers an outward-facing conformation of the transporters. Dissociation of the hydrolysis products of ATP appears to result in an inward-facing conformation. The TMDs are involved in substrate recognition and translocation. TMDs from Pgp are formed from a single peptide chain, whereas TMDs from BCRP are formed from a homodimer. A floppase model for Pgp-mediated efflux was proposed in 1992 and subsequently validated (Seelig, 2020). In brief, amphiphilic molecules constitute the substrates of Pgp; an amphiphilic substrate partitions from the aqueous intracellular environment to the adjacent lipid bilayer, where it binds to the transporter; with the hydrolysis of ATP, the transporter-substrate complex changes conformation and the substrate is released into the outer region of the bilayer; the substrate then partitions into the aqueous extracellular compartment. Presumably, one ATP molecule is hydrolyzed for every drug molecule transported. On dissociation of the hydrolysis products, the transporter returns to the inward-facing conformation, permitting the binding of ATP and substrate to repeat the cycle (Figure 4–6). Although some ABC superfamily transporters contain only a single ABC motif, they form homodimers (BCRP/ABCG2) or heterodimers (ABCG5 and ABCG8) that exhibit a transport function.

SLC Transporters

The SLC superfamily of transporters comprises a structurally diverse group that includes channels, facilitators, and secondary active transporters (Hediger et al., 2013). Although structurally diverse, most SLC transporters share certain structural characteristics. For example, all have TMDs, with most having between 7 and 12 TMDs (Garibsingh and Schlessinger, 2019; Pizzagalli et al., 2021). Crystal structures reveal that the TMDs have a pseudosymmetry and that SLC protein folds fall into

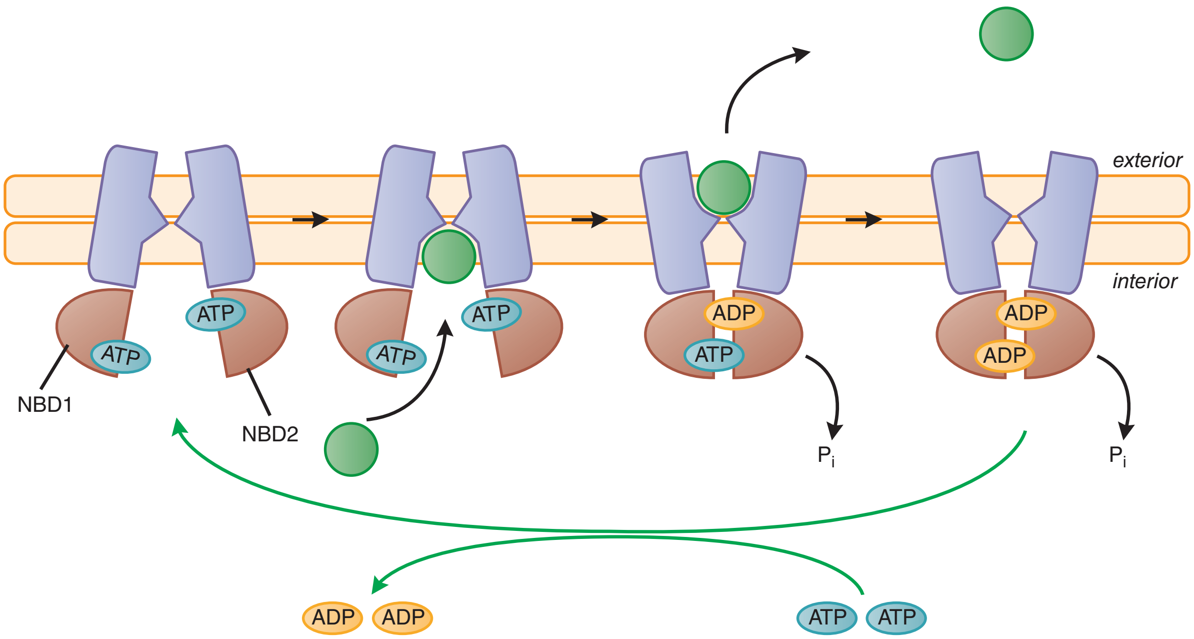

Figure 4–6 *Model of ABC transporter function.* The transporter accepts a solute molecule at the cytoplasmic membrane surface when its nucleotide NBDs are fully charged with ATP. Sequential hydrolysis of the ATP molecules produces steric change and leads to the translocation and release of the solute at the exterior membrane surface. Exchange of ADP for ATP on both NBDs completes the cycle and restores the system for readiness to transport another solute molecule.

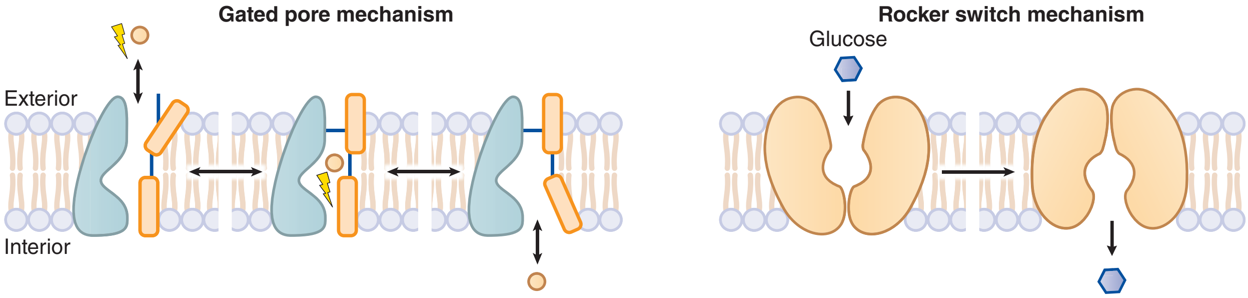

Figure 4–7 *Two alternating access models of membrane transport.* The gated pore represents a model that includes two domains, a stationary domain and a mobile domain that undergoes a hinge-like rearrangement to release the substrate to the intracellular side. The substrate is shown as a sphere and the lightning bolt represents an energizer such as sodium. This model is applicable to some sodium-dependent SLC transporters. The rocker switch represents the model by which major facilitator superfamily (MFS) proteins, such as Lac Y, work. This example models a facilitated glucose transporter, GLUT2.

two major groups, MFS and LeuT. Several members of the SLC2 family, glucose transporters (GLUTs), have been crystallized and have the MFS fold. Common drug transporters in the SLC22 family also have the MFS fold, which basically implies that they have two pseudo-repeats of six TMDs. The two pseudo-repeats serve in a rocker switch mechanism, alternating the substrate binding site to the intracellular or extracellular surface of the plasma membrane (Figure 4–7). Once a substrate binds to an accessible site (e.g., on the extracellular side) the TMDs shift, exposing the binding site to the other surface and releasing the substrate. The transport cycle is as follows: the substrate accesses the substrate binding site on one side of the membrane; substrate binding induces structural changes in the carrier protein, reorienting the opening of the binding site to the opposite side. The substrate dissociates from the transport site, allowing another substrate to be bound and transported in the opposite direction. Such a mechanism requires binding of different substrates (the "outbound" and "inbound" substrates) to be mutually exclusive; that is, there is a single reorienting binding site. Another common fold is the LeuT fold, which forms a rocker bundle or a gated pore (Garibsingh and Schlessinger, 2019; Pizzagalli et al., 2021). Transporters in the SLC5 and SLC6 families have the LeuT fold, which works in a slightly different way than the rocker switch. In brief, there are two pseudo-repeats of five TMDs, and only one arm is responsible for the alternating access of the binding site to the intracellular or extracellular surfaces of the plasma membrane. The other arm is stationary (Figure 4–7). SLC substrates include ionic and nonionic species and a variety of xenobiotics and drugs.

Vectorial Transport

Asymmetrical transport across a monolayer of polarized cells, such as the epithelial and endothelial cells of brain capillaries, is called *vectorial transport* (Figure 4–8). Vectorial transport is important for the absorption of nutrients and bile acids in the intestine and in the intestinal absorption of drugs (from lumen to blood). Vectorial transport also plays a major role in hepatobiliary and urinary excretion of drugs from the blood to the lumen. In addition, efflux of drugs from the brain via brain endothelial cells and brain choroid plexus epithelial cells involves vectorial transport. The ABC transporters mediate only unidirectional efflux, whereas SLC transporters mediate either drug uptake or drug efflux. For lipophilic compounds that have sufficient membrane permeability, ABC transporters alone are able to achieve vectorial transport without the help of influx transporters. For relatively hydrophilic organic anions and cations, coordinated uptake and efflux transporters in the polarized plasma membranes are necessary to achieve the vectorial movement of solutes across an epithelium. A typical configuration involves a primary or secondary active transporter at one membrane and a passive transporter at the other. In this way, common substrates of coordinated transporters are transferred efficiently across the epithelial barrier.

In the liver, a number of transporters with different substrate specificities are localized on the sinusoidal membrane (facing blood). These transporters are involved in the uptake of bile acids, amphipathic organic anions, and hydrophilic organic cations into the hepatocytes. Similarly, ABC transporters on the canalicular membrane (facing bile) export such

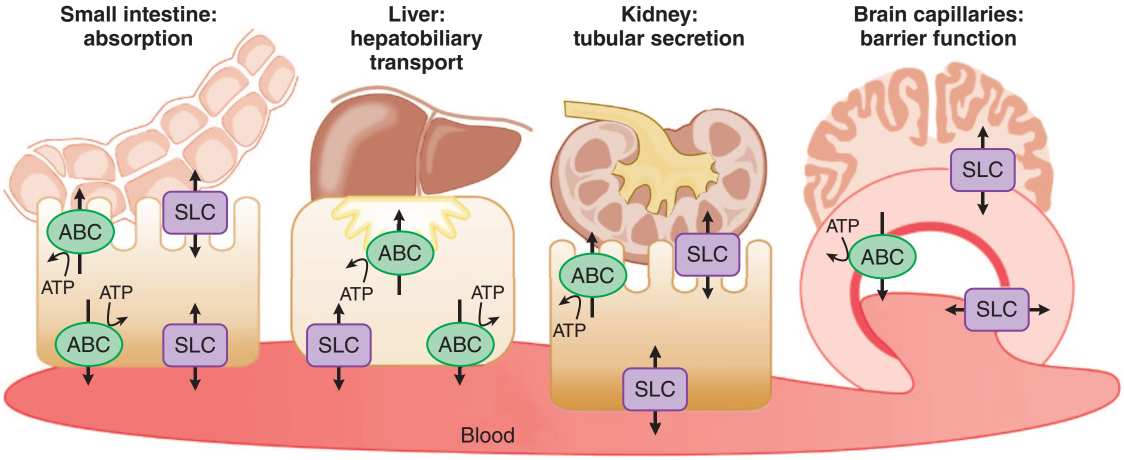

Figure 4–8 *Transepithelial and transendothelial flux.* Transepithelial or transendothelial flux of drugs requires distinct transporters at the two surfaces of the epithelial or endothelial barrier. These are depicted diagrammatically for transport across the small intestine (absorption), the kidney, liver (elimination), and brain capillary endothelial cells that compose the BBB.

compounds into the bile. Multiple combinations of uptake (OATP1B1, OATP1B3, OATP2B1) and efflux transporters (MDR1, MRP2, and BCRP) are involved in the efficient transcellular transport of a wide variety of compounds in the liver by using a system called "doubly transfected cells"; these cells express both uptake and efflux transporters on each side. In many cases, overlapping substrate specificities between the uptake transporters (OATP family) and efflux transporters (MRP family) make the vectorial transport of organic anions highly efficient. Similar transport systems also are present in the intestine, renal tubules, and endothelial cells of the brain capillaries (see Figure 4–8).

Transporter expression in various tissues can be regulated transcriptionally in response to drug treatment and pathophysiological conditions, resulting in induction or downregulation of transporter mRNAs. Type II nuclear receptors, which form heterodimers with the 9-*cis*-retinoic acid receptor (RXR), can regulate transcription of genes for drug-metabolizing enzymes and transporters (see Table 5–4, Figures 3–23, 5–13 and 5–14; see also Urquhart et al., 2007). Such receptors include PXR (NR1I2), CAR (NR1I3), FXR (NR1H4), PPARα, and RAR. Except for CAR, these are ligand-activated nuclear receptors that, as heterodimers with RXR, bind specific elements in the enhancer regions of target genes. CAR has constitutive transcriptional activity that is antagonized by inverse agonists, such as androstenol and androstanol, and induced by barbiturates. PXR, also referred to as SXR in humans, is activated by synthetic and endogenous steroids, bile acids, and drugs such as *clotrimazole, phenobarbital, rifampicin, sulfinpyrazone, ritonavir, carbamazepine, phenytoin, sulfadimidine, paclitaxel*, and *hyperforin* (a constituent of St. John's wort) (Guo and Zhou, 2015). The potency of activators of PXR varies among species, such that rodents are not necessarily a model for effects in humans. There is an overlap of substrates between CYP3A4 and Pgp, and PXR mediates coinduction of CYP3A4 and Pgp, supporting their synergy in efficient detoxification. Recent studies in human hepatocytes treated with an activator of PXR suggested that the expression levels of enzymes in the CYP family are much more highly increased than the levels of transporters in the SLC or ABC families (Smith et al., 2014). Table 4–1 summarizes the effects of drug activation of type II nuclear receptors on expression of transporters in the liver (Amacher, 2016).

DNA methylation is one mechanism underlying the epigenetic control of gene expression. Reportedly, the tissue-selective expression of transporters is achieved by DNA methylation (silencing in the transporter-negative tissues) as well as by transactivation in the transporter-positive tissues. Transporters subjected to epigenetic control in the liver and kidney include OATP1B1, OATP1B3, OCT2, OAT1, and OAT3. Other transporters that have been shown to be subject to epigenetic regulation in a variety of cell lines include MDR1 and BCRP in the ABC superfamily and OCT1, OCT3, and OCTN2 in the SLC22 family (Hirota et al., 2017).

Transporter Superfamilies in the Human Genome

The SLC Superfamily

The SLC superfamily includes 65 families and represents about 458 genes in the human genome, the products of which are membrane-spanning proteins, some of which are associated with genetic diseases (Table 4–2)

TABLE 4–1 ■ REGULATION OF TRANSPORTER EXPRESSION BY NUCLEAR RECEPTORS IN HUMANS

TRANSPORTER	TRANSCRIPTION FACTOR	LIGAND	EFFECT
MDR1 (P-gp)	PXR	Rifampin	↑ Transcription activity
			↑ Expression in duodenum
			↓ Oral bioavailability of digoxin
			↓ AUC of talinolol
			↑ Expression in primary hepatocyte
		St John's wort	↑ Expression in duodenum
			↓ Oral bioavailability of digoxin
	CAR	Phenobarbital	↓ Expression in primary hepatocyte
MRP2	PXR	Rifampin	↑ Expression in duodenum
		Rifampin/hyperforin	↑ Expression in primary hepatocyte
	FXR	GW4064/chenodeoxycholate	↑ Expression in HepG2-FXR
	CAR	Phenobarbital	↑ Expression in hepatocyte
BCRP	PXR	Rifampin	↑ Expression in primary hepatocyte
	CAR	Phenobarbital	
MRP3	PXR	Rifampin	↑ Expression in hepatocyte
OATP1B1	SHP1	Cholic acid	Indirect effect on HNF1α expression
	PXR	Rifampin	↑ Expression in hepatocyte
	FXR	Chenodeoxycholate	↑ Expression in hepatocyte
OATP1B3	FXR	Chenodeoxycholate	↑ Expression in hepatoma cells
	PXR	Rifampin	↓ Expression in hepatocyte
OCT1	PXR	Rifampin	↓ Expression in hepatocyte
	HNF4α	Berberine	↑ Expression in hepatocyte
BSEP	FXR	Chenodeoxycholate	↑ Transcription activity
OSTα/β	FXR	Chenodeoxycholate/GW4064	↑ Transcription activity
		Chenodeoxycholate	↑ Expression in ileal biopsies

CAR, constitutive androstane receptor; FXR, farnesoid X receptor; HNF4α, hepatocyte nuclear factor 4α; PXR, pregnane X receptor; SHP1, small heterodimer partner 1.

TABLE 4–2 ■ THE HUMAN SOLUTE CARRIER SUPERFAMILY

GENE	FAMILY	SELECTED DRUG SUBSTRATES	EXAMPLES OF LINKED HUMAN DISEASES
SLC1	Low-K_m glu/neutral aa T		Dicarboxylic aminoaciduria
SLC2	Facilitative GLUT		Fanconi-Bickel syndrome
SLC3	Heavy subunits, heteromeric aa Ts	Melphalan	Classic cystinuria type I
SLC4	Bicarbonate T		Distal renal tubule acidosis
SLC5	Na^+ glucose co-T	Dapagliflozin	Glucose-galactose malabsorption
SLC6	Na^+/Cl^--dependent neurotransmitter T	Paroxetine, fluoxetine	Cerebral creatine deficiency syndrome
SLC7	Cationic aa T	Melphalan	Lysinuric protein intolerance
SLC8	Na^+/Ca^{2+} Exch	Di-CH_3-arg	
SLC9	Na^+/H^+ Exch	Thiazide diuretics	Hypophosphatemic nephrolithiasis
SLC10	Na^+ bile salt co-T	Benzothiazepines (diltiazem)	Primary bile acid malabsorption
SLC11	H^+-coupled metal ion T		Hereditary hemochromatosis
SLC12	Electroneutral cation–Cl^- co-T		Gitelman syndrome
SLC13	Na^+–SO_4^-/COO^- co-T	SO_4^-/cys conjugates	
SLC14	Urea T		Kidd antigen blood group
SLC15	H^+–oligopeptide co-T	Valacyclovir	
SLC16	Monocarboxylate T	Salicylate, T_3/T_4, atorvastatin	Familial hyperinsulinemic hypoglycemia 7
SLC17	Vesicular glu T		Sialic acid storage disease
SLC18	Vesicular amine T	Reserpine	Myasthenic syndromes
SLC19	Folate/thiamine T	Methotrexate	Thiamine-responsive megaloblastic anemia
SLC20	Type III Na^+–PO_4^- co-T		
SLC21 (SLCO)	Organic anion T	Pravastatin	Rotor syndrome, hyperbilirubinemia
SLC22	Organic ion T	Pravastatin, metformin	Primary systemic carnitine deficiency
SLC23	Na^+-dependent ascorbate T	Vitamin C	
SLC24	$Na^+/(Ca^{2+}$-$K^+)$ Exch		Congenital stationary night blindness type 1D
SLC25	Mitochondrial carrier		Familial hypertrophic cardiomyopathy
SLC26	Multifunctional anion Exch	Salicylate, ciprofloxacin	Multiple epiphyseal dysplasia 4
SLC27	Fatty acid T		Ichthyosis prematurity syndrome
SLC28	Na^+-coupled nucleoside T	Gemcitabine, cladribine	
SLC29	Facilitative nucleoside T	Dipyridamole, gemcitabine	
SLC30	Zn efflux		Hypermanganesemia with dystonia
SLC31	Cu T	Cisplatin	
SLC32	Vesicular inhibitory aa T	Vigabatrin	
SLC33	Acetyl-CoA T		Congenital cataracts
SLC34	Type II Na^+-PO_4^-/ co-T		Hypercalciuric rickets
SLC35	Nucleoside-sugar T		Leukocyte adhesion deficiency II
SLC36	H^+-coupled aa T	D-Serine, cycloserine	Iminoglycinuria
SLC37	Sugar-phosphate/PO_4^- Exch		Glycogen storage disease
SLC38	Na^+-coupled neutral aa T		
SLC39	Metal ion T		Acrodermatitis enteropathica
SLC40	Basolateral Fe T		Hemochromatosis type IV
SLC41	MgtE-like Mg^{2+} T		
SLC42	Rh ammonium T		Rh-null regulator type disease
SLC43	Na^+-independent L-like aa T	Riboflavin	Oculocutaneous albinism type 4
SLC44	Choline-like transporter family		Neurodegeneration, childhood-onset, with ataxia, optic atrophy and cognitive decline, CONATOC

(*Continued*)

TABLE 4–2 ■ THE HUMAN SOLUTE CARRIER SUPERFAMILY (*CONTINUED*)

GENE	FAMILY	SELECTED DRUG SUBSTRATES	EXAMPLES OF LINKED HUMAN DISEASES
SLC45	H^+/sugar cotransporter family		Skin, hair, eye pigmentation variation
SLC46	Folate transporter family		Folate malabsorption
SLC47	Multidrug and toxin extrusion (MATE) family		
SLC48	Heme transporter family		
SLC49	FLVCR-related transporter family		
SLC50	Sugar efflux transporters		
SLC51	Transporters of steroid-derived molecules		
SLC52	Riboflavin transporter family		Riboflavin deficiency, Brown-Vialetto-Van Laere syndrome
SLC53	Phosphate carriers		
SLC54	Mitochondrial pyruvate carriers		
SLC55	Mitochondrial cation/proton exchangers		
SLC56	Sideroflexins		
SLC57	NiPA-like magnesium transporter family		
SLC58	MagT-like magnesium transporter family		
SLC59	Sodium-dependent lysophosphatidylcholine symporter family		
SLC60	Glucose transporters		
SLC61	Molybdate transporter family		
SLC62	Pyrophosphate transporters		
SLC63	Sphingosine-phosphate transporters		
SLC64	Golgi-Ca^{2+}/H^+ exchangers		
SLC65	NPC-type cholesterol transporters		

aa, amino acid; Exch, exchanger; T, transporter; T_3/T_4, thyroid hormone.

(Pizzagalli et al., 2021). Myriad substrates, including inorganic and organic ions, interact with SLC transporters. There are highly selective transporters that interact with structurally similar molecules, such as transporters in the SLC6 family that interact with specific monoamines, including dopamine (DAT, SLC6A2) and norepinephrine (NET, SLC6A3). On the other hand, there are transporters that accept a broad range of chemically diverse substrates, such as organic ion transporters in the SLC22 family. Unlike ABC transporters that rely on ATP hydrolysis to actively translocate their substrates, SLC transporters are mostly facilitative transporters, although some are secondary active transporters (see Figure 4–4). Knowledge of the SLC superfamily continues to grow; 13 new SLC families representing 63 genes have been identified since the prior edition of this text.

The physiologic roles of SLC transporters are important and diverse. For example, transporters in the SLC1, SLC3, SLC6, SLC7, SLC25, and SLC36 families, which are expressed in the intestine and kidney, among other organs, transport an array of amino acids critical in protein synthesis and energy homeostasis. Glucose and other sugars interact with transporters in the SLC2, SLC5, and SLC50 families for absorption, elimination, and cellular distribution. Proteins in the SLC11, SLC30, SLC39, and SLC40 families transport zinc, iron, and other metals. Members of the SLC19, SLC46, and SLC52 families transport water-soluble vitamins. Transporters in the SLC6 family move neurotransmitters across the plasma membrane; SLC18 family members transport neurotransmitters into storage vesicles.

Pharmacologically, SLC transporters have been characterized for their role in drug absorption, elimination, and tissue distribution and importantly as mediators of drug-drug interactions. Notably, transporters in the solute carrier organic anion family, SLCO, interact with diverse substrates, including statins and antidiabetic drugs. Transporters in the SLC22 family interact with anionic and cationic drugs, including many antibiotics and antiviral agents, to mediate active renal secretion. SLC transporters are increasingly being targeted for treatment of human disease. Over 100 SLC transporters are associated with monogenic disorders and therefore may be usefully targeted in the treatment of rare diseases (Lin et al., 2015). Several monogenic disorders associated with mutations in SLC transporters are detected in newborn screening programs, including carnitine transporter deficiency, an autosomal recessive disorder caused by mutations in SLC22A5 (OCTN2). Many SNPs in SLC transporters have reached a genome-wide level of significance in association studies of human disease. Notably, polymorphisms in *SLC30A8* are associated with type 1 diabetes mellitus, and polymorphisms in *SLC22A4* and *SLC22A5* are associated with inflammatory bowel disease.

An estimated 30% of SLC transporters in the human genome remain orphans, that is, they or their direct species orthologs have no known substrates. Recent efforts in transporter biology are aimed at discovering substrates for these transporters, and several such studies have been successful: SLC22A24 has been found to transport conjugates of steroids and to play a role in steroid homeostasis; SLC22A15 is a zwitterion transporter that transports endogenous and xenobiotic zwitterions such as ergothioneine and *gabapentin* (Yee et al., 2019, 2020).

The ABC Superfamily

The seven subfamilies of human ABC transporters are essential for many cellular processes, and mutations in at least 13 of the genes for ABC transporters cause or contribute to human genetic disorders (Table 4–3) (Arana

TABLE 4–3 THE HUMAN ATP BINDING CASSETTE (ABC) SUPERFAMILY

GENE	FAMILY	NUMBER OF MEMBERS	EXAMPLES OF LINKED HUMAN DISEASES
ABCA	ABC A	12	Tangier disease (defect in cholesterol transport; ABCA1), Stargardt syndrome (defect in retinal metabolism; ABCA4)
ABCB	ABC B	11	Bare lymphocyte syndrome type 1 (defect in antigen presenting; ABCB3 and ABCB4), progressive familial intrahepatic cholestasis type 3 (defect in biliary lipid secretion; MDR3/ABCB4), X-linked sideroblastic anemia with ataxia (a possible defect in iron homeostasis in mitochondria; ABCB7), progressive familial intrahepatic cholestasis type 2 (defect in biliary bile acid excretion; BSEP/*ABCB11*)
ABCC	ABC C	13	Dubin-Johnson syndrome (defect in biliary bilirubin glucuronide excretion; MRP2/*ABCC2*), pseudoxanthoma (unknown mechanism; *ABCC6*), cystic fibrosis (defect in Cl^- channel regulation; *ABCC7*), persistent hyperinsulinemic hypoglycemia of infancy (defect in inwardly rectifying K^+ conductance regulation in pancreatic B cells; SUR1/*ABCC8*)
ABCD	ABC D	4	Adrenoleukodystrophy (a possible defect in peroxisomal transport or catabolism of very-long-chain fatty acids; ABCD1)
ABCE	ABC E	1	
ABCF	ABC F	3	
ABCG	ABC G	5	Sitosterolemia (defect in biliary and intestinal excretion of plant sterols; ABCG5 and ABCG8)

and Altenberg, 2019). In addition to conferring multidrug resistance, an important pharmacological aspect of these transporters is xenobiotic export from healthy tissues. In particular, MDR1/*ABCB1*, MRP2/*ABCC2*, and BCRP/*ABCG2* are involved in overall drug disposition.

Tissue Distribution of Drug-Related ABC Transporters

Table 4–4 summarizes the distribution of human drug-related ABC transporters in tissues involved in the absorption, elimination, or distribution of drugs, along with information about typical substrates. MDR1 (*ABCB1*), MRP2 (*ABCC2*), and BCRP (*ABCG2*) are all expressed in the apical side of the intestinal epithelia, where they serve to pump out xenobiotics, including many orally administered drugs. MRP3 (*ABCC3*) is expressed in the basal side of the epithelial cells.

Key to the vectorial excretion of drugs into urine or bile, ABC transporters are expressed in the polarized tissues of kidney and liver: MDR1, MRP2, BCRP, and MRP4 (*ABCC4*) on the brush border membrane of renal epithelia; MDR1, MRP2, and BCRP on the bile canalicular membrane of hepatocytes; and MRP3 and MRP4 on the sinusoidal membrane of hepatocytes. Some ABC transporters are expressed specifically on the blood side (luminal) of the endothelial or epithelial cells that form barriers to the free entrance of toxic compounds into tissues: the BBB (MDR1 and MRP4 on the luminal side of brain capillary endothelial cells), the blood-CSF barrier (MRP1 and MRP4 on the basolateral blood side of choroid plexus epithelia), the blood-testis barrier (MRP1 on the basolateral membrane of mouse Sertoli cells and MDR1 in several types of human testicular cells), and the blood-placenta barrier (MDR1, MRP2, and BCRP on the luminal maternal side and MRP1 on the antiluminal fetal side of placental trophoblasts). BCRP is expressed on the apical membrane of mammary gland epithelium and is highly induced during lactation.

MRP/ABCC Family

The substrates of transporters in the MRP/ABCC family are mostly organic anions (see Table 4–4). Both MRP1 and MRP2 accept glutathione and glucuronide conjugates, sulfated conjugates of bile salts, and nonconjugated organic anions of an amphipathic nature (at least one negative charge and some degree of hydrophobicity). They also transport neutral or cationic anticancer drugs, such as vinca alkaloids and anthracyclines, possibly by means of a cotransport or symport mechanism with GSH. MRP3 also has a substrate specificity that is similar to that of MRP2 but with a lower transport affinity for glutathione conjugates compared with MRP1 and MRP2. MRP3 is expressed on the sinusoidal side of hepatocytes and is induced under cholestatic conditions. MRP3 functions to return toxic bile salts and bilirubin glucuronides into the blood circulation. MRP4 accepts negatively charged molecules, including cytotoxic compounds (e.g., *6-mercaptopurine* and *methotrexate*), cyclic nucleotides, antiviral drugs (e.g., *adefovir* and *tenofovir*), diuretics (e.g., *furosemide* and *trichlormethiazide*), and cephalosporins (e.g., *ceftizoxime* and *cefazolin*). Glutathione enables MRP4 to accept taurocholate and leukotriene B_4. MRP5 has a narrower substrate specificity and accepts nucleotide analogue and clinically important anti-HIV drugs. No substrates have been identified that explain the mechanism of the MRP6-associated disease pseudoxanthoma.

BCRP/ABCG2

BCRP accepts both neutral and negatively charged molecules, including cytotoxic compounds (e.g., *topotecan*, *flavopiridol*, and *methotrexate*); sulfated conjugates of therapeutic drugs and hormones (e.g., estrogen sulfate); antibiotics (e.g., *nitrofurantoin* and fluoroquinolones); statins (e.g., *pitavastatin* and *rosuvastatin*); and toxic compounds found in normal food (phytoestrogens, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, and pheophorbide A, a chlorophyll catabolite). In addition, genetic variants in the transporter have been implicated in hyperuricemia and gout and in the disposition of uric acid and the XOIs *allopurinol* and *oxypurinol*.

Physiological Roles of ABC Transporters

The physiological significance of the ABC transporters has been amply illustrated by studies involving knockout animals or patients with genetic defects in these transporters. Many ABC transporters serve in xenobiotic disposition. For instance, mice deficient in MDR1 function are viable and fertile and do not display obvious phenotypic abnormalities other than hypersensitivity to the toxicity of drugs. There are equally remarkable data for MRP1, MRP4, BCRP, and BSEP. The lesson is this: complete absence of these drug-related ABC transporters is not lethal and can remain unrecognized in the absence of exogenous perturbations due to food, drugs, or toxins. However, inhibition of physiologically important ABC transporters (especially those related directly to the genetic diseases described in Table 4–3) by drugs should be avoided to reduce the incidence of drug-induced side effects.

ABC Transporters in Drug Absorption and Elimination

With respect to clinical medicine, MDR1, also known as Pgp, is the most renowned ABC transporter yet identified. The systemic exposure to

TABLE 4–4 ■ ABC TRANSPORTERS INVOLVED IN DRUG ABSORPTION, DISTRIBUTION, AND EXCRETION PROCESSES

NAME Tissue Distribution[a]	SUBSTRATES
MDR1 (ABCB1) Liver, kidney, intestine, BBB, BTB, BPB	**Characteristics:** Bulky neutral or cationic compounds (many xenobiotics)—etoposide, doxorubicin, vincristine; diltiazem, verapamil; indinavir, ritonavir; erythromycin, ketoconazole; testosterone, progesterone; cyclosporine, tacrolimus; digoxin, quinidine, fexofenadine, loperamide
MRP1 (ABCC1) Ubiquitous	**Characteristics:** Negatively charged amphiphiles—vincristine (with GSH), methotrexate; GSH conjugate of LTC_4, ethacrynic acid; glucuronide of estradiol, bilirubin; estrone-3-sulfate; saquinavir; grepafloxacin; folate, GSH, GSSG
MRP2 (ABCC2) Liver, kidney, intestine, BPB	**Characteristics:** Negatively charged amphiphiles—methotrexate, vincristine; GSH conjugates of LTC_4, ethacrynic acid; glucuronides of estradiol, bilirubin; taurolithocholate sulfate; statins, AngII receptor antagonists, temocaprilat; indinavir, ritonavir; GSH, GSSG
MRP3 (ABCC3) Liver, kidney, intestine	**Characteristics:** Negatively charged amphiphiles—etoposide, methotrexate; GSH conjugates of LTC_4, PGJ_2; glucuronides of estradiol, etoposide, morphine, acetaminophen, hymecromone, harmol; sulfate conjugates of bile salts; glycocholate, taurocholate; folate, leucovorin
MRP4 (ABCC4) Ubiquitous, including BBB and BCSFB	**Characteristics:** Nucleotide analogues, 6-mercaptopurine, methotrexate; estradiol glucuronide; dehydroepiandrosterone sulfate; cyclic AMP/GMP; furosemide, trichlormethiazide; adefovir, tenofovir; cefazolin, ceftizoxime; folate, leucovorin, taurocholate (with GSH)
MRP5 (ABCC5) Ubiquitous	**Characteristics:** Nucleotide analogues 6-mercaptopurine; cyclic AMP/GMP; adefovir
MRP6 (ABCC6) Liver, kidney	**Characteristics:** Doxorubicin,[b] etoposide,[b] GSH conjugate of LTC_4; BQ-123 (cyclic penta peptide antagonist at the ETA endothelin receptor)
BCRP(MXR) (ABCG2) Liver, intestine, BBB	**Characteristics:** Neutral and anionic compounds—methotrexate, mitoxantrone, camptothecins, SN-38, topotecan, imatinib; glucuronides of 4-methylumbelliferone, estradiol; sulfate conjugates of dehydroepiandrosterone, estrone; nitrofurantoin, fluoroquinolones; pitavastatin, rosuvastatin; cholesterol, estradiol, dantrolene, prazosin, sulfasalazine, uric acid, allopurinol, oxypurinol
MDR3 (ABCB4) Liver	**Characteristics:** Phospholipids
BSEP (ABCB11) Liver	**Characteristics:** Bile salts
ABCG5, ABCG8 Liver, intestine	**Characteristics:** Plant sterols

BBB, blood-brain barrier; BCSFB, blood-cerebrospinal fluid barrier; BPB, blood-placenta barrier; BTB, blood-testis barrier; LTC, leukotriene C; PGJ, prostaglandin J.

[a] Tissue distribution refers to tissues that play a role in drug absorption, distribution, and elimination.

[b] Substrates and cytotoxic drugs with increased resistance (cytotoxicity with increased resistance is usually caused by the decreased accumulation of the drugs). Although MDR3 (ABCB4), BSEP (ABCB11), ABCG5, and ABCG8 are not directly involved in drug disposition, their inhibition will lead to unfavorable side effects.

orally administered *digoxin* is decreased by coadministration of *rifampin* (an MDR1 inducer) and is negatively correlated with the MDR1 protein expression in the human intestine. MDR1 is also expressed on the brush border membrane of renal epithelia, and its function can be monitored using *digoxin* (>70% excreted in the urine). MDR1 inhibitors (e.g., *quinidine, verapamil, valspodar, spironolactone, clarithromycin*, and *ritonavir*) all markedly reduce renal excretion of *digoxin*. Drugs with narrow therapeutic windows (e.g., *digoxin, cyclosporine, tacrolimus*) should be used with great care if MDR1-based drug-drug interactions are likely.

In the intestine, MRP3 can mediate intestinal absorption in conjunction with uptake transporters. MRP3 mediates sinusoidal efflux in the liver, decreasing the efficacy of the biliary excretion from the blood and excretion of intracellularly formed metabolites, particularly glucuronide conjugates. Thus, dysfunction of MRP3 results in shortening of the elimination $t_{1/2}$. MRP4 substrates also can be transported by OAT1 and OAT3 on the basolateral membrane of the epithelial cells in the kidney. The rate-limiting process in renal tubular secretion is likely the uptake process at the basolateral surface. Dysfunction of MRP4 enhances the renal concentration but has limited effect on the blood concentration.

Intestinal BCRP also plays a critical role in drug absorption and is the target for several clinically important drug-drug interactions. Consider the case of coadministration of *rosuvastatin* and *cyclosporin*. *Rosuvastatin* is a substrate for both BCRP and OATP1B1, both of which *cyclosporin* inhibits. Inhibition of BCRP by *cyclosporin* reduces efflux and enhances absorption of *rosuvastatin* from the GI tract, where the BCRP is abundantly expressed on the apical membrane of intestinal epithelia. Inhibition of OATP1B1 by *cyclosporin* reduces tissue uptake of *rosuvastatin*. The overall result is an increase in systemic plasma levels of *rosuvastatin*, which may result in increased susceptibility to statin-induced myopathy. Efficacy will depend upon the net effect on hepatic levels of *rosuvastatin*, driven by both the higher portal vein levels (from BCRP inhibition) and the lower hepatic uptake via OATP1B1 inhibition.

Transporters Involved in Pharmacokinetics

Drug transporters play a prominent role in pharmacokinetics (see Figure 4–1 and Table 4–4). Transporters in the liver and kidney have important roles in removal of drugs from the blood and hence in metabolism and excretion.

Hepatic Transporters

Hepatic uptake of organic anions (e.g., drugs, leukotrienes, and bilirubin), cations, and bile salts is mediated by SLC-type transporters in the basolateral (sinusoidal) membrane of hepatocytes: OATPs (SLCO)/OAT2 (SLC22A7), OCTs (SLC22), and NTCP (SLC10A1), respectively.

AL GRAWANY

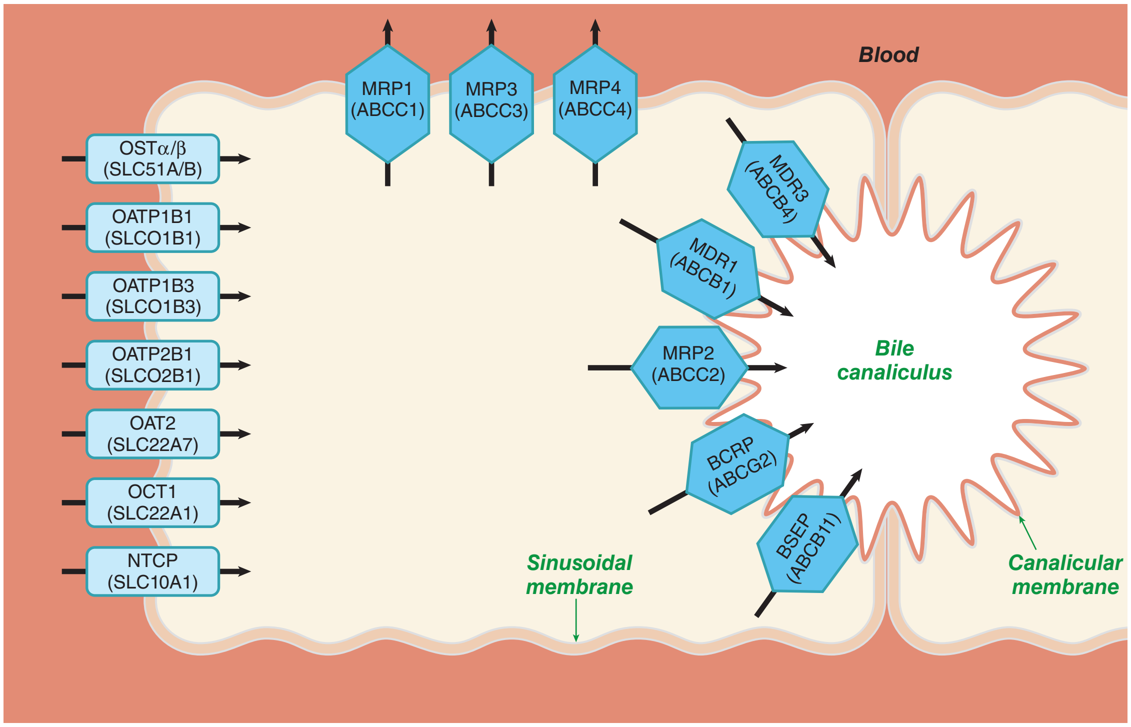

Figure 4–9 *Transporters in the hepatocyte that function in the uptake and efflux of drugs across the sinusoidal membrane and efflux of drugs into the bile across the canalicular membrane.* Arrows show the primary direction of transport. See text for details of the transporters pictured.

These transporters mediate uptake by either facilitated or secondary active mechanisms.

ABC transporters such as MRP2, MDR1, BCRP, BSEP, and MDR3 in the bile canalicular membrane of hepatocytes mediate the efflux (excretion) of drugs and their metabolites, bile salts, and phospholipids against a steep concentration gradient from liver to bile. This primary active transport is driven by ATP hydrolysis.

Vectorial transport of drugs from the circulating blood to the bile using an uptake transporter (OATP family) and an efflux transporter (MRP2, BCRP, MDR1) is important for determining drug exposure in the circulating blood and liver. Moreover, there are many other uptake and efflux transporters in the liver (Figure 4–9).

The following examples illustrate the importance of vectorial transport in determining drug exposure in the circulating blood and liver and the role of transporters in drug-drug interactions.

HMG-CoA Reductase Inhibitors

Statins are cholesterol-lowering agents that reversibly inhibit HMG-CoA reductase, which catalyzes a rate-limiting step in cholesterol biosynthesis (see Chapter 37). Most of the statins in their acid form are substrates of hepatic uptake transporters and undergo enterohepatic recirculation (see Figure 4–9). In this process, hepatic uptake transporters such as OATP1B1 and efflux transporters such as MRP2 act cooperatively to produce *bisubstrate vectorial transcellular transport*. The efficient first-pass hepatic uptake of these statins by OATP1B1 helps concentrate them in the liver where they produce their pharmacological effects, thus minimizing their systemic levels and adverse effects in muscle. Genetic polymorphisms of OATP1B1 also affect the function of this transporter (Meyer zu Schwabedissen et al., 2015).

Anti–Hepatitis C Virus Drugs

Drug therapy of hepatitis C virus (HCV) has made great strides through the discovery of direct-acting antiviral agents. Sustained virological response, an indicator of therapeutic effect, exceeds 90%, and it is now possible to cure hepatitis C infections (Hong et al., 2020). Several HCV NS3/4A protease inhibitors such as *simeprevir, glecaprevir*, and *grazoprevir* display nonlinear pharmacokinetics at therapeutic doses, at least partly due to the saturation of OATP1B-mediated hepatic uptake (Hong et al., 2020; Snoeys et al., 2016). The saturation of other components such as the ABC transporters and CYP enzymes may also contribute to the nonlinear pharmacokinetics. For these HCV drugs, their systemic exposure increases markedly when coadministered with a single dose of *rifampicin* or *cyclosporin A*, which suggests the occurrence of OATP1B-mediated drug-drug interactions (Hong et al., 2020; Snoeys et al., 2016). The likelihood of drug-drug interactions with HCV drugs is greater with NS3/4A protease inhibitors than NS5A inhibitors and NS5B polymerase inhibitors (Hong et al., 2020). The majority of clinically relevant drug-drug interactions are predictable, based on the known pharmacokinetic properties of transporters and enzymes. NS5A inhibitors and NS5B polymerase inhibitors are not substrates of OATP1B but are known to cause drug-drug interactions by inhibiting OATP1B (Hong et al., 2020).

Gemfibrozil, Pemafibrate

Gemfibrozil is a PPARα activator that is used to lower cholesterol levels (see Chapter 37). The drug can enhance toxicity (myopathy) to several statins at a therapeutic dose of 600 mg (bid) by a mechanism that involves a transporter (OATP1B) and a drug-metabolizing enzyme (CYP2C8). *Gemfibrozil* is metabolized to gemfibrozil 1-O-β-glucuronide. The parent compound and its glucuronide inhibit the uptake of the active acid forms of statins into hepatocytes by OATP1B1, and the glucuronide is also a potent mechanism based inhibitor of CYP2C8, an enzyme that metabolizes the statin such as cerivastatin. The net result is that coadministration of a statin and *gemfibrozil* causes an increase in the plasma concentrations of the statin and a concomitant increase in toxicity.

Fortunately, not all fibrates cause toxicity by these mechanisms. *Pemafibrate* also belongs to the class of fibrates (selective PPARα modulator) and displays efficacy and safety profiles superior to other conventional fibrates due to its high selectivity for PPARα. Like *gemfibrozil*, *pemafibrate* is a substrate for OATP1B and CYP enzymes. However, *pemafibrate* is effective at much lower blood levels than *gemfibrozil*, and a therapeutic dose is 0.1 mg; thus, *pemafibrate* does not increase tissue exposure to statins (typical OATP1B substrates). On the other hand, the blood exposure of *pemafibrate* is increased more than 10-fold by a single dose of *rifampicin* and *cyclosporin A*, suggesting the occurrence of drug-drug interactions via inhibition of hepatic OATP1Bs (Park et al., 2021).

Irinotecan

CPT-11 is a potent anticancer drug, but late-onset GI toxicities, such as severe diarrhea, make this a difficult agent to use safely. After intravenous administration of CPT-11, a carboxylesterase converts the drug to SN-38, an active metabolite. SN-38 is subsequently taken up by OATP1B followed by conjugation with glucuronic acid in the liver. SN-38 and SN-38 glucuronide are then excreted into the bile by MRP2, entering the GI tract and causing adverse effects (Toshimoto et al., 2017). The inhibition of MRP2-mediated biliary excretion of SN-38 and its glucuronide by coadministration of probenecid reduces the drug-induced diarrhea in experimental systems and may prove useful in humans (Horikawa et al., 2002). For additional details, see Figures 5–6, 5–8, and 5–9.

Bosentan

Bosentan is an endothelin antagonist used to treat pulmonary arterial hypertension. It is taken up in the liver by OATP1B1 and OATP1B3 and subsequently metabolized by CYP2C9 and CYP3A4. Transporter-mediated hepatic uptake can be a determinant of elimination of bosentan, and inhibition of its hepatic uptake by *cyclosporine* and *rifampicin* can affect its pharmacokinetics. Saturation of OATP1B-mediated hepatic uptake accounts for the nonlinear pharmacokinetics of *bosentan* following therapeutic doses (Sato et al., 2018).

Temocapril and Other ACE Inhibitors

Temocapril is an ACE inhibitor (see Chapter 30). Its active metabolite, temocaprilat, is excreted both in the bile and in the urine by the liver and kidney, respectively, whereas other ACE inhibitors are excreted mainly by the kidney. A special feature of *temocapril* among ACE inhibitors is that the plasma concentration of temocaprilat remains relatively unchanged even in patients with renal failure. However, the plasma AUC of *enalaprilat* and other ACE inhibitors is markedly increased in patients with renal disorders. Temocaprilat is a bisubstrate of the OATP family and MRP2, whereas other ACE inhibitors are not good substrates of MRP2 (although they are taken up into the liver by the OATP family). Taking these findings into consideration, the affinity for MRP2 may dominate in determining the biliary excretion of any series of ACE inhibitors. Drugs that are excreted into both the bile and urine to the same degree thus are expected to exhibit minimum interindividual differences in their pharmacokinetics.

Angiotensin II Receptor Antagonists

Angiotensin II receptor antagonists are used for the treatment of hypertension, acting on AT_1 receptors expressed in vascular smooth muscle, proximal tubule, adrenal medullary cells, and elsewhere. For most of these drugs, hepatic uptake and biliary excretion are important factors for their pharmacokinetics and pharmacological effects. *Telmisartan* is taken up into human hepatocytes in a saturable manner, predominantly via OATP1B3 (Ishiguro et al., 2006). On the other hand, both OATPs 1B1 and 1B3 are responsible for the hepatic uptake of *valsartan* and *olmesartan*, although the relative contributions of these transporters are unclear. Studies using doubly transfected cells with hepatic uptake transporters and biliary excretion transporters have clarified that MRP2 plays the most important role in the biliary excretion of *valsartan* and *olmesartan*.

Repaglinide, Nateglinide, and Glibenclamide

Repaglinide is a meglitinide analogue antidiabetic drug. Although it is eliminated almost completely by the metabolism mediated by CYPs 2C8 and 3A4, transporter-mediated hepatic uptake is one of the determinants of its elimination rate. In subjects with the OATP1B1 (*SLCO1B1*) 521CC genotype, a significant change in the pharmacokinetics of *repaglinide* was observed (Niemi et al., 2005). Genetic polymorphism in *SLCO1B1* 521T>C results in altered pharmacokinetics of *nateglinide* and *glibenclamide*, suggesting OATP1B1 is a determinant of their elimination, although they are subsequently metabolized by CYPs 2C9, 3A4, and others (Zhang et al., 2006b).

Renal Transporters

Organic Cation Transport

Structurally diverse organic cations are secreted in the proximal tubule. Many secreted organic cations are endogenous compounds (e.g., choline, *N*-methylnicotinamide, and DA), and renal secretion helps to eliminate excess concentrations of these substances. Another function of organic cation secretion is ridding the body of xenobiotics, including many positively charged drugs and their metabolites (e.g., *cimetidine, ranitidine, metformin, varenicline*, and *trospium*) and toxins from the environment (e.g., nicotine and paraquat). Organic cations that are secreted by the kidney may be either hydrophobic or hydrophilic. Hydrophilic organic drug cations generally have molecular weights less than 400 Da; a current model for their secretion in the proximal tubule of the nephron is shown in Figure 4–10 involving the transporters described next.

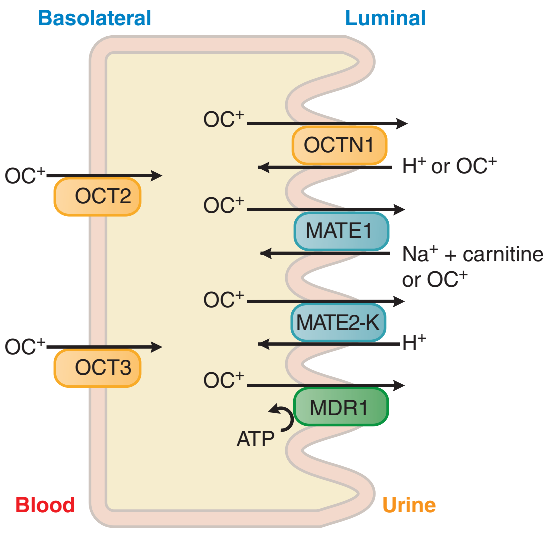

Figure 4–10 *Organic cation secretory transporters in the proximal tubule.* OC^+, organic cation. See text for details of the transporters pictured.

For the transepithelial flux of a compound (e.g., secretion), the compound must traverse two membranes sequentially, the basolateral membrane facing the blood side and the apical membrane facing the tubular lumen. Organic cations appear to cross the basolateral membrane in the human proximal tubule by two distinct transporters in the SLC family 22 (SCL22): OCT2 (*SLC22A2*) and OCT3 (*SLC22A3*). Organic cations are transported across this membrane down an electrochemical gradient.

Transport of organic cations from cell to tubular lumen across the apical membrane occurs through an electroneutral proton–organic cation exchange, which is mediated by transporters in the SLC47 family, which comprises members of the MATE family. Transporters in the MATE family, assigned to the apical membrane of the proximal tubule, appear to play a key role in moving hydrophilic organic cations from tubule cell to lumen. In addition, OCTNs, located on the apical membrane, appear to contribute to organic cation flux across the proximal tubule. In humans, these include *OCTN1* (SLC22A4) and *OCTN2* (SLC22A5). These bifunctional transporters are involved not only in organic cation secretion but also in carnitine reabsorption. In the reuptake mode, the transporters function as Na^+ cotransporters, relying on the inwardly driven Na^+ gradient created by Na^+/K^+-ATPase to move carnitine from tubular lumen to cell. In the secretory mode, the transporters appear to function as proton–organic cation exchangers. That is, protons move from tubular lumen to cell interior in exchange for organic cations, which move from cytosol to tubular lumen. The inwardly directed proton gradient (tubular lumen → cytosol) is maintained by transporters in the SLC9 family, which are Na^+/K^+ exchangers (NHEs, antiporters). Of the two steps involved in secretory transport, transport across the luminal membrane appears to be rate limiting.

OCT2 (SLC22A2). Human, mouse, and rat orthologs of OCT2 are expressed in abundance in human kidney and to some extent in neuronal tissue, such as choroid plexus. In the kidney, OCT2 is localized in the proximal and distal tubules and collecting ducts. In the proximal tubule, OCT2 is restricted to the basolateral membrane. OCT2-mediated transport of model organic cations MPP^+ (1-methyl-4-phenylpyridinium) and TEA (tetraethylammonium) is electrogenic, and both OCT2 and OCT1 can support organic cation–organic cation exchange. OCT2 generally accepts a wide array of monovalent organic cations with molecular weights below 400 Da. OCT2 is also present in neuronal tissues; however, monoamine neurotransmitters have low affinities for OCT2.

***OCT3* (SLC22A3).** The OCT3 gene is located in tandem with genes for OCT1 and OCT2 on chromosome 6. Tissue distribution studies suggest that human OCT3 is expressed in liver, kidney, intestine, placenta, skeletal muscle, and adipose tissue, although in the kidney it appears to be expressed in considerably less abundance than OCT2, and in the liver it is less abundant than OCT1. Like OCT1 and OCT2, OCT3 appears to support electrogenic potential-sensitive organic cation transport. OCT3 plays a role in both the renal elimination and the intestinal absorption of metformin.

***OCTN1* (SLC22A4).** OCTN1 seems to operate as an organic cation–proton exchanger. OCTN1-mediated influx of model organic cations is enhanced at alkaline pH, whereas efflux is increased by an inwardly directed proton gradient. OCTN1 contains a nucleotide-binding sequence motif, and transport of its substrates appears to be stimulated by cellular ATP. OCTN1 also can function as an organic cation–organic cation exchanger. OCTN1 functions as a bidirectional pH- and ATP-dependent transporter at the apical membrane in renal tubular epithelial cells and appears to be important in renal transport of *gabapentin*.

***OCTN2* (SLC22A5).** OCTN2 is a bifunctional transporter; it functions as both a Na^+-dependent carnitine transporter and a Na^+-independent OCT. OCTN2 transport of organic cations is sensitive to pH, suggesting that OCTN2 may function as an organic cation exchanger. The transport of L-carnitine by OCTN2 is a Na^+-dependent electrogenic process. Mutations in OCTN2 can result in insufficient renal reabsorption of carnitine and appear to be the cause of primary systemic carnitine deficiency (Tamai, 2013).

***MATE1 and MATE2-K* (SLC47A1, SLC47A2).** Multidrug and toxin extrusion family members MATE1 and MATE2-K interact with structurally diverse hydrophilic organic cations, including the antidiabetic drug *metformin*, the H_2 antagonist *cimetidine*, and the anticancer drug *topotecan*. In addition to cationic compounds, the transporters recognize some anions, including the antiviral agents *acyclovir* and *ganciclovir*. The zwitterions *cephalexin* and *cephradine* are specific substrates of MATE1. The herbicide paraquat, a bis-quaternary ammonium compound that is nephrotoxic in humans, is a high-affinity substrate of MATE1. Both MATE1 and MATE2-K have been localized to the apical membrane of the proximal tubule. MATE1, but not MATE2-K, is also expressed on the canalicular membrane of the hepatocyte. These transporters appear to be the long-searched-for organic cation–proton antiporters on the apical membrane of the proximal tubule; that is, an oppositely directed proton gradient can drive the movement of organic cations via MATE1 or MATE2-K. The antibiotics *levofloxacin* and *ciprofloxacin*, though potent inhibitors, are not translocated by either MATE1 or MATE2-K.

Polymorphisms of OCTs and MATEs. OCT1 exhibits the greatest number of amino acid polymorphisms, followed by OCT2 and then OCT3. Recent studies suggest that genetic variants of OCT1 and OCT2 are associated with alterations in the renal elimination and response to the antidiabetic drug metformin. MATEs have fewer amino acid polymorphisms; however, recent studies suggested that noncoding region variants of SLC47A1 and SLC47A2 are associated with variation in response to *metformin*.

Organic Anion Transport

As with organic cation transport, a primary function of organic anion secretion appears to be the removal of xenobiotics from the body. The candidate substrates are structurally diverse and include many weakly acidic drugs (e.g., *pravastatin, captopril*, PAH, and penicillins) and toxins (e.g., ochratoxin). OATs not only move both hydrophobic and hydrophilic anions but also may interact with cations and neutral compounds.

Figure 4–11 shows a current model for the transepithelial flux of organic anions in the proximal tubule. Two primary transporters on the basolateral membrane mediate the flux of organic anions from interstitial fluid to tubule cell: OAT1 (*SLC22A6*) and OAT3 (*SLC22A8*). Energetically, hydrophilic organic anions are transported across the basolateral membrane against an electrochemical gradient, exchanging with intracellular α-ketoglutarate, which moves down its concentration gradient from cytosol to blood. The outwardly directed gradient of α-ketoglutarate is maintained at least in part by a basolateral Na^+-dicarboxylate transporter (NaDC3), using the Na^+ gradient established by Na^+,K^+-ATPase. Transport of low-molecular-weight organic anions by the cloned transporters OAT1 and OAT3 can be driven by α-ketoglutarate; coupled transport of α-ketoglutarate and low-molecular-weight organic anions (e.g., PAH) occurs in isolated basolateral membrane vesicles. The molecular pharmacology and molecular biology of OATs have been reviewed (Srimaroeng et al., 2008).

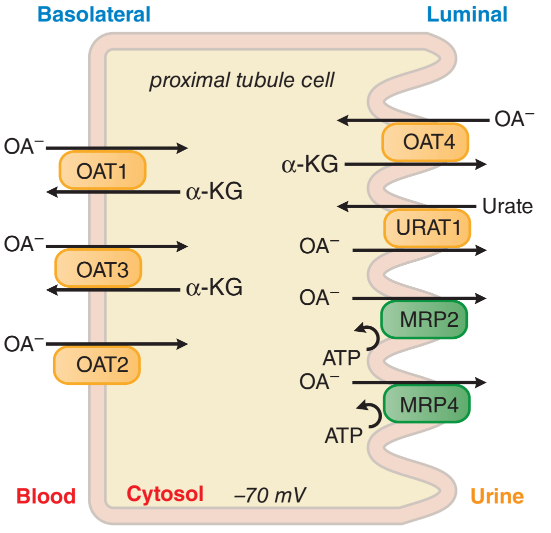

Figure 4–11 *Organic anion (OA) secretory transporters in the proximal tubule.* Two primary transporters on the basolateral membrane mediate the flux of OAs from interstitial fluid to tubule cell: OAT1 (*SLC22A6*) and OAT3 (*SLC22A8*). Hydrophilic OAs are transported across the basolateral membrane against an electrochemical gradient in exchange with intracellular α-ketoglutarate (α-KG), which moves down its concentration gradient from cytosol to blood. The outwardly directed gradient of α-KG is maintained at least in part by a basolateral Na^+-dicarboxylate uptake transporter (NaDC3). The Na^+ gradient that drives NaDC3 is maintained by Na^+,K^+-ATPase.

The mechanism responsible for the apical membrane transport of organic anions from tubule cell cytosol to tubular lumen remains controversial. OAT4 may serve as the luminal membrane transporter for organic anions, but the movement of substrates via this transporter can be driven by exchange with an α-ketoglutarate, suggesting that OAT4 may function in the reabsorptive, rather than secretory, flux of organic anions. NaPT1, originally cloned as a phosphate transporter, can support the low-affinity transport of hydrophilic organic anions such as PAH. MRP2 and MRP4, multidrug resistance transporters in the ABCC family, can interact with some organic anions and may actively pump them from tubule cell cytosol to tubular lumen.

***OAT1* (SLC22A6).** Mammalian isoforms of OAT1 are expressed primarily in the kidney, with some expression in brain and skeletal muscle. Immunohistochemical studies suggest that OAT1 is expressed on the basolateral membrane of the proximal tubule in humans, with highest expression in the middle segment, S2 (see Figure 25–1). Based on quantitative polymerase chain reaction, OAT1 is expressed at a third of the level of OAT3. OAT1 exhibits saturable transport of organic anions such as PAH. This transport is transstimulated by other organic anions, including α-ketoglutarate. Thus, the inside negative-potential difference drives the efflux of the dicarboxylate α-ketoglutarate, which in turn supports the influx of monocarboxylates such as PAH. Sex steroids regulate expression of OAT1 in the kidney. OAT1 generally transports low-molecular-weight organic anions, either endogenous (e.g., PGE_2 and urate) or exogenous (ingested drugs and toxins). Some neutral compounds are also transported by OAT1 at a lower affinity (e.g., *cimetidine*).

OAT2 (SLC22A7). OAT2 is present in both kidney and liver; renal OAT2 is localized to the basolateral membrane of the proximal tubule. OAT2 functions as a transporter for nucleotides, particularly guanine nucleotides such as cyclic GMP, for which it is a bidirectional facilitative transporter (Cropp et al., 2008). Cellular studies indicate that OAT2 functions in both the influx and the efflux of guanine nucleotides. OAT2 transports organic anions such as PAH and *methotrexate* with low affinity, PGE_2 with high affinity, and some neutral compounds but with lower affinity (e.g., *cimetidine*).

OAT3 (SLC22A8). Human OAT3 is confined to the basolateral membrane of the proximal tubule. This protein consists of two variants, one of which transports a wide variety of organic anions, including PAH, estrone sulfate, and many drugs (e.g., *pravastatin, cimetidine, 6-mercaptopurine*, and *methotrexate*) (Srimaroeng et al., 2008). The longer variant does not support transport. The specificities of OAT3 and OAT1 overlap, although kinetic parameters differ: Estrone sulfate is transported by both but by OAT3 with a much higher affinity; OAT1 transports the H_2 receptor antagonist *cimetidine* with high affinity.

OAT4 (SLC22A11). Human OAT4 is expressed in placenta and kidney (on the luminal membrane of the proximal tubule). Organic anion transport by OAT4 can be stimulated by transgradients of α-ketoglutarate, suggesting that OAT4 may be involved in the reabsorption of organic anions from tubular lumen into cell (see Figure 4–11). The specificity of OAT4 includes the model compounds estrone sulfate and PAH, as well as *zidovudine, tetracycline,* and *methotrexate*. Collectively, emerging studies suggest that OAT4 may be involved not in secretory flux of organic anions but in reabsorption instead.

Other Anion Transporters. URAT1 (*SLC22A12*) is a kidney-specific transporter confined to the apical membrane of the proximal tubule. URAT1 is primarily responsible for urate reabsorption, mediating electroneutral urate transport that can be transstimulated by Cl^- gradients. NPT1, Na^+-dependent phosphate transport protein 1 (SLC17A1), is expressed on the luminal membrane of the proximal tubule as well as in the brain. NPT1 transports PAH, *probenecid*, and *penicillin G*. It appears to be involved in organic anion efflux from tubule cell to lumen and interacts with uric acid. Figure 42–2 depicts renal urate transport.

MRP2 (*ABCC2*) is considered to be the primary transporter involved in efflux of many drug conjugates (e.g., GSH conjugates) across the canalicular membrane of the hepatocyte. MRP2 is also found on the apical membrane of the proximal tubule, where it is thought to play a role in the efflux of organic anions into the tubular lumen. In general, MRP2 transports larger, bulkier compounds than do most of the OATs in the SLC22 family. MRP4 (*ABCC4*), localized on the apical membrane of the proximal tubule, transports a wide array of conjugated anions, including glucuronides and GSH conjugates. MRP4 appears to interact with *methotrexate*, cyclic nucleotide analogues, and antiviral nucleoside analogues. BCRP (ABCG2) is localized to the apical membrane of the proximal tubule and duodenum and is involved in uric acid secretion and secretion of the XOIs *allopurinol* and *oxypurinol*.

Polymorphisms in OAT1 and OAT3 have been identified in ethnic human subpopulations (see https://www.pharmgkb.org). Notably, polymorphisms in ABCG2 have been associated with reduced response to *allopurinol* and *oxypurinol*.

Transporters and Pharmacodynamics: Drug Action in the Brain

Biogenic amine neurotransmitters are packaged in vesicles in presynaptic neurons, released in the synapse by fusion of the vesicles with the plasma membrane, and then taken back into the presynaptic neurons or postsynaptic cells (see Chapters 10 and 16). Plasma membrane transporters involved in the neuronal reuptake of the neurotransmitters and the regulation of their levels in the synaptic cleft belong to two major superfamilies, SLC1 and SLC6. Transporters in both families play roles in reuptake of GABA, glutamate, and the monoamine neurotransmitters NE, 5HT, and DA. These transporters may serve as pharmacologic targets for neuropsychiatric drugs. SLC6 family members localized in the brain and involved in the reuptake of neurotransmitters into presynaptic neurons include NET (*SLC6A2*), DAT (*SLC6A3*), SERT (*SLC6A4*), and several GATs (GAT1, GAT2, and GAT3). Each of these transporters appears to have 12 transmembrane (TM) regions and a large extracellular loop with glycosylation sites between TM3 and TM4.

SLC6 family members are secondary active transporters, depending on the Na^+ gradient to transport their substrates into cells. Cl^- is also required, although to a variable extent depending on the family member. Through their reuptake mechanisms, the neurotransmitter transporters in the SLC6A family regulate the concentrations and dwell times of neurotransmitters in the synaptic cleft. The extent of transmitter uptake also influences subsequent vesicular storage of transmitters, which occurs via transporters in the SLC17 (vesicular glutamate) and SLC18 (vesicular monoamine) families. Many of the transporters in the SLC6, SLC17, and SLC18 families are present in other tissues (e.g., intestine, kidney, and platelets) and may serve other roles. Further, the transporters can function in the reverse direction; that is, the transporters can export neurotransmitters in a Na^{2+}-independent fashion.

GABA Uptake: GAT1 (*SLC6A1*), GAT3 (*SLC6A11*), GAT2 (*SLC6A13*), and BGT1 (*SLC6A12*)

GAT1 is the most important GABA transporter in the brain, expressed in GABAergic neurons and found largely on presynaptic neurons. GAT1 is abundant in the neocortex, cerebellum, basal ganglia, brainstem, spinal cord, retina, and olfactory bulb. GAT3 is found only in the brain, largely in glial cells. GAT2 is found in peripheral tissues, including the kidney and liver, and within the CNS in the choroid plexus and meninges. Physiologically, GAT1 appears to be responsible for regulating the interaction of GABA at receptors. The presence of GAT2 in the choroid plexus and its absence in presynaptic neurons suggest that this transporter may play a primary role in maintaining the homeostasis of GABA in the CSF. GAT1 is the target of the antiepileptic drug *tiagabine* (a nipecotic acid derivative), which presumably acts to prolong the dwell time of GABA in the synaptic cleft of GABAergic neurons by inhibiting the reuptake of GABA. A fourth GAT, BGT1, occurs in extrasynaptic regions of the hippocampus and cortex (Madsen et al., 2011).

Catecholamine Uptake: NET (*SLC6A2*)

NET is found in central and peripheral nervous tissues as well as in adrenal chromaffin tissue. NET colocalizes with neuronal markers, consistent with a role in reuptake of monoamine neurotransmitters. NET provides reuptake of NE (and DA) into neurons, thereby limiting the synaptic dwell time of NE and terminating its actions, salvaging NE for subsequent repackaging. NET serves as a drug target for the antidepressant *desipramine*, other tricyclic antidepressants, and cocaine. NET is also a target for serotonin-norepinephrine reuptake inhibitor antidepressants (e.g., *duloxetine* and *venlafaxine*), which also target SERT (*SLC6A4*). Orthostatic intolerance, a rare familial disorder characterized by an abnormal blood pressure and heart rate response to changes in posture, has been associated with a mutation in NET.

Dopamine Uptake: DAT (*SLC6A3*)

DAT is located primarily in the brain in dopaminergic neurons. The primary function of DAT is the reuptake of DA, terminating its actions. Although present on presynaptic neurons at the neurosynaptic junction, DAT is also present in abundance along the neurons, away from the synaptic cleft. Physiologically, DAT is involved in functions attributed to the dopaminergic system, including mood, behavior, reward, and cognition. Drugs that interact with DAT include cocaine and its analogues, amphetamines, and the neurotoxin MPTP (methylphenyltetrahydropyridine).

Serotonin Uptake: SERT (*SLC6A4*)

SERT is responsible for the reuptake and clearance of 5HT in the brain. Like the other SLC6A family members, SERT transports its substrates in a Na^+-dependent fashion and is dependent on Cl^- and possibly on the countertransport of K^+. Substrates of SERT include 5HT, various tryptamine derivatives, and neurotoxins such as MDMA (ecstasy) and *fenfluramine*. SERT is the specific target of the SSRI antidepressants (e.g., *fluoxetine* and *paroxetine*) and one of several targets of tricyclic antidepressants (e.g., *amitriptyline*). Genetic variants of SERT have been associated with an array of behavioral and neurological disorders. The precise mechanism by which reduced activity of SERT, caused by either a genetic variant or an antidepressant, ultimately affects behavior, including depression, is not known.

Transporters and Pharmacodynamics: Antidiabetic Drug Action

Glucose requires transporters to cross plasma membranes. The homoeostatic mechanisms for glucose are controlled primarily by glucose transporters in the SLC2 (facilitated GLUT transporter family) and SLC5 (sodium glucose co-transporter family) families. Within the SLC family, SLC5A1, commonly known as SGLT1, is expressed in the intestine, where it mediates glucose absorption, and in the kidney, where it participates in reabsorption. SGLT2 (SLC5A2) is primarily expressed in the renal proximal tubule, where it plays an almost exclusive role in glucose reabsorption. In fact, patients with genetic mutations in SLC5A2 have profound glucosuria, whereas those with mutations in SLC5A1 have only mild glucosuria. These observations spurred the development of SGLT2 inhibitors, the gliflozins, in the treatment of type 2 diabetes. Through targeting of SGLT2, the gliflozins inhibit glucose reabsorption in the kidney, leading to reduced systemic blood levels of glucose in patients with type 2 diabetes. This inhibition effect is particularly beneficial in patients with type 2 diabetes, in whom SGLT2 expression in the kidney and reabsorption of filtered glucose are increased. Though the mechanisms are not completely understood, the inhibition of glucose reabsorption and, consequently, renal glucose metabolism appears to contribute to the ancillary benefits of gliflozins on diabetic nephropathy. SGLT2 inhibitors are also finding utility in the treatment of heart failure with reduced ejection fraction (see Chapter 33).

The Blood-Brain Barrier: A Pharmacological View

The CNS is well protected from circulating neurotransmitters, well supplied with necessary nutrients and ions, and able to exclude many toxins, bacteria, and xenobiotics. This careful set of conditions is achieved by a barrier called the BBB, the blood-brain barrier. Chapter 17 describes the BBB in considerable detail. From the viewpoint of membrane transport, a short introduction to the BBB follows below.

The BBB results from the specialized properties of the microvasculature of the CNS, which consists of endothelial cells that limit permeability and various neural and immune cells (Profaci et al., 2020). Functionally, the BBB is partly physical, partly a consequence of selective permeability (export of undesirable molecules and import of necessary molecules), and partly a consequence of the enzymatic destruction of certain permeants by enzymes in the barrier. There are some neurosensory and neurosecretory regions of the brain that lack the barrier: posterior pituitary, median eminence, area postrema, subfornical organ, subcommissural organ, and laminar terminalis.

The *physical part* of the BBB derives from the distinctive structure of the capillary endothelium in the brain and choroid plexus. Unlike the endothelial cells of peripheral microvasculature that have gaps between them that permit flow of water and small molecules to the interstitial space, endothelial cells in the BBB have tight junctions that limit paracellular flow and generally have very low rates of vesicular transport (transcytosis) compared to peripheral endothelium. Moreover, the luminal surface of CNS endothelium is covered by a glycocalyx, which is denser than the glycocalyx of peripheral microvasculature. This glycocalyx prevents large molecules from interacting with the endothelial cell itself. Further, the abluminal surface is wrapped by a basement membrane, pericytes, and the pseudopodial processes of astroglia. Lipophilic molecules and gases such as O_2 and CO_2 can readily diffuse across these layers from blood to brain. Hydrophilic molecules (nutrients, ions, charged molecules, many drugs) cannot cross these multiple membrane barriers by diffusion at sufficient rates.

Thus, the system relies on *selective permeability*. For instance, certain transporters in the two major superfamilies, ABC and SLC, are enriched in the endothelial cells of the BBB (Profaci et al., 2020). ABC transporters that are expressed in abundance on the luminal membrane include ABCB1 (MDR1) and ABCG2 (BCRP). These transporters (see Table 4–4) are involved in the efflux of numerous small molecules and endogenous molecules such as steroids, extruding their substrates across the luminal membrane of the brain capillary endothelial cells into the blood, thereby limiting penetration into the brain. Many transporters in the SLC superfamily are involved in nutrient flux, ensuring that the brain receives nutrients such as glucose (SLC2A1/GLUT1), amino acids (SLC7A1 and SLC7A5/LAT1), and folic acid (SLC19A1, RFC). Metabolic salvage products also cross the BBB by transporters in the SLC16 family (SLC16A1 and SLC16A2). Members of the SLC22 family including both organic anion transporters (SLC22A6 [OAT1] and SLC22A8 [OAT3]) and organic cation transporters (SLC22A1 [OCT1] and SLC22A3 [OCT3]) are expressed in the endothelial cells. There are receptor-mediated transport systems for ferritin and insulin, and there is a low level of transcytosis (caveolin-dependent vesicle trafficking). Thus, the selective permeability of the BBB, conferred through its complement of transporters, allows necessary physiological compounds to enter the brain while limiting the entry of various xenobiotics including many drugs.

There is a *metabolic barrier* for some compounds. For instance, circulating catecholamines are inactivated by MAO in the endothelial cells and endothelial MAO, and dopa decarboxylase (aromatic amino acid decarboxylase; see Chapter 10) metabolizes L-dopa to 3,4-dihydroxyphenylacetate (hence the necessity of including a dopa decarboxylase inhibitor when giving L-dopa to treat Parkinson's disease). The metabolic barrier enzyme γ-glutamyl transpeptidase cleaves LTC_4, the leukotriene mediator produced by the 5-lipoxygenase pathway (see Chapter 41), and other glutathione adducts.

What about drug molecules? Once they reach the systemic circulation, delivery to the general region of the brain is not a problem: The brain receives about 15% of cardiac output (see Table 2–2). What about crossing the BBB? Small drugs can diffuse across the BBB as a function of their lipid solubility (oil/water partition coefficient). Thus, anesthetics such as nitrous oxide and *thiopental* move readily across the BBB. Some drugs may resemble substrates that are transported into the brain (e.g., amino acids, nucleosides) and thereby gain entry. LAT1 (SLC7A5) is involved in the influx of several drugs, such as L-dopa and *gabapentin*, across the BBB. OAT1 and OAT3, which generally play a role in the efflux of drugs from the CSF, mediate the uptake of organic compounds such as β-lactam antibiotics, statins, and H_2 receptor antagonists. Charged and large drugs, on the other hand, generally do not penetrate so easily into the brain. The transport proteins, especially MDR1, BCRP, and MRP4, actively extrude many drugs; clearly, recognition by these transporters is a major disadvantage for a drug used to treat CNS disease. Further, there is ongoing research suggesting that MDR1 and BCRP expression levels in the BBB may be modulated in neurological diseases, which may represent an important pathological mechanism for certain neurodegenerative diseases.

There are methods of permeation under development, which largely consist of emerging developments in nanotechnology: nanoparticles and liposomes containing drugs, drugs adducted to ferritin, and development of drug forms with suitable lipophilicity. Basic biomedical research is advancing our understanding of the role of nuclear receptors in the regulation of drug transporters and enzymes in the BBB and of the development of the BBB and the interaction of its cellular and subcellular components to maintain barrier function (Profaci et al., 2020). Kim and Bynoe (2016) reported that activation of the adenosine A_{2A} receptor in an *in vitro* human brain endothelial barrier model reversibly decreased expression of Pgp (MDR1), suggesting that adenosine A_{2A} receptor agonists may be useful in modulating permeability of the BBB. Such studies and techniques may provide progress in putting the control of BBB permeability into the hands of physicians. Chapter 17 reviews developing strategies for providing regulated drug entry into the CNS.

The Extended Clearance Concept and Physiologically Based Pharmacokinetic (PBPK) Modeling

Based on the “extended clearance concept,” hepatic clearance consists of some intrinsic processes, such as hepatic uptake PS_1, backflux from hepatocytes to blood PS_2, hepatic metabolism CL_{met}, and biliary sequestration PS_3 (Figure 4–12) (Shitara et al., 2006, 2013).

The overall hepatic intrinsic clearance $CL_{int,all}$ is expressed as

$$CL_{int,all} = PS_1 \cdot \frac{CL_{met} + PS_3}{PS_2 + CL_{met} + PS_3} \qquad \text{(Equation 4–6)}$$

If the sum of the intrinsic clearance of metabolism and biliary sequestration is much larger than the backflux clearance [$PS_2 << (CL_{met} + PS_3)$], $CL_{int,all}$ approximates PS_1, and uptake is a rate-determining process of the overall hepatic intrinsic clearance. In general, many transporter substrates are efficiently excreted into bile or extensively metabolized rather than fluxed back into blood, so their uptake clearances often determine their overall intrinsic hepatic clearance. Assuming that an orally administered drug is completely absorbed from the small intestine and predominantly cleared by the liver, its blood AUC based on the “well-stirred model” can be described as

$$\mathrm{AUC}_{blood} = \frac{\mathrm{Dose}}{f_B \cdot CL_{int,all}} = \frac{\mathrm{Dose}}{f_B \cdot PS_1 \cdot \dfrac{CL_{met} + PS_3}{PS_2 + CL_{met} + PS_3}} \qquad \text{(Equation 4–7)}$$

where f_B represents the unbound fraction in blood.

The AUC_{liver} is described as (Shitara et al., 2013)

$$\begin{aligned}\mathrm{AUC}_{liver} &= \frac{PS_1}{PS_2 + CL_{met} + PS_3} \cdot \mathrm{AUC}_{blood} \\ &= \frac{PS_1}{PS_2 + CL_{met} + PS_3} \cdot \frac{\mathrm{Dose}}{f_B \cdot PS_1 \cdot \dfrac{CL_{met} + PS_3}{PS_2 + CL_{met} + PS_3}} \qquad \text{(Equation 4–8)} \\ &= \frac{\mathrm{Dose}}{f_B \cdot (CL_{met} + PS_3)}\end{aligned}$$

Equations 4–6 through 4–8 suggest that if the uptake clearance PS_1 is decreased, AUC_{blood} is increased in inverse proportion to PS_1, while AUC_{liver} is not affected. On the other hand, if drug uptake is a rate-determining process of the overall hepatic intrinsic clearance, the decrease in the function of metabolism or biliary sequestration causes the increase in AUC_{liver}, but not AUC_{blood}. Therefore, if the molecular targets of pharmacological effect and adverse effect induced by drugs are located inside and outside hepatocytes, respectively, as in the case of statins, decrease in the hepatic uptake clearance of drugs caused by drug-drug interaction or genetic polymorphism of transporters affects mainly adverse effect and not so much pharmacological effect.

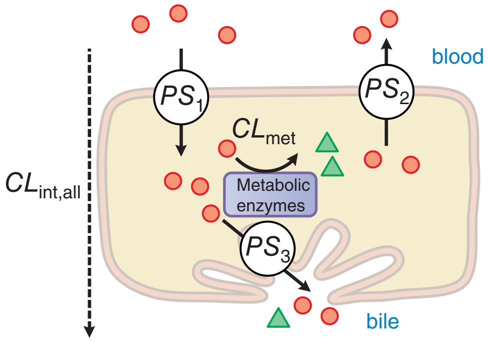

Figure 4–12 *Extended clearance concept: hepatic uptake, backflux into blood, metabolism, and efflux into bile.* The red circles represent parent drugs; the green triangles represent drug metabolites.

To simulate the impact of variations in the transporter activities on the systemic and liver exposure of a statin, which is eliminated mainly via OATP1B1 and MRP2, a PBPK model has been used (Jamei et al., 2014; Watanabe et al., 2009).

In a PBPK model, compartments representing actual tissues are connected by blood flow to predict the time course of drug disposition in the body. A PBPK model allows deep insight into the factors governing the systemic exposure and tissue distribution of drugs and simulates the impact of variations in physiological or drug-dependent parameters on drug disposition. Sensitivity analyses based on the PBPK model indicate that the variation in OATP1B1 activities will have a minimal impact on the therapeutic efficacy but a large impact on the side effect (myopathy) of *pravastatin*; the opposite will be true for variations in MRP2/BCRP activities: a large impact on efficacy, a small impact on the side effect (Watanabe et al., 2009) (Figure 4–13). Such characteristics have been demonstrated for some statins (e.g., *simvastatin* and *rosuvastatin*): Pharmacogenomic variation of OATP1B1 activity is associated with the risk of adverse reactions, whereas variation of biliary excretion and intestinal absorption mechanisms results in variation in therapeutic response (Chasman et al., 2012; SEARCH Collaborative Group, 2008).

Genetic Variation in Membrane Transporters: Implications for Clinical Drug Response

There are inherited defects in SLC transporters (see Table 4–2) and ABC transporters (see Table 4–3) that lead to disease. However, more common genetic polymorphisms in membrane transporters play roles in drug response and are yielding new insights into pharmacogenetics and pharmacology (see Chapter 7).

Clinical studies reveal that polymorphisms in three transporters, *ABCG2* (BCRP), *SLCO1B1* (OATP1B1), and *SLC22A1* (OCT1), contribute to interindividual variation in the response to many drugs and should be considered in drug development (Yee et al., 2018). For example, a common missense polymorphism (rs2231142), p.Gln141Lys in BCRP, is associated with increased response to *rosuvastatin*. BCRP provides for intestinal efflux of *rosuvastatin*. The p.Gln141Lys polymorphism results in reduced function of BCRP and hence reduced intestinal efflux of *rosuvastatin* and a concomitant increase in the bioavailability of the drug. The BCRP polymorphism has also been associated with poor response to *allopurinol* in several genome-wide association studies, albeit the mechanisms for the association are unclear. A common missense polymorphism (rs4149056) in OATP1B1, p.Val174Ala, is associated with elevated blood levels of statins and the resultant muscle toxicities of statins (see Chapter 37) and with interindividual variation in the pharmacokinetics of *methotrexate* and *ticagrelor* (Yee et al., 2018). Myriad studies indicate that multiple missense genetic variants in transporters in the SLC22A family are associated with interindividual variation in clearance and response to various drugs. For example, there are significant associations between SLC22A1 missense variants (reduced function variants) and systemic levels of the antidiabetic drug *metformin*, the antimigraine drug *sumatriptan*, the opiate analgesic *morphine*, and the antinausea drug ondansetron (Yee et al., 2018). Transporters in addition to OATP1B1, ABCG2, and OCT1 harbor polymorphisms that have been associated with *interindividual* variation in drug response (Rungtivasuwan et al., 2015). For example, the altered disposition of *tenofovir*, an antiviral agent, has been associated with polymorphisms in both ABCC2 (MRP2) and ABCC4 (MRP4).

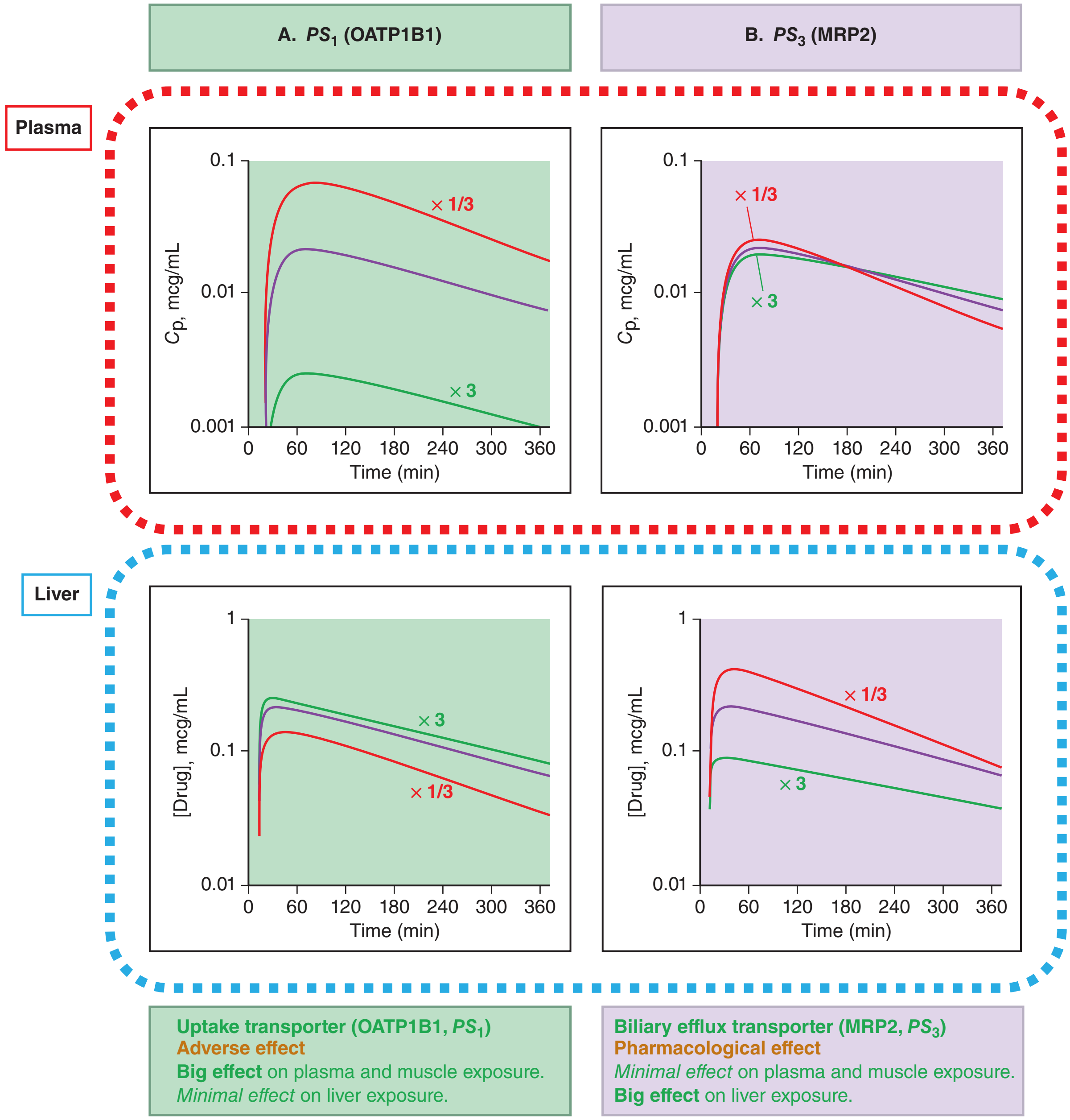

Figure 4–13 *Sensitivity analysis of the effect of functional changes of hepatic uptake clearance PS_1 (A) and biliary excretion clearance PS_3 (B) on the plasma and liver concentrations of pravastatin (Watanabe et al., 2009).* These sensitivity analyses were made based on the PBPK model, which connected five sequential liver compartments by blood flow so that this model can be used for drugs exhibiting transporter-mediated high clearance. Plasma and liver concentrations after oral administration (40 mg) were simulated with varying hepatic transport activities over a 1/3- to 3-fold range of the initial values.

Transporters in Regulatory Sciences

Because of their importance in drug disposition and action, transporters are major determinants of variation in therapeutic and adverse drug reactions. As a result, transporters may mediate drug-drug interactions that result in drug safety issues. A notable example is the interaction between *gemfibrozil* and *cerivastatin*. Gemfibrozil glucuronide formed in hepatocytes reduces the hepatic uptake and metabolism of *cerivastatin*; the result is a high C_p for *cerivastatin*. Elevated statin levels result in statin-induced myopathies, including rhabdomyolysis, a life-threatening adverse effect. This interaction resulted in the removal of *cerivastatin* from the market because of deaths due to rhabdomyolysis. The U.S. FDA has issued a clinical pharmacology guidance on performing drug-drug interaction studies during clinical drug development (FDA, 2020). The guidance presents information on how to use *in vitro* data for transporter studies to make decisions about whether to conduct a clinical drug-drug interaction study. For example, if a new molecular entity (NME) inhibits the *in vitro* transport of a canonical substrate of OCT2 and MATE1 at clinically relevant (unbound) concentrations, the guidance recommends that the sponsor consider performing a clinical drug-drug interaction study to determine whether the NME inhibits the renal clearance of an OCT2 and MATE1 substrate (e.g., metformin) *in vivo* (Nishiyama et al., 2019). On the other hand, if the NME neither inhibits OCT2 nor MATE1 mediated transport in *in vitro* assays at therapeutic concentrations, the guidance does not recommend a clinical study. Although only a handful of transporters (OATP1B1, OATP1B3, Pgp, BCRP, OCT2, MATE1, OAT1, and OAT3) are included in the FDA guidance, an increasing number of studies are being performed to identify and characterize transporters that mediate clinical drug-drug interactions.

Various endogenous biomarkers have been identified as *in vivo* probes for a number of hepatic and renal drug transporters. Available clinical data indicate the utility of endogenous biomarkers in phase I trials in facilitating subject phenotyping and drug-drug interaction

TABLE 4–5 ■ ENDOGENOUS BIOMARKERS TO ASSESS THE DRUG-DRUG INTERACTION RISK

TARGET TRANSPORTER	COMPOUND	FORMATION	PK PARAMETER
OATP1B1/OATP1B3	CP-I/III	Byproduct of heme biosynthesis	C_{max} or AUC
	GCDCA-S, GCDCA-G, CDCA-24G	Bile acid metabolites	C_{max} or AUC
	HDA and TDA	Fatty acid metabolites	C_{max} or AUC
	Unconjugated biluribins/conjugated bilirubins[a]	Heme metabolites	C_{max} or AUC
OAT1/OAT3	Taurine	Amino acid	CL_R
	6β-Hydroxycortisol	Cortisol metabolite	AUC, CL_R
	GCDCA-S	Bile acid metabolite	CL_R
	Pyridoxic acid	Vitamin B_6 metabolite	AUC, CL_R
OCT2/MATEs	Creatinine	Creatine metabolite	AUC, CL_R
	N-methylnicotinamide	Nicotinamide metabolite	CL_R
	N-methyladenosine	degradation product of tRNA,rRNA and snRNA	CL_R

PK, pharmacokinetics; CL_R, renal clearance; C_{max}, maximum concentration; CP, coproporphyrin; HDA, hexadecanedioate; TDA, tetradecanedioate ; GCDCA-S, glycochenodeoxycholate-3-sulfate, a surrogate endogenous substrate for OATP1B1.

[a] Quantified as total and direct bilirubins in the clinical studies.

Sources: Chu et al., 2018; Miyake et al., 2021; Rodrigues et al., 2018.

assessment (Table 4–5) (Chu et al., 2018; Rodrigues et al., 2018). The use of such endogenous biomarkers offers benefits in downstream planning of clinical studies. The two most commonly used endogenous biomarkers are coproporphyrin-I for OATP1B-mediated hepatic uptake and *N*-methylnicotinamide for the renal uptake and excretion transporters (OCT2/MATEs) (Chu et al., 2018; Rodrigues et al., 2018). A useful PBPK model (Yoshikado et al., 2018) and a population pharmacokinetic model (Barnett et al., 2018) for the OATP1B biomarker (coproporphyrin-I) have been proposed. The models support the translation of the effect of a new chemical entity on changes in plasma coproporphyrin-I level to that on clinically used drugs (e.g., statins). Thus, the modeling can complement existing OATP1B-mediated drug-drug interaction risk assessment approaches based on regulatory agency guidelines.

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Chapter 5

Drug Metabolism

Frank J. Gonzalez and Michael Coughtrie

COPING WITH XENOBIOTICS

THE PHASES OF DRUG METABOLISM

SITES OF DRUG METABOLISM

PHASE 1 REACTIONS
- CYPs: The Cytochrome P450 Superfamily
- Flavin-Containing Monooxygenases
- Hydrolytic Enzymes

PHASE 2 REACTIONS: CONJUGATING ENZYMES
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- Sulfation
- Glutathione Conjugation
- *N*-Acetylation
- Methylation

ROLE OF XENOBIOTIC METABOLISM IN SAFE AND EFFECTIVE USE OF DRUGS

INDUCTION OF DRUG METABOLISM
- The Aryl Hydrocarbon Receptor
- Type 2 Nuclear Receptors

ROLE OF DRUG METABOLISM IN DRUG DEVELOPMENT

ANIMAL MODELS FOR PRECLINICAL DRUG DEVELOPMENT

Coping With Xenobiotics

Humans come into contact with thousands of foreign chemicals or xenobiotics (substances foreign to the body) through diet and exposure to environmental contaminants. Fortunately, humans have developed a means to rapidly eliminate xenobiotics so that they do not accumulate in the tissues and cause harm. Plants are a common source of dietary xenobiotics, providing many structurally diverse chemicals, some of which are associated with pigment production and others that are toxins (called *phytoalexins*) that protect plants against predators. Poisonous mushrooms are a common example: They have many toxins that are lethal to mammals, including amanitin, gyromitrin, orellanine, muscarine, ibotenic acid, muscimol, psilocybin, and coprine. Animals must be able to metabolize and eliminate such chemicals to consume vegetation. While humans can now choose their dietary sources, a typical animal does not have this luxury and as a result is subject to its environment and the vegetation that exists in that environment. Thus, the ability to metabolize unusual chemicals in plants and other food sources is critical for adaptation to a changing environment and ultimately the survival of animals.

Enzymes that metabolize xenobiotics have historically been called drug-metabolizing enzymes by pharmacologists; however, these enzymes are involved in the metabolism of many foreign chemicals to which humans are exposed and are more appropriately called *xenobiotic-metabolizing enzymes*. Myriad diverse enzymes have evolved in animals to metabolize foreign chemicals. Dietary differences amongst species during the course of evolution could account for the marked species variation in the complexity of the xenobiotic-metabolizing enzymes. Additional diversity within these enzyme systems has also derived from the necessity to "detoxify" a host of endogenous chemicals that would otherwise prove harmful to the organism, such as bilirubin, steroid hormones, and catecholamines. Many of these endogenous compounds are detoxified by the same or closely related xenobiotic-metabolizing enzymes.

Drugs are xenobiotics, and the capacity to metabolize and clear drugs involves the same enzymatic pathways and transport systems that are used for normal metabolism of dietary constituents. Indeed, many drugs are derived from chemicals found in plants, some of which have been used in traditional medicines for thousands of years. Of the prescription drugs in use today for cancer treatment, some are also derived from plants (see Chapters 69 and 73); investigating folkloric claims led to the discovery of many drugs. The capacity to metabolize xenobiotics, although largely beneficial, has made development of drugs more time consuming and costly due in part to:

- Species differences in expression of enzymes that metabolize drugs and thereby limit the utility of animal models to predict drug effects in humans
- Interindividual variations in the capacity of humans to metabolize drugs
- Drug-drug interactions involving xenobiotic-metabolizing enzymes
- Metabolic activation of chemicals to toxic and carcinogenic derivatives

Today, most xenobiotics to which humans are exposed come from sources that include environmental pollution, food additives, cosmetic products, agrochemicals, processed foods, and drugs.

In general, most xenobiotics are hydrophobic chemicals; in the absence of metabolism, these would not be efficiently eliminated and thus would accumulate in the body, potentially resulting in toxicity. With few exceptions, all xenobiotics are subjected to one or multiple enzymatic pathways that constitute *phase 1 oxidation* and *phase 2 conjugation*. As a general paradigm, metabolism serves to convert these hydrophobic chemicals into more hydrophilic derivatives that can easily be eliminated from the body through the urine or the bile.

To enter cells and reach their sites of action, drugs generally must possess physical properties that allow them to move down a concentration gradient and across cell membranes. Many drugs are hydrophobic, a property that allows entry via diffusion across lipid bilayers into the systemic circulation and then into cells. With some compounds, transporters on the plasma membrane facilitate entry (see Chapter 4). Hydrophobic drugs are difficult to eliminate because, in the absence of metabolism, they accumulate in fat and cellular phospholipid bilayers. The xenobiotic-metabolizing enzymes convert drugs and other xenobiotics into derivatives that are more hydrophilic and thus easily eliminated via excretion into the aqueous compartments of the tissues and ultimately into the urine. High-molecular-weight drugs are preferentially eliminated via the bile into the intestine and feces.

Abbreviations

ADR: adverse drug reaction
AHR: aryl hydrocarbon receptor
AUC: area under the plasma concentration–time curve
CAR: constitutive androstane receptor
CYP: cytochrome P450
EH: epoxide hydrolase
ER: endoplasmic reticulum
FMO: flavin-containing monooxygenase
GI: gastrointestinal
GSH and GSSG: reduced and oxidized glutathione
GST: glutathione-*S*-transferase
HGPRT: hypoxanthine guanine phosphoribosyl transferase
HIF: hypoxia-inducible factor
HIV: human immunodeficiency virus
INH: isonicotinic acid hydrazide (isoniazid)
MAPK: mitogen-activated protein kinase
mEH: microsomal epoxide hydrolase
6-MP: 6-mercaptopurine
MT: methyltransferase
NADPH: nicotinamide adenine dinucleotide phosphate
NAPQI: *N*-acetyl-*p*-benzoquinone imine
NAT: *N*-acetyltransferase
PAPS: 3′-phosphoadenosine-5′-phosphosulfate
PPAR: peroxisome proliferator–activated receptor
PXR: pregnane X receptor
SULT: sulfotransferase
TPMT: thiopurine methyltransferase
UDP-GA: uridine diphosphate–glucuronic acid
UGT: uridine diphosphate–glucuronosyltransferase

Metabolism of a drug can begin even before a drug is absorbed: Gut bacteria represent the first metabolic interface between orally administered drugs and the body (see Chapter 6). The microbiome of the gastrointestinal (GI) tract can metabolize xenobiotics; interindividual differences in composition of the gut flora could influence drug action and contribute to differences in drug response. Indeed, diurnal oscillations in GI bacteria and their metabolic capacity, superposed on host clock gene oscillations, appear to affect drug disposition and effect (Fitzgerald et al., 2015).

The process of drug metabolism that leads to elimination also plays a major role in diminishing the biological activity of a drug. For example, *(S)-phenytoin*, an anticonvulsant used in the treatment of epilepsy, is virtually insoluble in water. Metabolism by the phase 1 CYPs followed by phase 2 UGTs (uridine diphosphate–glucuronosyltransferases) produces a metabolite that is highly water soluble and readily eliminated from the body (Figure 5–1). Metabolism also terminates the biological activity of *(S)-phenytoin*. Because conjugates are generally hydrophilic, elimination via the bile or urine is dependent on the actions of many efflux transporters to facilitate transmembrane passage (see Chapter 4).

While xenobiotic-metabolizing enzymes facilitate the elimination of chemicals from the body, paradoxically these same enzymes can also convert certain chemicals to highly reactive, toxic, and carcinogenic metabolites. This occurs when an unstable intermediate is formed that has reactivity toward other compounds in the cell. Chemicals that can be converted by xenobiotic metabolism to cancer-causing derivatives are called carcinogens. Depending on the structure of the chemical substrate, xenobiotic-metabolizing enzymes can produce electrophilic metabolites that react with nucleophilic cellular macromolecules such as DNA, RNA, and protein. This can cause cell death and organ toxicity. Most drugs and other xenobiotics that cause hepatotoxicity damage mitochondria, leading to hepatocyte death. Reaction of these electrophiles with DNA can sometimes result in cancer through the mutation of oncogenes or tumor suppressor genes. It is generally believed that most human cancers are due to exposure to chemical carcinogens.

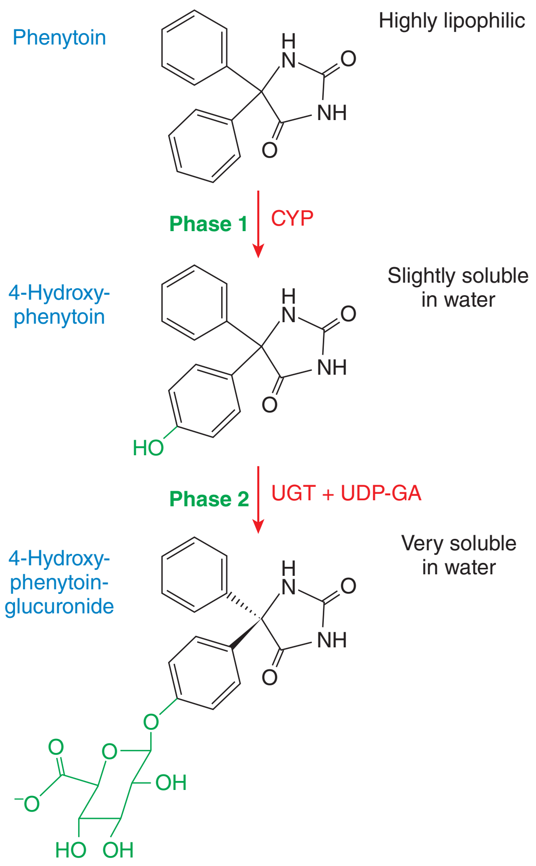


Figure 5–1 *Metabolism of phenytoin.* In phase 1, CYP facilitates 4-hydroxylation of *phenytoin* to yield HPPH. In phase 2, the hydroxy group serves as a substrate for UGT, which conjugates a molecule of glucuronic acid using UDP-GA as a cofactor. Together, phase 1 and phase 2 reactions convert a very hydrophobic molecule to a larger hydrophilic derivative that is eliminated via the bile. HPPH, 5-(-4-hydroxyphenyl)-5-phenylhydantoin.

This potential for carcinogenic activity makes testing the safety of drug candidates vitally important. Testing for cancer-causing potential is particularly critical for drugs that will be used for the treatment of chronic diseases. Because each species has evolved a unique combination of xenobiotic-metabolizing enzymes, nonprimate models (mostly rodents) cannot be solely used for testing the safety of new drug candidates targeted for human diseases. Nevertheless, testing in rodent models (e.g., mice and rats) can usually identify potential carcinogens. Fortunately, there are no instances of drugs that test negative in rodents but cause cancer in humans, albeit some chemicals that cause cancer in rodents are not associated with human cancer. Many cytotoxic drugs used to treat cancer also have the potential to cause cancer; this risk is minimized by their acute, rather than chronic, use in cancer therapy.

The Phases of Drug Metabolism

Xenobiotic-metabolizing enzymes have historically been categorized as

- *phase 1 reactions*, which include oxidation, reduction, and hydrolytic reactions; or
- *phase 2 reactions*, in which enzymes catalyze conjugation of the substrate (the phase 1 product) with a second molecule.

The *phase 1 enzymes* lead to the introduction of functional groups, such as –OH, –COOH, –SH, –O–, or NH_2 (Table 5–1). The addition of functional groups does little to increase the water solubility of the drug

TABLE 5–1 ■ XENOBIOTIC-METABOLIZING ENZYMES

ENZYMES	REACTIONS
Phase 1 enzymes (CYPs, FMOs, EHs)	
Cytochrome P450s (P450 or CYP)	C and O oxidation, dealkylation, others
Flavin-containing monooxygenases (FMOs)	N, S, and P oxidation
Epoxide hydrolases (EHs)	Hydrolysis of epoxides
Phase 2 "transferases"	
Sulfotransferases (SULT)	Addition of sulfate
UDP-glucuronosyltransferases (UGTs)	Addition of glucuronic acid
Glutathione-*S*-transferases (GSTs)	Addition of glutathione
N-Acetyltransferases (NATs)	Addition of acetyl group
Methyltransferases (MTs)	Addition of methyl group
Other enzymes	
Alcohol dehydrogenases	Reduction of alcohols
Aldehyde dehydrogenases	Reduction of aldehydes
NADPH-quinone oxidoreductase (NQO)	Reduction of quinones

mEH and sEH, microsomal and soluble epoxide hydrolase, respectively; NADPH, reduced nicotinamide adenine dinucleotide phosphate; UDP, uridine diphosphate.

but can dramatically alter the biological properties of the drug. Reactions carried out by phase 1 enzymes usually lead to the inactivation of a drug. However, in certain instances, metabolism, usually the hydrolysis of an ester or amide linkage, results in bioactivation of a drug. Inactive drugs that undergo metabolism to become active drugs are called prodrugs (Rautio et al., 2018). Prodrugs can be activated by gut bacterial enzymes, enzymes in blood, or intracellular enzymes such as cytochromes P450 (CYPs), flavin-containing monooxygenases (FMOs), and hydrolytic enzymes. Examples of prodrugs bioactivated by CYPs are the antitumor drug *cyclophosphamide*, which is bioactivated to a cell-killing electrophilic derivative (see Chapter 70), and the anti-thrombotic agent *clopidogrel*, which is activated to 2-oxo-clopidogrel and further metabolized to an irreversible inhibitor of platelet ADP $P2Y_{12}$ receptors. Through conjugation reactions, *phase 2 enzymes* produce metabolites with improved water solubility, a property that facilitates drug elimination from the tissue, normally via efflux transporters described in Chapter 4. Thus, in general, phase 1 reactions result in biological inactivation of a drug, and phase 2 reactions facilitate the drug elimination and the inactivation of electrophilic and potentially toxic metabolites produced by oxidation.

Superfamilies of evolutionarily related enzymes and receptors are common in the mammalian genome. Superfamilies are designated based on similar structure and function. Within the superfamilies are more closely related subfamilies of genes that are usually co-localized on a chromosome. The enzyme systems responsible for drug metabolism are good examples. The phase 1 oxidation reactions are carried out by CYPs, FMOs, and epoxide hydrolases (EHs). The CYPs and FMOs are composed of superfamilies and subfamilies encoded by multiple genes. The phase 2 enzymes include several superfamilies of conjugating enzymes. Among the more important are the glutathione-*S*-transferase (GSTs), UGTs, sulfotransferases (SULTs), *N*-acetyltransferases (NATs), and methyltransferases (MTs) (Table 5–1).

The conjugation reactions of the phase 2 enzymes usually require the substrate to have oxygen (hydroxyl or epoxide groups), nitrogen, or sulfur atoms that serve as acceptor sites for a hydrophilic moiety, such as glutathione, glucuronic acid, sulfate, or an acetyl group, that can be covalently conjugated to an acceptor site on the substrate. The metabolism of phenytoin (Figure 5–1) illustrates the two-phase metabolic sequence. The oxidation by phase 1 enzymes either adds or exposes a functional group, permitting the products of phase 1 metabolism to serve as substrates for the phase 2 conjugating or synthetic enzymes. In the case of UGTs, glucuronic acid is delivered to the functional group, forming a glucuronide metabolite that is more water soluble and is targeted for excretion in the urine or bile. When the substrate is a drug, these reactions usually convert the original drug to a form that is not able to bind to its target receptor, thus attenuating the biological response to the drug.

Sites of Drug Metabolism

Xenobiotic-metabolizing enzymes are found in most tissues in the body, with the highest levels located in the GI tract (liver, small and large intestines). The small intestine plays a crucial role in drug metabolism. Orally administered drugs are first exposed to the GI flora, which can metabolize some drugs (see Chapter 6). During absorption, drugs are exposed to xenobiotic-metabolizing enzymes in the epithelial cells of the GI tract; this is the initial site of drug metabolism. Once absorbed, drugs enter the portal circulation and reach the liver, where they may be extensively metabolized (the "first-pass effect") before entering the general circulation. The liver is the major "metabolic clearinghouse" for both endogenous chemicals (e.g., cholesterol, steroid hormones, fatty acids, and proteins) and xenobiotics. Subsequent passes through the liver result in more metabolism of the parent drug until the agent is eliminated. Drugs that are poorly metabolized remain in the body for longer periods of time, and their pharmacokinetic profiles show much longer elimination half-lives than drugs that are rapidly metabolized.

During drug development, desirable compounds are those that have a favorable pharmacokinetic profile and are eliminated over the course of 24 h after administration. This allows the use of daily single dosing. If a compound with a favorable efficacy cannot be modified to improve its pharmacokinetic profile, twice-a-day or even three-times-a-day dosing needs to be used. Other organs that contain significant xenobiotic-metabolizing enzymes include tissues of the nasal mucosa and lung, which play important roles in the metabolism of drugs that are administered through aerosol sprays. These tissues are also the first line of contact with hazardous substances that are airborne.

Within the cell, xenobiotic-metabolizing enzymes are found in the intracellular membranes and in the cytosol. The phase 1 CYPs, FMOs, and EHs and some phase 2 conjugating enzymes, notably the UGTs, are all located in the endoplasmic reticulum (ER) of the cell (Figure 5–2). The ER consists of phospholipid bilayers organized as tubes and sheets throughout the cytoplasm. This network, which is very extensive in liver hepatocytes where most xenobiotic metabolism occurs, has an inner lumen that is physically distinct from the rest of the cytosolic components of the cell and has connections to the plasma membrane and nuclear envelope. This membrane localization is ideally suited for the metabolic function of these enzymes: Hydrophobic molecules enter the cell and become embedded in the lipid bilayer, where they come into direct contact with the phase 1 enzymes. Once subjected to oxidation, drugs can be directly conjugated by the UGTs (in the lumen of the endoplasmic reticulum) or by the cytosolic transferases, such as GSTs and SULTs. The UGT co-substrate UDP-GA (uridine diphosphate–glucuronic acid) must be transported in the lumen of the ER, from which the glucuronide conjugates must be exported. After metabolism in the liver, metabolites are transported across the plasma membrane into the bloodstream and eliminated via the kidney or transported into the bile via the bile canaliculus, from which they are deposited in the gut and eliminated in the feces (as shown for the topoisomerase inhibitor SN-38 in Figure 5–9). Whether a compound is targeted to the kidneys or to the bile depends in part on its molecular weight, with high-molecular-weight compounds preferentially eliminated through the bile, and also on the substrate specificities of the membrane transporters involved.

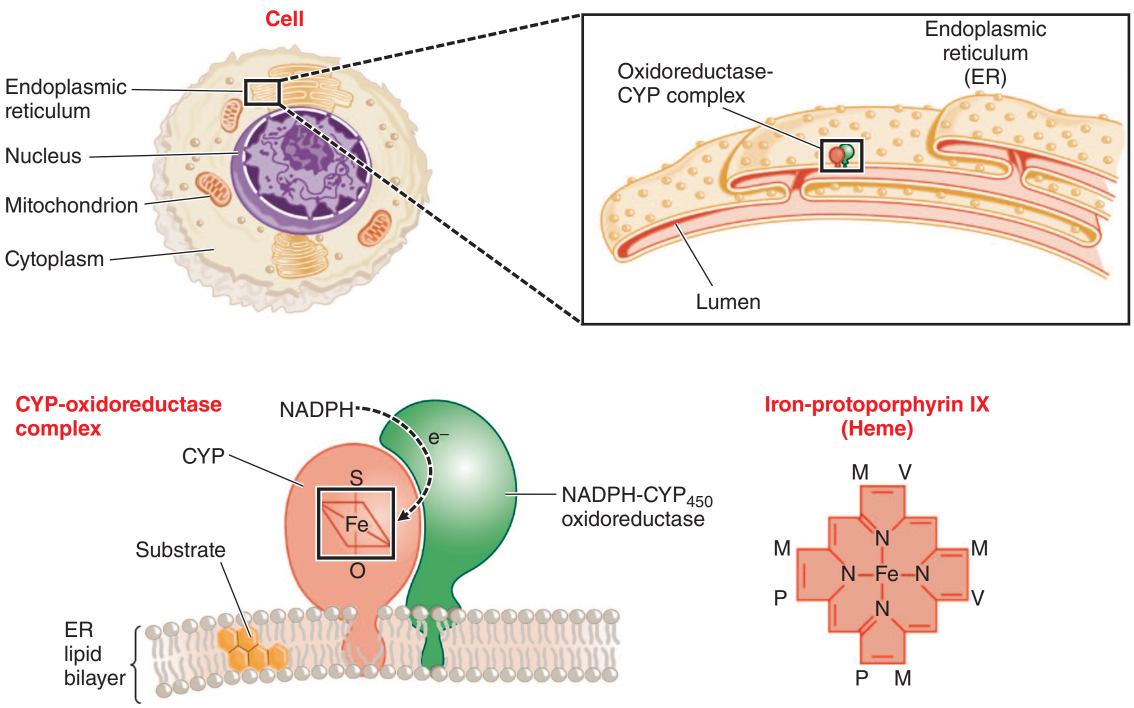

Cell
Endoplasmic reticulum
Nucleus
Mitochondrion
Cytoplasm
Oxidoreductase-CYP complex
Endoplasmic reticulum (ER)
Lumen
CYP-oxidoreductase complex
NADPH
e⁻
CYP
S
Fe
O
Substrate
NADPH-CYP450 oxidoreductase
ER lipid bilayer
Iron-protoporphyrin IX (Heme)
M
V
M
M
N
N
Fe
N
P
V
N
P
M

Figure 5–2 *Location of CYPs in the cell.* Increasingly microscopic levels of detail are shown, sequentially expanding the areas within the black boxes. CYPs are embedded in the phospholipid bilayer of the ER. Most of the enzyme is located on the cytosolic surface of the ER. A second enzyme, NADPH-CYP oxidoreductase, transfers electrons to the CYP where it can, in the presence of O_2, oxidize xenobiotic substrates, many of which are hydrophobic and dissolved in the ER. A single NADPH-CYP oxidoreductase species transfers electrons to all CYP isoforms in the ER. Each CYP contains a molecule of iron-protoporphyrin IX that functions to bind and activate O_2. Substituents on the porphyrin ring are methyl (M), propionyl (P), and vinyl (V) groups.

Phase 1 Reactions

CYPs: The Cytochrome P450 Superfamily

The CYPs are a superfamily of enzymes, each of which contains a molecule of heme bound noncovalently to the polypeptide chain (Figure 5–2). Many enzymes that use O_2 as a substrate for their reactions contain heme, and heme is the oxygen-binding moiety in hemoglobin. Heme contains one atom of iron in a hydrocarbon cage that functions to bind O_2 in the active site of the CYP as part of the catalytic cycle of these enzymes. CYPs use O_2, plus H^+ derived from the cofactor-reduced NADPH, to carry out the oxidation of substrates. The H^+ is supplied through the enzyme NADPH-CYP oxidoreductase. Metabolism of a substrate by a CYP consumes one molecule of O_2 and produces an oxidized substrate and a molecule of H_2O as a by-product. However, for most CYPs, depending on the nature of the substrate, the reaction is "uncoupled," consuming more O_2 than substrate metabolized and producing what is called activated oxygen or O_2^-. The O_2^- is usually converted to water by the enzyme superoxide dismutase. However, when elevated by excess oxidation and metabolism of certain substrates, O_2^-, also called a reactive oxygen species (ROS), can cause oxidative stress that is detrimental to cellular physiology and is associated with diseases such as hepatic cirrhosis.

Substrate Specificity and Promiscuity Among CYPs

Among the diverse reactions carried out by mammalian CYPs are *N*-dealkylation, *O*-dealkylation, aromatic hydroxylation, *N*-oxidation, *S*-oxidation, deamination, and dehalogenation (Table 5–2). Fifty-seven individual CYPs have been identified in humans. Metabolism of dietary and xenobiotic chemicals is not the only role that CYPs play. Other members of the CYP family are involved in the synthesis of endogenous compounds such as steroids, fatty acid signaling molecules (e.g., epoxyeicosatrienoic acids; see Figure 5–5), and bile acids.

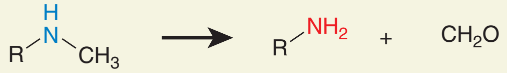
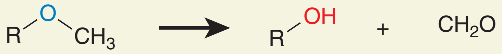
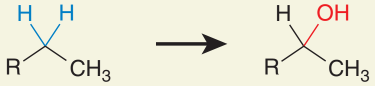
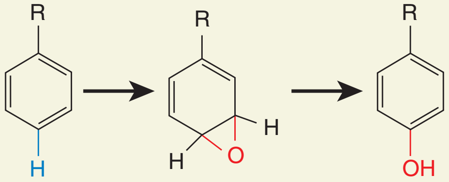
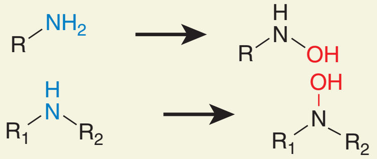
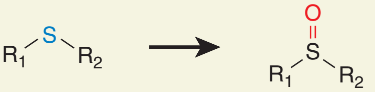
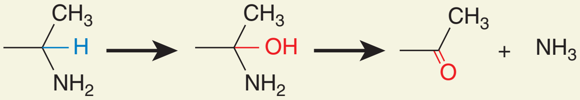
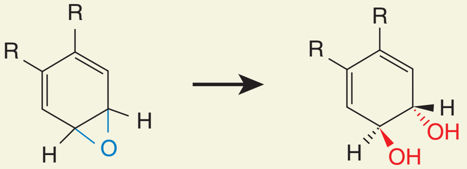
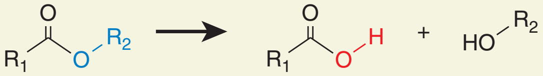
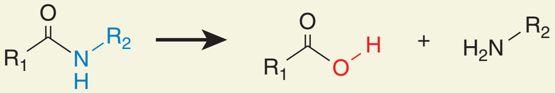
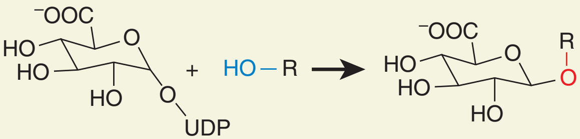
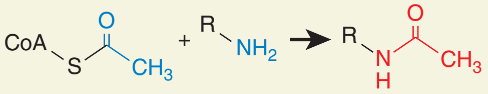
The CYPs that catalyze steroid and bile acid synthesis have specific substrate preferences. For example, the CYP that produces estrogen from testosterone, CYP19 or aromatase, can metabolize only testosterone or androstenedione and does not metabolize xenobiotics. Specific inhibitors for aromatase, such as *anastrozole*, are used in the treatment of estrogen-dependent tumors (see Chapters 48 and 73).

The synthesis of bile acids from cholesterol occurs in the liver, where, subsequent to CYP-catalyzed oxidation, the bile acids are conjugated with amino acids and transported through the bile duct and gallbladder into the small intestine. Bile acids are emulsifiers that facilitate the elimination of high-molecular-weight conjugated drugs from the liver and serve to enhance the absorption of fatty acids and vitamins from the diet. More than 90% of bile acids are reabsorbed by the gut and transported back to hepatocytes in a tightly regulated process of enterohepatic transport (Gonzalez, 2012). Similar to the steroid biosynthetic CYPs, CYPs involved in bile acid production have strict substrate requirements and do not participate in xenobiotic or drug metabolism.

In contrast to CYPs that carry out highly specific reactions, the xenobiotic-metabolizing CYPs are promiscuous in their capacity to bind and metabolize multiple substrates (Table 5–2). As a group, the CYPs that carry out xenobiotic metabolism have the capacity to metabolize a large number of structurally diverse chemicals. This is due to the multiplicity of CYPs and their isoforms and to the capacity of a single CYP to metabolize many structurally diverse chemicals. A single compound may also be metabolized, albeit at different rates, by different CYPs. In addition, CYPs can metabolize a single compound at different positions on the molecule, and two CYPs can carry out the metabolism of a single compound at distinct oxidation sites.

This promiscuity of CYPs is due to large and fluid substrate-binding sites in CYPs, a feature that sacrifices enzymatic turnover rates for broad specificity: CYPs metabolize substrates at a fraction of the rate of more typical enzymes involved in intermediary metabolism and mitochondrial electron transfer. As a result, drugs generally have half-lives on the order of 3 to 30 h, while endogenous compounds have half-lives on the order of seconds or minutes when exogenously administered (e.g., catecholamines and glucose). Even though CYPs have slow catalytic rates, their activities are sufficient to metabolize drugs that are administered at high concentrations.

TABLE 5–2 ■ MAJOR REACTIONS INVOLVED IN DRUG METABOLISM

	REACTION	EXAMPLES
I. Oxidative reactions		
N-Dealkylation		Imipramine, diazepam, codeine, erythromycin, morphine, tamoxifen, theophylline, caffeine
O-Dealkylation		Codeine, indomethacin, dextromethorphan
Aliphatic hydroxylation		Tolbutamide, ibuprofen, phenobarbital, meprobamate, cyclosporine, midazolam
Aromatic hydroxylation		Phenytoin, phenobarbital, propanolol, ethinyl estradiol, amphetamine, warfarin
N-Oxidation		Chlorpheniramine, dapsone, meperidine
S-Oxidation		Cimetidine, chlorpromazine, thioridazine omeprazole
Deamination		Diazepam, amphetamine
II. Hydrolysis reactions		
		Carbamazepine (see Figure 5-4)
		Procaine, aspirin, clofibrate, meperidine, enalapril, cocaine
		Lidocaine, procainamide, indomethacin
III. Conjugation reactions		
Glucuronidation		Acetaminophen, morphine, oxazepam, lorazepam
Sulfation	$\mathrm{PAPS} + \mathrm{HO{-}R} \longrightarrow \mathrm{HO_3S{-}O{-}R} + \mathrm{PAP}$	Acetaminophen, steroids, methyldopa
Acetylation		Sulfonamides, isoniazid, dapsone, clonazepam
Methylation*	$\mathrm{R{-}OH} + \mathrm{AdoMet} \longrightarrow \mathrm{R{-}O{-}CH_3} + \mathrm{AdoHomCys}$	L-dopa, methyldopa, mercaptopurine, captopril
Glutathionylation	$\mathrm{GSH} + \mathrm{R} \longrightarrow \mathrm{R{-}GSH}$	Adriamycin, fosfomycin, busulfan

PAPS, 3′-phosphoadenosine-5′-phosphosulfate; PAP, 3′-phosphoadenosine-5′-phosphate; AdoMet, S-adenosylmethionine; AdoHomCys, S-adenosylhomocysteine.

*also for [illegible] S- RN[illegible]

The metabolism of a particular drug can often be ascribed to the activity of a single CYP. However, when two coadministered drugs are both metabolized by a single CYP, they compete for binding to the enzyme's active site. This can result in the inhibition of metabolism of one or both of the drugs, leading to elevated plasma drug levels. If there is a narrow therapeutic index for the drugs, the elevated serum levels may elicit unwanted toxicities. Drug-drug interactions are among the leading causes of adverse drug reactions (ADRs). Prescription drug labeling provides warnings about drug interactions, including identity of the CYPs most prominently involved in the metabolism of a particular drug. Preclinical studies must take into consideration any drug on the market that might be coadministered with the drug under development. For example, treatment of syndrome X (metabolic syndrome) would involve statins and antidiabetic drugs. If these drugs are metabolized by the same CYP, drug interactions could be predicted and adverse responses avoided by suitable drug choices or dosage adjustments.

The Naming of CYPs

The CYPs, responsible for metabolizing the vast majority of therapeutic drugs, are the most actively studied of the xenobiotic-metabolizing enzymes. CYPs are complex and diverse in their regulation and catalytic activities. Genome sequencing has revealed the existence of 102 putatively functional CYP genes and 88 pseudogenes in the mouse and 57 putatively functional genes and 58 pseudogenes in humans. These genes are grouped, based on amino acid sequence similarity, into a superfamily comprising families and subfamilies with increasing sequence similarity. CYPs are named with the root CYP followed by a number designating the family, a letter denoting the subfamily, and another number designating the CYP form. Thus, CYP3A4 is family 3, subfamily A, and gene number 4.

A Small Number of CYPs Metabolize the Majority of Drugs

A limited number of CYPs from familes 1, 2, and 3 account for a preponderance of xenobiotc metabolism in humans. Twelve CYPs - CYP1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5) - are mentioned repeatedly in discussions of drug metabolism in this book. Moreover, because a single CYP can metabolize a large number of structurally diverse compounds, these enzymes can collectively metabolize not just pharmaceuticals but also scores of chemicals found in the diet and the environment. The liver contains the greatest abundance of xenobiotic-metabolizing CYPs, thus ensuring efficient first-pass metabolism of drugs. CYPs are also expressed throughout the GI tract and in lower amounts in lung, kidney, and even in the CNS.

The expression of the CYPs can differ markedly as a result of dietary and environmental exposure to inducers or through interindividual heritable differences in CYP structure that can affect overall drug metabolism and clearance. The most active CYPs involved in drug metabolism are those in the CYP2C, CYP2D, and CYP3A subfamilies. CYP3A4, the most abundantly expressed in liver, is involved in the metabolism of over 50% of clinically used drugs (Figure 5–3A). The CYP1A, CYP1B, CYP2A, CYP2B, and CYP2E subfamilies are not significantly involved in the metabolism of most therapeutic drugs, but they can catalyze the metabolic activation of protoxins and procarcinogens found in the diet and environment to their reactive metabolites that can cause toxicity and cancer.

CYP Polymorphism

There are large differences in levels of expression of each CYP between individuals as assessed by both clinical pharmacologic studies and analysis of expression in human liver samples. This large interindividual variability in CYP expression is due to the presence of genetic polymorphisms and differences in gene regulation (see discussion that follows). Several human CYP genes exhibit polymorphisms, including *CYP2A6*, *CYP2C9*, *CYP2C19*, and *CYP2D6*. Polymorphisms in CYPs are significant contributors to interindividual differences in drug metabolism, and marked ethnic differences exist in the extent of the genetic polymorphisms. For instance, up to 10% and 25% of Caucasians and Asians lack expression of CYP2D6 and CYP2C19, respectively. The CYP2D6 polymorphism has led to the withdrawal of several clinically used drugs (e.g., *debrisoquine* and *perhexiline*) and more cautious use of other drugs that are known CYP2D6 substrates (e.g., *encainide* and *flecainide* [antiarrhythmics], *desipramine* and *nortriptyline* [antidepressants], and *codeine*). In the case of codeine, individuals who lack CYP2D6 activity experience lower or no analgesic effects due to lack of codeine *O*-demethylation to morphine.

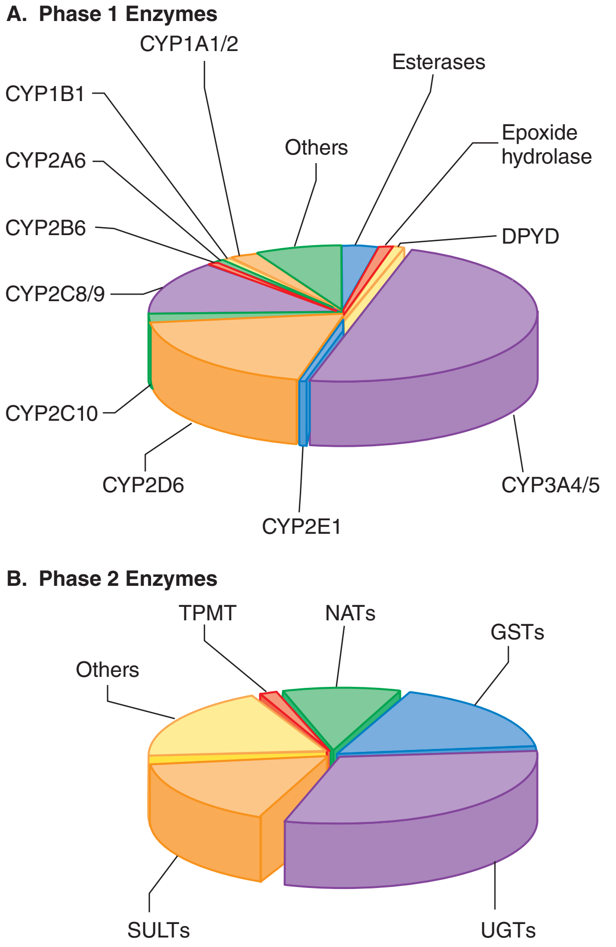


Figure 5–3 *The fraction of clinically used drugs metabolized by the major phase 1 and phase 2 enzymes.* The relative size of each pie section represents the estimated percentage of drugs metabolized by the major phase 1 (**A**) and phase 2 (**B**) enzymes, based on studies in the literature. In some cases, more than a single enzyme is responsible for metabolism of a single drug.

Allelic variants have been found in the *CYP1B1* and *CYP3A4* genes, but they are present at low frequencies in humans and appear not to have a major role in interindividual levels of expression of these enzymes. However, homozygous mutations in the *CYP1B1* gene, although rare, are associated with primary congenital glaucoma.

Drug-Drug Interactions

Differences in the rate of metabolism of a drug can be due to drug interactions. Most commonly, this occurs when two drugs (e.g., a statin and a macrolide antibiotic or antifungal agent) are coadministered and subjected to metabolism by the same enzyme. Because most of these drug-drug interactions are due to CYPs, it is important to determine the identity of the CYP that metabolizes a particular drug and to avoid coadministration of drugs that are metabolized by the same enzyme. The common antifungal agent *ketoconazole* is metabolized by CYP3A4 and other CYPs, and coadministration of ketoconazole with the anti-HIV (human immunodeficiency virus) protease inhibitors that are also CYP3A4 substrates reduces the clearance of the protease inhibitors and increases their plasma concentrations and the risks of toxicity. If a drug has a narrow therapeutic index, that is, a narrow range between the concentration required for therapeutic efficacy and the lowest concentration that causes toxicity, then consequential drug interactions are more likely.

If a drug has a narrow therapeutic index and coadministration with other drugs sharing the CYP cannot be avoided, then medicinal chemists may try to modify the drug to eliminate the CYP oxidation site while retaining the drug's efficacy (receptor binding affinity).

Some drugs are CYP inducers that not only can increase their own rates of metabolism but also can induce metabolism of other coadministered drugs (see the following discussion and Figure 5–12). Steroid hormones and herbal products such as St. John's wort (*Hypericum perforatum*) can increase hepatic levels of CYP3A4, thereby increasing the metabolism of many orally administered drugs. Drug metabolism can also be influenced by diet. CYP inhibitors and inducers are sometimes found in foods and can influence the toxicity and efficacy of a drug through induction or inhibition of CYPs. For most drugs, information found on the package insert lists the CYP that carries out its metabolism and the potential for drug interactions. Components found in grapefruit juice (e.g., naringin, furanocoumarins) are potent inhibitors of CYP3A4, and thus some drug inserts recommend not taking medication with grapefruit juice because it could increase the bioavailability of a drug.

Terfenadine, a once-popular antihistamine, was removed from the market because its metabolism was inhibited by CYP3A4 substrates such as *erythromycin* and grapefruit juice. *Terfenadine* is actually a prodrug that requires oxidation by CYP3A4 to its active metabolite, and at high doses, the parent compound caused arrhythmias. Elevated plasma levels of the parent drug resulting from CYP3A4 inhibition caused potentially fatal ventricular tachycardia in some individuals, an adverse response that led to the withdrawal of *terfenadine* from the market. The CYP3A4 metabolite of *terfenadine*, *fexofenadine*, which is not cardiotoxic, was developed as a replacement for *terfenadine*.

Flavin-Containing Monooxygenases

The FMOs are another superfamily of phase 1 enzymes involved in drug metabolism. Similar to CYPs, the FMOs are expressed at high levels in the liver and are bound to the ER, a site that favors interaction with and metabolism of hydrophobic drug substrates. There are six families of FMOs, with FMO3 the most abundant in liver. FMO3 is able to metabolize nicotine, as well as H_2 receptor antagonists (*cimetidine* and *ranitidine*), antipsychotics (*clozapine*), and antiemetics (*itopride*). Trimethylamine *N*-oxide (TMAO) occurs in high concentrations, up to 15% by weight, in marine animals, where it acts as an osmotic regulator. In humans, FMO3 normally metabolizes TMAO to TMA (trimethylamine), but a rare genetic deficiency of FMO3 causes the fish-odor syndrome, in which unmetabolized TMAO accumulates in the body and causes a socially offensive fish odor. This can be controlled by limiting dietary intake of foods containing TMAO.

FMOs are considered minor contributors to drug metabolism, and they almost always produce benign nonelectrophilic metabolites. In addition, FMOs are not readily inhibited and are not induced by any of the *xenobiotic receptors* (see discussion that follows); thus, in contrast to CYPs, FMOs would not be expected to be involved in drug-drug interactions. In fact, this has been demonstrated by comparing the pathways of metabolism of two drugs used in the control of gastric motility: *itopride* and *cisapride*. *Itopride* is metabolized by FMO3; *cisapride* is metabolized by CYP3A4. As predicted, *itopride* is less likely to be involved in drug-drug interactions than is *cisapride*. CYP3A4 participates in drug-drug interactions through both induction and inhibition of metabolism, whereas FMO3 is not induced or inhibited by any clinically used drugs. It is possible that FMOs may be important in the development of new drugs. A candidate drug could be designed by introducing a site for oxidation by FMOs with the knowledge that selected metabolism and pharmacokinetic properties could be accurately predicted for efficient drug-based biological efficacy.

Hydrolytic Enzymes

Epoxide Hydrolases

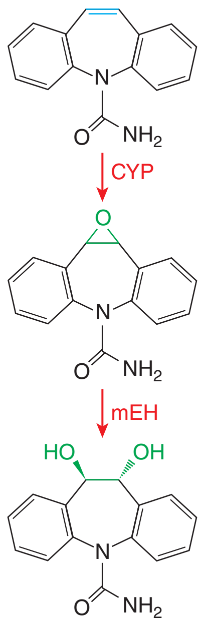


Figure 5–4 *Metabolism of carbamazepine by CYP and mEH.* *Carbamazepine* is oxidized to the pharmacologically active metabolite carbamazepine-10,11-epoxide by CYP. The epoxide is converted to a trans-dihydrodiol by mEH. This metabolite is biologically inactive and can be conjugated by phase 2 enzymes.

Two forms of epoxide hydrolase (EH) carry out hydrolysis of epoxides, most of which are produced by CYPs. Soluble epoxide hydrolase (sEH) is expressed in the cytosol; mEH (microsomal epoxide hydrolase) is localized to the membrane of the ER. Epoxides are highly reactive electrophiles produced by CYPs that can bind to cellular nucleophiles found in protein, RNA, and DNA, resulting in cell toxicity and transformation. Thus, EHs participate in the deactivation of potentially toxic metabolites.

There are a few examples of the influence of mEH on drug metabolism. The antiepileptic drug *carbamazepine* is a prodrug that is converted to its pharmacologically active derivative carbamazepine-10,11-epoxide, by CYP3A4. This metabolite is efficiently hydrolyzed to a dihydrodiol by mEH, resulting in inactivation of the drug (Figure 5–4). Inhibition of mEH can cause an elevation in plasma concentrations of the active metabolite and consequent side effects. The tranquilizer *valnoctamide* and anticonvulsant *valproate* inhibit mEH, resulting in clinically significant drug interactions with *carbamazepine*. This has led to efforts to develop new antiepileptic drugs, such as *gabapentin* and *levetiracetam*, that are metabolized by CYPs and not by EHs.

In general, the sEH complements the mEH in terms of substrate selectivity, with mEH degrading epoxides on cyclic systems and sEH having a high V_m and low K_m for fatty acid epoxides. Fatty acid epoxides are chemical mediators in the CYP branch of the arachidonic acid cascade. Simplistically, they can be thought of as balancing the generally proinflammatory and hypertensive prostaglandins, thromboxanes, and leukotrienes. The epoxides of arachidonic acid and docosahexaenoic acid reduce inflammation, hypertension, and pain but are normally degraded quickly by sEH to vicinal diols that are generally less biologically active (Figure 5–5). Thus, by inhibiting sEH, one can obtain dramatic biological effects. Recent work has focused on pain, where sEH inhibitors reduce both inflammatory and neuropathic pain and synergize with nonsteroidal anti-inflammatory drugs (Kodani and Hammock, 2015). In experimental systems, epoxides of dietary omega-3 and omega-6 fatty acids have anti-inflammatory properties, moderating inflammation and autophagy in insulin-sensitive tissues, effects that inhibitors of sEH promote (Lopez-Vicario et al., 2015).

Carboxylesterases

The carboxylesterases comprise a superfamily of enzymes that catalyze the hydrolysis of ester- and amide-containing chemicals. These enzymes are found in both the ER and the cytosol of many cell types and are involved in detoxification or metabolic activation of various drugs, environmental toxicants, and carcinogens. Carboxylesterases also catalyze

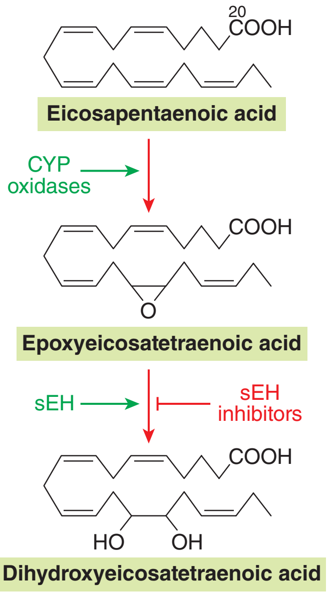


Figure 5–5 *Production and metabolism of an omega-3 fatty acid epoxide.* Fatty acid epoxides, such as the epoxide of the omega-3 fatty acid shown here, have a variety of anti-inflammatory and antinociceptive properties in test systems but are usually evanescent, metabolized to biologically less-active dihydroxy forms by sEH. Inhibition of sEH may promote the salutary effects of these epoxides.

the activation of prodrugs to their respective free acids. For example, the prodrug and cancer chemotherapeutic agent *irinotecan* is a camptothecin analogue that is bioactivated by intracellular carboxylesterases to the potent topoisomerase inhibitor SN-38 (Figure 5–6). Similar to FMOs, carboxylesterases are not involved in significant drug interactions.

Phase 2 Reactions: Conjugating Enzymes

There are a large number of phase 2 conjugating enzymes, all of which are considered to be synthetic in nature because they result in the formation of metabolites with increased molecular mass. Phase 2 reactions also normally terminate the biological activity of the drug, although there are exceptions. For *morphine* and *minoxidil,* glucuronide and sulfate conjugates, respectively, are more pharmacologically active than the parent. The relative contributions of different phase 2 reactions to drug metabolism are shown in Figure 5–3B.

Two of the phase 2 reactions, glucuronidation and sulfation, result in the formation of metabolites with significantly increased water-to-lipid partition coefficients. Sulfation and glucuronidation generally terminate the biological activity of drugs, and the minor change in overall charge increases the aqueous solubility of the metabolite. The enhanced hydrophilicity facilitates metabolite transport into the aqueous compartments of the cell and the body. Characteristic of the phase 2 reactions is the dependency of the catalytic reactions on cofactors (or, more correctly, cosubstrate): UDP-GA for UGT and 3′-phosphoadenosine-5′-phosphosulfate (PAPS) for SULTs, which react with available functional groups on the substrates. The reactive functional groups are often generated by the phase 1 CYPs, although there are many drugs (e.g., *acetaminophen*) for which glucuronidation and sulfation occur directly without prior oxidative metabolism. All of the phase 2 reactions are carried out in the cytosol of the cell, with the exception of glucuronidation, which is localized to the luminal side of the endoplasmic reticulum.

The catalytic rates of phase 2 reactions are significantly faster than the rates of the CYPs. Thus, if a drug is targeted for phase 1 oxidation through the CYPs, followed by a phase 2 conjugation reaction, usually the rate of elimination will depend on the initial (phase 1) oxidation reaction. Because the rate of conjugation is faster and the process leads to an increase in hydrophilicity of the drug, phase 2 reactions are generally considered to ensure efficient elimination and detoxification of most drugs.

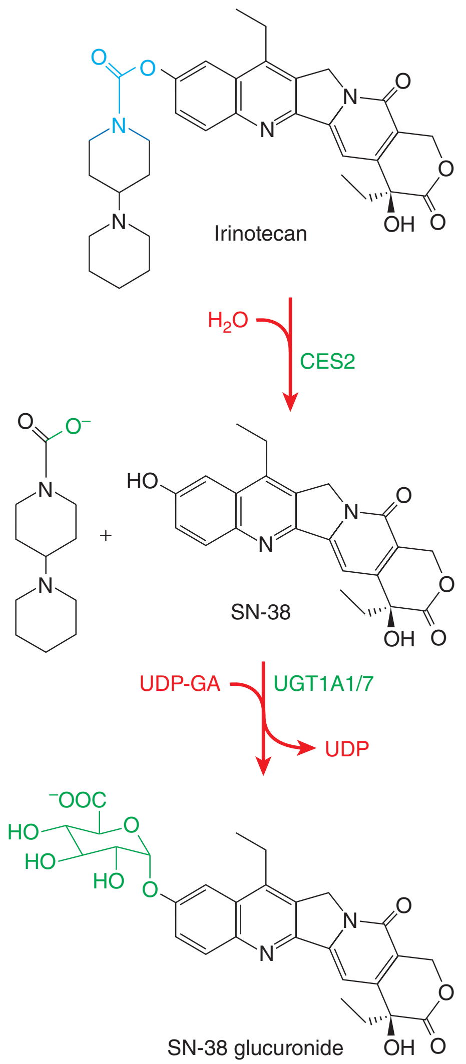


Figure 5–6 *Metabolism of irinotecan (CPT-11).* The prodrug CPT-11 is initially metabolized by a serum carboxylesterase 2 (CES2) to the topoisomerase inhibitor SN-38, which is the active camptothecin analogue that slows tumor growth. SN-38 is then subject to glucuronidation, which results in loss of biological activity and facilitates elimination of SN-38 in the bile.

Glucuronidation

Among the more important of the phase 2 reactions in drug metabolism are those carried out by uridine diphosphate–glucuronosyltransferases (UGTs) (Figure 5–3B). These enzymes catalyze the transfer of glucuronic acid from the cofactor UDP-GA to a substrate to form β-d-glucopyranosiduronic acids (glucuronides), metabolites that are sensitive to cleavage by β-glucuronidase. Glucuronides can be formed at alcoholic and phenolic hydroxyl groups; carboxyl, sulfuryl, and carbonyl moieties; and primary, secondary, and tertiary amine sites on molecules. UGT substrates include hundreds of chemically unique pharmaceuticals; dietary substances; environmental agents; humoral agents such as circulating hormones (androgens, estrogens, mineralocorticoids, glucocorticoids, thyroxine); bile acids; retinoids; and bilirubin, the end product of heme catabolism.

Examples of glucuronidation reactions are shown in Table 5–2 and Figures 5–1 and 5–6. The structural diversity of the drugs and other xenobiotics that are processed through glucuronidation ensures that many clinically efficacious therapeutic agents will be excreted as glucuronides.

The UGTs are expressed in a highly coordinated, tissue-specific, and often inducible fashion, with the highest concentration found in the GI tract and liver. Per tissue weight, there are a greater number and higher concentration of the UGTs in the small intestine than in liver, so efficient first-pass metabolism plays a role in predicting bioavailability of many orally administered medications. Formation of glucuronides and their increased polarity can result in their passage into the circulation, from which they are excreted into the urine. Alternatively, as xenobiotics enter the liver and are absorbed into hepatocytes, glucuronide formation provides substrates for active transport into the bile canaliculi and ultimate excretion with components of the bile (see, for example, Figure 5–9). Many of the glucuronides that are excreted into the bile eventually become substrates for soluble microbial β-glucuronidase in the large intestine, resulting in the formation of free glucuronic acid and the initial substrate. The colon actively absorbs water and a variety of other compounds (see Figure 54–3); depending on its solubility, the glucuronide or the original substrate may be reabsorbed via passive diffusion or by apical transporters in the small intestine and colon to reenter systemic circulation. This process of *enterohepatic recirculation* is responsible for reuptake of bile acids and can extend the half-life of a xenobiotic that is conjugated in the liver because the compound's ultimate excretion is delayed (see Figure 5–8).

There are 19 human genes that encode the UGT proteins. Nine are encoded by the *UGT1A* locus on chromosome 2q37 (1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9, and 1A10), while 10 genes are encoded by the *UGT2* family of genes on chromosome 4q13.2 (2A1, 2A2, 2A3, 2B2, 2B4, 2B7, 2B10, 2B11, 2B15, and 2B17). Of these proteins, the major UGTs involved in drug metabolism are UGT1A1, 1A3, 1A4, 1A6, 1A9, and 2B7 (for a list of common UGT drug substrates, see Rowland et al., 2013). Although both families of proteins are associated with metabolism of drugs and xenobiotics, the UGT2 family of proteins appears to have greater specificity for the glucuronidation of endogenous substances.

The *UGT1* locus on chromosome 2 (Figure 5–7) spans nearly 200 kb, with over 150 kb of a tandem array of cassette exonic regions that encode approximately 280 amino acids of the amino terminal portion of the UGT1A proteins. Four exons are located at the 3′ end of the locus; these encode the carboxyl 245 amino acids that combine with one of the consecutively numbered arrays of first exons to form the individual *UGT1A* gene products. Because exons 2 to 5 encode the same sequence for each UGT1A protein, the variability in substrate specificity for each of the UGT1A proteins results from the significant divergence in sequence encoded by the exon 1 regions. Reduced UGT activity resulting from allelic mutations in exons 2 to 5 affect all of the UGT1A proteins, whereas inactivating mutations in the exon 1 region lead to reduced glucuronidation by only the affected UGT1A protein. Over 100 allelic variants targeting the divergent exon 1 regions have been identified, many of which result in lowered UGT activity.

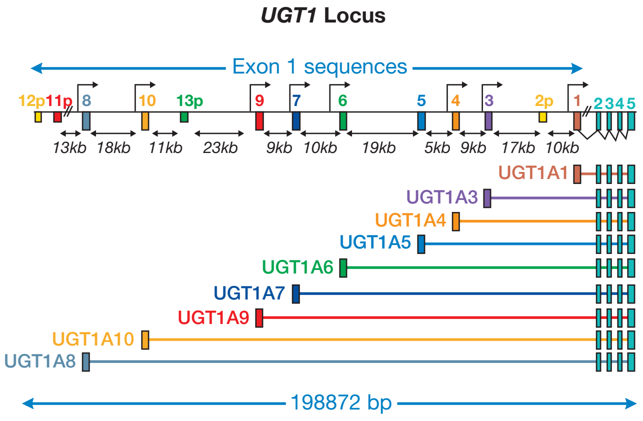


Figure 5–7 *Organization of the UGT1A locus.* Transcription of the *UGT1A* genes commences with the activation of PolII, which is controlled through tissue-specific events. Conserved exons 2 to 5 are spliced to each respective exon 1 sequence, resulting in the production of unique *UGT1A* sequences. The *UGT1A* locus encodes nine functional proteins.

From a clinical perspective, the expression of UGT1A1 assumes an important role in drug metabolism because the glucuronidation of bilirubin by UGT1A1 is the rate-limiting step in ensuring efficient bilirubin clearance, and this rate can be affected by both genetic variation and competing substrates (drugs). Deficiency in CYP1A1 activity and bilirubin clearance results in Gilbert's syndrome, usually an autosomal recessive disorder, but sometime autosomal dominant, depending on the type of mutation (Strassburg, 2008). Bilirubin is the breakdown product of heme, 80% of which originates from circulating hemoglobin and 20% from other heme-containing proteins, such as the CYPs. Bilirubin is hydrophobic, associates with serum albumin, and must be metabolized further by glucuronidation to ensure its elimination. The failure to efficiently metabolize bilirubin by glucuronidation leads to elevated serum levels and a clinical symptom called hyperbilirubinemia or jaundice. Delayed expression of the *UGT1A1* gene in newborns is the primary reason for neonatal hyperbilirubinemia.

There are more than 40 genetic lesions in the *UGT1A1* gene that can lead to inheritable unconjugated hyperbilirubinemia. Crigler-Najjar syndrome type 1 (CN-1) is diagnosed as a complete lack of bilirubin glucuronidation and results from inactivating mutations in exon 1 or in the common exons of the *UGT1A1* gene. CN-2 is differentiated by the detection of low amounts of bilirubin glucuronides in duodenal secretions and is linked to promoter mutations or reading frame mutations in the *UGT1A1* gene that lead to greatly reduced glucuronide formation. The danger associated with CN-1 and CN-2 is the accumulation of toxic levels of unconjugated bilirubin, which can lead to CNS toxicity. Children diagnosed with CN-1 require immediate and extensive blue light therapy to break down circulating bilirubin; these patients eventually require liver transplantation. Agents that induce *UGT1A1* gene expression, such as phenobarbital, can improve the glucuronidation and elimination of bilirubin in patients with CN-2. The *UGT1A1* gene is the only gene associated with xenobiotic metabolism that is essential for life because there is an absolute requirement for the daily elimination of serum bilirubin. Allelic variants associated with other xenobiotic-metabolizing genes (phase 1 and phase 2) can enhance disease and toxicity associated with drug use but show few or no phenotypic effects.

Gilbert syndrome is a generally benign condition that is present in 8% to 23% of the population, based on ethnic diversity. It is diagnosed clinically by circulating bilirubin levels that are 100% to 300% higher than normal. There is increasing epidemiological evidence to suggest that Gilbert syndrome may be protective against cardiovascular disease, potentially as a result of the antioxidant properties of bilirubin. The most common genetic polymorphism associated with Gilbert syndrome is a mutation in the *UGT1A1* gene promoter, identified as the *UGT1A1*28* allele, that leads to an $A(TA)_7TAA$ promoter sequence that differs from the more common $A(TA)_6TAA$ sequence. The elevated total serum bilirubin levels are associated with significantly reduced expression levels of hepatic UGT1A1.

Subjects diagnosed with Gilbert syndrome may be predisposed to ADRs (Table 5–3) resulting from a reduced capacity to metabolize drugs by UGT1A1. If a drug undergoes selective metabolism by UGT1A1, competition for drug metabolism with bilirubin glucuronidation will exist, resulting in pronounced hyperbilirubinemia as well as reduced clearance of the metabolized drug. *Tranilast* [*N*-(3′4′-demethoxycinnamoyl)-anthranilic acid] is an investigational drug used for the prevention of restenosis in patients who have undergone transluminal coronary revascularization (intracoronary stents). *Tranilast* therapy in patients with Gilbert syndrome can lead to hyperbilirubinemia, as well as potential hepatic complications resulting from elevated levels of *tranilast*.

Gilbert syndrome also alters patient responses to *irinotecan*. *Irinotecan*, a prodrug used in chemotherapy of solid tumors (see Chapter 70) is metabolized to its active form, SN-38, by tissue carboxylesterases (Figure 5–6). SN-38, a potent topoisomerase inhibitor, is inactivated by UGT1A1 and excreted in the bile (Figures 5–8 and 5–9). Once in the lumen of the intestine, the SN-38 glucuronide undergoes cleavage by bacterial β-glucuronidase and reenters the circulation through intestinal absorption. Elevated levels of SN-38 in the blood lead to hematological toxicities

TABLE 5–3 ■ DRUG TOXICITY AND GILBERT SYNDROME

PROBLEM	FEATURE
Gilbert syndrome	UGT1A1*28 (main variant in Caucasians)
Established toxicity reactions	Irinotecan, atazanavir
UGT1A1 substrates (potential risk?)	Gemfibrozil,[a] ezetimibe Simvastatin, atorvastatin, cerivastatin[a] Ethinylestradiol, buprenorphine, fulvestrant Ibuprofen, ketoprofen

[a] A severe drug reaction owing to the inhibition of glucuronidation (UGT1A1) and CYP2C8 and CYP2C9 when both drugs were combined led to the withdrawal of cerivastatin.

Source: Reproduced with permission from Strassburg CP. Pharmacogenetics of Gilbert's syndrome. *Pharmacogenomics*, **2008**, *9*:703–715. Copyright © 2008 Future Medicine Ltd. All rights reserved. Permission conveyed through Copyright Clearance Center, Inc.

characterized by leukopenia and neutropenia, as well as damage to the intestinal epithelial cells, resulting in acute and life-threatening ileocolitis. Patients with Gilbert syndrome who are receiving *irinotecan* therapy are predisposed to the hematological and GI toxicities resulting from elevated serum levels of SN-38, the net result of insufficient UGT1A activity and the consequent accumulation of a toxic drug in the GI epithelium.

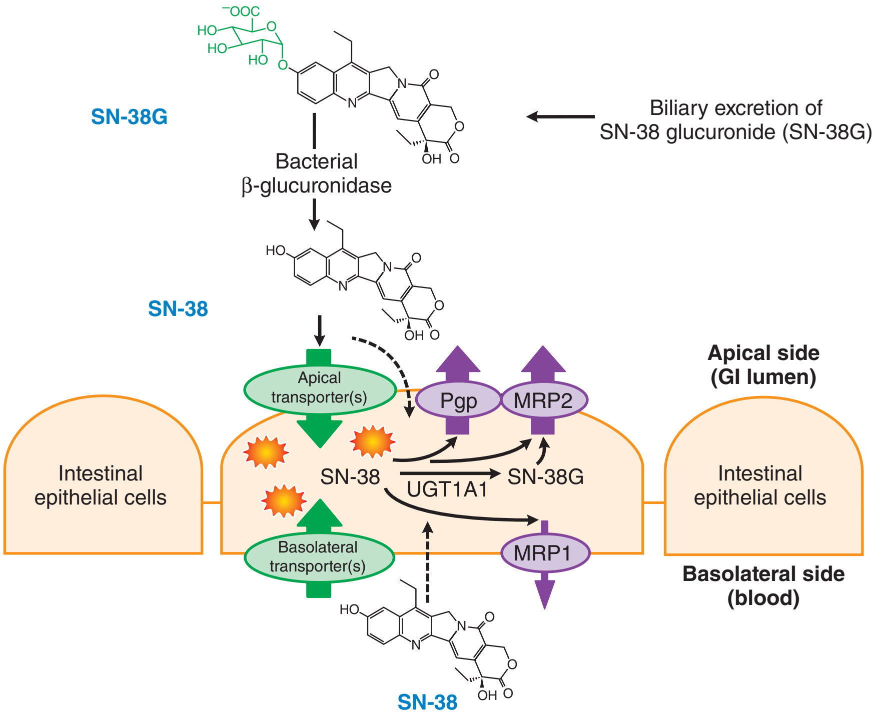
While most of the drugs that are metabolized by UGT1A1 compete for glucuronidation with bilirubin, patients with Gilbert syndrome who are HIV positive and on protease inhibitor therapy with *atazanavir* develop hyperbilirubinemia because *atazanavir* inhibits UGT1A1 function even though *atazanavir* is not a substrate for glucuronidation. Severe hyperbilirubinemia can develop in patients with Gilbert syndrome who contain enzyme-inactivating mutations in the *UGT1A3* and *UGT1A7* genes. Clearly, drug-induced side effects attributed to the inhibition of the UGT enzymes can be a significant concern and can be complicated in the presence of gene-inactivating mutations.

Sulfation

The sulfotransferases (SULTs), located in the cytosol, conjugate sulfate derived from PAPS to hydroxyl and, less frequently, amine groups of aromatic and aliphatic compounds. Like all of the xenobiotic-metabolizing enzymes, the SULTs metabolize a wide variety of endogenous and exogenous substrates. In humans, 13 SULT isoforms have been identified; based on sequence comparisons, they are classified into four families: the SULT1 family (SULT1A1, 1A2, 1A3/4, 1B1, 1C2, 1C3, 1C4, 1E1); the SULT2 family (SULT2A1, SULT2B1a, SULT2B1b); and the SULT4 (4A1) and SULT6 (6A1) families. There are major interspecies differences in the expressed complement of SULTs, which makes extrapolation of data on xenobiotic sulfation in animals to humans particularly unreliable.

SULTs play an important role in normal human homeostasis. For example, SULT2B1b is a predominant form expressed in skin, carrying out the catalysis of cholesterol. Cholesterol sulfate is an essential metabolite in regulating keratinocyte differentiation and skin development. SULT2A1 is highly expressed in the fetal adrenal gland, where it produces the large quantities of dehydroepiandrosterone sulfate that are required for placental estrogen biosynthesis during the second half of pregnancy. SULTs 1A3 and 1A4 (identical proteins produced from different genes) are highly selective for catecholamines, while estrogens (17β-estradiol in particular) are sulfated by SULT1E1. In humans, significant fractions of circulating catecholamines, estrogens, iodothyronines, and dehydroepiandrosterone (DHEA) exist in the sulfated form.

Some human SULTs display unique substrate specificities, whereas others are promiscuous. Members of the SULT1 family are the major isoforms involved in xenobiotic metabolism, with SULT1A1 quantitatively and qualitatively the most important in the liver (Riches et al., 2009).


⁻OOC
HO
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O
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N
N
O
O
OH
O
SN-38G
Biliary excretion of SN-38 glucuronide (SN-38G)
Bacterial β-glucuronidase
HO
SN-38
Apical transporter(s)
Pgp
MRP2
Apical side (GI lumen)
Intestinal epithelial cells
SN-38
UGT1A1
SN-38G
Intestinal epithelial cells
Basolateral transporter(s)
MRP1
Basolateral side (blood)
SN-38

Figure 5–8 *Routes of SN-38 transport and exposure to intestinal epithelial cells.* SN-38 is transported into the bile following glucuronidation by liver UGT1A1 and extrahepatic UGT1A7. Following cleavage of luminal SN-38 glucuronide (SN-38G) by bacterial β-glucuronidase, reabsorption into epithelial cells can occur by passive diffusion (indicated by the dashed arrows entering the cell) as well as by apical transporters. Movement into epithelial cells may also occur from the blood by basolateral transporters. Intestinal SN-38 can be transported into the lumen by P-glycoprotein (Pgp) and multidrug resistance protein 2 (MRP2) and into the blood via multidrug resistance protein (MRP1). Excessive accumulation of the SN-38 in intestinal epithelial cells, resulting from reduced glucuronidation, can lead to cellular damage and toxicity. (Modified and reproduced with permission from Tukey RH et al. Pharmacogenomics of human UDP-glucuronosyltransferases and irinotecan toxicity. *Mol Pharmacol,* **2002**, *62*:446–450. Copyright © 2002 The American Society for Pharmacology and Experimental Therapeutics.)

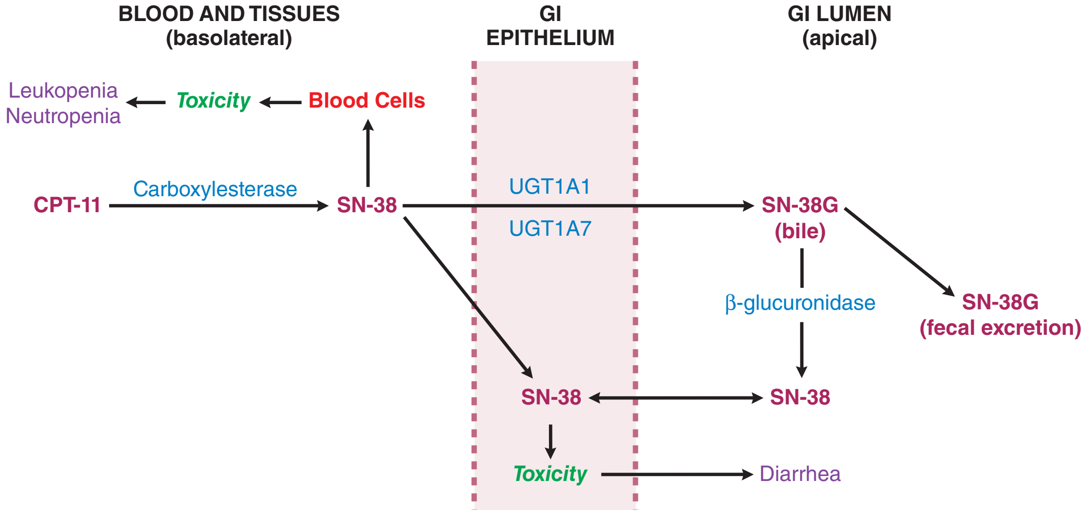

Figure 5–9 *Cellular targets of SN-38 in the blood and intestinal tissues.* Excessive accumulation of SN-38 can lead to blood toxicities, such as leukopenia and neutropenia, as well as damage to the intestinal epithelium. These toxicities are pronounced in individuals who have reduced capacity to form the SN-38 glucuronide, such as patients with Gilbert syndrome. Note the different body compartments and cell types involved. (Modified and reproduced with permission from Tukey RH et al. Pharmacogenomics of human UDP-glucuronosyltransferases and irinotecan toxicity. *Mol Pharmacol*, **2002**, *62*:446–450. Copyright © 2002 The American Society for Pharmacology and Experimental Therapeutics.)

SULT1A1 displays extensive diversity in its capacity to catalyze the sulfation of a broad variety of structurally heterogeneous xenobiotics with high affinity. Enzymes in the SULT1 family are recognized as phenol SULTs; they catalyze the sulfation of phenolic molecules such as *acetaminophen*, *minoxidil*, and *17α-ethinyl estradiol*. SULT1B1 is similar to SULT1A1 in its wide range of substrates, although it is much more abundant in the intestine than the liver. Three SULT1C enzymes exist in humans, but little is known of their substrate specificity. In rodents, SULT1C enzymes are capable of sulfating the hepatic carcinogen *N*-OH-2-acetylaminofluorene and are responsible for the bioactivation of this and related carcinogens. Their role in this pathway in humans is not clear. SULT1C enzymes are expressed abundantly in human fetal tissues; however, abundance declines significantly in adults. SULT1E catalyzes the sulfation of endogenous and exogenous steroids and is localized in liver and in hormone-responsive tissues such as the testis, breast, adrenal gland, and placenta. In the upper GI tract, SULT1A3/4 and SULT1B1 are particularly abundant, whereas SULT1A3/4 is absent from the adult liver.

The conjugation of drugs and xenobiotics is considered primarily a detoxification step, ensuring that the metabolites enter the aqueous compartments of the body and are targeted for elimination. However, drug metabolism through sulfation often leads to the generation of chemically reactive metabolites, wherein the sulfate is electron withdrawing and may be heterolytically cleaved, leading to the formation of an electrophilic cation. Most examples of the generation by sulfation of a carcinogenic or toxic response in animal or mutagenicity assays have been documented with chemicals derived from the environment or from heterocyclic arylamine food mutagens generated from well-cooked or burnt meat. Thus, it is important to understand whether genetic linkages can be made by associating known human SULT polymorphisms to cancers that are believed to originate from environmental sources. Because SULT1A1 is the most abundant SULT form in human tissues and displays broad substrate specificity, the polymorphic profiles associated with this gene and their associations with various human cancers are of considerable interest.

Copy number polymorphisms within the *SULT1A1*, *SULT1A3*, and *SULT1A4* genes have been identified, which may help explain much of the interindividual variation in the expression and activity of these enzymes. Knowledge of the structure, activities, regulation, and polymorphisms of the *SULT* superfamily will aid in understanding the linkages between sulfation and cancer susceptibility, reproduction, and development. Structural data, the results of kinetic studies, and molecular dynamics simulations are beginning to provide a picture of the mechanisms by which the SULTs express their unique patterns of substrate specificity (Tibbs et al., 2015).

Glutathione Conjugation

The glutathione-*S*-transferases (GSTs) catalyze the transfer of glutathione to reactive electrophiles, a function that serves to protect cellular macromolecules from interacting with electrophiles that contain electrophilic heteroatoms (–O, –N, and –S) and in turn protects the cellular environment from damage (Vaish et al., 2020). The cosubstrate in the reaction is glutathione, a tripeptide consisting of γ-glutamic acid, cysteine, and glycine (Figure 5–10). Glutathione exists in the cell in oxidized (GSSG) and reduced (GSH) forms, and the GSH:GSSG ratio is critical in maintaining a cellular environment in the reduced state. In addition to affecting xenobiotic conjugation with GSH, a severe reduction in GSH content can predispose cells to oxidative damage, a state that has been linked to a number of human health issues.

In the formation of glutathione conjugates, the GST reaction generates a thioether linkage with a drug or xenobiotic to the cysteine moiety of the tripeptide. Characteristically, all GST substrates contain an electrophilic atom and are hydrophobic by nature; they will associate with cellular proteins. Because the concentration of glutathione in cells is usually high, typically 7 μmol/g of liver or in the 10 mM range, many drugs and xenobiotics can react nonenzymatically with glutathione. However, the GSTs occupy up to 10% of the total hepatocellular protein concentration,

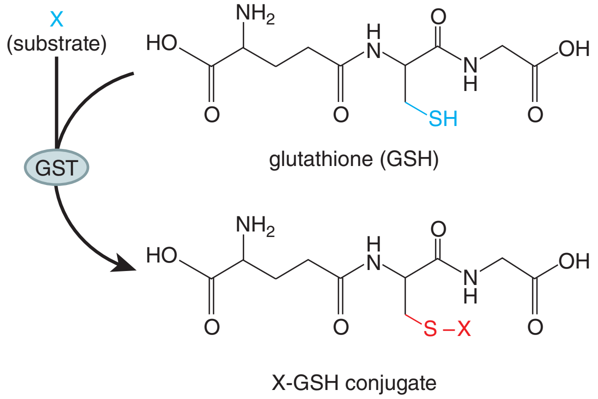

Figure 5–10 *Glutathione is a cosubstrate in the conjugation of a xenobiotic (X) by GST.*

a property that ensures efficient conjugation of glutathione to reactive electrophiles. The high concentration of GSTs also provides the cells with a sink of cytosolic protein, a property that facilitates noncovalent and sometimes covalent interactions with compounds that are not substrates for glutathione conjugation. The cytosolic pool of GSTs, once identified as *ligandin*, binds steroids, bile acids, bilirubin, cellular hormones, and environmental toxicants, in addition to complexing with other cellular proteins.

There are in excess of 20 human GSTs, divided into two subfamilies: the *cytosolic* and the *microsomal* forms. The major differences in function between the microsomal and cytosolic GSTs reside in the selection of substrates for conjugation: the cytosolic forms have more importance in the metabolism of drugs and xenobiotics, whereas the microsomal GSTs are important in the endogenous metabolism of leukotrienes and prostaglandins. The cytosolic GSTs comprise seven classes termed alpha (GSTA1 and 2), mu (GSTM1 through 5), omega (GSTO1), pi (GSTP1), sigma (GSTS1), theta (GSTT1 and GSTT2), and zeta (GSTZ1). Those in the alpha and mu classes can form heterodimers, allowing for a large number of active transferases to form. The cytosolic forms of GST catalyze conjugation, reduction, and isomerization reactions.

The high concentration of GSH in the cell, as well as the overabundance of GSTs, means that few reactive molecules escape detoxification. Despite the appearance of overcapacity of enzyme and reducing equivalents, there is always concern that some reactive intermediates will escape detoxification and, by nature of their electrophilicity, will bind to cellular components and cause havoc. The potential for such an occurrence is heightened if GSH is depleted or if a specific form of GST is polymorphic and dysfunctional. While it is difficult to deplete cellular GSH levels, therapeutic agents that require large doses to be clinically efficacious have the greatest potential to lower cellular GSH levels.

Acetaminophen, normally metabolized by glucuronidation and sulfation, is also a substrate for oxidative metabolism by CYP2E1 and CYP3A4, which generate the toxic metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI), which, following normal dosing, is readily neutralized through conjugation with GSH. However, an overdose of *acetaminophen* can deplete cellular GSH levels and thereby increase the potential for NAPQI to interact with other cellular components, resulting in toxicity and cell death. *Acetaminophen* toxicity, with its increased levels of NAPQI and potential hepatic necrosis, may be treated in a time- and drug concentration–dependent manner by administration of *N*-acetylcysteine, which replenishes the depleted GSH, permitting detoxification of the excess NAPQI before it can cause more cellular damage (see Figure 9–4).

All of the GSTs are polymorphic. The mu (_GSTM1*0_) and theta (_GSTT1*0_) genotypes express a null phenotype; thus, individuals who are polymorphic at these loci are predisposed to toxicities by agents that are selective substrates for these GSTs. For example, the mutant _GSTM1*0_ allele is observed in 50% of the Caucasian population and links genetically to human malignancies of the lung, colon, and bladder. Null activity in the *GSTT1* gene associates with adverse side effects and toxicity in cancer chemotherapy with cytostatic drugs; the toxicities result from insufficient clearance of the drugs via GSH conjugation. Expression of the null genotype can be as high as 60% in Chinese and Korean populations. Thus, GST polymorphisms may influence efficacies and severity of adverse side effects of drugs.

While the GSTs play an important role in cellular detoxification, their activities in cancerous tissues have been linked to the development of drug resistance toward chemotherapeutic agents that are both substrates and nonsubstrates for the GSTs. Many anticancer drugs are effective because they initiate cell death or apoptosis, which is linked to the activation of mitogen-activated protein kinases (MAPKs) such as JNK and p38. Overexpression of GSTs is associated with resistance to apoptosis and the inhibition of MAPK activity. In a variety of tumors, GSTs are overexpressed, leading to a reduction in MAPK activity and reduced efficacy of chemotherapy. Taking advantage of the relatively high levels of GST in tumor cells, inhibition of GST activity has been exploited as a therapeutic strategy to modulate drug resistance by sensitizing tumors to anticancer drugs. TLK199, a glutathione analogue, is a prodrug that plasma esterases convert to a GST inhibitor, TLK117, which potentiates the toxicity of different anticancer agents (Figure 5–11).

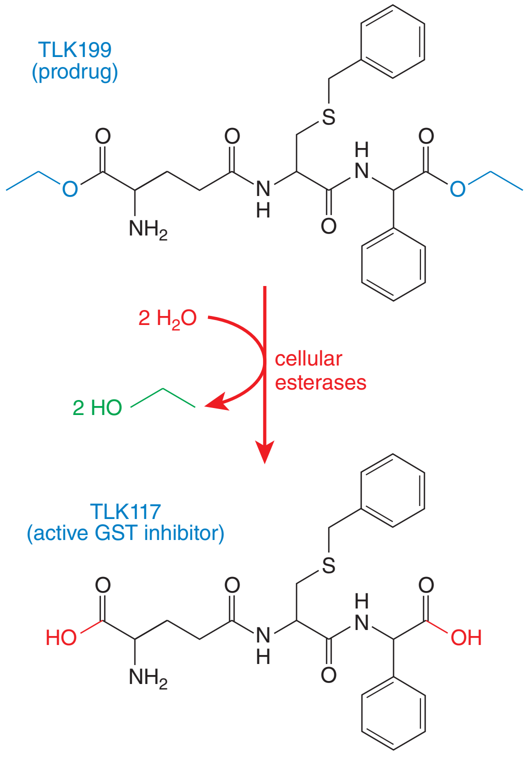

Figure 5–11 *Activation of TLK199 to TLK117, a GST inhibitor.*

Alternatively, the elevated GST activity in cancer cells has been the basis for the development of prodrugs that can be activated by the GSTs to form electrophilic intermediates. For example, TLK286 is a substrate for GST that undergoes a β-elimination reaction, forming a glutathione conjugate and a nitrogen mustard (Figure 5–12) that is capable of alkylating cellular nucleophiles, resulting in cell killing and antitumor activity (Townsend and Tew, 2003).

N-Acetylation

The cytosolic NATs are responsible for the metabolism of drugs and environmental agents that contain an aromatic amine or hydrazine group (Mitchell, 2020). Addition of the acetyl group from the cofactor acetyl-coenzyme A often leads to a metabolite that is *less* water soluble because the potential ionizable amine is neutralized by the covalent addition of the acetyl group. NATs are among the most polymorphic of all the human xenobiotic drug-metabolizing enzymes.

The characterization of an acetylator phenotype in humans was one of the first hereditary traits identified and was responsible for the development of the field of pharmacogenetics (see Chapter 7). Following the discovery that *isoniazid* (isonicotinic acid hydrazide, INH) could be used to treat tuberculosis, a significant proportion of the patients (5%–15%) experienced toxicities that ranged from numbness and tingling in their fingers to CNS damage. After finding that INH is metabolized by acetylation and excreted in the urine, researchers noted that individuals who experienced the toxic effects of the drug excreted the largest amount of unchanged drug and the least amount of acetylated INH. Pharmacogenetic studies led to the classification of "rapid" and "slow" acetylators, with the slow phenotype predisposed to toxicity (see Figure 65–4). The NAT alleles for slow and fast acetylators were characterized, revealing genetic changes that correspond to the slow acetylator phenotype.

There are two functional NAT genes in humans, *NAT1* and *NAT2*. Over 25 allelic variants of *NAT1* and *NAT2* have been characterized. In individuals in whom acetylation of drugs is compromised, homozygous genotypes for at least two variant alleles are required to predispose a patient to slower drug metabolism. Polymorphism in the *NAT2* gene

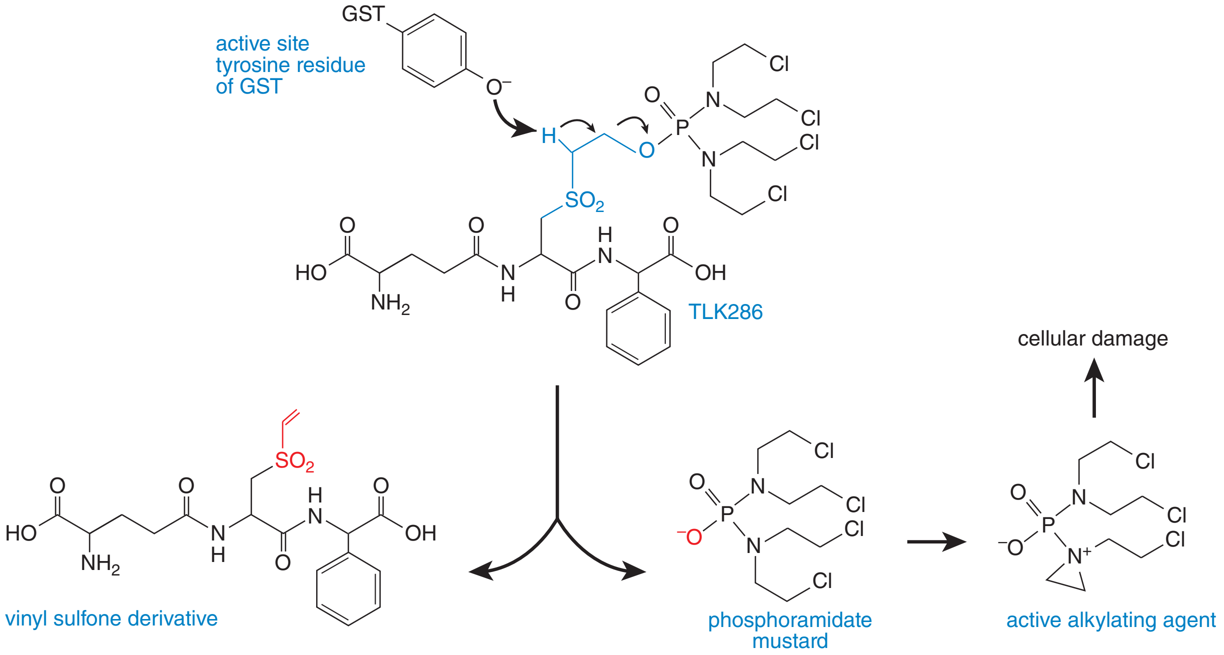

Figure 5–12 *Generation of the reactive alkylating agent following the conjugation of glutathione to TLK286.* GST interacts with the prodrug and GSH analogue TLK286 via a tyrosine in the active site of GST. The GSH portion is shown in blue. The interaction promotes β-elimination and cleavage of the prodrug to a vinyl sulfone and an active alkylating fragment.

and its association with the slow acetylation of INH were one of the first completely characterized genotypes shown to affect drug metabolism, thereby linking pharmacogenetic phenotype to a genetic polymorphism. Although nearly as many mutations have been identified in the *NAT1* gene as the *NAT2* gene, the frequency of the slow acetylation patterns is attributed mostly to the polymorphism in the *NAT2* gene.

Some common drug substrates of NAT and their known toxicities are listed in Table 5–4 (see Meisel, 2002, for details). The therapeutic relevance of NAT polymorphisms is in avoiding drug-induced toxicities. The adverse drug response in a slow acetylator resembles a drug overdose; thus, reducing the dose or increasing the dosing interval is recommended. Aromatic amine or hydrazine groups exist in many classes of clinically used drugs, and if a drug is known to be subjected to metabolism through acetylation, determining an individual's phenotype can be important in maximizing a positive therapeutic outcome. For example, *hydralazine*, a once-popular orally active antihypertensive (vasodilator) drug, is metabolized by NAT2. The administration of therapeutic doses of *hydralazine* to a slow acetylator can result in extreme hypotension and tachycardia.

Several known targets for acetylation, such as the sulfonamides, have been implicated in idiosyncratic hypersensitivity reactions; in such instances, an appreciation of a patient's acetylation phenotype is particularly important. Sulfonamides are transformed into hydroxylamines that interact with cellular proteins, generating haptens that can elicit autoimmune responses, to which slow acetylators are predisposed.

Tissue-specific expression patterns of NAT1 and NAT2 have a significant impact on the fate of drug metabolism and the potential for

TABLE 5–4 ■ THERAPEUTIC USES AND ADVERSE EFFECTS OF COMMON *N*-ACETYLTRANSFERASE SUBSTRATES

NAT SUBSTRATE	THERAPEUTIC USES	ADVERSE EFFECTS
Acebutolol	Adrenal cortex carcinoma, breast cancer	Drowsiness, weakness, insomnia
Aminoglutethimide	β Blockade, arrhythmias, hypertension	Clumsiness, nausea, dizziness, agranulocytosis
Aminosalicylic acid	Ulcerative colitis	Allergic fever, itching, leukopenia
Amrinone	Positive inotrope in heart failure	Thrombocytopenia, arrhythmias
Benzocaine	Local anesthesia	Dermatitis, itching, rash, methemoglobinemia
Caffeine	Neonatal respiratory distress syndrome	Dizziness, insomnia, tachycardia
Clonazepam	Seizures, anxiety	Drowsiness, ataxia, dizziness, slurred speech
Dapsone	Leprosy, dermatitis	Hemolysis, methemoglobinemia, nausea, dermatitis
Hydralazine	Hypertension (acts via vasodilation)	Hypotension, sympathetic baroreceptor reflex effects
Isoniazid	Tuberculosis	Peripheral neuritis, hepatotoxicity
Nitrazepam	Insomnia	Dizziness, somnolence
Phenelzine	Depression (acts via MAO inhibition)	Dizziness, CNS excitation, insomnia, orthostatic hypotension, hepatotoxicity
Procainamide	Ventricular tachyarrhythmia	Hypotension, bradycardia, lupus erythematosus
Sulfonamides	As bacteriostatic agents	Hypersensitivity, acute hemolytic anemia, reversible bone marrow suppression (with AIDS or myelosuppressive chemotherapy)

eliciting a toxic episode. NAT1 is ubiquitously expressed among most human tissues, whereas NAT2 is found predominantly in liver and the GI tract. Characteristic of both NAT1 and NAT2 is the capacity to form *N*-hydroxy–acetylated metabolites from bicyclic aromatic hydrocarbons, a reaction that leads to the nonenzymatic release of acetyl groups and the generation of highly reactive nitrenium ions. Thus, *N*-hydroxy acetylation is thought to activate certain environmental toxicants. In contrast, direct *N*-acetylation of bicyclic aromatic amines is stable and leads to detoxification. Individuals who are NAT2 fast acetylators are able to efficiently metabolize and detoxify bicyclic aromatic amines through liver-dependent acetylation. However, slow acetylators (NAT2 deficient) accumulate bicyclic aromatic amines that become substrates for CYP-dependent *N*-oxidation. These N-OH metabolites are eliminated in the urine. In tissues such as bladder epithelium, NAT1 is highly expressed and can efficiently catalyze the *N*-hydroxy acetylation of bicyclic aromatic amines, a process that leads to deacetylation and the formation of the mutagenic nitrenium ion, especially in NAT2-deficient subjects. Epidemiological studies have shown that slow acetylators are predisposed to bladder cancer if exposed environmentally to bicyclic aromatic amines.

Methylation

In humans, drugs and xenobiotics can undergo *O*-, *N*-, and *S*-methylation catalyzed by methyltransferases. Humans express two COMTs, three *N*-methyl transferases, a phenol-*O*-methyltransferase (POMT), a thiopurine methyltransferase (TPMT), and a thiol methyltransferase (TMT). All of the MTs exist as monomers and use SAM (*S*-adenosylmethionine, AdoMet) as the methyl donor. With the exception of a signature sequence that is conserved among the MTs, there is limited similarity among these enzymes, indicating that each MT has evolved to display a unique catalytic function. Although the common theme among the MTs is the generation of a methylated product, substrate specificity is high and distinguishes the individual enzymes.

Among the *N*-methyl transferases, nicotinamide *N*-methyltransferase (NNMT) methylates serotonin and tryptophan as well as pyridine-containing compounds such as nicotinamide and nicotine. Phenylethanolamine *N*-methyltransferase (PNMT) is responsible for methylation of the neurotransmitter norepinephrine to form epinephrine; histamine *N*-methyltransferase (HNMT) metabolizes drugs containing an imidazole ring. Catechol-*O*-methyltransferase (COMT), which exists as two isoforms generated by alternate exon usage, methylates neurotransmitters containing a catechol moiety, such as dopamine and norepinephrine, and drugs such as *methyldopa* and *ecstasy* (3,4-methylenedioxymethamphetamine, MDMA).

From a clinical perspective, the most important MT may be TPMT, which catalyzes the *S*-methylation of aromatic and heterocyclic sulfhydryl compounds, including the thiopurine drugs *azathioprine (AZA)*, *6-mercaptopurine (6-MP)*, and *thioguanine*. AZA and 6-MP are used for the management of inflammatory bowel disease (see Chapter 55), as well as autoimmune disorders such as systemic lupus erythematosus and rheumatoid arthritis. *Thioguanine* is used in the treatment of acute myeloid leukemia, and 6-MP is used worldwide for the treatment of childhood acute lymphoblastic leukemia (see Chapter 70). Because TPMT is responsible for the detoxification of 6-MP, a genetic deficiency in TPMT can result in severe toxicities in patients taking the drug (see metabolic scheme in Figure 55–5). When given orally at clinically established doses, 6-MP serves as a prodrug that is metabolized by hypoxanthine guanine phosphoribosyl transferase (HGPRT) to 6-thioguanine nucleotides (6-TGNs), which become incorporated into DNA and RNA, resulting in arrest of DNA replication and cytotoxicity.

Toxic side effects arise when a lack of 6-MP methylation by TPMT causes a buildup of 6-MP and the consequent generation of toxic levels of 6-TGNs. The identification of the inactive TPMT alleles and the development of a genotyping test to identify homozygous carriers of the defective allele permit identification of individuals who may be predisposed to the toxic side effects of 6-MP therapy. Simple adjustments in the dosage regimen are a lifesaving intervention for patients with TPMT deficiencies.

Role of Xenobiotic Metabolism in Safe and Effective Use of Drugs

Any xenobiotics entering the body must be eliminated through metabolism and excretion via the urine or bile/feces. Mechanisms of metabolism and excretion prevent foreign compounds from accumulating in the body and possibly causing toxicity. In the case of drugs, metabolism normally results in inactivation of their therapeutic effectiveness and facilitates their elimination. The rate and extent of metabolism can determine the efficacy and toxicity of a drug by controlling its biological half-life. Among the most serious considerations in the clinical use of drugs are ADRs. If a drug is metabolized too quickly, it rapidly loses its therapeutic efficacy. This can occur if specific enzymes involved in metabolism are overly active or are induced by dietary or environmental factors. If a drug is metabolized too slowly, the drug can accumulate in the bloodstream; as a consequence, the plasma clearance of the drug is decreased, the area under the plasma concentration–time curve (AUC; see Figure 2–9) is elevated, and exposure to the drug may exceed clinically appropriate levels. An increase in AUC often results when specific xenobiotic-metabolizing enzymes are inhibited. Such an event may occur when an individual is taking a combination of therapeutic agents and two or more of the agents target a specific enzyme involved in drug metabolism; similarly, a drug and a dietary product may interact. For example, the consumption of grapefruit juice can inhibit intestinal CYP3A4, blocking the metabolism of numerous drugs. Among the components of grapefruit juice that inhibit CYP3A4 are *naringin* and *furanocoumarins*. The inhibition of specific CYPs in the gut by dietary consumption of grapefruit juice alters the oral bioavailability of many classes of drugs, including certain antihypertensives, immunosuppressants, antidepressants, antihistamines, and the statins, among others.

While environmental factors can alter the steady-state levels of specific enzymes or inhibit their catalytic potential, these phenotypic changes in drug metabolism are also observed clinically in groups of individuals who are genetically predisposed to ADRs because of pharmacogenetic differences in the expression of xenobiotic-metabolizing enzymes (see Chapter 7). Most of the xenobiotic-metabolizing enzymes display polymorphic differences in their expression, resulting from heritable changes in the structure of the genes. For example, hyperbilirubinemia can result from a reduction in the ability to glucuronidate circulating bilirubin due to a lowered expression of the *UGT1A1* gene (Gilbert syndrome). Drugs that are subject to glucuronidation by UGT1A1, such as the topoisomerase inhibitor SN-38 (Figures 5–6, 5–8, and 5–9), will display an increased AUC in individuals with Gilbert syndrome because such patients cannot detoxify these drugs. Most cancer chemotherapeutic agents have a narrow therapeutic index; thus, increases in the circulating levels of the active form, due to a deficiency in drug clearance, can result in significant toxicities.

Nearly every class of therapeutic agent has been reported to initiate an ADR. In the United States, ADRs annually cost an estimated at $100 billion and cause over 100,000 deaths. An estimated 56% of drugs associated with ADRs are substrates for xenobiotic-metabolizing enzymes. Because many of the CYPs and UGTs are subject to induction as well as inhibition by drugs, dietary factors, and other environmental agents, these enzymes play an important role in most ADRs. Thus, prior to filing a New Drug Application (NDA), a new drug's route of metabolism must be known. It is now routine practice in the pharmaceutical industry to establish which enzymes are involved in metabolism of a drug candidate and to identify the metabolites and determine their potential toxicity. In consideration of the major role of CYPs in the generation of ADRs, there is likely to be a move to avoid the major oxidative routes of metabolism when developing new small-molecule drugs.

Induction of Drug Metabolism

Xenobiotics can influence drug metabolism by activating transcription and inducing the expression of genes encoding drug-metabolizing enzymes. Thus, a foreign compound may induce its own metabolism, as may certain drugs. One potential consequence of this is a decrease in

TABLE 5–5 ■ NUCLEAR RECEPTORS THAT INDUCE DRUG METABOLISM

RECEPTOR	LIGANDS
Aryl hydrocarbon receptor (AHR)	Omeprazole
Constitutive androstane receptor (CAR)	Phenobarbital
Pregnane X receptor (PXR)	Rifampin
Farnesoid X receptor (FXR)	Bile acids
Vitamin D receptor (VDR)	Vitamin D
Peroxisome proliferator–activated receptor (PPARs)	Fibrates
Retinoic acid receptor (RAR)	All-*trans*-retinoic acid
Retinoid X receptor (RXR)	9-*cis*-Retinoic acid

plasma drug concentration over the course of treatment, resulting in loss of efficacy as the autoinduced metabolism of the drug exceeds the rate at which new drug enters the body. A list of ligands and the receptors through which they induce drug metabolism is shown in Table 5–5. A particular receptor, when activated by a ligand, can induce the transcription of a series of target genes. Among these target genes are certain CYPs and drug transporters; induction of CYPs and transport proteins could lead to drug interactions. Figure 5–13 shows the scheme by which a drug may interact with nuclear receptors to induce its own metabolism.

The Aryl Hydrocarbon Receptor

The aryl hydrocarbon receptor (AHR) is a member of a superfamily of transcription factors with diverse roles in mammals, such as serving a regulatory role in the development of the mammalian CNS and modulating the response to chemical and oxidative stress. This superfamily of transcription factors includes Per (Period) and Sim (Simpleminded), two transcription factors involved in development of the CNS, and hypoxia-inducible factor 1α (HIF1α), HIF2α, and their dimerization partner HIF1β. Under hypoxic conditions, a cytosolic HIFα subunit translocates to the nucleus and forms a dimer with HIF1β; the dimer binds to the hypoxia response element to activate gene transcription.

The AHR induces expression of genes encoding CYP1A1, CYP1A2, and CYP1B1, which are able to metabolically activate chemical carcinogens, including environmental contaminants and carcinogens derived from food. Many of these substances are inert unless metabolized by CYPs. Thus, induction of these CYPs by a drug could potentially result in an increase in the toxicity and carcinogenicity of procarcinogens. For example, *omeprazole*, a proton pump inhibitor used to treat gastric and duodenal ulcers (see Chapter 53), is a ligand for the AHR and can induce CYP1A1 and CYP1A2, with the possible consequences of toxin/carcinogen activation as well as drug-drug interactions in patients receiving agents that are substrates for either of these CYPs.

Type 2 Nuclear Receptors

Another important induction mechanism is due to type 2 nuclear receptors that are in the same superfamily as the steroid hormone receptors. Many of these receptors, identified on the basis of their structural similarity to steroid hormone receptors, were originally termed *orphan receptors* because no endogenous ligands were known to interact with them. We now know that some of these receptors are activated by xenobiotics, including drugs. The type 2 nuclear receptors of most importance to drug metabolism and drug therapy include the pregnane X receptor (PXR), the constitutive androstane receptor (CAR), and peroxisome proliferator–activated receptors (PPARs).

PXR, discovered because it is activated by the synthetic steroid pregnenolone-16α-carbonitrile, is also activated by a number of other drugs, including antibiotics (*rifampicin* and *troleandomycin*), Ca^{2+} channel blockers (*nifedipine*), statins (*mevastatin*), antidiabetic drugs (*troglitazone*), HIV protease inhibitors (*ritonavir*), and anticancer drugs (*paclitaxel*) (Nicolussi et al., 2020).

Hepatotoxicity during antiretroviral therapy provides a good example of the role of CYP regulation in drug toxicity. Patients treated with the antibiotic *rifampicin* (for tuberculosis) or *efavirenz* (for HIV), followed by *ritonavir*-containing cocktails for HIV, develop liver toxicity with high frequency. The mechanism of toxicity appears to involve PXR activation

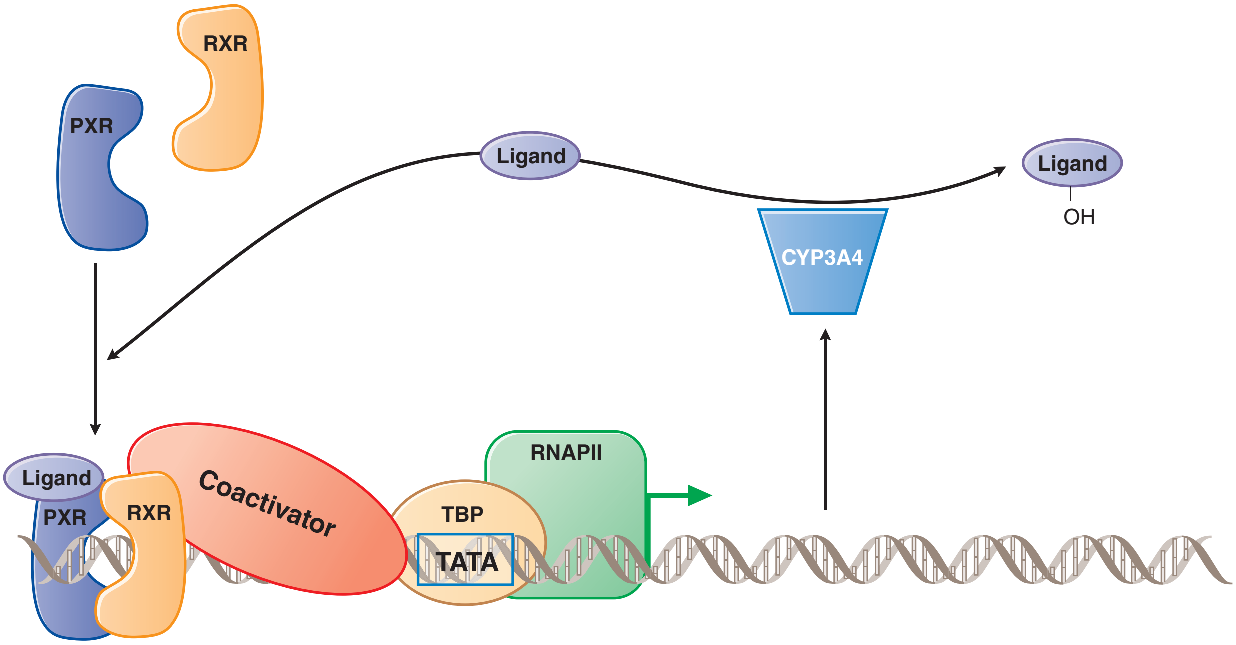

Figure 5–13 *Induction of drug metabolism by nuclear receptor–mediated signal transduction.* When a drug such as *atorvastatin* (Ligand) enters the cell, it can bind to a nuclear receptor such as the PXR. The PXR then forms a complex with the RXR (retinoid X receptor), binds to DNA upstream of target genes, recruits coactivator (which binds to the TATA box–binding protein, TBP), and activates transcription. Among PXR target genes is *CYP3A4,* which can metabolize *atorvastatin* and decrease its cellular concentration. Thus, *atorvastatin* induces its own metabolism. *Atorvastatin* undergoes both ortho- and parahydroxylation.

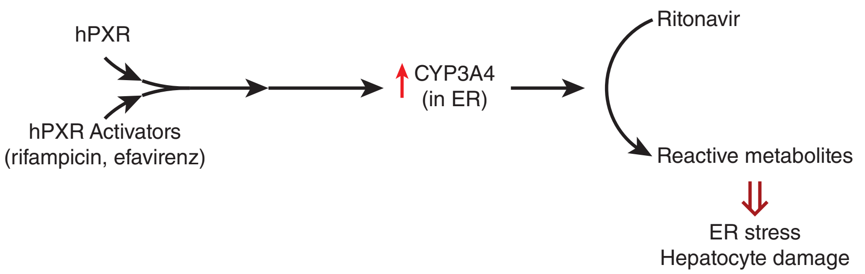

Figure 5–14 *Potentiation of ritonavir hepatotoxicity by activators of hPXR. Ritonavir*, a potent inhibitor and substrate of CYP3A4, is used in HIV-AIDS therapy as a pharmacokinetic enhancer of HIV protease inhibitors that are also substrates of CYP3A4 (see Table 64–4). *Ritonavir* is metabolized to reactive metabolites that can damage hepatic cells, and the hepatotoxicity of *ritonavir* is markedly increased in patients treated with *rifampicin* (for tuberculosis) or *efavirenz* (a nonnucleoside reverse transcriptase inhibitor used to treat HIV). The drug-induced potentiation of *ritonavir* toxicity may be explained by the activation of hepatic hPXR by either *rifampicin* or *efavirenz* that leads to induction of high levels of CYP3A4, which catalyzes increased production of toxic reactive metabolites of *ritonavir* that stress the ER and cause hepatocyte damage (Shehu et al., 2019). AIDS, acquired immunodeficiency syndrome; hPXR, human pregnane X receptor.

(Figure 5–14): Metabolism of *ritonavir* by CYP3A4 produces reactive metabolites that cause stress to the ER and cellular toxicity; *rifampicin* and *efavirenz* activate PXR and induce high levels of CYP3A4; metabolism of *ritonavir* by CYP3A4 is thereby increased, resulting in elevated levels of hepatotoxic metabolites (Shehu et al., 2019). This drug interaction could be decreased by developing strategies to decrease PXR activation, lower CYP3A4 metabolism, or mitigate downstream pathways to improve the safe use of *ritonavir* in the clinic.

Hyperforin, a component of St. John's wort, an over-the-counter herbal remedy used for depression, also activates PXR. This activation is thought to be the basis for the increase in failure of oral contraceptives in individuals taking St. John's wort: Activated PXR is an inducer of CYP3A4, which can metabolize steroids found in oral contraceptives and thereby lower the concentration of steroids below the range of effective contraception. PXR also induces the expression of genes encoding certain drug transporters and phase 2 enzymes, including SULTs and UGTs. Thus, PXR can facilitate the metabolism and elimination of numerous xenobiotics, sometimes with notable consequences.

The nuclear receptor CAR was discovered based on its ability to activate genes in the absence of ligand. Steroids such as *androstanol*, the antifungal agent *clotrimazole*, and the antiemetic *meclizine* are inverse agonists that inhibit gene activation by CAR; the steroid 5-β-pregnane-3,20-dione (and probably other endogenous compounds) and the pesticide 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene are agonists that activate gene expression when bound to CAR. Genes induced by CAR include those encoding several CYPs (2B6, 2C9, and 3A4); various phase 2 enzymes (including GSTs, UGTs, and SULTs); and drug and endobiotic transporters. CYP3A4 is induced by both PXR and CAR; thus, its level is highly influenced by a number of drugs and other xenobiotics. In addition to inducing the degradation of drugs, CAR and PXR appear to function in the control of multiple aspects of hepatic physiology, including bilirubin degradation, energy metabolism, and cell proliferation (Cai et al., 2021).

Clearly, PXR and CAR can be activated or inhibited by a great variety of ligands. As with the xenobiotic-metabolizing enzymes, species differences also exist in the ligand specificities of these receptors. For example, low clinically relevant concentrations of *rifampicin* activate human PXR, while only very high concentrations of the drug activate mouse or rat PXR; pregnenolone-16α-carbonitrile preferentially activates the mouse and rat PXR but not human PXR. Paradoxically, *meclizine* activates mouse CAR but inhibits gene induction by human CAR. These findings further underscore that rodent model systems do not always reflect the response of humans to drugs.

The PPAR family is composed of three members: α, β/δ, and γ. PPARα is the target for the fibrate class of hyperlipidemic drugs, including the widely prescribed *gemfibrozil* and *fenofibrate*. Activation of PPARα results in induction of target genes encoding fatty acid–metabolizing enzymes, resulting in lowering of serum triglycerides. In addition, activation of PPARα induces CYP4 enzymes that carry out the oxidation of fatty acids and drugs with fatty acid–containing side chains, such as *leukotriene* and arachidonate analogues. PPARγ is the target for the thiazolidinedione class of anti–type 2 diabetic drugs, including *rosiglitazone* and *pioglitazone*. PPARγ and PPARβ/δ do not induce enzymes involved in xenobiotic metabolism.

The UGT genes, in particular *UGT1A1*, are targets for AHR, PXR, CAR, PPARα, and NRF2 (nuclear factor 2–erythroid-derived 2-like factor, a major transcriptional regulator of cytoprotective genes induced by an antioxidant response). Because the UGTs are abundant in the GI tract and liver, regulation of the UGTs by drug-induced activation of these nuclear receptors would be expected to play a role concerning the pharmacokinetic parameters of many orally administered therapeutic agents.

Role of Drug Metabolism in Drug Development

There are two key elements associated with successful drug development: *efficacy* and *safety*. Both depend on drug metabolism. It is necessary to determine which enzymes metabolize a new drug candidate to predict whether the compound may cause drug-drug interactions or be susceptible to marked interindividual variation in metabolism due to genetic polymorphisms.

For determination of metabolism, the compound under development that shows efficacy in preclinical models is subjected to analysis by human hepatocytes, human liver, or liver extracts that contain all the drug-metabolizing enzymes. Such studies can predict how humans will metabolize a particular drug and, to a limited extent, predict the rate of metabolism. If a CYP is involved, a panel of recombinant CYPs can be used to determine which CYP predominates in the metabolism of the drug. If a single CYP, such as CYP3A4, is found to be the sole CYP that metabolizes a drug candidate, then a decision can be made about the likelihood of drug interactions. Prior to entering clinical trials, the structure of a drug candidate can be modified to change the sites on the molecule that are metabolized, particularly by CYPs, in order to mitigate potential rapid degradation and toxicities.

Interactions become a problem when multiple drugs are simultaneously administered, for example, in elderly patients, who on a daily basis may take prescribed anti-inflammatory drugs, cholesterol-lowering drugs, blood pressure medications, a gastric acid suppressant, an anticoagulant, and a number of over-the-counter medications. An ideal drug candidate would be metabolized by several CYPs so that variability in expression levels of one CYP or drug-drug interactions would not significantly affect its metabolism and pharmacokinetics.

Similar studies can be carried out with phase 2 enzymes and drug transporters to predict the metabolic fate of a drug. In addition to the use of recombinant human xenobiotic-metabolizing enzymes in predicting drug metabolism, human receptor-based systems or cell lines expressing nuclear receptors are used to determine whether a particular drug candidate is a ligand or activator of PXR, CAR, or PPARα. For example, a drug that activates PXR may result in rapid clearance of other drugs that are CYP3A4 substrates, thus decreasing their bioavailability and efficacy.

Computer-based computational (*in silico*) prediction of drug metabolism is a prospect for the near future. The structures of several CYPs have been determined, including those of CYPs 2A6, 2C9, and 3A4. These structures may be used to predict metabolism of a drug candidate by fitting the compound to the enzyme's active site and determining oxidation potentials of sites on the molecule. However, the structures, determined by X-ray analysis of crystals of enzyme-substrate complexes, are static, whereas enzymes are flexible; this vital distinction may be limiting. The large size of the CYP active sites, which permits them to metabolize many different compounds, also renders them difficult to model. The potential for modeling ligand or activator interactions with nuclear receptors also exists with limitations similar to those discussed for the CYPs.

Determining the potential of a drug candidate to produce acute toxicity in preclinical studies is vital and routine in drug development. This is typically done by administering escalating doses of the drug candidate to rodents, usually above the predicted human therapeutic dose. For drug candidates proposed for chronic use in humans, such as for lowering serum triglycerides and cholesterol or for treatment of type 2 diabetes, long-term carcinogenicity studies are carried out in rodent models. Signs of toxicity are monitored and organ damage assessed by postmortem pathologies. This process is not high throughput and can be a bottleneck in development of lead compounds.

A new technology of high-throughput screening for biomarkers of toxicity is being adopted for drug development using *metabolomics* (Jacob et al., 2019). Metabolomics is the systematic identification and quantification of all metabolites in a given organism or biological sample. Analytical platforms such as ^{1}H nuclear magnetic resonance and liquid chromatography or gas chromatography coupled to mass spectrometry, in conjunction with chemometric and multivariate data analysis, allow the simultaneous determination and comparison of thousands of chemicals in biological fluids such as serum and urine, as well as the chemical constituents of cells and tissues. This technology can screen for drug toxicity in whole-animal systems during preclinical drug development and can obviate the need for time-consuming and expensive necropsies and pathologies on thousands of animals.

Using metabolomics, animals, treated and not treated with a drug candidate, can be analyzed for the presence of one or more metabolites in urine that correlate with drug efficacy or toxicity. Urine metabolites that are fingerprints for liver, kidney, and CNS toxicity have been identified using known chemical toxicants. Metabolic fingerprints of specific compounds that are elevated in urine can be used to determine whether a particular drug causes toxicity and can also be employed in early clinical trials to monitor for potential toxicities. Metabolomics can be used to find biomarkers for drug efficacy and toxicity that can be of value in clinical trials to identify responders and nonresponders. Drug metabolism can be studied in whole-animal model systems and in humans to determine the metabolites of a drug or indicate the presence of a polymorphism in drug metabolism that might signal an adverse clinical outcome. Finally, biomarkers developed from experimental metabolomics could eventually be developed for routine monitoring for signs of toxicity in patients receiving pharmacotherapy.

Animal Models for Preclinical Drug Development

By use of the *in silico*, *in vitro*, and cell culture tools described above, drug companies can determine the human CYPs and other enzymes that carry out metabolism of a drug candidate, thereby facilitating prediction of drug interactions that might occur in the clinic. These techniques can also establish whether a compound has the potential to be metabolically activated to a cytotoxic or carcinogenic derivative. A more accurate assessment on how a drug will behave in humans can be obtained with small animal models that have the advantage of a higher throughput analysis of many derivatives of a potential drug. However, mouse and rat CYPs markedly differ from CYPs found in humans. To this end, humanized mice that express human CYPs and the receptors that regulate CYPs, such as PXR as described above, have been developed (Gonzalez et al., 2015). UGTs that exhibit species differences have also been humanized (Fujiwara et al., 2018). In most cases, the endogenous mouse genes have been replaced by the corresponding human genes. Since drug transporters, FMOs, EHs, and phase 2 enzymes do not display large species differences in regulation and catalytic activities, the CYP-PXR humanized mice can be widely employed to estimate drug stability and bioavailability prior to launching phase I clinical trials. These humanized mice can also be used for long-term toxicity and carcinogenicity studies. Another model that has been developed is the human liver replacement strategy in which human liver is used to replace mouse liver (Naratomi et al., 2019). However, this model requires immunocompromised mice and has the issue that each human liver used to replace the mouse liver may express different levels of individual CYPs due to the genetic (polymorphisms) and dietary (inducers in food from the donor) factors.

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Chapter 6

The Gastrointestinal Microbiome and Drug Response

Shirley M. Tsunoda, Pieter C. Dorrestein, and Rob Knight

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INDIRECT DRUG METABOLISM BY GUT MICROBES

ENTEROHEPATIC RECYCLING

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DRUG EFFECTS ON THE MICROBIOME

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- Probiotics
- Designer Microbiota
- Phage Therapy

The Human Microbiome

What Is It?

The microbiome is the genetic repertoire of the ecosystem of microbes (bacteria, viruses including phages, and sometimes archaea, fungi, and microbial eukaryotes) that coexist at a given site within each human. The human microbiota consists of the collection of microbes that exist in a specific area within the body, such as the oral cavity, esophagus, skin, gut, vagina, and other sites. The composition, regulation, and dynamics of the microbiomes and microbiota inhabiting these body areas are individualized and compartmentalized. The organisms in the oral cavity and gut are more diverse than those in other body sites (Costello et al., 2009; Human Microbiome Project Consortium, 2012). Establishment of the microbiome begins at delivery, with the infant inheriting microbes from the mother and the environment. The mode of delivery affects the infants' microbial colonization, with babies born via cesarean section dominated by epidermal bacterial species with predisposition to asthma and allergies later in life (Bager et al., 2008; Dominguez-Bello et al., 2010). After the child is weaned, the microbiome is established with an individual signature that persists long term (Faith et al., 2013), possibly throughout life (Maynard et al., 2012). Genes from the microbiome far outnumber human genes, with estimates of over 200 million microbial genes compared to 20,000 human germline genes (Qin et al., 2010). Microbial cells (mainly gut bacteria), on the other hand, are in approximately 1:1 ratio with human cells (Sender et al., 2016). Although an individual's microbiome appears to be generally stable over that individual's lifetime, numerous environmental factors can alter the microbial composition, including geographic location, diet, lifestyle, and xenobiotics (Human Microbiome Project Consortium, 2012; Kaplan et al., 2019; Kurilshikov et al., 2021). Other influences include circadian rhythms, age, and season of the year.

More than 90% of the gut microbiota are members of two bacterial phyla, *Bacteroidetes* and *Firmicutes* (Turnbaugh et al., 2007). Previously, it was thought that human gut microbiomes could be categorized into discrete "enterotypes" with enrichment of *Bacteroides*, *Prevotella*, or *Ruminococcus* forming the basis for three different enterotypes. However, it is now thought that the variability of the gut microbiome across the human population likely forms a continuum as opposed to discrete groups (Jeffery et al., 2012). The person-to-person variability in the gut microbiome is expansive, with individual microbiomes differing by greater than 90% (Parfrey and Knight, 2012).

Disease and the Microbiome

Although the mechanisms await definition, the gut microbiome is implicated in numerous poor health outcomes and diseases including autoimmune conditions, autism, cardiovascular diseases, cancer, and liver diseases such as nonalcoholic fatty liver disease (Gilbert et al., 2016). The National Cancer Institute estimates that 16% of all cancers in the U.S. are demonstrably due to components of the microbiome (e.g., *Helicobacter pylori*, human papillomavirus, hepatitis C). Not yet counted among the causes of that 16% are colibactin-producing *Escherichia coli*, which can cleave human DNA (Li et al., 2019) and also induce colorectal cancer. One challenge for the future will be to determine whether functional changes in the gut microbiome can serve as biomarkers or targets for therapeutic intervention or whether the microbiome itself can serve as therapeutic intervention in disease states.

Drugs and the Microbiome

The gut microbiome is a prominent contributor to variation in drug response. Gut bacteria directly metabolize xenobiotics, indirectly affect drug metabolism by influencing the activities of phase I and phase II enzymes and intestinal drug transporters, play a role in enterohepatic recycling of drugs, and are involved in pharmacodynamic responses to drugs (Lam et al., 2019; Tsunoda et al., 2021). In today's era of precision medicine, understanding how and to what extent the gut microbiome interacts with drugs and their actions will be key to individualizing therapy. With its distribution throughout the gastrointestinal (GI) tract, the gut microbiome interfaces with many of the chemicals encountered in daily life, including endogenous ligands, environmental sources, food, inhaled substances, and exogenous chemicals such as drugs. There is a bidirectional nature to this interaction between drugs and the microbiota. Drugs alter the microbiome; one needs only to consider the example of antibiotics that disrupt the quantity and variety of gut microbes.

Abbreviations

5-ASA: 5-aminosalicylic acid
cgr: cardiac glycoside reductases
CYP: cytochrome P450
FMT: fecal microbiota transplantation
FXR: farnesoid X receptor
GI: gastrointestinal
ICI: immune checkpoint inhibitor
LPS: lipopolysaccharide
OAT: organic anion transporter
OATP: organic anion-transporting polypeptide
PD-1: programmed cell death protein 1
Pgp: P-glycoprotein
PPI: proton pump inhibitor
PXR: pregnane X receptor
UGT: uridine diphosphate glucuronosyltransferase

However, many nonantibiotic compounds can impact gut microbiota, with 240 drugs showing inhibition of at least one bacterial strain *in vitro* (Maier et al., 2018); such a result may have implications for antibiotic resistance from drugs traditionally characterized as nonantibiotic agents. Conversely, it is known that the microbiome can directly modify drugs. Gastroenterologists depend on the actions of gut microbiota when administering the prodrug *sulfasalazine* to treat inflammatory bowel disease. Gut bacteria reduce the azo bond of *sulfasalazine*, releasing the metabolite sulfapyridine and the active drug 5-aminosalicylate (*mesalamine*) (see Figure 55–3). Recent animal and human studies show evidence for indirect metabolism by gut microbes via modification of the machinery of host drug metabolism (Selwyn et al., 2016; Toda et al., 2009b). Gut microbes also play a role in the gut-liver cross talk that affects enterohepatic recycling of drugs (see later section on enterohepatic recycling).

Therapeutic Interventions Using the Microbiome

Once we have a deeper understanding of what constitutes a "healthy" microbiome, how the microbes regulate and coordinate function, and factors that influence the variability within and between individuals, we will be better poised to rationally design therapeutic interventions to manipulate the microbiome for health. One such intervention, fecal microbiota transplantation (FMT), is already being used in the clinical setting to treat *Clostridioides difficile* (formerly, *Clostridium difficile*) colitis and involves a dramatic reshaping of the microbiome from an unhealthy state to a state resembling the healthy donor (Weingarden et al., 2015). Other prebiotics, probiotics, postbiotics, and living microbes are being studied for a variety of indications such as inflammatory bowel disease, autism (Kang et al., 2017), and phenylketonuria. New guidelines for studying and approving clinical use of these "living microbes" will need to be created and agreed upon by the scientific and clinical community.

Techniques for Studying the Microbiome

The ability to study the microbiome has improved greatly in the last few decades due to the advancement of technologies to interrogate and characterize microbes. One technological advancement that has been instrumental is the ability to sequence and inventory the microbiome. Early in the microbiome field, the focus was on taking inventories using 16S rRNA, allowing genus information. With lowered cost and improved throughput of sequencing, it is now becoming routine to get species information using metagenomic sequencing. The 16S rRNA gene is shared by all bacteria and archaea and has regions that can be used as primer sites for polymerase chain reaction. Microbes can be identified at the family, genus, and sometimes near-species level. The most notable technology to revolutionize the field is the higher-level resolution and functional-based DNA assay, shotgun metagenomics (Figure 6–1), which is rapidly becoming the standard method of studying the microbiome. Shotgun metagenomics relies on extracting total DNA from a sample, breaking it into small fragments, sequencing those fragments, and interpreting the fragments or "assembled" longer sequences in terms of the taxa represented (sometimes to the level of species or strain) and the gene functions contained in the assembly. Thus, in addition to higher taxonomic resolution, shotgun metagenomics helps describe and identify microbial genes and pathways. For expression-level information, metatranscriptomics, metaproteomics, and metabolomics provide information at the levels of RNA, protein, and metabolite, information that can be utilized in tandem with DNA-based assays. The availability of gnotobiotic mouse models (i.e., mice without a microbiome) has enabled more mechanistic studies to be performed such as introducing single microbial species or groups of microbes and testing their influences on various parameters of disease and pharmacotherapy, studies that provide greater information about causality.

Pharmacomicrobiomics

An orally administered drug encounters many obstacles *en route* to the systemic circulation. There are physicochemical barriers within the stomach and small intestine that can alter absorption, metabolizing enzymes such as intestinal and hepatic CYPs (cytochromes P450), and membrane

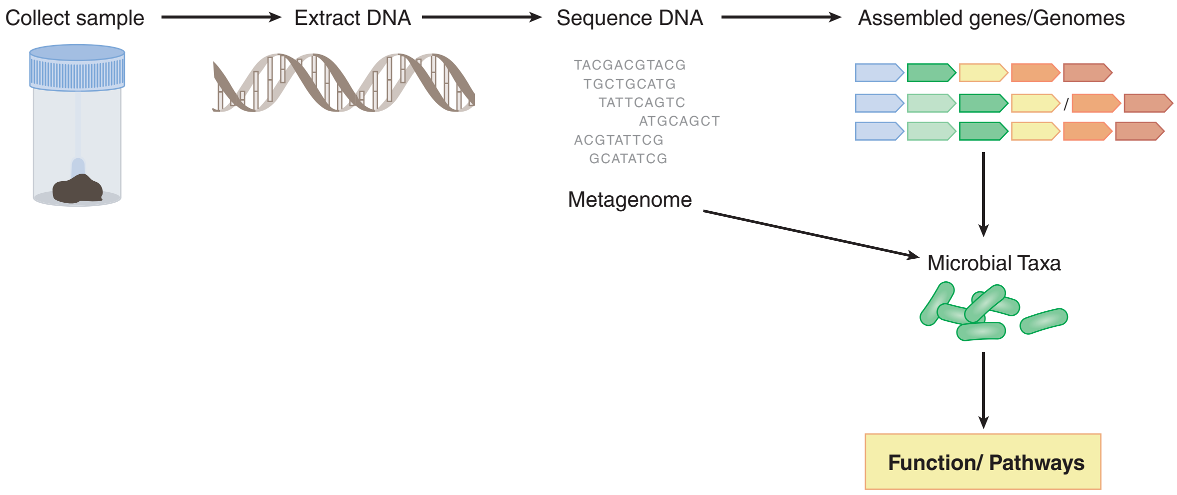

Figure 6–1 *Shotgun metagenomics.*

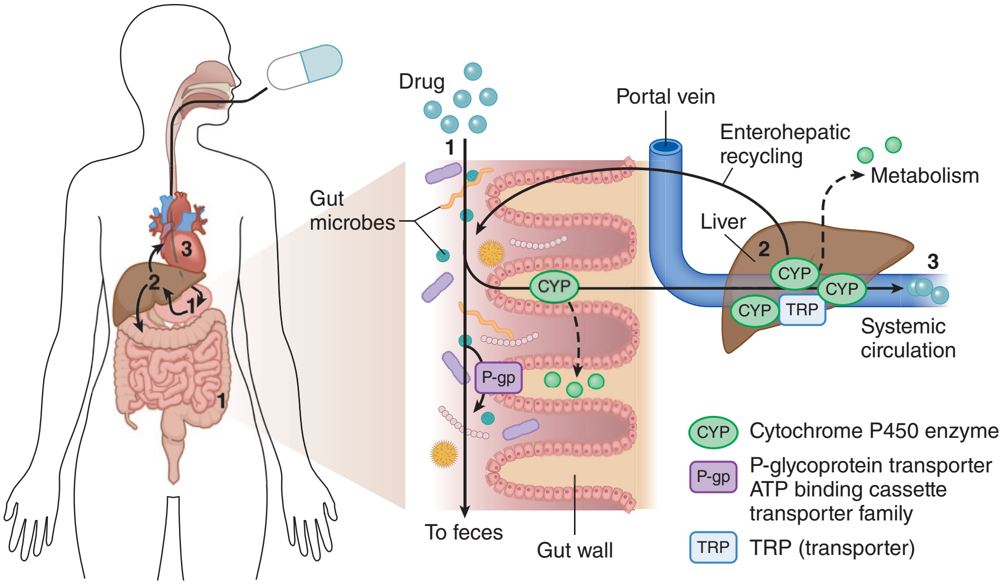

Figure 6–2 *Pathway of an orally administered drug.* When a drug is administered orally (1), it encounters gut microbes, CYPs, and transporters such as P-glycoprotein in the small and large intestine. Some drug will be lost in the feces in these processes. Drug that remains unmetabolized in the small intestine is absorbed and then travels through the portal vein to the liver where it encounters more transporters and CYPs, whereby more drug may be lost to metabolism (2). The amount of drug that enters the systemic circulation is often a fraction of what was originally ingested (3). Many absorbed drugs are subsequently metabolized in the liver and excreted into the GI tract via the bile as polar conjugates with UDP-glucuronic acid, glutathione, or sulfate and are not sufficiently lipophilic to be reabsorbed. The intestinal microbiome plays a role in deconjugation, hydrolyzing glucuronides and sulfates and rendering them more lipophilic; the more lipophilic moieties are reabsorbed and reenter the portal circulation, as though they had been administered orally, and begin the cycle again. Thus, enterohepatic cycling can prolong the elimination half-life of a xenobiotic.

transporters such as the intestinal multidrug-efflux transporter Pgp (P-glycoprotein; MDR1, ABCB1) that interact with drugs but can also be inhibited by competing solute such as other drugs and can be induced by drugs. The picture can get complex, especially when we realize that gut bacteria can modify drugs directly and also indirectly by modifying host metabolic processes (Figure 6–2). Such interactions are not confined to orally administered agents. Parenterally administered drugs and their metabolites may reach the intestine through biliary secretion and thus also interact with the gut microbiota.

The myriad processes acting on a drug—acidic environment of the stomach, metabolism in the gut and liver before distribution throughout the body, membrane barriers and selective permeabilities, extrusion from intestinal cells into the lumen of the GI tract, and effects of the microbiome that may alter a drug's solubility or chemical identity and efficacy as a drug—affect the fraction of an orally administered drug that actually enters the systemic circulation. This fraction (total drug administered/total drug actually entering the systemic circulation) is called the bioavailability of the drug, or F, a fraction that varies between 0 and 1, $0 < F < 1$ (see Chapter 2).

CYPs and drug-efflux transporters are key drivers of bioavailability. The variability in bioavailability within and between individuals is a major cause of therapeutic failure and toxicity. CYP enzymes metabolize 70% to 80% of all marketed drugs (see Figure 5–3). CYP3A is responsible for over 50% of CYP-mediated drug metabolism. In the intestine, CYP3A enzymes metabolize substrates prior to their entering the portal circulation, and Pgp pumps drugs out of the intestine, thus limiting bioavailability. Hepatic CYP3A can further metabolize substrates. Considering the extended time that orally administered drugs spend in the small and large intestines where the bulk of the gut microbiome resides, it is important to understand the impact of the gut microbiota on drug efficacy and toxicity.

The new field that studies interactions of the microbiome with xenobiotics is called pharmacomicrobiomics. Among the complexities of studying the contributions of the microbiome to drug effect and toxicity are an individual's disease state and genetic, environmental, and lifestyle characteristics. Interindividual variability in response to drug therapy is a major cause for therapeutic inefficacy and toxicity with resulting hospitalizations and morbidity/mortality. Much progress has been made in determining genetic variability in drug-metabolizing enzymes, drug transporters, and drug target genes, resulting in clinically actionable guidelines for select drugs (Relling and Klein, 2011). Gut microbes may impact drugs by influencing pharmacokinetics and pharmacodynamics. Bacteria may metabolize drugs before absorption, after absorption through the intestinal epithelia, and after biliary excretion from the liver, which may alter or lead to reabsorption of the drug through enterohepatic recycling. The gut microbiome may impact the activity of transporters (Walsh et al., 2020) and drug-metabolizing enzymes that affect a drug's pharmacokinetics. The microbiome can also bioaccumulate drugs, actually storing them without altering them (Klünemann et al., 2021), thereby reducing the drugs' bioavailability in the GI tract. Data are emerging on the gut microbiome's effect on pharmacodynamics and the complex intersection with the gut-brain axis as well as the gut–immune system axis. It will be important to incorporate the contribution of the gut microbiome to drug efficacy and toxicity along with other factors such as genetics, age, sex, circadian rhythms, inflammation, disease, and coadministered drugs.

Direct Drug Metabolism by Gut Microbes

Bacterial metabolism consists of distinct types of reactions that differ in significant ways from human host metabolism. Bacterial metabolism of drugs often results in increased hydrophobicity of the compounds, enhancing their lipid solubility and potentially increasing their toxicity, whereas host metabolism generally produces more hydrophilic compounds, decreasing their toxicity and facilitating excretion. Modifications performed by bacteria include reduction, hydrolysis, hydroxylation, dealkylation, and demethylation, among others. Bacteria also can add or remove functional groups through hydrolysis and deconjugation (Sousa et al., 2008) (Table 6–1). Reduction and hydrolysis appear to predominate because these two reactions reflect the energetic demands of the largely anaerobic gut microbes. Reduction reactions facilitate anaerobic respiration by providing a wider range of electron acceptors available, while

TABLE 6–1 ■ REPRESENTATIVE METABOLISM OF DRUGS MEDIATED BY THE MICROBIOME

MODIFICATION	TYPE	EXAMPLE DRUGS	INDICATION	MICROBES	EFFECT ON ACTIVITY
Reduction	Azoreduction	Sulfasalazine	Anti-inflammatory	Many; e.g., *Bacteroides fragilis, Streptococcus faecium, Streptococcus faecalis*	Activates
	Nitroreduction	Chloramphenicol	Antibiotic	*Escherichia coli, Haemophilus influenzae, Neisseria meningitides, Bacteroides fragilis*	Deactivates
	Reduction	Digoxin	Cardiovascular	*Eggerthella lenta*	Deactivates
	Reduction	Fluorouracil–once attached to ribose	Antineoplastic		Deactivates
	Sulfoxide reduction	Omeprazole	Gastric acid–related disorders	*Bacillus megaterium*	Deactivates
Acylation	Proprionylation	Tobramycin	Antibiotic	Cutured cystic fibrosis communities	Unknown
	Acetylation	Aminoglycosides (e.g., kanamycin A)	Antibiotic	*Mycobacterium tuberculosis*	Deactivates
	Hydroxylation	Simvastatin	Antihypolipidemic	Cultured gut community	Deactivates
Hydrolysis	Ester hydrolysis	Lovastatin	Antihypolipidemic		Activates
	Deglucuronidation	Irinotecan (SN-38-G)	Antineoplastic	*E. coli, Lactobacillus rhamnosus, Ruminococcus gnavus, Faecalibacterium prausnitzii*	Activates
	Desulfation	Sodium picosulfate	Laxative	*Eubacterium rectale*	Deactivates
	Dephosphorylation	5-Fluorouracil	Antineoplastic	Gut community	Activates
	Amide hydrolysis	Methotrexate	Antimetabolite	Firmicutes association	Activates
	Deacetylation	Diltiazem	Antihypertensive	*Bacteroides thetaiotaomicron*	Reduced activity
Demethylation	Demethylation	Altretamine	Antineoplastic	Pooled fecal microbial culture	Activates
Decarboxylation	Decarboxylation	Levodopa	Parkinson's disease	Cultured gut community	Deactivates

Tabulated data drawn from: Wilson and Nicholson, 2017; Guthrie et al., 2019; Letertre et al., 2020; Onuora, 2021; Aura et al., 2011; Swanson, 2015; Pellock and Redinbo, 2017; Biernat et al., 2019; Tsodikov et al., 2014; Jarmusch et al., 2020; Jang et al., 2017; Guo et al., 2020; Zimmermann et al., 2019; Clarke et al., 2019; Guthrie and Kelly, 2019; Koppel et al., 2017; Sun et al., 2019.

hydrolysis provides substrates for microbial growth. These bacterial metabolic modifications complement those performed by the host's CYPs that include *N*- and *S*-oxidation, *N*- and *O*-dealkylation, aromatic hydroxylation, deamination, and dehalogenation (Zanger and Schwab, 2013).

Although direct biotransformation of drugs by bacteria has been known for over a century, only recently have we appreciated its widespread nature, reflecting advances in tools to identify and characterize these bacterial transformations. Gut microbiota can directly metabolize drugs into active, inactive, or toxic metabolites. Microbial azoreductases are ubiquitous across several bacterial phyla found in the gut microbiome. The therapeutic effects of several prodrugs are activated by the reduction of the azo bond following oral administration. For example, the ulcerative colitis drug *sulfasalazine* is activated by gut microbial azoreductases to cleave into sulfapyridine and 5-aminosalicylic acid (5-ASA). 5-ASA is the active drug that inhibits inflammation within the colon of ulcerative colitis patients. 5-ASA can be inactivated by bacterial arylamine *N*-acetyltransferases. The activity of these enzymes can vary over a 10-fold range (Deloménie et al., 2001), leading to significant interindividual variability in the metabolism and possibly the efficacy of *sulfasalazine* among patients.

Unlike human drug biotransformation, for which phase I and II drug-metabolizing enzymes are well characterized, a comprehensive knowledge base of drug-metabolizing bacteria is lacking. The field is progressing, however. Using a combination of *in vitro* drug incubations and untargeted metabolomics, 76 gut bacterial species were shown to metabolize 176 nonantibiotic drugs spanning a diverse set of clinical indications (Zimmermann et al., 2019). Some patterns of direct bacterial drug metabolism have emerged. Bacterial isolates share phylum-specific metabolic activities and predictably metabolize drugs with specific functional groups. For example, drugs metabolized by *Bacteroidetes* contain ester or amide groups that can be hydrolyzed. Drugs with lactones, nitro, azo, and urea groups are more susceptible to microbial metabolism. In addition, results suggest that identification to the species level is insufficient to explain bacterial metabolism and that identification of gene markers directly associated with microbial enzymatic drug metabolism may be necessary (Zimmermann et al., 2019).

To date, there are few examples of direct gut microbiota drug metabolism that are clinically significant enough to change drug efficacy or toxicity, but a few drugs with narrow ranges demonstrate the issue nicely. The cardiac glycoside *digoxin*, which is used clinically for atrial fibrillation and heart failure, is a good example. Signs of *digoxin* toxicity can occur at around twice the level required for effective treatment. Staying within this narrow therapeutic window requires therapeutic drug monitoring and careful titration of dosage. In approximately 10% of patients, high levels of an inactive metabolite, dihydrodigoxin, are produced that lead to a significant decrease in the systemic concentration of the active drug. The gut microbe *Eggerthella lenta* is responsible for the reduction of *digoxin*'s α,β-unsaturated lactone ring, leading to the formation of dihydrodigoxin. More precisely, only a specific strain of *E. lenta* (DMS2243) that induces the expression of a two-gene operon, cardiac glycoside reductases (cgr) 1 and 2, reduces the lactone ring of *digoxin* (Haiser et al., 2013). Interestingly, a diet high in arginine inhibits cgr, thus mitigating the bacterial reduction (Haiser et al., 2013). This demonstrates the highly specific nature of interindividual variability that can influence the therapeutic efficacy of *digoxin*. *Tacrolimus*, an immunosuppressive with a narrow therapeutic range, also exhibits variability of efficacy that is linked to a member of the microbiota, *Faecalibacterium prausnitzii*. Kidney transplant patients who required higher doses of *tacrolimus* had gut microbiomes enriched in *F. prausnitzii*, a nonmotile gram-positive bacterium (Lee et al., 2015). Further investigation showed that incubation of *tacrolimus* with *F. prausnitzii* produces a keto-reduction product of *tacrolimus* that was not found when incubated in hepatic microsomes, suggesting a direct and unique biotransformation of *tacrolimus* by gut microbes (Guo et al., 2019).

Indirect Drug Metabolism by Gut Microbes

In addition to direct enzymatic transformation by gut microbes, intestinal bacteria can also indirectly affect drug biotransformation by regulating host drug-metabolizing genes. Animal studies have demonstrated that gene expression, protein levels, and activity of drug-metabolizing enzymes are altered. For instance, gene expression for Cyp3a (the mouse ortholog to human *CYP3A*) is markedly downregulated in both intestine and liver of germ-free mice (Toda et al., 2009b) compared to wild-type controls. This was correlated with decreased nuclear binding of the pregnane X receptor (PXR), a transcriptional upregulator of Cyp3a in the liver (Björkholm et al., 2009). Conventionalization of the germ-free mice restored Cyp3a to near normal levels (Selwyn et al., 2016). Treatment of conventional mice with the quinolone antibiotic *ciprofloxacin* reduced hepatic Cyp3a expression and decreased metabolism of the Cyp3a substrate *triazolam*; *ciprofloxacin* caused no changes to Cyp3a activity in germ-free mice (Toda et al., 2009a). These data suggest that microbes or microbial products may bind to nuclear factors such as PXR to downregulate the expression of drug-metabolizing enzymes such as CYP3A.

Other phase I and phase II enzymes and transporters are altered by gut microbes in animals. Germ-free mice had higher intestinal mRNA expression of the phase I enzymes alcohol dehydrogenase and aldehyde dehydrogenase, the phase II enzyme UDP-glucuronosyltransferase 1a1 (Ugt1a1), two bile acid transporters (Fu et al., 2017), and the hepatic Mdr1b, the mouse ortholog to human *MDR*, the gene encoding Pgp (Walsh et al., 2020). By contrast, germ-free mice had decreased hepatic expression of organic anion transporter (OAT) protein Oatp1a1 (the ortholog to human OATP1A1), the breast cancer resistance protein Bcrp1 (ortholog to human BCRP), and the organic cation transporter Oct1 (ortholog to human OCT1) (Kuno et al., 2016). Human studies with respective protein levels and activities of these enzymes and transporters will be important to determine the clinical relevance of these results.

The available human data are limited but are consistent with the data from studies with animals. A study in healthy volunteers showed decreases in the activity of CYPs 1A2, 2C19, and 3A4 after a 7-day course of the cephalosporin *cefprozil* (Jarmusch et al., 2020); the substrates used were caffeine for CYP1A2, *omeprazole* for CYP2C19, and *midazolam* for CYP3A4 (Table 6–2). Analysis of the microbial community showed decreased alpha diversity (a measure of the variance of organisms within a given sample) and a correlation between loss of alpha diversity and increased drug and metabolite formation for all three probe substrates (Jarmusch et al., 2020). Altering the microbiome with antibiotic therapy modestly decreased enzyme activity, suggesting that a healthy and diverse microbiome may be necessary for optimal functioning of drug-metabolizing enzymes. Future investigations into the mechanism of this effect as well as those of other antibiotics may provide additional clinically actionable information.

The analgesic *acetaminophen* provides an example of gut microbes altering host phase II hepatic metabolism (see Table 6–2). *Acetaminophen* undergoes glucuronidation, and bacterial glucuronidases can deconjugate the glucuronide metabolite, allowing for reabsorption of the parent *acetaminophen* or further metabolism to sulfate and/or glucuronide conjugates. With antibiotic treatment, there is a decrease in the sulfate conjugate of *acetaminophen* (Malfatti et al., 2020). In addition, gut bacteria produce a metabolite of aromatic amino acid metabolism, p-cresol, that competes with *acetaminophen* for binding to the sulfotransferase (SULT1A1). Individuals who produce high levels of p-cresol have lower capacity to sulfonate *acetaminophen* (Clayton et al., 2009). Therefore, antibiotic therapy and high levels of the bacterially derived metabolite p-cresol could predispose individuals to the hepatotoxic effects of *acetaminophen*.

Enterohepatic Recycling

Enterohepatic recycling occurs when xenobiotics or endogenous substances are absorbed through enterocytes, processed by hepatocytes, and then secreted into the bile where they are then reabsorbed by intestinal cells (see Figure 6–1). Enterohepatic recycling can often be accompanied by hepatic conjugation and intestinal deconjugation. This process can occur continuously and results in a prolonged mean residence time for the substrate. Many drugs and endogenous substances are modified by phase II enzymes such as uridine diphosphate glucuronosyltransferases (UGTs), which add a glucuronic acid moiety to make a more water-soluble metabolite that is more easily excreted into urine or bile. In the intestine, the metabolites can encounter bacterial enzymes such as β-glucuronidase, β-glucosidase, demethylase, desulfase, and other enzymes with phase II reversing activity that cleave off the small molecules such as glucuronide, making the parent compound available again for reabsorption.

β-Glucuronidases are among the most studied gut-derived microbial enzymes. In addition to cleaving glucuronide moieties from drugs, they also breakdown complex carbohydrates, thereby providing a source of carbon for bacterial growth (Dabek et al., 2008). Members of multiple bacterial phyla can catalyze the hydrolysis of glycosidic bonds, including *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (Pellock and Redinbo, 2017), highlighting the widespread phylogenetic distribution of this process. Notably, β-glucuronidases from opportunistic (as opposed to commensal) bacteria may play the larger role in xenobiotic-induced toxicity (Dashnyam et al., 2018). One clinically important example of the role of β-glucuronidases occurs with *irinotecan*, a chemotherapy agent used in the treatment of colorectal cancer. Severe diarrhea is a dose-limiting adverse effect that occurs in approximately 50% of patients. *Irinotecan* is a prodrug that is metabolized to the active compound SN-38, which is then glucuronidated in the liver by UGT1A1. Subsequently, the inactive SN-38 glucuronide is secreted into the bile and reabsorbed by intestinal cells via enterohepatic recycling. SN-38 glucuronide is then subjected to gut bacterial β-glucuronidases that remove the glucuronide, producing active SN-38. High intestinal levels of SN-38 are responsible for the diarrhea (see Figures 5–8 and 5–9). Inhibitors of β-glucuronidases such as *ciprofloxacin* may exert a protective effect from the diarrhea (Kodawara et al., 2016; Wallace et al., 2010). Other medications metabolized by β-glucuronidases include *morphine*, *estrogen*, *ibuprofen*, and *midazolam*. The activity of β-glucuronidases may vary with sex, age, and bacterial species (Elmassry et al., 2021); that variation may have consequences on drug bioavailability.

Bile acids, produced in the liver, mediate significant cross talk between the intestine and liver. Bile acids, modified by bacteria in the gut, are important signaling molecules that regulate host metabolism. Microbes can modify host-derived bile acids (Gentry EC et al., 2021; Foley et al., 2019). Microbes from several genera including *Lactobacillus*, *Clostridium*, and *Bifidobacterium* populate the upper intestine, where they deconjugate taurine and glycine conjugates via the action of dedicated hydrolases. Other microbe-catalyzed modifications of bile acids include de-hydroxylation (e.g., converting cholic acid to deoxycholic acid) and epimerization and

TABLE 6–2 ■ INDIRECT DRUG METABOLISM BY MICROBES AND THEIR EFFECT ON PHARMACOKINETIC (PK) PARAMETERS

INDIRECT METABOLISM						
DRUG	**ANIMAL/ HUMAN**	***IN VIVO/IN VITRO***	**EFFECT ON PK (INTACT GUT MICROBIOME)**	**ENZYME**	**DIVERSITY**	**COMMENTS**
Acetaminophen (Malfatti et al., 2020)	Animal	*In vivo*	↑ AUC and C_{max}	SULT1A1	NA	P-cresol competes with acetaminophen binding to SULT1A1 → prevents host from detoxifying acetaminophen
Caffeine (Jarmusch et al., 2020)	Human	*In vivo*	↓ CL	CYP1A2	↓ α ↑ β	↓ CYP activity when treated with cefprozil
Metformin (Wu et al., 2017)	Animal	*In vivo*	↓ C_{max} and ↑ half-life	Oct1	NA	PK changes likely due to ↓ Oct1 expression in the liver → altered hepatic uptake of metformin *in vivo*
Midazolam (Jarmusch et al., 2020; Togao et al., 2020)	Animal Human	*In vitro* *In vivo*	↓ C_{max}, AUC, half-life ↓ CL	Cyp3a; Ugt	NA ↓ α ↑ β	Low levels of Cyp3a activity in GF mice ↓ drug metabolism *in vivo* ↓ CYP activity when treated with cefprozil
Omeprazole (Jarmusch et al., 2020)	Human	*In vivo*	↓ AUC metabolite ratio	CYP2C19	↓ α ↑ β	↓ CYP activity when treated with cefprozil
Progestogens (Coombes et al., 2020)	Human	*In vivo*	MPA had longest half-life	CYP450	NA	Hydroxylation of progestins are likely CYP mediated
Triazolam (Toda et al., 2009a, 2009b)	Animal	*In vivo*	↑ metabolite to parent drug ratio in SPF vs. GF mice	Cyp3a Cyp3a11 Cyp3a25	NA	↑ hepatic Cyp activity in SPF mice (*Bacteroides* and *Escherichia coli*) Ciprofloxacin administration to SPF mice → significant ↓ mRNA expression of hepatic Cyp3a11

AUC, area under the curve; CL, clearance; GF, germ-free; MPA, medroxyprogesterone; NA, not applicable; SPF, specific pathogen free.

oxidation of alcohols. In 2020, it was discovered that microbiota also have the ability to deconjugate with different amino acids, vastly expanding the diversity of bile acids made by microbes (Gentry et al., 2021; Guzior and Quinn, 2021).

Bile acids achieve their signaling properties by binding to nuclear receptors such as the farnesoid X receptor (FXR), and TGR5, a G protein-coupled receptor activated by bile acids (Pols et al., 2011). Binding of bile acids to FXR modulates CYP3A (Gnerre et al., 2004) and transporter activity (Ananthanarayanan et al., 2001). Other microbial products such as the secondary bile acid lithocholic acid, lipopolysaccharides (LPS) produced from gram-negative bacteria, and indole-3-propionic acid can activate PXR (Staudinger et al., 2001). This is a plausible mechanism by which bacterially derived bile acids could regulate host drug metabolism.

Pharmacodynamics

The role of the microbiome in modifying pharmacodynamics—what the drug does to the body—is emerging. Two examples of drugs used in cancer are highlighted here: immune checkpoint inhibitors (ICIs) and a panel of cytotoxic agents.

Immune checkpoint inhibitors are used for a variety of solid and hematological malignancies. They induce an immune response by suppressing pathways targeting cytotoxic T-lymphocyte–associated antigen (CTLA-4) and the programmed cell death protein 1 (PD-1) involved in negative regulation of the immune system (see Figure 39–5 and Chapter 72). The responses to ICIs are often variable and not durable. Gut microbiome composition is associated with ICI efficacy. An abundance of *Faecalibacterium* was observed in melanoma patients who responded to ICI therapy and had significantly longer progression-free survival. Conversely, patients with higher relative abundance of *Bacteroidales* had a shorter progression-free survival. In fact, the strongest microbial predictors of response to ICI therapy were alpha diversity and an abundance of *Faecalibacterium* and *Bacteroidales* (Gopalakrishnan et al., 2018). Loss of microbial diversity from antibiotic exposure has been shown to decrease survival in cancer patients on ICI therapy (Derosa et al., 2018; Routy et al., 2018). Animal and human studies suggest that a gut microbiome with high diversity and an abundance of *Faecalibacterium* plays a role in enhancing the antitumor immune responses mediated by antigen presentation and effector T cells.

Chemotherapy resistance is a major challenge in oncology that often leads to therapeutic inefficacy and significant mortality (see Chapter 71). The gut microbiome may play a role in limiting the efficacy of some chemotherapy agents. A specific gut microbe, *Fusobacterium nucleatum*, is associated with shorter survival in colorectal cancer patients (Mima et al., 2016) and is increased in patients with postchemotherapy recurrence compared to patients without recurrence. *F. nucleatum* targets innate immune signaling and specific microRNAs to activate the autophagy pathway and control chemoresistance of *5-fluorouracil*, *capecitabine*, and *oxaliplatin*, drugs commonly used in colorectal cancer (Yu et al., 2017). In addition, *Gammaproteobacteria* can metabolize the chemotherapeutic agent *gemcitabine* to its inactive form, 2′,2′-difluorodeoxyuridine. In a colon cancer mouse model, intratumor *Gammaproteobacteria* induced *gemcitabine* resistance. Resistance was ameliorated by the antibiotic *ciprofloxacin* (Geller et al., 2017). The potential for modifying specific gut bacteria or communities of gut bacteria to enhance drug efficacy or minimize drug toxicity is a compelling goal.

Drug Effects on the Microbiome

Antibiotics have varying but profound effects on the gut microbiome. The effects of antibiotics at birth and early infanthood may have permanent consequences on the developing microbiome (Nobel et al., 2015). In addition, emerging evidence suggests that antibiotics interfere with the microbiome and the immune system resulting in immunological disorders (Mårild et al., 2013). Not surprisingly, antibiotic use is associated with a lower abundance of susceptible GI microbes; in one recent *in vitro* high-throughput drug screen, 78% of 156 antibacterials tested had activity against at least one gut microbial species. Interestingly, sulfonamides, aminoglycosides, and antimycobacterial agents had no activity against gut commensals (Maier et al., 2018). A notable consequence of antibiotic use on the gut microbial community is the increased risk for *C. difficile* infection, a bacterial infection of the colon that causes significant morbidity and mortality. *C. difficile* infections are difficult to treat, with up to 65% of patients relapsing. Although the risk of *C. difficile* infection varies with the particular antibiotic, the metabolic effects of antibiotics on the gut microbiome may depend more on the concentration of antibiotics in the gut. For example, in mice, high concentrations of antibiotics reduce or eliminate most products of microbial metabolism such as short-chain fatty acids and secondary bile acids, whereas precursors such as oligosaccharides, sugar alcohols, and primary bile acids accumulate (Jump et al., 2014; Zhao et al., 2013).

There are also many non-antibiotic pharmaceuticals that influence the gut microbiota. Proton pump inhibitors (PPIs) and *metformin* are good examples. In a large *in vitro* study, PPIs were one of four drug classes (PPIs, *metformin*, laxatives, and antibiotics) associated with the largest impact on gut microbial taxa, with a total of 40 altered taxa. The impact of PPIs on gut microbes may be mediated by the changes in GI pH and also by direct inhibition of certain commensal gut bacteria such as *Dorea* and *Ruminococcus* species (Maier et al., 2018). Microbiome functional changes associated with PPIs include an increase in fatty acid and lipid biosynthesis, fermentation, nicotinamide adenine dinucleotide metabolism, and biosynthesis of L-arginine. These functional changes are likely explained by changes to taxa. For example, *Streptococcus mutans* is enriched in PPI users, and this taxa is associated with biosynthesis of L-arginine (Vich Vila et al., 2020). PPIs are also associated with *C. difficile* infection. Long-term PPI use may decrease bacterial diversity (Seto et al., 2014) and may increase Enterococcaceae and Streptococcaceae taxa (Freedberg et al., 2015), which have been associated with antibiotic exposure and increased risk for *C. difficile* infection. Additionally, PPIs induce hypochlorhydria, which permits increased gastric and fecal populations of *Streptococcus,* leading to an increased risk for *C. difficile* infection.

Metformin is often used to treat type 2 diabetes. In metagenomic studies of gut flora and alterations associated with type 2 diabetes, data were conflicting. Further investigation showed that the results were confounded by the effect of *metformin* on the gut microbiome. *Metformin* significantly altered the relative abundance of 86 bacterial strains and 48 microbial pathways (Forslund et al., 2015; Wu et al., 2017). This was associated with changes in the metabolic potential of the microbiome, including increases in butanoate production, quinone biosynthesis, degradation of sugar derivatives, and polymyxin resistance pathways. Analysis of metagenomic pathways and gene families showed that *E. coli* was the major contributor to these functional changes associated with *metformin* (Vich Vila et al., 2020).

The therapeutic effects of *metformin* on glucose metabolism may be mediated by microbes. *Metformin* can alter the composition and function of the gut microbiota in concert with its effects on blood glucose and hemoglobin A_{1c}. *Metformin* treatment promoted changes in the abundance of *Escherichia* and *Intestinibacter* and increased LPS biosynthesis and short-chain fatty acid metabolism (Wu et al., 2017), two factors that may play a role in glucose homeostasis. When these *metformin*-treated microbiota were transferred to germ-free mice, glucose tolerance was improved and hemoglobin A_{1c} was reduced (Wu et al., 2017). Thus, the gut microbiota may play a role in *metformin*'s therapeutic effects in type 2 diabetes.

Diet and the Microbiome

Food has a strong effect on the microbiome, but the microbiome also has a strong effect on the health outcomes of diet (Rowland et al., 2018). Microbiota use food to biosynthesize and supply many of our key vitamins, essential amino acids, and essential lipids and to help digest diet-derived molecules such as complex carbohydrates, polyphenols, and proteins to allow uptake of the metabolized molecules as nutrients. Because diet can be changed, microbiome-mediated therapy is a viable strategy to develop interventions to change the microbiome and affect health outcomes. When fruits and vegetables are consumed, the microbiome has a higher diversity, whereas consumption of a meat-based diet leads to decreased diversity, thus resulting in large changes in metabolism. There are studies that have mechanistically revealed the importance of diet and the microbiome in relation to health outcomes. For example, in mice, *Clostridium orbiscindens* metabolizes flavonoids, a class of molecules found in fruits and vegetables, to make desaminotyrosine via hydrolysis from the desaminotyrosine-conjugated flavonoids. Desaminotyrosine, in turn, protects the host from *Haemophilus influenzae* by boosting the immune system through type I interferon signaling (Steed et al., 2017). Another example is the production of trimethylamine oxide in the liver from ammonia. Trimethylamine oxide is damaging to the cardiovascular and renal systems, leading to increased risk of stroke, myocardial infarction, and other cardiovascular diseases. Its production is mediated by the microbiome via release of ammonia from foods rich in choline and choline-containing lipids such as eggs, fish, and meat.

The interconnections of nutrition, diet, and the microbiome are significant to our understanding of health and disease and are a key component of the 2020–2030 strategic plan for the National Institutes of Health as part of their nutritional health initiative (National Institutes of Health, 2020). Dietary influence of microbiota or microbiota-mediated digestion of food has been linked to growth stunting in infants, metabolic disease, neurological decline, and cardiovascular and renal problems, among others. For example, the PREDICT (Personalised Responses to Dietary Composition Trial) study found that *Prevotella copri* and *Blastocystis* species largely associated with a healthy and plant-based diet improved cardiometabolic blood markers (postprandial glycemic, lipemic, and inflammatory indices) (Asnicar et al., 2021). Similarly, a Mediterranean diet resulted in a decreased cardiovascular risk and also a lowered abundance of *P. copri*, an organism that is also protectively associated with rheumatoid arthritis inflammation pathology (Wang et al., 2021). Indeed, diet can significantly reduce or even eliminate rheumatoid arthritis through alteration of the microbiome. A ketogenic diet, however, leads to a decrease of *Bifidobacterium* due to the production of ketone bodies that are inhibitory to *Bifidobacterium*. Because *Bifidobacterium* species are proinflammatory via stimulating T_H17 cells, inflammation was also decreased. Thus, diet-mediated microbiome alterations might usefully be explored as a strategy to control chronic inflammatory diseases such as Crohn's disease, ulcerative colitis, and other T_H17 cell–mediated skin and lung disorders. Consequently, diet interventions targeting the microbiome as a therapeutic strategy to improve health are a viable option. As the National Institutes of Health nutritional health initiative advances evidence-based rules of diet, nutrition, microbiome, and health connections, diet as a therapeutic strategy will become more commonplace and clinically routine.

Future of Drugs and the Microbiome

Fecal Transplants

In livestock, transfaunation, the practice of transferring rumen microorganisms from a healthy donor to a sick recipient, usually provided orally, has been performed for well over a century, with documented examples going back to the 1700s. Transfaunation has been successfully used to treat horses, cattle, goats, and many other animals with indigestion, diarrhea, and drop in milk production, among other conditions. The related process in humans is the fecal transplant. The first documented human fecal transplant was delivered via enema in 1958 to treat fulminant pseudomembranous colitis (antibiotic-induced syndrome), now known to be a result of *C. difficile* infection. Despite the success of these fecal transplants, there was not a wide adoption of the practice. Only in 2013 was a randomized clinical trial performed on patients for whom antibiotic therapy for *C. difficile* had failed. The trial was halted when the 90% cure rate among the fecal transplant recipients with *C. difficile* infections made it unethical to withhold treatment from the control group. Fecal transplant, sometimes referred to as bacteriotherapy, is still controversial but has become more widely used as an option for treatment of *C. difficile* infections. Fecal transplant therapy is not approved by the FDA (U.S. Food and Drug Administration); rather, the current guidance by the FDA on fecal transplant is "enforcement discretion under limited conditions." These conditions are as follows: "1) the licensed health care provider treating the patient obtains adequate consent from the patient or his or her legally authorized representative for the use of FMT products. The consent should include, at a minimum, a statement that the use of FMT products to treat *C. difficile* is investigational and a discussion of its reasonably foreseeable risks; 2) the FMT product is not obtained from a stool bank; and 3) the stool donor and stool are qualified by screening and testing performed under the direction of the licensed health care provider for the purpose of providing the FMT product for treatment of the patient" (FDA, 2020).

The microbiome has now been connected not only to intestinal infections but also immunological disorders and the brain-gut-microbiome axis. Currently, FMT is being used or evaluated to treat myriad diseases including Crohn's disease, ulcerative colitis, irritable bowel syndrome, obesity, steatohepatitis, autism, some neurological disorders (Vendrik et al., 2020), establishment of normal flora for infants delivered via cesarean section (Korpela et al., 2020), pediatric cytomegalovirus disease, and as anti-PD-1 co-therapy for cancers.

Because there is no standardized, controlled process to prepare fecal transplants, a fecal transplant cannot be guaranteed to be safe and is not without risk and side effects. For this reason, the FDA has released warnings about the possible transmission of parasites; bacterial pathogens; viruses, including HIV (human immunodeficiency virus) and SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2); and other infectious diseases. One death has been reported after a fecal transplant. Side effects observed with successful FMT include constipation, diarrhea, cramping, bloating, and changes in stool consistency. Sometimes, anecdotally, the recipient also takes on metabolic characteristics associated with the donor, such as gain or loss of weight due to changes in the microbiome metabolic capacity. For example, the transfer of intestinal microbiota from lean donors increases insulin sensitivity (Bibbò et al., 2020). Other characteristics such as obesity, dietary preferences, and energy consumption have been observed to be transferred, albeit documented infrequently. The long-term effects of a fecal transplant are unknown.

Defined Microbial Communities

Fecal transplant relies on donors, and since donor microbiomes differ, fecal transplants vary considerably in efficacy. For this reason, controlled and defined consortium of microbes and single-strain microbiota, including probiotics or (bacterio)phage therapy, are being explored (Weiman, 2018). Currently, no single defined microbial community is FDA approved for therapy; however, clinical trials with defined communities are ongoing and include *C. difficile* treatments (phase III) and treatment of ulcerative colitis (phase IIb).

Probiotics

The use of single microbial treatments has a long history and is a $6 billion industry in the U.S. alone. An early and important probiotic is *E. coli* Nissle 1917, discovered in World War I from a corporal that, unlike his fellow soldiers, did not get sick with *Shigella* (Sonnenborn, 2016). *E. coli* Nissle was also found to be beneficial against *Salmonella* and other GI infections. It is now manufactured under Good Manufacturing Practice conditions and licensed in Germany as an active pharmaceutical ingredient. Another example is *Bacillus subtilis*, isolated from camel dung, that treated dysentery in World War II (Ayalah, 2010). Capsules of *B. subtilis* were commonly used for human consumption worldwide until the 1960s, when single antibiotics became favored. Elsewhere (e.g., in Germany, France, and Israel), *B. subtilis* is still a commercial product for human (and animal) consumption under a doctor's supervision (Casula and Cutting, 2002). In the U.S., *B. subtilis* has become widely used in agriculture to prevent crop infections and as an active ingredient of fertilizers due to its crop growth-promoting properties. Other probiotics such as *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces* are taking its place, especially in treatments of GI disorders and infections. Single microbial treatments have remained on the periphery of therapeutic options due to the limited evidence of their efficacy. Due to the increasing appreciation of health-related roles the microbiota may play, both the Food and Agriculture Organization/World Health Organization and the American Gastroenterological Association have provided clinical practice guidelines for the evaluation of probiotics for treating GI disorders and for general probiotic use (Brüssow, 2019; Su et al., 2020). Currently, numerous clinical trials are evaluating single microbial species as treatments for diseases associated with the intestine (e.g., *C. difficile*, autism), oral cavity (e.g., reduction in *S. mutans*) (Twetman and Stecksén-Blicks, 2008), and skin (e.g., atopic dermatitis) (Hsiao et al ., 2013; Sharon et al., 2019).

Designer Microbiota

One emerging interest of the scientific community is the use of genetically engineered microbial strains or synthetic ecosystems (Ainsworth, 2020). These designer microbiota can be engineered to deliver specific therapeutics, to make up for deficiencies, or to focus in specific areas. One example is in phenylketonuria, in which the patient cannot produce functional phenylalanine hydroxylase or has a deficiency in the cofactor tetrahydrobiopterin that converts the amino acid phenylalanine to tyrosine, resulting in the accumulation of phenylalanine. Excess phenylalanine leads to neurological defects (autism-like behavior, motor deficiencies, seizures) or even death. The treatment is a diet low in phenylalanine. Both *E. coli* and *Lactococcus lactis*, two common microbiota, have been engineered to contain phenylalanine hydroxylase and were shown to significantly reduce phenylalanine levels. This is an encouraging example where engineered strains may, one day, serve as probiotics to overcome errors in metabolism.

Phage Therapy

The viriome (including both host-associated viruses and phages that attack bacteria) comprises an often-overlooked part of the microbiome. Viruses that infect bacteria and archaea are known as bacteriophages or phages. Phages infect and kill selected hosts and are a key component of our innate immune system (Barr et al., 2013; Carroll-Portillo and Lin, 2019), protecting mucus-covered epithelial layers from microbial invasion. The ability of phages to kill bacterial pathogens and provide protection against infection is the main driver behind the development of phage therapy. Phage therapy is being evaluated in clinical trials as a strategy to treat drug-resistant urinary tract infections due to pathogenic *E. coli*, *Klebsiella pneumoniae*, *Burkholderia cenocepacia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus,* and other multidrug-resistant pathogens, and also to treat inflammatory bowel disease, lung infections, chronic wounds, osteomyelitis, mastoiditis, prosthetic joint infections, and other chronic diseases. The field got a boost from the widely publicized example of phage therapy used to treat a professor who was comatose after becoming infected with a multidrug-resistant form of *Acinetobacter baumannii* after vacationing in Egypt. The patient's wife, an infectious disease epidemiologist, and a team of physicians and researchers located candidate phages active against *A. baumannii*. With emergency approval from the FDA, a phage cocktail was administered intravenously and the professor ultimately recovered, apparently the first person in the U.S. to be cured from a multidrug-resistant infection by phage therapy (Tara Rava Zolnikov, 2019). At least 16 other patients have been successfully treated in a similar phage therapy to treat *A. baumannii* infections and at least 39 other people have been treated for nine other multidrug-resistant microbes using different phages. The FDA has also provided emergency clearance to treat COVID-19 (coronavirus disease 2019) patients coinfected with multidrug-resistant *A. baumannii*. For more on phages and phage therapy, see the section on bacteriophages in Chapter 59 and also Figure 59–4.

BOX 6–1 ■ Phage Therapy Is Not a New Idea

Twort (1915) and d'Hérelle (1917) discovered phages and their capacity to kill bacteria during World War I. These researchers had observed that some bacterial cultures from patients with dysentery lysed and then cleared, and addition of a bacterium-free supernatant from a clear culture promoted clearing in infected cultures. There were reports of such "blooms" of very tiny microbes shortly before soldiers recovered from *Shigella* infection, with the microbes apparently killing the *Shigella*. d'Hérelle presented his observations to the Academy of Sciences in Paris, describing "an invisible microbe, an antagonist of the dysentery bacillus," which he termed a *bacteriophage,* a "bacteria eater." These and other observations stimulated considerable interest. In the period between World War I and World War II, d'Hérelle and others developed several therapies using phages, and phage therapies for bacterial infections became commercially available in Europe and the U.S. Meanwhile, basic scientists debated the nature of the bacteriophage: an "ultramicrobe" (virus) or an "autocatalytic bacterial enzyme." In the U.S.S.R., Russian scientists developed strategies for harvesting phages for therapeutic purposes up to World War II. From the 1940s onward, phages became the focus of much basic research as work on molecular genetics and the basis of heredity progressed, exemplified by the work of Lwoff and Delbrück. In the West, interest in phage therapy waned with Fleming's discovery of *penicillin*, its isolation and purification by Chain and Florey, and its mass production by the U.S. and Britain during the war. There was a proliferation of antibiotics during the postwar and Cold War periods. East of the Iron Curtain, however, interest in phages and their therapeutic potential persisted and flourished, especially in the Georgian capital of Tbilisi, at the bacteriophage institute now known as the Eliava Institute, named after its founder, George Eliava, a protégé of d'Hérelle. This history, especially work in the U.S.S.R., is well summarized by Myelnikov (2018).

In Western medicine, there is a burgeoning rediscovery of bacteriophages as antimicrobial therapies. At the same time, new techniques for customizing genomes have become available that can help to direct the vigorous reciprocal interplay between the defense mechanisms of bacteria (e.g., restriction-modification, CRISPR-Cas9 [clustered regularly interspaced short palindromic repeats–CRISPR-associated endonuclease 9; a gene editing tool]) and the counterdefense mechanisms of bacteriophages (covalent genome modification) (Liu et al., 2020). As a result, biotech companies have begun phase I/II clinical trials on multiphage cocktails to treat the most common multidrug-resistant pathogens. Chapter 59 presents some aspects of the current use of phages as antimicrobials.

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Chapter 7

Pharmacogenetics and Pharmacogenomics

Dan M. Roden and Sara L. Van Driest

IMPORTANCE OF PHARMACOGENETICS TO VARIABILITY IN DRUG RESPONSE

A BRIEF HISTORY OF PHARMACOGENETIC DISCOVERY

FOUNDATIONS OF PHARMACOGENETICS AND PHARMACOGENOMICS

- Types of Genetic Variation That Alter Drug Response
- Pharmacogenomic Terminology Used to Describe Effects of Genetic Variation
- Scenarios for Clinically Important Pharmacogenetic Interactions

METHODS FOR PHARMACOGENOMIC DISCOVERY

- Associating Genetic Variation with Variable Drug Actions
- Candidate Gene Versus Agnostic Approaches
- Methods to Strengthen the Associations Between Genetic Variation and Variable Drug Actions
- Polygenic Approaches

GENOMICS AS A PATHWAY TO IDENTIFY NEW DRUG TARGETS

PHARMACOGENETICS IN CLINICAL PRACTICE

Patients vary in their responses to drug therapy. Some patients derive striking and sustained benefits from drug administration, others may display no benefit, and still others display mild, severe, or even fatal adverse drug reactions (ADRs). Common sources of such variability include noncompliance, medication errors, clinical factors, drug interactions (see Chapter 4 and Appendices I and II), and genetic factors. Pharmacogenetics is the study of the genetic basis for variation in drug response and often implies large effects of a small number of DNA variants. Pharmacogenomics, on the other hand, studies larger numbers of variants, in an individual or across a population, to explain the genetic influences on drug response. Discovering which variants or combinations of variants have functional consequences for drug effects, validating those discoveries, and ultimately applying them to patient care and to drug discovery are the tasks of modern pharmacogenetics and pharmacogenomics.

Importance of Pharmacogenetics to Variability in Drug Response

An individual's response to a drug depends on the complex interplay of drug factors (e.g., dose, route, formulation), environmental factors (e.g., diet, infections, other drugs, exercise level, exposure to toxins), clinical factors (e.g., age, indication for drug, organ function), and genetic factors. Key genes known to be involved in driving variable drug actions, called *pharmacogenes*, may influence drug response via variable drug concentrations (*pharmacokinetics*) or variable drug effects (*pharmacodynamics*). Pharmacogenes include those encoding drug-metabolizing enzymes, drug transport molecules, drug targets, and a host of other genes that modulate the molecular context within which drugs act, notably genes dysregulated in the disease for which the drug is administered. In some situations, variation in non-germline genomes (e.g., in cancer cells or in infectious agents) can be critical determinants of drug response.

Many well-established pharmacogenes encode drug-metabolizing enzymes. Drug metabolism is highly heritable, as assessed using drug exposures in monozygotic versus fraternal twins, drug exposures in cell lines from related subjects, and analysis of very large data sets using technologies such as genome-wide genotyping, discussed further in this chapter. Some drug metabolism traits behave in a conventional "monogenic" fashion, with clearly definable (and separable) groups of drug response phenotypes: poor metabolizers who inherit two alleles with no function, intermediate metabolizers who have one functional allele and one nonfunctional allele (heterozygotes), and normal metabolizers who have two functional alleles. The study of these traits has helped define key genetic variants that contribute to the individual variability in responses described in this chapter. However, drug responses predicted by a small number of large effect size variants in a single gene are the exception. For most drug responses, understanding the influence of genetic variability will require a truly pharmacogenomic approach. A major challenge to the field is to accrue large numbers of subjects with well-characterized drug responses to enable discovery, and subsequent replication and validation, of multigene effects and of interactions of genomic predictors with environmental factors. The important variants within pharmacogenes often differ across different populations; this chapter includes several examples of the potential influence of ancestry on drug response. Thus, an additional major challenge to the field is to accrue data from diverse populations to ensure generalizability of findings to populations worldwide.

A Brief History of Pharmacogenetic Discovery

In the early 20th century, Garrod (Box 7–1) proposed that specific enzyme defects could not only cause "inborn errors of metabolism" such as alkaptonuria but also could account for variability in drug responses (Roden et al., 2019). Interestingly, however, the first examples of genetically determined variable drug responses were not based on variable drug metabolism but were pharmacodynamic. The widespread use of antimalarials in the Pacific theater during World War II led to the recognition that certain individuals, predominantly African-Americans, were susceptible to hemolysis due to glucose-6-phosphate dehydrogenase (G6PD) deficiency. Subsequent studies identified malignant hyperthermia following anesthetic exposure as a second genetically determined pharmacodynamic trait. Not long thereafter, Garrod's hypothesis was confirmed when the first examples of genetically determined variable drug responses due to pharmacokinetic variability were described: prolonged paralysis after *succinylcholine* resulting from

Abbreviations

ACE: angiotensin-converting enzyme
ADR: adverse drug reaction
CNV: copy number variant
EGFR: epidermal growth factor receptor
EHR: electronic health record
FH: familial hypercholesterolemia
G6PD: glucose-6-phosphate dehydrogenase
GWAS: genome-wide association studies
HER2: human epidermal growth factor receptor 2
HLA: human leukocyte antigen
iPSC: induced pluripotent stem cell
LDL: low-density lipoprotein
MHC: major histocompatibility complex
NAT: *N*-acetyl transferase
RCT: randomized clinical trial
SNV: single-nucleotide variant
TYMS: thymidylate synthase

pseudocholinesterase deficiency and isoniazid hepatotoxicity due to a deficiency of *N*-acetyl transferase (NAT).

In the 1970s, using analysis of plasma drug concentrations, two groups identified striking variability in response to *debrisoquine* (an antihypertensive) and sparteine (an antiarrhythmic) with side effects attributable to defective drug metabolism and consequent accumulation of high (toxic) drug concentrations. Subsequent work established that the same enzymatic defect—decreased CYP2D6 enzyme function—was responsible in both cases. Decades of research then established the role of variable CYP2D6-mediated metabolism in response to dozens of other drugs, identified specific common genetic variants determining variable metabolism, linked unusually high drug concentrations due to pharmacogenetic defects for myriad drug-metabolizing enzymes such as TPMT, UGT1A1, CYP2C19, and others (see Tables 7–2 and 7–3), and defined the role of pharmacogenetic traits in mediating responses to prodrugs such as *codeine* or *clopidogrel* due to variable metabolic bioactivation. As described below, the application of methods in contemporary genome science, such as genome-wide association and sequencing, is helping to expand our understanding of the genetic basis of variable drug responses.

BOX 7–1 ■ Origins of Pharmacogenetics

As an assistant physician at the Hospital for Sick Children in London, Archibald Edward Garrod (1857–1936) pursued his interest in metabolism, heredity, and disease. Through diligent collection of family histories and urine samples of affected patients, Garrod identified the pattern of inheritance of alkaptonuria, his paper becoming the first published account of an autosomal recessive disease (Garrod, 1902). Continuing this line of thinking with studies of albinism and cystinuria, he developed the concept of "inborn [genetically transmitted] errors of metabolism" (Garrod, 1909). His work showed the relationship among biochemistry, genetics, and medical practice; Garrod described alkaptonuria as follows: "the splitting of the benzene ring of homogentisic acid in normal metabolism is the work of a special enzyme [and] . . . in congenital alcaptonuria this enzyme is wanting" (Garrod, 1923). In *The Inborn Factors in Disease* (Garrod, 1931), Garrod proposed that inborn errors of metabolism are "extreme examples of variations of chemical behaviour which are probably everywhere present in minor degrees" and put forward the concept of "chemical individuality," postulating that the aberrant metabolism of exogenous substances could account for unusual reactions to food or drugs, a foundational principle of pharmacogenetics and precision medicine.

After World War II, genetically determined drug responses began to be well characterized. In North America, the field was advanced by two German expatriates, Arno Motulsky (1923–2018) and Werner Kalow (1917–2008). Motulsky, generally considered the father of pharmacogenetics, established the Division of Medical Genetics within the Department of Medicine at the University of Washington and defined the field of pharmacogenetics (Motulsky, 1957). He had broad interests, studying the genetics of G6PD deficiencies, blood groups, and sundry protein polymorphisms. With one trainee, Joseph Goldstein, Motulsky studied familial hyperlipidemia, suggesting that familial hypercholesterolemia was a monogenic disorder. Thirteen years later, Goldstein and Michael Brown won a Nobel prize "for their discoveries concerning the regulation of cholesterol metabolism" (Motulsky, 1986).

Joining the Department of Pharmacology at the University of Toronto in 1951, Werner Kalow studied the metabolism of *succinylcholine* and inherited differences in serum cholinesterase activity. He concluded that the observed differences in cholinesterase activity resulted from different affinities for the substrate, implying different enzyme structures and hence different amino acid sequences. His analysis of multiple families confirmed high, intermediate, and low enzyme activities with a Mendelian pattern of inheritance. Based on these and other studies, Kalow proposed that individual differences in metabolic function due to genetic variation could lead to unique outcomes following drug therapy (Kalow, 1961). Kalow, himself, homozygous for low CYP2D6 activity, described his career as "the rich life of a poor metabolizer" (Grant and Tyndale, 2008).

Foundations of Pharmacogenetics and Pharmacogenomics

Types of Genetic Variation That Alter Drug Response

Genetic variations can be categorized by many characteristics, including frequency in a population, number of base pairs involved, location in the encoded gene, and the effect on the encoded protein. Pharmacogenetic variants include variants in each of these categories. In some cases, important pharmacogene variants are unusually common compared to disease genes in which sequence variations with large potential adverse effects are rare. The high population frequency is thought to reflect the lack of selection pressure. Since pharmacogenetic traits are often imperceptible until drug is administered, the frequencies of variant alleles in pharmacogenes are not reduced in populations over time. Important variants in pharmacogenes can be so rare as to be documented only in a single individual or so common that it is difficult to define which allele is the "wild type." The frequency of specific alleles is often highly variable across populations. For example, most individuals of European descent have no CYP3A5 enzymatic activity (two *no function* alleles), whereas in many populations of African descent, most individuals have one or two alleles expressing functional CYP3A5 protein. The mechanism underlying loss of function in this case is described below.

Pharmacogenetic variants also span the spectrum with respect to size. Single base pairs to large stretches of DNA (including entire genes or chromosomes) can be altered by insertions, deletions, inversions, and duplications of DNA. The smallest genetic variants are substitutions of a single base pair of DNA, called *single-nucleotide variants* (SNVs). Large deletions or duplications are called *copy number variants* (CNVs). For example, the amount of functional CYP2D6 enzyme in an individual depends on the interplay of over 100 possible SNVs, many insertion/deletions, and CNVs deleting or duplicating the entire gene.

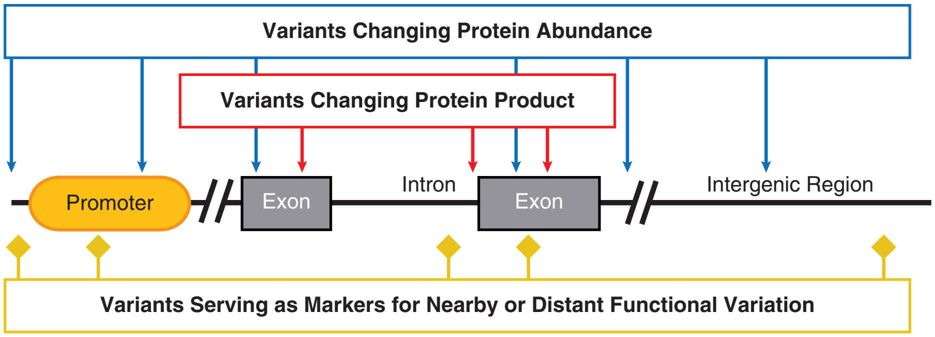
Variants Changing Protein Abundance
Variants Changing Protein Product
Promoter
Exon
Intron
Exon
Intergenic Region
Variants Serving as Markers for Nearby or Distant Functional Variation

Figure 7–1 *Many types of genetic variants can lead to drug response outcomes.* A simplified gene, including a promotor sequence (orange oval), exons (gray boxes), and introns (black line between exons), is shown. The DNA between genes is referred to as intergenic. Variants that change protein abundance, indicated by blue arrows, can be found throughout all these regions. Variants that change the protein sequence, indicated by red arrows, are generally found in or near exons. Variants that serve as markers for other functional variations in linkage disequilibrium (which may affect protein abundance or product and may be nearby or distant) can be found throughout all of these regions. These variants are indicated in yellow diamonds.

Regardless of size, some variants in pharmacogenes alter the sequence of the encoded protein, for example, by substituting one amino acid for another, deleting or adding an amino acid, changing the start or stop codon, or introducing a frameshift affecting the remainder of the transcribed protein. Variants within or outside the coding sequence can also affect protein abundance (Figure 7–1). The number of TA repeats in the _UGT1A1_ promoter affects the level of expression of this important glucuronosyltransferase in liver; the most common allele has six repeats, and the seven-repeat variant (_UGT1A1*28_) decreases _UGT1A1_ expression. The frequency of the _UGT1A1*28_ allele is up to 30%, with up to 10% of subjects (depending on ancestry) being homozygous. Decreased _UGT1A1_ transcription can modulate drug actions and also accounts for a common form of mild hyperbilirubinemia (Gilbert's syndrome; see Table 5–3 and Figure 5–7).

Many pharmacogenetic variants can be identified through association studies, including genome-wide association studies (GWAS; see below). These association studies, unless coupled with functional characterization, do not prove the causal link between the genetic variant(s) and the pharmacogenetic trait. Genetic variants are often not inherited in isolation, but as a constellation of variants frequently found together (termed *linkage disequilibrium*). An association study may identify a variant in linkage disequilibrium with a functional variant, rather than the functional variant itself. These patterns of linkage disequilibrium are population specific. As a result, a variant associated with a particular drug outcome by GWAS in one population may not replicate in another population. This highlights the importance of functional studies and population-specific replication of pharmacogenetic associations.

Pharmacogenomic Terminology Used to Describe Effects of Genetic Variation

A *haplotype*—a series of alleles found at a linked locus on a chromosome—specifies the DNA sequence variation in a gene or a gene region. A haplotype represents the constellation of variants that occur together. For any gene, individuals will have two haplotypes, one maternal and one paternal in origin. In some cases, this constellation of variants, rather than the individual variant or allele, may be functionally important. In others, however, a single variant may be functionally important regardless of other linked variants within the haplotype(s). Selected common _CYP2C19_ variants and haplotypes are illustrated in Figure 7–2.

For many important pharmacogenes (but not all), a shorthand nomenclature for haplotypes has been adopted. This "star allele" nomenclature allows communication of the entire haplotype by stating the gene name followed by an asterisk and the specific haplotype designation. By convention, *1 designates a functional allele; for example, _CYP3A5*1_ and _CYP2C19*1_ encode functional enzymes. In _CYP3A5_, a common noncoding intronic variant creates an alternative splice site, resulting in a transcript with an early stop codon (Figure 7–3). This allele, designated as _CYP3A5*3_ generates a *no function* protein and is the predominant allele in European ancestry individuals, whereas _CYP3A5*1_ is the predominant allele in African ancestry individuals (Figure 7–4). Star allele designations often include information about several variants across an allele, as seen in the example of _CYP2C19_ in Figure 7–2. Web-based resources such as PharmGKB and PharmVar (Table 7–1) provide information on specific star alleles.

Standard nomenclature has also been developed for specifying drug metabolism enzyme function. The terms poor, intermediate, normal, rapid, and ultrarapid metabolizer are used to describe individuals who, based on the combination of maternal and paternal alleles present in their genome, have no enzyme activity (poor metabolizer) to greatly increased enzyme activity (ultrarapid metabolizer).

Scenarios for Clinically Important Pharmacogenetic Interactions

Given the complexity and redundancy of most pharmacokinetic and pharmacodynamic pathways, variations in a single pharmacogene will often have a small effect. There are specific scenarios that lead to large effects of variation in a single pharmacogene. Recognition of these scenarios led to the fundamental early discoveries in pharmacogenetics discussed above and form the basis for most of the pharmacogenetic predictors clinically implemented at this time.

Scenarios leading to clinically important pharmacogenetic interactions include:

- Drugs for which a key step in their metabolism or transport depends on single enzymes or transporters with functional variability (*pharmacokinetic alterations*), such as *debrisoquine*, *sparteine*, *codeine*, and other drugs metabolized by CYP2D6.
- Drugs interacting with proteins with functional variability, either as the intended drug target or as an off-target effect (*pharmacodynamic alterations of the receptor/target*), such as some aminoglycosides, β blockers, and antiarrhythmic drugs.
- Drugs with differential effect depending on the broad biologic milieu (*pharmacodynamic alterations beyond the receptor/target*), such as antimalarial drugs and *rasburicase*, where G6PD deficiency predicts ADRs.

Some drug responses are associated with more than one gene and may be described as multigenic traits. This section summarizes important examples of well-established pharmacokinetic, pharmacodynamic, and multigenic traits, but cannot be all inclusive. Web-based resources such as PharmGKB (see Table 7–1) provide information on specific genes, variants, drugs, and diseases.

Pharmacokinetic Alterations

Variations in genes that encode drug-metabolizing enzymes and transporters affect drug concentrations and are therefore major determinants of drug response (at the end of the chapter, see Table 7–3 for additional

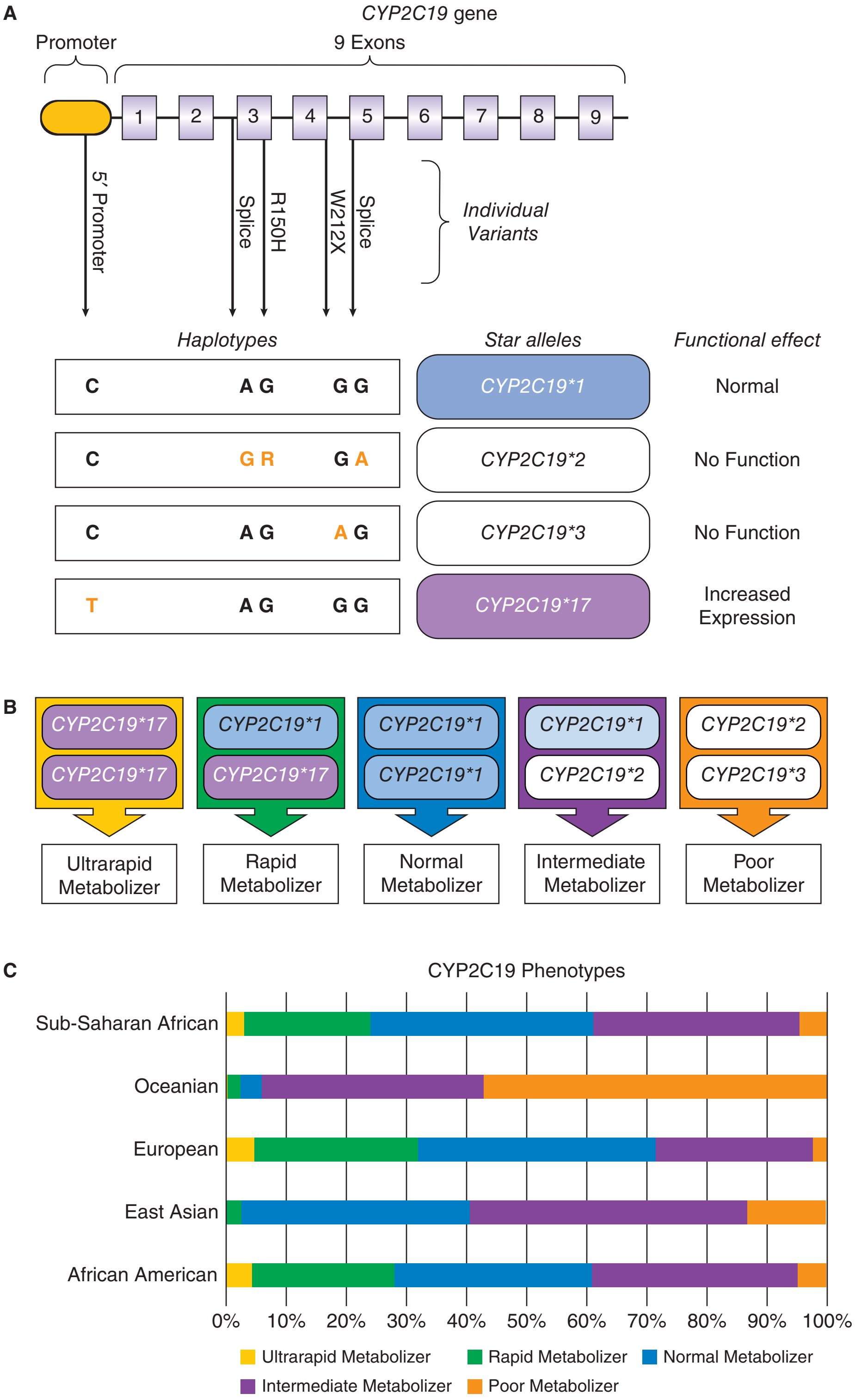
A
CYP2C19 gene
Promoter
9 Exons
1
2
3
4
5
6
7
8
9
5′ Promoter
Splice
R150H
W212X
Splice
Individual Variants
Haplotypes
Star alleles
Functional effect
C A G G G
CYP2C19*1
Normal
C G R G A
CYP2C19*2
No Function
C A G A G
CYP2C19*3
No Function
T A G G G
CYP2C19*17
Increased Expression
B
CYP2C19*17
CYP2C19*17
Ultrarapid Metabolizer
CYP2C19*1
CYP2C19*17
Rapid Metabolizer
CYP2C19*1
CYP2C19*1
Normal Metabolizer
CYP2C19*1
CYP2C19*2
Intermediate Metabolizer
CYP2C19*2
CYP2C19*3
Poor Metabolizer
C
CYP2C19 Phenotypes
Sub-Saharan African
Oceanian
European
East Asian
African American
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Ultrarapid Metabolizer
Rapid Metabolizer
Normal Metabolizer
Intermediate Metabolizer
Poor Metabolizer

Figure 7–2 *Common CYP2C19 variants, haplotypes, star alleles, functional effects, and metabolizer status.* The gene encoding CYP2C19 has nine exons, shown in gray boxes in panel **A**. Common variants are found in the 5′ promoter, the introns, and the exons. The patterns of individual variants define haplotypes, which in turn are designated using specific star alleles. *CYP2C19*1* has normal enzyme function, **2* and **3* have no enzyme function, and **17* has increased function (due to a promoter variant that increases expression of the enzyme). Note that there are many additional known variants and star alleles in *CYP2C19* than those depicted in the figure. Each individual inherits two *CYP2C19* alleles (one maternal and one paternal), as shown in panel **B**. The combination of alleles determines the total amount of CYP2C19 enzyme functional activity or metabolizer status. The distribution of CYP2C19 metabolizer status varies by ancestral population, as shown in panel **C**. In Oceanian populations, poor metabolizers are very common, whereas this phenotype is uncommon among those of European ancestry.

examples of genetic polymorphisms influencing drug response). A particularly high-risk situation is a drug with a narrow therapeutic margin where a step in the metabolic pathway is dependent on a single enzyme. Loss of enzyme function will lead to increases in exposure to the parent drug and any metabolites upstream in the metabolic pathway. There will also be lower concentrations of metabolites downstream of the critical enzyme. Variants that increase enzymatic function have the opposite effect, leading to low concentrations of parent compound and upstream

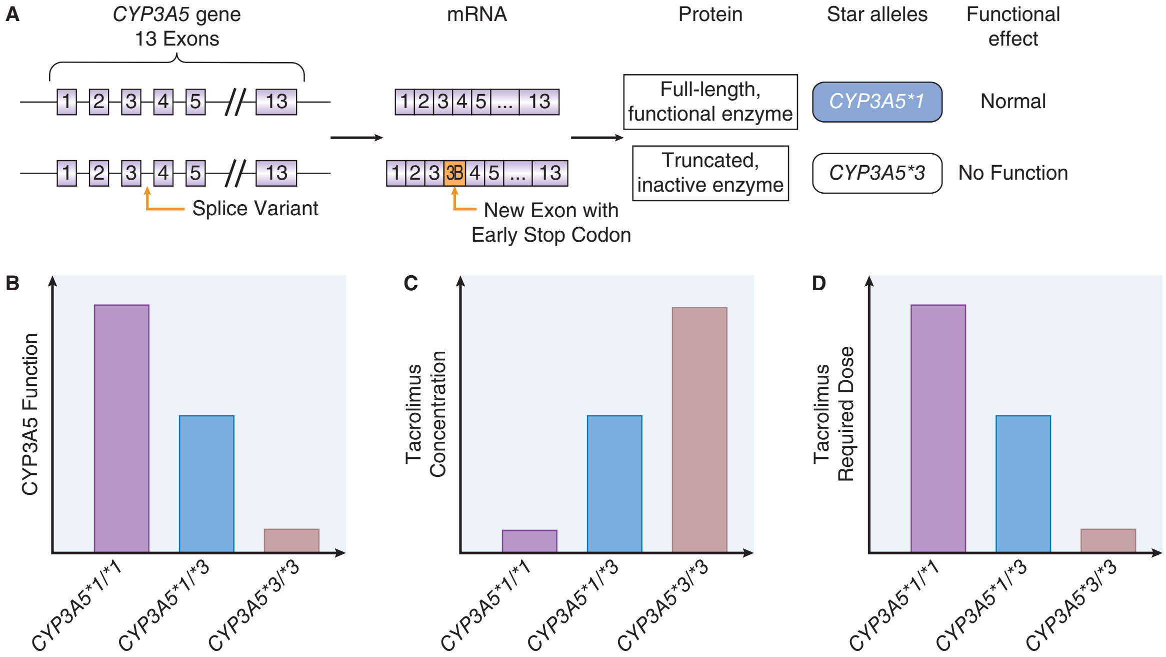
A
CYP3A5 gene
13 Exons
mRNA
Protein
Star alleles
Functional effect
1 2 3 4 5 13
1 2 3 4 5 ... 13
Full-length, functional enzyme
CYP3A5*1
Normal
1 2 3 4 5 13
1 2 3 3B 4 5 ... 13
Truncated, inactive enzyme
CYP3A5*3
No Function
Splice Variant
New Exon with Early Stop Codon
B
CYP3A5 Function
CYP3A5*1/*1
CYP3A5*1/*3
CYP3A5*3/*3
C
Tacrolimus Concentration
CYP3A5*1/*1
CYP3A5*1/*3
CYP3A5*3/*3
D
Tacrolimus Required Dose
CYP3A5*1/*1
CYP3A5*1/*3
CYP3A5*3/*3

Figure 7–3 *A splice variant in the CYP3A5 gene affects protein function, drug concentrations, and required dose to achieve therapeutic levels.* Panel **A** depicts the *CYP3A5* gene. A common variant in the intron between exons 3 and 4 creates an alternate splice site. Transcription of this allele leads to an mRNA with a new, early stop codon. Translation of the mRNA results in truncated, inactive enzyme. This variant is referred to as the *CYP3A5*3* allele. Individuals with two alleles coding for functional CYP3A5 enzyme (e.g., **1/*1*) have the highest CYP3A5 function, whereas those with two alleles coding for enzyme with no function (e.g., **3/*3*) have the least CYP3A5 function (**B**). *Tacrolimus*, a calcineurin inhibitor/immunosuppressant (see Chapter 39), is a CYP3A5 substrate; thus, when administered the same dose of drug, those with the *CYP3A5*1/*1* genotype will have lower plasma concentrations than those with **1/*3* or **3/*3* genotypes (**C**). To achieve a therapeutic plasma drug concentration, those with the *CYP3A5*1/*1* genotype require higher doses than those with **1/*3* or **3/*3* genotypes (**D**).

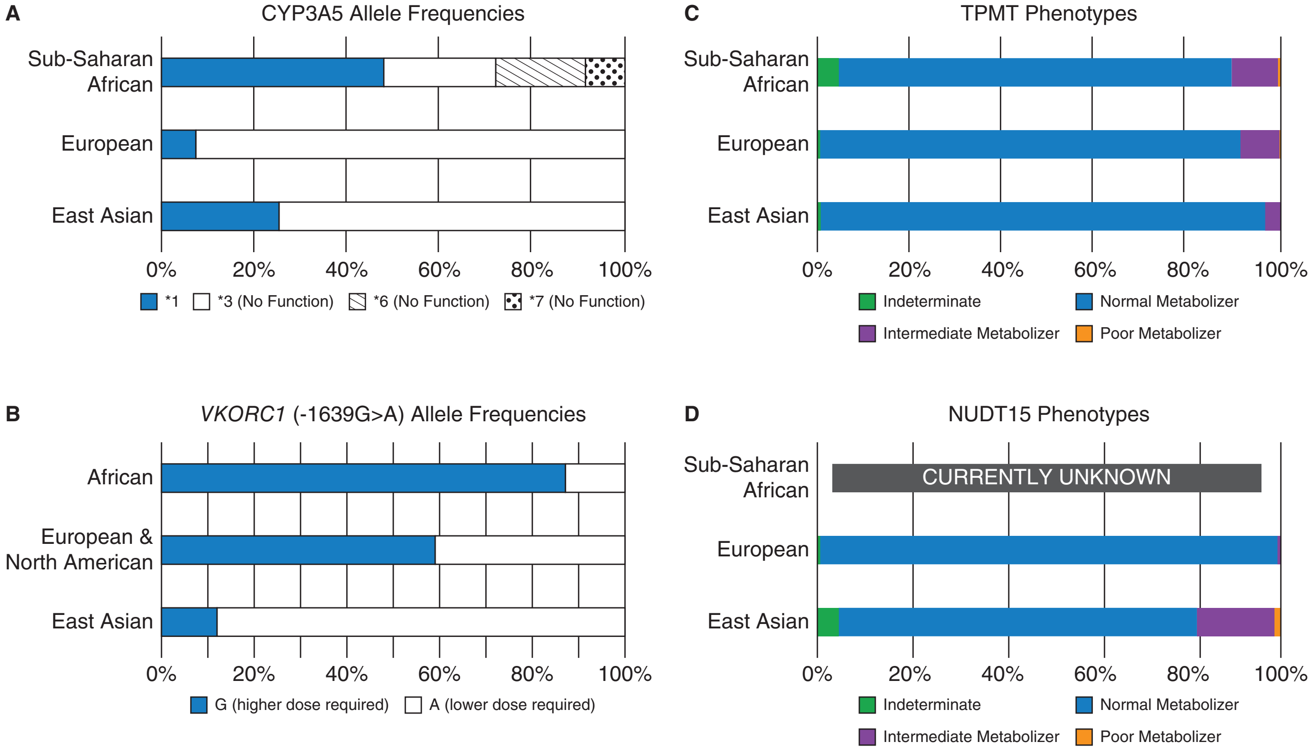

Figure 7–4 *The frequencies of pharmacogenetic variants and metabolizer phenotypes vary across populations.* Panels **A** and **B** depict the allele frequencies for common variants in *CYP3A5* and *VKORC1*, respectively. Of note, the most common *CYP3A5* allele in populations of European ancestry is the no function **3* allele (see Figure 7–3). In contrast, in many African ancestry populations, the functional **1* allele is the most common. In addition, there are variants observed in African ancestry populations that are very rare in other populations (e.g., **6* and **7*). A promoter variant that determines hepatic *VKORC1* expression varies widely by ancestry (**B**). Panels **C** and **D** show the phenotypes resulting from variation in the *TPMT* and *NUDT15* genes, respectively. Poor metabolizer status for either gene can increase risk of toxicity from thiopurine drugs. Of note, TPMT poor metabolizers are rare in East Asian populations, but this same population has a higher frequency of NUDT15 poor and intermediate metabolizers. The frequency of *NUDT15* variants in those of African ancestry has not been well characterized, highlighting the need to include diverse worldwide populations in research efforts.

TABLE 7–1 ■ ENABLING RESOURCES FOR PHARMACOGENETICS AND PHARMACOGENOMICS

DATABASE NAME	URL	DESCRIPTION OF CONTENTS
Broad Institute Software	www.broadinstitute.org/data-software-and-tools	Software tools for the analysis of genetic studies
Clinical Pharmacogenetics Implementation Consortium	cpicpgx.org/	Peer-reviewed, evidence-based, updatable, and detailed gene/drug clinical practice guidelines
dbSNP	www.ncbi.nlm.nih.gov/snp	Single-nucleotide variants and frequencies in various populations
Flockhart Table	drug-interactions.medicine.iu.edu/MainTable.aspx	Tables of drugs that are substrates, inducers, and inhibitors of cytochrome P450 enzymes
Genome Aggregation Database	gnomad.broadinstitute.org	Variants identified by exome and whole-genome sequencing of thousands of individuals from worldwide populations
GTEx	www.gtexportal.org/home/	Genetics of gene expression
GWAS Central	www.gwascentral.org	Genotype/phenotype associations
IMGT/HLA database	hla.alleles.org	HLA allele information and nomenclature
Online Mendelian Inheritance in Man	www.ncbi.nlm.nih.gov/omim	Human genes and genetic disorders
PharmGKB	www.pharmgkb.org	Genotype and phenotype data related to drug response
PharmVAR	www.pharmvar.org/	Pharmacogene variation with focus on haplotype structure and star allele nomenclature
University of California Santa Cruz Genome Browser	http://genome.ucsc.edu	Sequence of the human genome; variant alleles

TABLE 7–2 ■ EXAMPLES OF SELECTED WELL-ESTABLISHED, CLINICALLY ACTIONABLE PHARMACOGENES

GENE	DRUG(S)	RESPONSES AFFECTED
Drug metabolism and transport		
CYP2B6	Efavirenz	Plasma levels, risk for toxicity
CYP2C19	Amitriptyline, citalopram, clopidogrel, escitalopram, lansoprazole, omeprazole, pantoprazole, voriconazole	Drug efficacy
CYP2C9	Celecoxib, flurbiprofen, fosphenytoin, ibuprofen, lornoxicam, meloxicam, phenytoin, piroxicam, siponimod, tenoxicam, warfarin	Drug efficacy, toxicity, or required dose
CYP2D6	Amitriptyline, atomoxetine, codeine, nortriptyline, ondansetron, paroxetine, pitolisant, tamoxifen, tramadol, tropisetron	Drug efficacy, toxicity, or required dose
CYP3A5	Tacrolimus	Required dose
CYP4F2	Warfarin	Required dose
DPYD	Capecitabine, fluorouracil	Toxicity
NUDT15 and *TPMT*	Azathioprine, mercaptopurine, thioguanine	Toxicity and efficacy, risk of second cancers
SLC01B1	Simvastatin	Plasma levels, risk for toxicity (myopathy)
Targets and receptors		
CFTR	Ivacaftor	Efficacy
VKORC1	Warfarin	Anticoagulant effect, bleeding risk
Modifiers		
CACNA1S and *RYR1*	Desflurane, enflurane, halothane, isoflurane, methoxyflurane, sevoflurane, succinylcholine	Risk for toxicity (malignant hyperthermia)
G6PD	Rasburicase, tafenoquine	Risk for toxicity (hemolytic anemia, methemoglobinemia)
HLA-A	Carbamazepine	Risk for toxicity (hypersensitivity reactions)
HLA-B	Abacavir, allopurinol, carbamazepine, fosphenytoin, oxcarbazepine, phenytoin	Risk for toxicity (hypersensitivity reactions)
IFNL3 and *IFNL4*	Peginterferon alfa-2a, peginterferon alfa-2b	Efficacy
UGT1A1	Atazanavir, irinotecan	Risk for toxicity (hyperbilirubinemia)

metabolites and higher concentrations downstream. When the active compound is the parent drug or a metabolite formed upstream of the defect, low enzyme function leads to higher risk for toxicity or ADRs, mimicking an overdose. Increased enzyme function leads to loss of efficacy, as the exposure to active drug is decreased (Roden and Stein, 2009).

The immunosuppressive drug *tacrolimus*, which is metabolized by the CYP3A5 enzyme, provides an example. This drug is known to have a narrow therapeutic index, leading to clinical therapeutic drug monitoring. Individuals with one or two functional copies of *CYP3A5* require higher doses of *tacrolimus* to achieve therapeutic levels of drug (see Figure 7–3) (Birdwell et al., 2015). Because *CYP3A5*1* alleles are more common in individuals of African ancestry and relatively rare in those of European ancestry, it has been suggested that increased rates of transplant rejection in those of African descent may reflect decreased plasma concentrations (Birdwell et al., 2012). Similarly, several proton pump inhibitors, including *omeprazole* and *lansoprazole*, are inactivated by CYP2C19. Thus, CYP2C19 poor metabolizers have higher exposure to active parent drug, a greater pharmacodynamic effect (higher gastric pH), and a higher probability of ulcer cure than CYP2C19 rapid or ultrarapid metabolizers (Furuta et al., 1998; Lima et al., 2021).

A variation on this theme comes from drugs that require bioactivation to achieve pharmacological effect. When that bioactivation is achieved through the action of a single enzyme, variants that decrease enzymatic function can lead to inefficacy, and variants that increase enzymatic function can lead to ADRs (Roden and Stein, 2009). *Clopidogrel*, bioactivated by CYP2C19, is an example (see Table 7–2). CYP2C19 poor metabolizers display decreased antiplatelet effects and increased stent thrombosis during *clopidogrel* treatment (Claassens et al., 2019; Mega et al., 2010; Pereira et al., 2020; Shuldiner et al., 2009). CYP2C19 intermediate metabolizers (~20% of European ancestry populations and >45% of East Asian ancestry populations) receiving *clopidogrel* may achieve adequate antiplatelet effects by increasing the dose. Poor metabolizers (2%–3% of European ancestry populations and >10% of East Asian ancestry populations) should be treated with an alternate antiplatelet drug, because even large dose increases do not lead to drug effect. *Codeine* (a prodrug bioactivated to *morphine* by CYP2D6) provides another example. In CYP2D6 poor metabolizers, analgesia is absent. Perhaps more important, excess *morphine* is generated by CYP2D6 ultrarapid metabolizers, and overdose symptoms, including respiratory depression and death, have been reported (Crews et al., 2021).

Pharmacodynamic Alterations of the Receptor/Target

Genetic alterations leading to changes in the target protein for a drug can lead to variability in drug response. In some cases, drugs are specifically designed to target proteins affected by these changes (see Cancer as a Special Case); in these cases, genetic testing is used to determine the gene sequence and select the appropriate drug. In other instances, variations in the target (whether that is the intended target or a potential off-target interaction) can lead to differences in drug efficacy and toxicity. An example is provided by the aminoglycoside antibiotic drugs and variation in the mitochondrial *MT-RNR1* gene. Aminoglycosides act by binding to the A-site of the 16S rRNA of the 30S ribosomal subunit and altering its conformation, thereby disrupting bacterial protein synthesis (see Chapter 59). Hearing loss is a known dose-related toxicity of aminoglycosides, likely due to off-target binding of drug to human mitochondrial ribosomes in the inner ear, where drug concentrations are high. However, some individuals experience partial or profound aminoglycoside-induced hearing loss after a single dose of drug and with "nontoxic" serum levels. Pharmacogenetic studies have revealed associations of variants in the *MT-RNR1* gene to ototoxicity. These variants are thought to alter the structure of human mitochondrial rRNA, making it more closely resemble bacterial rRNA and increasing affinity for aminoglycosides (Barbarino et al., 2016).

Other examples of drug target variants affecting drug response are presented in Table 7–2. In addition, serotonin receptor polymorphisms have been implicated as predictors of responsiveness to antidepressants and of the overall risk of depression. β Adrenergic receptor polymorphisms have been linked to asthma responsiveness, changes in renal function following ACE (angiotensin-converting enzyme) inhibitors, sinus heart rate following β blockers, and the incidence of atrial fibrillation during β blocker therapy. The degree of lowering of LDL (low-density lipoprotein) cholesterol by statins has been linked to polymorphisms in HMG-CoA reductase, the statin target (see Chapter 37). Ion channel polymorphisms have been linked by both candidate gene and exome sequencing approaches to a risk of cardiac arrhythmias in the presence and absence of drug triggers (Kaab et al., 2012; Weeke et al., 2014).

Pharmacodynamic Alterations Beyond the Receptor/Target

There are genetic variants that influence drug outcomes without affecting pharmacokinetics or the pharmacodynamics of the drug's interaction with its target. Instead, these variants alter the outcome of drug exposure through changes in the biologic milieu. One example is seen with G6PD and *rasburicase*, a recombinant enzyme used to prevent and treat hyperuricemia by increasing the breakdown of uric acid to allantoin and hydrogen peroxide (see Chapter 42). As described above, individuals with G6PD deficiency are more susceptible to ADRs to antimalarial drugs. Individuals with G6PD deficiency are unable to handle the increased oxidative stress from the hydrogen peroxide formation and are susceptible to drug-induced lysis of red blood cells (hemolytic anemia) if treated with *rasburicase* (Relling et al., 2014). Further, oxidation of the iron in hemoglobin leads to the formation of methemoglobin, which cannot carry oxygen or carbon dioxide. Methemoglobinemia can lead to arrhythmias, seizures, and death. Although G6PD is not involved in the metabolism of *rasburicase* and is not the target of *rasburicase* action, *G6PD* genotype is highly predictive of ADRs with antimalarials and *rasburicase*. Thus, *rasburicase* is contraindicated in individuals with G6PD deficiency.

Multigenic Pharmacogenomic Traits

Many drug responses have genetically mediated influences on both pharmacokinetics and pharmacodynamics. *Warfarin* is one example (Figure 7–5). The more active *S*-enantiomer of *warfarin* is metabolized by CYP2C9. CYP2C9 poor and intermediate metabolizers require lower steady-state *warfarin* dosages and are at increased risk of bleeding (Aithal et al., 1999; Kawai et al., 2014) (see also Table 36–2). *Warfarin* exerts its anticoagulant effect by interfering with the synthesis of vitamin K–dependent clotting factors, and the target molecule with which *warfarin* interacts to exert this effect is encoded by *VKORC1*, an enzyme in the vitamin K cycle (see Figures 7–5 and 36–6). There are variants that alter the encoded VKORC1 protein and that lead to partial or complete *warfarin* resistance; interestingly, these variants are rare in

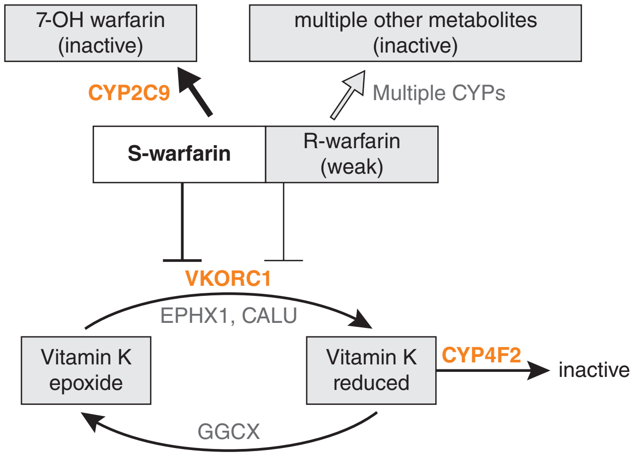

Figure 7–5 *Simplified pharmacokinetic and pharmacodynamic pathways for warfarin, with important pharmacogenes highlighted. Warfarin* is primarily metabolized by CYP2C9 to inactive metabolites and exerts its anticoagulant effect partly via inhibition of VKORC1, an enzyme necessary for reduction of inactive to active vitamin K. Reduced vitamin K is metabolized by CYP4F2. Common polymorphisms in all three genes, *CYP2C9*, *VKORC1*, and *CYP4F2*, affect *warfarin* pharmacokinetics and pharmacodynamics.

many populations, but relatively common (5% allele frequency) in Ashkenazi patients and may account for high dosage requirements in carrier subjects. On the other hand, there are promoter variants that lead to low levels of expression of VKORC1; individuals with these variants require lower steady-state *warfarin* doses. These variants are more common in those of Asian ancestry than in European or African ancestry populations (see Figure 7–4). Vitamin K is removed from the vitamin K cycle by the metabolism of reduced vitamin K to hydroxyvitamin K_1, a process that is catalyzed by CYP4F2, and variation in the *CYP4F2* gene has been shown to influence *warfarin* response (Caldwell et al., 2008). Together, inherited variation in *CYP2C9, VKORC1,* and *CYP4F2* accounts for more than 50% of the variability in *warfarin* doses needed to achieve the desired coagulation level (Johnson et al., 2017).

Another example of a drug with multiple genes influencing response is the antiepileptic drug *phenytoin* (and its prodrug *fosphenytoin*). *Phenytoin* is metabolized by CYP2C9; CYP2C9 poor metabolizers are at increased risk for toxicity compared to normal metabolizers due to higher plasma concentrations (see Box 2–3). *Phenytoin* ADRs include Stevens-Johnson syndrome and toxic epidermal necrolysis, which can be life threatening. Risk for these severe ADRs appears to be especially high in patients who carry variants in *CYP2C9* as well as specific human leukocyte antigen B (*HLA-B*) alleles, including *HLA-B*15:02* (see discussion of immunopharmacogenomics below). Thus, knowledge of the genotypes for both *CYP2C9* and *HLA-B* pharmacogenes for an individual can assist in predicting response to *phenytoin* (Karnes et al., 2021).

Cancer as a Special Case

As with many other drugs, variants in the germline (inherited) genome can affect anticancer drug effects. In addition, variants in the tumor (somatic) genome have emerged as a critical determinant of anticancer drug effects.

An example of a germline effect is genetic variation reducing UGT1A1 activity (e.g., in Gilbert's syndrome), which is associated with higher levels of the active metabolite SN-38 of the cancer chemotherapeutic agent *irinotecan* (see Chapter 70), and this increased concentration has been associated with an increased risk of serious toxicities (see Figures 5–6, 5–8, and 5–9). Similarly, for thiopurine drugs, variants in the *TPMT* and *NUDT15* genes alter concentrations of active metabolites and influence risk of bone marrow toxicity (Relling et al., 2013).

Tumor sequencing to identify somatic variants is becoming standard of care for choosing among anticancer drugs in certain settings (see Chapters 69–73). For example, patients with lung cancer with activating mutations in the gene encoding the epidermal growth factor receptor (*EGFR*), display increased responses to the EGFR inhibitor *gefitinib* (Maemondo et al., 2010). Thus, patients with the activating mutation have, in treatment terms, a distinct pharmacogenetic category of lung cancer. Further, EGFR inhibitors are not indicated in patients with tumors that do not carry these EGFR mutations. The human epidermal growth factor receptor 2 (HER2) antibody *trastuzumab* can produce cardiomyopathy in exposed patients. Patients with breast cancer whose tumors express the HER2 antigen may benefit from *trastuzumab*, whereas those whose tumors do not express HER2 do not benefit but are nevertheless susceptible to cardiomyopathy. Similarly, only patients with melanoma whose tumors express the mutant BRAF V600E respond to *vemurafenib*, an inhibitor of BRAF (V600E) kinase; interestingly, *vemurafenib* may also be effective in other tumors (e.g., thyroid cancer, hairy cell leukemia) that express BRAF V600E. Some genetic alterations affect both tumor and host: the presence of two instead of three copies of a thymidylate synthase (*TYMS*) enhancer repeat polymorphism not only increases the risk of host toxicity but also increases the chance of tumor susceptibility to TYMS inhibitors, and the level of TYMS expression in multiple tumor types has been associated with outcome (Johnston et al., 1994; Marsh, 2005).

Immunopharmacogenomics as a Special Case

There are several drugs where toxicity has been associated with HLA alleles. *HLA-B* is one of three genes that comprise the human major histocompatibility complex (MHC) class I cluster. *HLA-A, HLA-B*, and *HLA-C* genes encode a cell surface protein that enables the immune system to distinguish self-proteins from foreign proteins. The HLA genes, and specifically *HLA-B*, are among the most polymorphic genes in the entire genome, and a specific nomenclature system has been developed for them. HLA nomenclature is posted on the IMGT/HLA database, available online (Robinson et al., 2015) (see Table 7–1). As described above, toxicity of *phenytoin* has been linked to a specific allele of the *HLA-B* locus, designated as *HLA-B*15:02*.

The frequencies of specific HLA alleles differ across populations, similar to many pharmacogenetic variants. The *phenytoin* risk allele *HLA-B*15:02* is most prevalent in East Asian and Central/South Asian populations. In some of these populations, up to 20% of individuals carry the risk allele. In contrast, this allele is absent or very rare in populations of European and sub-Saharan African descent. Depending on the population, other *HLA* alleles have been reported to increase risk for ADRs, including the related *HLA-B*15:21, HLA-B*15:11,* and *HLA-B*15:08* alleles in other Asian populations, and the more distinct *HLA-B*56:02* allele in Aboriginal Australian populations (Somogyi et al., 2019). The ability to confidently rule out risk for a *phenytoin* ADR based on HLA testing depends on testing the correct complement of *HLA* alleles for the target population. Incomplete knowledge will lead to false reassurance from a pharmacogenomic test result. Other drugs with ADR risk associated to *HLA* genes include *allopurinol* and *HLA-B*58:01*, *abacavir* and *HLA-B*57:01*, *oxcarbazepine* and *HLA-B*15:02*, and *carbamazepine* and *HLA-B*15:02* (in East Asian individuals) and *HLA-A*31:01* (in European ancestry individuals). The mechanism of action for these HLA-associated ADRs has been proposed to include noncovalent binding of the drug or a metabolite to immune receptors such as HLA or the T-cell receptor; however, the details of the ensuing immunologic response have not been characterized.

Methods for Pharmacogenomic Discovery

Associating Genetic Variation with Variable Drug Actions

Initial studies with pseudocholinesterase deficiency or the poor metabolizer trait used biochemical methods to establish that individuals with aberrant drug responses displayed anomalous *in vitro* behaviors. Thus, for example, a biochemical assay was used to establish the lack of succinylcholine esterase activity in patients with pseudocholinesterase deficiency. In the case of G6PD, it was found that antimalarials increase red blood cell fragility, probably by reducing the levels of the antioxidant glutathione in individuals with G6PD deficiency, leading to profound hemolytic anemia. Drug level assays were used to identify poor metabolizers because of unusually high drug concentrations; further studies, often using liver microsomes, were used to identify common substrates and inhibitors for specific metabolic pathways such as CYP2D6 or CYP2C19. As mentioned above, these methods were then applied to relatives of affected patients or in twin studies to establish that the traits were in fact familial and thus likely genetic.

It was only with the cloning of individual genes in the 1980s and subsequently the cloning of whole genomes that the field could move to identifying specific variants underlying increasingly well-recognized pharmacogenetic traits. Initial studies demonstrated that surprisingly common genetic variants identified in poor metabolizer patients, or liver preparations, did in fact reduce or eliminate enzymatic activity and, thus, were definitively linked to the poor metabolizer trait. Other methods to strengthen the association between a genetic variant and a variable drug response are discussed below. Further studies identified variants responsible for rapid metabolizer phenotypes, including specific missense alleles that increase enzymatic function, CNVs resulting in multiple copies of functional enzyme (e.g., *CYP2D6* duplication, described above), and promotor variants leading to increased expression.

Candidate Gene Versus Agnostic Approaches

In many domains of genome science, associations between logically chosen variants in candidate genes and variable human traits have not been replicated (Ioannidis et al., 2001) and thus the candidate gene approach has fallen into disfavor. Key pharmacogenetic variants constitute an exception to this general rule. This likely reflects the idea that candidate pharmacogenes were initially identified using clear understanding of the mechanisms of action and disposition pathways of drugs found to exert strikingly variable effects in patients, often through highly variable drug concentrations. This biologic understanding then naturally led to the identification of variation in genes such as *CYP2D6* and pseudocholinesterase as key candidate modulators of variable drug actions; such variants can have relatively large effects, and these associations have been repeatedly replicated.

While the traditional starting point for identifying pharmacogenes has been an understanding of the mechanisms underlying variability in drug action, the agnostic approach of GWAS has also been applied to identify pharmacogenes that mediate important drug responses, notably those underlying ADRs (Motsinger-Reif et al., 2013). Unlike GWAS for common diseases, these studies may generate clear signals at genome-wide significance even with small numbers of subjects. One example is the development of Stevens-Johnson syndrome during treatment with the anticonvulsant *carbamazepine*. Previous studies had implicated the *HLA-B*15:02* as a risk allele in subjects of Asian origin. A GWAS in 65 subjects of European ancestry with Stevens-Johnson syndrome–related ADRs and 3987 controls found an association with *HLA-A*31:01* (McCormack et al., 2011). Studies of dozens of immune-function-related genes assayed with an "immunochip" and a subsequent GWAS have not only validated *TPMT* variants as mediators of thiopurine-related bone marrow suppression (previously identified using candidate gene approaches) but have also identified variants in *NUDT15* (Yang et al., 2014; Yang et al., 2015). This is especially important in subjects of Asian ancestry in whom thiopurine-related toxicity is more common than in other ancestries but *TPMT* variants are less common (see Figure 7–4). Similarly, a GWAS in a small number ($n = 85$) of subjects with *simvastatin*-related myotoxicity and 90 controls identified a strong association with a nonsynonymous variant in *SLCO1B1* (Link et al., 2008), which encodes a transporter (OATP1B1) important for modulating intracellular drug concentrations, although the exact mechanism underlying the toxicity remains undefined. GWAS has validated other associations previously established by candidate gene approaches; examples include *CYP2C19*2* and failure of *clopidogrel* to inhibit platelet function (Shuldiner et al., 2009) and *CYP2C9*, *VKORC1*, and *CYP4F2* variants and variability in changes in *warfarin* maintenance dose (Cooper et al., 2008; Takeuchi et al., 2009).

These GWAS have been enabled by the collection of large numbers of patients exposed to the drugs of interest and phenotyped in some fashion for drug response. These collections can be developed during the course of clinical trials, as in the case of *simvastatin-SLCO1B1*; collected in healthcare systems (*HLA-carbamazepine*); or extracted from very large cohorts developed for epidemiologic study or derived from electronic health records (EHRs). These resources are also enabling GWAS of drug response phenotypes known to be variable but where the candidate gene approach has not yielded large effect size variants. Examples include renal dysfunction during *vancomycin* administration (Van Driest et al., 2015), *heparin*-induced thrombocytopenia (Karnes et al., 2015), ACE inhibitor–related cough (Mosley et al., 2016), and anthracycline-related heart failure (Wells et al., 2017).

Methods to Strengthen the Associations Between Genetic Variation and Variable Drug Actions

With increasing application of exome or whole-genome sequencing in populations, millions of DNA variants are being identified, and methods to establish their function are evolving. *In silico* methods to predict the effects of amino acid substitutions on protein function have been developed using sequence comparisons across species, assuming that mutations of highly conserved residues are more likely to alter protein function. Integrating increasingly well-developed structural models will complement and extend these approaches. Further, while single experimental approaches can suggest a relationship between variable drug responses and a variant in a specific locus or gene, the use of multiple complementary approaches provides the strongest evidence supporting such relationships.

In some cases, the effect of candidate pharmacogenetic variants can be approached in well-defined *in vitro* systems. The activity of nonsynonymous coding region variants in drug-metabolizing or drug transport molecules can be studied using heterologous expression and compared to that of wild type. These approaches can be extended to study the effect of variants on drug interactions with target molecules such as G protein-coupled receptors or ion channels, and in these cases, the assays are tailored to the specific pharmacology of the drug target.

Different approaches must be developed to establish the effect of candidate variants that do not disrupt pharmacogene coding sequence. Liver microsomes expressing wild-type or variant promoter sequences have been used to establish the extent to which promoter variants drive variability in expression of key pharmacogenes, as was done for *VKORC1* (Rieder et al., 2005). A synonymous coding region variant in the drug transport molecule P-glycoprotein encoded by *ABCB1* is associated with variable drug concentrations of substrates such as *digoxin*; *in vitro* studies suggested that the variant results in altered folding of the nascent protein and its insertion into the cell membrane (Kimchi-Sarfaty et al., 2007). It is now possible to generate very large numbers of variants across genes of interest and to use novel high-throughput approaches to simultaneously assess effects on functional properties such as enzymatic activity or cell surface expression in the case of targets such as ion channels (Esposito et al., 2019; Matreyek et al., 2018; Starita et al., 2017).

The effect of drug administration to genotyped humans can be used to define the consequences of variation in candidate pharmacogenes using outcomes such as drug concentrations or effects. The same approach can be used in genetically modified mice or other model systems. An emerging complementary approach is to generate induced pluripotent stem cells (iPSCs) from humans with specific genetic variants of interest; these can then be matured into specific cell types of interest (e.g., hepatocytes, cardiomyocytes) to study the effects of candidate variants. The ability to easily and reliably edit DNA in iPSCs allows the study of specific variants even if human carriers are not available and to control for the genetic background, providing evidence for functional consequence.

Very large DNA biobanks that also capture drug response phenotypes are another emerging tool. These biobanks often capture target phenotypes, including drug response, using EHRs and may complement EHR-based interrogation with direct patient-provided information (Allen et al., 2014; Denny et al., 2019; Roden et al., 2008). Such biobanks have been used to validate and extend (e.g., to other ancestries) known high effect size associations such as *clopidogrel-CYP2C19* and *warfarin-CYP2C19/VKORC1/CYP4F2* (Delaney et al., 2012; Ramirez et al., 2012) and to enable GWAS, as described above.

Cell-based transcriptomics and proteomics have also been used to study modulators of drug response. For example, studying the transcriptomic consequences of exposure to *simvastatin* in cell lines identified six potential modulators of *simvastatin* effect; variants in one of these genes, glycine amidinotransferase, were associated with *simvastatin* myotoxicity in a clinical trial (Mangravite et al., 2013). Large repositories of cell-specific transcriptional profiles (e.g., the GTEx project, see Table 7–1) will enable further studies in this area.

Polygenic Approaches

The traditional view of pharmacogenetics has focused on common variants in one or occasionally a few genes that mediate substantial variability in drug action (e.g., thiopurines, *warfarin*). However, this approach does not capture variability in drug response that arises because of the effects of many small effect size variants across multiple genes, such as those captured by GWAS. GWAS for common disease has enabled the development of polygenic risk scores that may identify individuals at substantially increased disease risk over a lifetime, even in the absence of individual high effect size variants (Khera et al., 2018). A number of studies have now used this approach to analyze polygenic drug responses.

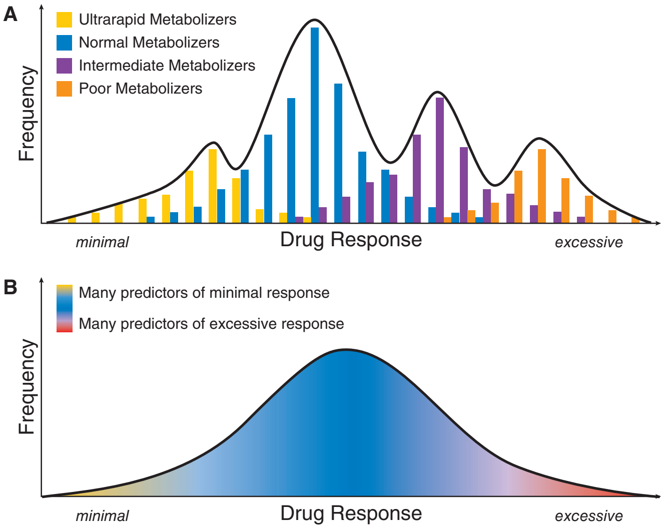

Figure 7–6 *Monogenic and polygenic effects of pharmacogene variation on drug response.* Panel **A** depicts the drug responses that may be observed across a population when the response is predominantly influenced by one or very few genes. Discrete groups may be evident in the population (e.g., representing ultrarapid to poor metabolizers). In contrast, Panel **B** depicts observed drug responses for a drug influenced by many genes. A normal distribution with no clear subgroups is observed, as individuals are on a spectrum from one extreme (many predictors of minimal response) to another (many predictors of excessive response).

Using idealized data, Figure 7–6 depicts the effects of pharmacogene variation on drug response when the variation involves a small number versus a large number of genes.

A polygenic risk score for baseline QTc interval, created by 62 individual variants derived from a large GWAS, predicted both QTc interval prolongation during drug exposure and the risk for the associated arrhythmia torsades de pointes (Strauss et al., 2017). Similarly, polygenic risk scores for coronary artery disease identified patients most likely to derive benefit from newer LDL cholesterol–lowering drugs (Damask et al., 2020; Marston et al., 2020), possibly reflecting the fact that individuals with a higher genetic risk for disease are more likely to respond than those with a high burden of conventional risk factor such as diabetes, hypertension, or smoking. This result may be useful in selecting patients most likely to benefit from newer drugs that are much more expensive than statins. One recent study reported that the extent of variation explained by small and medium effect size variants derived from GWAS accounted for 38% to 98% of variability for 11 drug response phenotypes (Muhammad et al., 2020).

Genomics as a Pathway to Identify New Drug Targets

The identification of genetic pathways in normal physiology and in disease can provide important clues to new drug targets. Indeed, studies from the drug development community suggest that when the actions of a candidate drug are supported by human genetics studies, that drug is three times more likely to be marketed compared to drug candidates for which such evidence is absent (Nelson et al., 2015).

Seminal studies by Brown and Goldstein of patients with the rare disease familial hypercholesterolemia (FH) identified a defect in the regulation of HMG-CoA reductase, the key rate-limiting enzyme in LDL cholesterol biosynthesis (Schekman, 2013). The statins, which inhibit that enzyme, are now among the most effective and widely used medications in cardiovascular therapy (see Chapter 37). PCSK9 contributes to the degradation of LDL receptors, which are responsible for removing LDL cholesterol from the circulation; rare gain-of-function variants in *PCSK9* thereby increase LDL cholesterol and cause FH. Conversely, work in the Dallas Heart Study and in the Atherosclerosis Risk in Communities epidemiologic study showed that relatively common nonsense (i.e., loss-of-function) variants in *PCSK9* in African American subjects associated with lower LDL cholesterol values decreased risk for coronary artery disease (Cohen et al., 2006). This result, in turn, identified PCSK9 as a potential drug target for high LDL in all ancestries, again highlighting the importance of ancestral diversity to enable genomic discovery. In 2015, two antibodies that target PCSK9, *alirocumab* and *evolocumab*, were approved by the U.S. Food and Drug Administration for clinical use in FH and other lipid disorders. These PCSK9 inhibitors prevent degradation of LDL receptors and their recycling to the hepatocyte membrane, thereby facilitating removal of LDL cholesterol and lowering blood LDL cholesterol levels (see Figure 37–5).

In a similar fashion, new drug targets have been identified by work showing that loss-of-function variants reduce risk for common diseases; examples are *APOC3*, where variants lower triglycerides and reduce the risk of coronary artery disease (Stitziel et al., 2014); *SLC30A8,* where variants reduce risk for type 2 diabetes (Flannick et al., 2014); and *HSD17B13,* where variants reduce risk of chronic liver disease (Abul-Husn et al., 2018). Patients homozygous for *SCN9A* loss-of-function variants are pain insensitive (Cox et al., 2006); inhibitors of SCN9A might be useful analgesics.

Hundreds of mutations in the chloride transporter encoded by *CFTR* cause cystic fibrosis, but through diverse mechanisms. *Ivacaftor* partially corrects abnormal gating of certain rare variants of *CFTR* (G551D and others), while *lumacaftor* improves cell surface expression of the most common variant, ΔF508. *Ivacaftor* (Whiting et al., 2014) and the *ivacaftor/lumacaftor* combination (Wainwright et al., 2015) improve symptoms and outcomes in patients with cystic fibrosis. These agents, and other combinations with similar mechanisms of action, have now been approved for genotype-guided use.

One interesting approach using DNA biobanks described above is to turn the GWAS paradigm "on its head" and to ask with what human phenotype a particular genetic variant associates. This "phenome-wide association study" (PheWAS) approach can be used to replicate a GWAS result or to identify entirely new associations (Denny et al., 2016). New associations with variants in genes known to encode drug targets have then been used to suggest new indications for existing drugs ("repurposing") (Diogo et al., 2018; Pulley et al., 2017; Rastegar-Mojarad et al., 2015) and to predict adverse effects of available or new drugs (Jerome et al., 2018).

Genetic variant information is the key driver for the prescribing of newer, targeted anticancer therapies, as described above. GWAS data have also been proposed as a route to identify a target population in whom a new therapy may be especially effective. One example may be the high-density lipoprotein–elevating drug *dalcetrapib*. A large phase III trial showed no difference in clinically important endpoints between treatment and placebo groups, but a subsequent GWAS identified a variant in *ADCY9* (adenylyl cyclase type 9) as a predictor of efficacy (Tardif et al., 2015). Follow-up *in vitro* and mouse studies (Rautureau et al., 2018) supported this suggestion, and a large trial randomizing only subjects with the genotype predicting a favorable drug effect has been mounted.

Pharmacogenetics in Clinical Practice

The increasing understanding of genetic contributors to variable drug actions raises questions of how these data might be used by healthcare providers to choose among drugs, doses, and dosing regimens. One approach is point-of-care testing, in which genotyping is ordered at the time of drug prescription; platforms that reliably deliver relevant genotypes rapidly (often in less than an hour) now make such approaches feasible. However, one difficulty with this approach is that each drug requires a separate assay. An alternate approach envisions genotyping at multiple loci relevant for responses to large numbers of drugs, embedding this information in each patient's EHR, and using clinical decision support to advise on drug selection and dosing when a relevant drug is prescribed to a patient with a variant genotype. This approach is being tested in a number of "early adopter" sites (Rasmussen-Torvik et al., 2014; Van Driest et al., 2014).

TABLE 7–3 ■ ADDITIONAL GENETIC VARIANTS INFLUENCING DRUG RESPONSE

GENE PRODUCT (*GENE*)	DRUGS	EXAMPLES OF RESPONSES AFFECTED
Drug Metabolism and Transport		
CYP2C9 (*CYP2C9*)	Tolbutamide, warfarin*, phenytoin*, nonsteroidal anti-inflammatory drugs*	Anticoagulant effect of warfarin; exposure-related toxicity to phenytoin
CYP2C19 (*CYP2C19*)	Mephenytoin, omeprazole*, voriconazole*, hexobarbital, mephobarbital, proguanil, phenytoin*, clopidogrel*, citalopram*	Peptic ulcer response to omeprazole; cardiovascular events after clopidogrel
CYP2D6 (*CYP2D6*)	β-Blockers*, antidepressants*, anti-psychotics*, codeine*, debrisoquine, atomoxetine*, dextromethorphan*, encainide, flecainide, fluoxetine, guanoxan, *N*-propylajmaline, perhexiline, phenacetin, phenformin, propafenone*, sparteine, tamoxifen*	β-Blocker effect, tardive dyskinesia from antipsychotics, narcotic side effects, codeine efficacy and toxicity, imipramine dose requirement, breast cancer recurrence after tamoxifen
CYP3A4/3A5/3A7 (*CYP3A5/3A5/3A7*)	Macrolides, cyclosporine, tacrolimus, Ca^{2+} channel blockers, midazolam, terfenadine, lidocaine, dapsone, quinidine, triazolam, etoposide, teniposide, lovastatin, alfentanil, tamoxifen, steroids	Efficacy of immunosuppressive effects of tacrolimus
CYP2B6 (*CYP2B6*)	Methadone, cyclophosphamide	Arrhythmia during methadone, ovarian failure during cyclophosphamide
CYP2A6 (*CYP2A6*)	Nicotine	Smoking addiction risk
Dihydropyrimidine dehydrogenase (*DPYD*)	Fluorouracil*, capecitabine*	5-Fluorouracil toxicity; capecitabine toxicity
N-acetyltransferase (*NAT2*)	Isoniazid, hydralazine, sulfonamides, amonafide, procainamide, dapsone, caffeine	Hypersensitivity to sulfonamides, amonafide toxicity, hydralazine- and procainamide-induced lupus, isoniazid neurotoxicity
Glutathione transferases (*GSTM1, GSTT1, GSTP1*)	Several anticancer agents	↓response in breast cancer, ↑toxicity and worse response in acute myelogenous leukemia
Thiopurine methyltransferase (*TPMT*)	Mercaptopurine*, thioguanine*, azathioprine*	Thiopurine toxicity and efficacy, risk of second cancers
UDP-glucuronosyl-transferase (*UGT1A1*)	Irinotecan*, atazanavir	Irinotecan toxicity, hyperbilirubinemia during atazanavir
P-glycoprotein (*ABCB1*)	Natural product anticancer drugs, HIV protease inhibitors, digoxin	↓ CD4 response in HIV-infected patients, ↓ digoxin concentration, drug resistance in epilepsy
UGT2B7 (*UGT2B7*)	Morphine	Morphine plasma levels
Organic anion transporter (*SLCO1B1*)	Statins*, methotrexate, ACE inhibitors	Statin plasma levels, myopathy; methotrexate plasma levels, mucositis
COMT (*COMT*)	Levodopa	Enhanced drug effect
Organic cation transporter (*SLC22A1, OCT1*)	Metformin	Pharmacologic effect and pharmacokinetics
Organic cation transporter (*SLC22A2, OCT2*)	Metformin	Renal clearance
Novel organic cation transporter (*SLC22A4, OCTN1*)	Gabapentin	Renal clearance
Drug Targets		
Thymidylate synthase (*TYMS*)	5-Fluorouracil	Colorectal cancer response
Chemokine receptor 5 (*CCR5*)	Antiretrovirals, interferon	Antiviral response
β_2 Adrenergic receptor (*ADBR2*)	β_2 Antagonists (e.g., albuterol, terbutaline)	Bronchodilation, susceptibility to agonist-induced desensitization, cardiovascular effects (e.g., increased heart rate, cardiac index, peripheral vasodilation)
β_1 Adrenergic receptor (*ADBR1*)	β_1 Antagonists	Blood pressure and heart rate after β_1 antagonists
5-Lipoxygenase (*ALOX5*)	Leukotriene receptor antagonists	Asthma response

(*Continued*)

TABLE 7–3 ■ ADDITIONAL GENETIC VARIANTS INFLUENCING DRUG RESPONSE (*CONTINUED*)

GENE PRODUCT (*GENE*)	DRUGS	EXAMPLES OF RESPONSES AFFECTED
Dopamine receptors D_2, D_3, D_4 (*DRD2, DRD3, DRD4*)	Antipsychotics (e.g., haloperidol, clozapine, thioridazine, nemonapride)	Antipsychotic response (D_2, D_3, D_4), antipsychotic-induced tardive dyskinesia (D_3) and acute akathisia (D_3), hyperprolactinemia in females (D_2)
Estrogen receptor α (*ESR1*)	Estrogen hormone replacement therapy	High-density lipoprotein cholesterol
Serotonin transporter 1 (*SLC6A4, 5-HTT*)	Antidepressants (e.g., clomipramine, fluoxetine, paroxetine, fluvoxamine)	Clozapine effects, 5HT neurotransmission, antidepressant response
Serotonin receptor (*HTR2A*, $5\text{-}HT_{2A}$)	Antipsychotics	Clozapine antipsychotic response, tardive dyskinesia, paroxetine antidepression response, drug discrimination
HMG-CoA reductase (*HMGCR*)	Statins	Reduction in serum cholesterol
Vitamin K oxidoreductase (*VKORC1*)	Warfarin*	Anticoagulant effect, bleeding risk
Corticotropin releasing hormone receptor (*CRHR1*)	Corticosteroids	Response to corticosteroids in asthma
Ryanodine receptor (RYR1)	General anesthetics*	Malignant hyperthermia
Modifiers		
Apolipoprotein E (*APOE*)	Statins (e.g., simvastatin)	Lipid-lowering
Human leukocyte antigen (*HLA-A, HLA-B*)	Abacavir*, carbamazepine*, phenytoin*, allopurinol*	Hypersensitivity reactions
G6PD deficiency (*G6PD*)	Rasburicase*, dapsone*	Methemoglobinemia, hemolytic anemia
Cholesteryl ester transfer protein (*CETP*)	Statins	Slowing atherosclerosis progression
Cardiac ion channels (*KCNH2, KCNQ1, KCNE1, KCNE2*)	QT prolonging antiarrhythmics (e.g. sotalol, dofetilide, quinidine), many other drugs (e.g. erythromycin, methadone, thioridazine, haloperidol, pentamidine)	Drug-induced polymorphic ventricular tachycardia (*torsades de pointes*), increased QT interval
Coagulation factor V (*F5*)	Oral contraceptives	Venous thrombosis
Voltage-gated K^+ channel interacting protein 4 (*KCNIP4*)	ACE inhibitors	Cough

*Drug-gene pair included on U.S. Food and Drug Administration Table of Pharmacogenomic Biomarkers in Drug Labeling, available at *https://www.fda.gov/drugs/science-and-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling*; accessed 29 May 2022.

There are several barriers that must be addressed if either approach is to become widely adopted. *First*, the evidence linking a variant to a variable drug response must be solid, the variable outcome must be clinically important, and some form of genetically guided advice should be provided (e.g., choose another drug, choose another dose). Drug-gene pairs such as *clopidogrel-CYP2C19* or *warfarin-CYP2C19/VKORC1/CYP4F2* may fall into this category; the Clinical Pharmacogenomics Implementation Consortium provides guidelines on such advice by genotype across multiple drugs (Table 7–1) (Relling et al., 2020). *Second,* the strength of the evidence supporting a genotype-specific prescribing strategy varies. The strongest level of evidence comes from randomized clinical trials (RCTs), in which a clinically important, genotype-guided treatment strategy is compared to a standard of care. Using this approach, genotyping for *HLA-B*57:01* has been shown to eliminate the risk for severe skin reactions (e.g., Stevens-Johnson syndrome) during treatment with the antiretroviral agent *abacavir* (Mallal et al., 2008). RCTs comparing *clopidogrel* to other antiplatelet agents have shown fewer bleeding events and no increase in stent thrombosis (the ADR associated with failure of *clopidogrel* bioactivation) with a genotype-guided approach (Claassens et al., 2019; Pereira et al., 2020). A number of trials have studied the utility of genotyping for *CYP2C9* and *VKORC1* variants during *warfarin* therapy. When the main outcome metric has been duration of drug exposure in therapeutic range during the first 30 to 90 days of therapy, the results have been inconsistent, with none showing a huge effect (Kimmel et al., 2013; Pirmohamed et al., 2013). These studies have few bleeding events, and an RCT that focused on bleeding as a primary endpoint did show a significant benefit of a genotyping approach to therapy (Gage et al., 2017). EHR-based case-control studies looking at *warfarin*-related bleeding have implicated variants in *CYP2C9* or *CYP4F2* as risk alleles (Kawai et al., 2014; Roth et al., 2014). Nonrandomized study designs are weaker than RCTs, but performing RCTs to target small subsets of patients carrying uncommon variants may not be feasible. There is debate about the level of evidence required for clinical implementation of pharmacogenetics, since adjustments to drug choice and dosing are routinely pursued by clinicians for other factors, without supporting evidence from RCTs (e.g., drug choice based on ADR risk and dose reduction for renal impairment).

An 8100-patient European RCT (van der Wouden et al., 2017) is comparing usual therapy for 43 target drugs to therapy guided using a multiplexed assay that interrogates variants in 13 key pharmacogenes. The study has randomized hospital systems in seven countries using a crossover design (18 months using standard therapy; 18 months genotype-guided). The results should be available in 2022.

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Chapter 8

Postmarketing Drug Safety

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INTRODUCTION

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Introduction

Initiatives to improve the safety of drugs after they are marketed arose from the recognition that rare potentially serious adverse drugs reactions (ADRs) are usually identified only after a drug is in clinical use. One has only to recall the example of phocomelia in newborns from exposure to *thalidomide*, a drug not approved for use in the U.S. at the time but used widely elsewhere from 1957 to 1961 to treat morning sickness in pregnant women and that caused congenital malformations in over 10,000 children (Vargesson, 2015). As a result of this tragedy and other ADRs, many countries have developed postmarketing surveillance systems to identify ADRs and to determine their prevalence and impact.

Approaches to drug safety have evolved in response to increased recognition of three important principles. First, the safety and efficacy of a drug are not fixed at the time of its approval but must be reassessed over its life cycle. Second, drug safety after marketing is affected not only by rare ADRs but also (and perhaps more importantly) by increased risk of common events not recognized initially as ADRs (e.g., myocardial infarction with *rofecoxib*). Third, strategies that mitigate risk effectively can allow drugs with serious adverse effects to remain on the market (e.g., use of *thalidomide* for multiple myeloma).

Counterfeit, substandard, and adulterated drugs; incorrect or unsafe clinical use; overdose; and accidental exposure to drugs also impact drug safety (Figure 8–1). This chapter focuses on efforts to improve drug safety by identifying and managing risks associated with the drug and its appropriate use in clinical practice after it is marketed, and on subsequent reevaluation of the drug's risk-benefit ratio. Many countries implement variations of the approaches we describe; for simplicity, we describe the U.S. system.

History of Postmarketing Drug Safety

Landmark Laws That Improved Drug Safety

Three landmark laws in the U.S. have been enacted in response to public health disasters. These laws focused on improving evidence of preclinical safety, clinical efficacy, and postmarketing safety, respectively.

1. The Food, Drug, and Cosmetic Act of 1938 was passed in response to the death of more than 100 patients who received a preparation of *sulfanilamide* elixir with diethylene glycol as a solvent (Paine, 2017). The legislation required that manufacturers test new drugs for toxicity before marketing to prove their safety.
2. The Kefauver-Harris amendment to the Food, Drug, and Cosmetic Act of 1962 was a response to the aforementioned *thalidomide* disaster. The U.S. avoided that disaster because a reviewer for the U.S. Food and Drug Administration (FDA), Francis Kelsey, did not allow the approval of *thalidomide* due to concerns about insufficient safety data and risk of peripheral neuropathy (Ross and Kesselheim, 2015). The legislation required proof of drug efficacy in well-controlled clinical studies prior to marketing and report of serious side effects (Greene and Podolsky, 2012; Ross and Kesselheim, 2015).
3. The FDA Amendments Act of 2007 was developed after a reexamination of postmarketing drug safety resulted in an Institute of Medicine (IOM) report (IOM, 2007) in response to the withdrawal of *rofecoxib* from the market for increased risk of myocardial infarction and stroke after millions of people had been exposed and potentially tens of thousands suffered serious adverse events (Topol, 2004). The legislation instructed the FDA to build a population-based surveillance system; allowed authorities to require changes to the label, postapproval studies, and risk evaluation and mitigation strategies (REMS); and mandated that the parties responsible for clinical trials post information about the trials and their results in a public database (Avorn et al., 2018).

Why We Need Postmarketing Drug Safety Information

Premarketing Clinical Trials Do Not Adequately Define the Safety of a Drug

Premarketing clinical trials are essential to determine if the benefits of a medication outweigh its immediate risks, but the idea that a drug is safe because it has passed the threshold required for marketing fails to recognize the limitations of premarketing trials.

Abbreviations

ADR: adverse drug reaction
CI: confidence interval
DSC: drug safety communication
FAERS: FDA Adverse Event Reporting System
HR: hazard ratio
HRT: hormone replacement therapy
ICSR: individual case safety report
IOM: Institute of Medicine
RCT: randomized controlled trial
REMS: risk evaluation and mitigation strategy

TABLE 8–1 ■ FIVE ILLUSTRATIVE DRUG WITHDRAWALS

These examples illustrate that drugs may be approved based on studies in a small number of patients in clinical trials yet be later withdrawn when postmarketing surveillance of a much larger patient cohort reveals safety issues.

DRUG	NUMBER OF PATIENTS EXPOSED TO THE DRUG IN PREMARKETING STUDIES	NUMBER OF PATIENTS EXPOSED POSTMARKETING BEFORE THE DRUG WAS WITHDRAWN
Terfenadine	5000	7,500,000
Fenfluramine	340	6,900,000
Dexfenfluramine	1200	2,300,000
Mibefradil	3400	600,000
Bromfenac	2400	2,500,000

Source: Adapted from Friedman MA, et al. The safety of newly approved medicines: do recent market removals mean there is a problem? *JAMA*. **1999**, *281*:1728–1734.

Premarketing Trials Are Small

The total number of patients exposed to a new drug in the clinical trials that lead to approval is often in the hundreds or low thousands. In the 253 pivotal clinical trials for 109 new drugs and biologics approved between 2015 and 2017, the median number of patients studied was 467 (interquartile range, 209–722) (Zhang et al., 2020). For several medications withdrawn from the market, the contrast between the small number of patients studied in premarketing trials and the large number exposed prior to withdrawal is striking (Table 8–1) (Friedman et al., 1999).

The small number of patients studied in most premarketing clinical trials makes it impossible to detect uncommon side effects and accurately define the prevalence of common ones (Table 8–2). For example, the antidepressant *nefazodone* causes liver damage at the rate of one case of death or transplant per 250,000 to 300,000 patient-years, an event that could not be captured reliably even in a large clinical trial. Also, with small premarketing studies, there is little chance of identifying subgroups of patients more likely to suffer particular adverse effects. For example, the increased risk of angioedema after treatment with an angiotensin-converting enzyme (ACE) inhibitor in patients of African ancestry was recognized only long after the drugs were marketed (Brown et al., 1996).

Genetic variation is an important risk factor for adverse drug responses (ADRs); in small clinical trials, there will be few patients with uncommon, but important, genetic variants. For example, approximately 0.3% of people of European ancestry are homozygous for genetic variants in *TPMT*, the gene that encodes the enzyme thiopurine methyltransferase that metabolizes thiopurine drugs such as *azathioprine* and *6-mercaptopurine*. These people are at risk of life-threatening myelosuppression if they receive thiopurines (Osanlou et al., 2018). The genetic mechanisms underlying an ADR may be unknown during premarketing drug trials (e.g., *abacavir* hypersensitivity in patients carrying HLA-B*5701), and there may be a delay in recognizing the clinical importance of patient genetics. Consider the interaction of CYP2D6 with *codeine*: CYP2D6 metabolizes *codeine* to *morphine*, the active analgesic. *CYP2D6* polymorphisms give rise to a range of activities, from absent or very slow to ultrarapid metabolization. As a result, children who are ultrarapid metabolizers of *codeine* are at risk of respiratory depression, a symptom of opiate excess, whereas very slow metabolizers may be undertreated for pain (see Chapters 7 and 23).

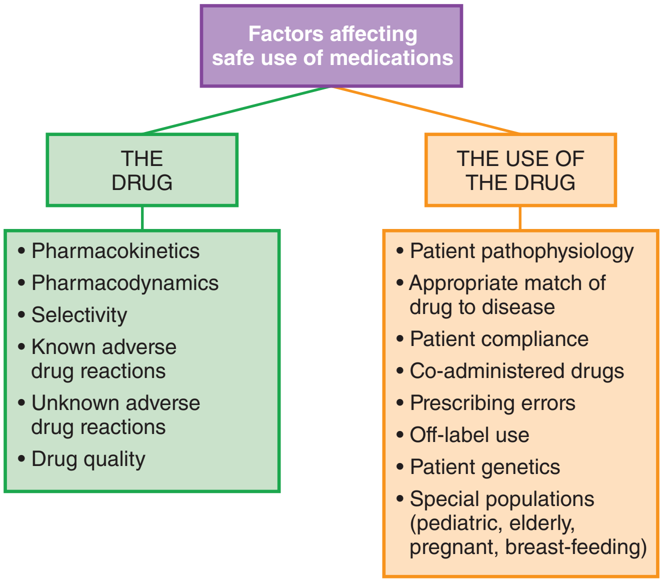
Factors affecting safe use of medications
THE DRUG
• Pharmacokinetics
• Pharmacodynamics
• Selectivity
• Known adverse drug reactions
• Unknown adverse drug reactions
• Drug quality
THE USE OF THE DRUG
• Patient pathophysiology
• Appropriate match of drug to disease
• Patient compliance
• Co-administered drugs
• Prescribing errors
• Off-label use
• Patient genetics
• Special populations (pediatric, elderly, pregnant, breast-feeding)

Figure 8–1 *Postmarketing drug safety is affected by many factors intrinsic to the drug itself and the way it is used.*

Premarketing Trials Are Short

Although some drugs are used by patients for years, even for life, premarketing clinical trials are relatively short. The median duration of pivotal clinical trials for drugs and biologics approved between 2015 and 2017 in the U.S. was 24.0 weeks (interquartile range, 12.0–37.6 weeks); only 46.2% of drugs were supported by one or more clinical trials of at least 6 months in duration (Zhang et al., 2020). Serious adverse effects may only manifest after long-term use of a drug or after a long latent period and thus would not emerge in premarketing studies. For example,

TABLE 8–2 ■ EXAMPLES OF THE INCREASES IN RELATIVE RISK THAT ARE DETECTABLE FOR OUTCOMES OF VARYING FREQUENCY IN CLINICAL TRIALS OF DIFFERENT SIZE COMPARING A DRUG TO A CONTROL

EVENT PROPORTION (RISK) IN CONTROLS	SAMPLE SIZE (BOTH ARMS)		
	2000 PATIENTS	4000 PATIENTS	8000 PATIENTS
	DETECTABLE RELATIVE RISK		
5 per 100	1.73	1.50	1.34
1 per 100	3.04	2.31	1.86
5 per 1000	4.32	3.05	2.31
1 per 1000	13.13	7.77	4.95

Calculations assume a trial in which the follow-up for both the control and treatment arms is fixed (e.g., 30 days or 1 year) and the outcome is binary. They assume 90% power, a two-sided type 1 error of 0.05, a 1:1 ratio between patients receiving control and study drug, and a null hypothesis that the true relative risk is 1.0. Relative risk is the ratio of risk in the treatment group to that in the control group; a relative risk of 1.50 indicates a 50% increase in risk.

bisphosphonates—used to prevent and treat osteoporosis, often a lifelong condition—have infrequent side effects such as avascular necrosis of the jaw and atypical femoral shaft fractures that typically occur after long-term therapy. Moreover, because premarketing studies are short, the relationship between duration of therapy and long-term efficacy is poorly defined. Thus, despite lack of rigorous studies, current practice for treating osteoporosis has evolved to recommending bisphosphonate drug holidays for some patients with the aim of decreasing the risk of long-term side effects while maintaining protection against fractures (Camacho et al., 2020).

Premarketing Trials Study Select Populations

The primary goal of premarketing clinical trials is to demonstrate efficacy and safety (i.e., acceptable benefit vs. risk). The most efficient approach is to study a homogeneous population selected to maximize the efficacy signal and minimize the risk of adverse events. Thus, many premarketing studies exclude or have small numbers of children, pregnant women, patients older than 75 years of age, subjects with comorbidities such as impaired renal function, and patients at high risk of adverse events or drug-drug interactions. Consequently, new information about such patient groups frequently only emerges after marketing. For example, even for statins, one of the most intensively studied and heavily prescribed classes of drugs, the cardiovascular benefits of treating elderly patients (Gencer et al., 2020) and the increased risk of diabetes (Swerdlow et al., 2015) were shown only recently.

As a result of the premarketing approval process, a drug that has been studied in a few hundred or thousand carefully selected people can be prescribed rapidly to millions of people that include patient populations and indications not studied in premarketing trials. For example, the high dose of *rofecoxib* (50 mg/day) that subsequently was found to confer a 5-fold increased risk of myocardial infarctions (Curfman et al., 2005) was approved only for the treatment of acute pain for a maximum of 5 days but was prescribed widely off-label to patients with chronic pain for long periods of time (Griffin et al., 2004), substantially increasing their risk of adverse cardiovascular effects.

Premarketing Trials Often Use Surrogate Endpoints

The goal of treatment with a drug is to improve direct measures of patient health such as survival, prevention of disease, symptoms, or function. To reduce trial size or shorten its duration, premarketing studies often use surrogate endpoints—biomarkers or other measures thought to mediate or be predictors of the ultimate clinical endpoint (Fleming and DeMets, 1996). Examples of surrogate endpoints include blood pressure, lipid levels, hemoglobin A_{1c}, and CD4 counts. Some surrogate endpoints such as HIV viral load (plasma HIV ribonucleic acid [RNA] levels) are excellent predictors of clinical outcomes and response to drug therapy; however, others have been misleading. For example, ventricular premature depolarizations are a risk factor for mortality after myocardial infarction; the antiarrhythmic drugs *flecainide* and *encainide* reduced ventricular premature depolarizations (the surrogate endpoint); thus, these two agents were widely used to prevent death after myocardial infarction until the CAST clinical trial showed they increased rather than decreased mortality in this setting (Echt et al., 1991).

Surrogate endpoints are frequently used for drug approval, particularly for drugs evaluated through one of the special expedited programs of the FDA (Priority Review, Accelerated Approval, Fast Track, Breakthrough Therapy) designed for drugs that address unmet medical needs for serious or life-threatening conditions. An analysis of 253 pivotal U.S. premarketing trials supporting new drugs and biologics approved from 2015 to 2017 found that, overall, 59% used surrogate endpoints. The rate was 67% in 128 trials for drugs that used any expedited regulatory approval program and 51% in 125 trials for drugs that did not use such programs (Zhang et al., 2020). Thus, when a drug is marketed, its effect on the endpoints of major interest may not be known.

Experience with medications for diabetes illustrates the pitfalls inherent in surrogate endpoints. Although the effect of an antidiabetic drug on blood glucose or the hemoglobin A_{1c} fraction is important, a major goal of treatment is to prevent the complications of diabetes. However, the assumption that a drug's hypoglycemic effect predicts its clinical outcomes has been shown to be flawed. The thiazolidinedione antidiabetic medication *rosiglitazone* was approved in 1999 based on short-term trials of glycemic control; however, in 2007, a meta-analysis of randomized controlled trials found an increased risk of myocardial infarctions due to *rosiglitazone* (Psaty and Furberg, 2007). The ensuing controversy led the FDA to recommend that trials for new antidiabetic drugs should be large enough to exclude an increase in cardiovascular risk of 30% or larger. In order not to delay drug introduction, the guidelines indicated that a two-stage approach was permissible, with premarketing trials powered to exclude an increase of 80% or greater (Chong et al., 2020) and subsequent postmarketing trials powered to exclude an increase of 30% or greater. The mandate resulted in large postmarketing cardiovascular outcomes studies that identified a reduction in cardiovascular risk for the newer sodium-glucose cotransporter-2 inhibitor (SGLT2i) and glucagon-like peptide-1 receptor agonist (GLP-1 RA) antidiabetic drugs.

More recently, the FDA has proposed a new approach for approval of antidiabetic drugs to replace the previously required postmarketing studies to establish cardiovascular safety. Rather than postmarketing studies of cardiovascular drug safety, large premarketing studies will be required that ensure adequate exposure (both the number of patients and the duration of exposure) to the new drug and include patients with common diabetes comorbidities such as cardiovascular disease and impaired renal function (Chong et al., 2020). Table 8–2 illustrates examples of risk for any ADR that such an approach could exclude in large clinical trials of *different* sizes. It is striking that even for a relatively common ADR (e.g., occurring at a rate of 1 event per 100 controls) a study of 8000 patients (4000 each receiving new and control drug) would be unable to exclude an increase in relative risk smaller than 1.86 (i.e., an 86% increase in relative risk) with 90% confidence.

Premarketing Trials Seldom Use Active Comparators

The basis of the FDA requirement for premarketing trials is the language of the 1962 Kefauver-Harris amendments requiring "adequate and well-controlled investigations" to demonstrate efficacy. This requirement has been interpreted to permit placebo-controlled studies because trials that compare a new agent to the best available therapy generally require substantially greater sample size. Consequently, premarketing studies frequently compare a new drug to a placebo; only 29% of pivotal U.S. premarketing studies from 2015 to 2017 used an active comparator (Zhang et al., 2020). As a result, when a drug is marketed, prescribers and patients may not know how the efficacy and safety of the new drug compare to that for drugs already on the market.

New Serious Adverse Drug Effects Are Commonly Discovered After a Drug Is Marketed

As newly approved medications are administered to large numbers of patients over prolonged periods of time, adverse effects are frequently identified by postmarketing surveillance. Between 2001 and 2010, the FDA approved 222 novel drugs and biologics; there were 123 postmarketing safety events (3 withdrawals from the market, 61 boxed warnings, and 59 safety communications) affecting 71 of the new therapies (Downing et al., 2017). Safety-related changes to the drug label are even more common, affecting 70% of drugs after marketing: For 278 new molecular entities approved between 2002 and 2014, there were 703 label changes addressing 2505 safety issues (Pinnow et al., 2018). Label updates occurred throughout the follow-up period of up to 13.2 years and included the addition of 51 (2%) and 842 (33.6%) safety issues to the "boxed warnings" and "warnings and precautions" sections of the label, respectively.

A Life Cycle Approach to Drug Safety

Historically, a drug was often considered "safe" because it had been approved for marketing until events occurred that showed it was "unsafe." However, experience shows that it is dangerous to consider a drug safe because it has passed the safety threshold required for marketing. Given the many limitations of premarketing trials, a critical experiment takes place after the drug is marketed. We increasingly recognize that information about safety and efficacy evolves over the life cycle of the drug as new knowledge emerges, a central theme that guides modern approaches to drug safety. These approaches include organized systems for reporting and monitoring cases of potential adverse events, controlled postmarketing studies, and vigorous regulatory efforts to assure that new safety information is reflected in clinical practice (Avorn et al., 2018; IOM, 2007).

Approaches to Postmarketing Surveillance

Case Reports and Spontaneous Adverse Event Reporting Systems

Reports of suspected cases of adverse drug effects, including those from spontaneous adverse event reporting systems, can quickly identify potential drug safety problems when drugs are administered to large populations. Such reporting systems are the foundation of postmarketing drug safety surveillance and have rapidly identified many prominent serious ADRs. For example, case reports identified the teratogenicity of *thalidomide* (Avorn, 2011) and the cardiac valvular abnormalities related to *fenfluramine-phentermine* (Connolly et al., 1997). To improve detection of ADRs beyond case reports published in the medical literature, many countries have developed adverse event reporting systems that collect and interpret spontaneous reports of adverse events, such as the FDA Adverse Event Reporting System (FAERS) in the U.S.

The FAERS database is large (Table 8–3), currently receives more than 2 million reports a year, and is accessible to the public (FDA, 2021). Healthcare providers and consumers can report adverse events to the FDA voluntarily through the MedWatch system; reporting these is mandatory for drug manufacturers. The FDA monitors reports in FAERS, issues a quarterly report of new safety information and potential signals of serious risk, and undertakes additional studies if warranted.

Limitations of Spontaneous Reporting Systems

Calculation of the frequency of a particular adverse reaction requires accurate knowledge of the numerator (occurrences of the adverse response) and the denominator (patients exposed to the drug). Neither of these numbers is easy to obtain accurately. The circumstances surrounding the adverse reaction—including severity, duration and dose of the suspect drug, concomitant medications, comorbidities, and patients at greatest risk—may be critical to evaluate causality. Thus, the limitations of spontaneous reporting systems are numerous.

Small Fraction of Cases Reported. Spontaneous reporting systems cannot define how often a particular adverse event occurs with a particular drug (the numerator) because there is substantial underreporting and reporting patterns can change unpredictably over the life cycle of a drug. An analysis across 12 countries found a median underreporting rate of 94% (Hazell and Shakir, 2006). Furthermore, factors such as media coverage, litigation, and how long a drug has been on the market influence reporting. Thus, comparisons of the relative frequency of adverse event reports for a new drug to those of an established drug usually will be misleading (Hazell and Shakir, 2006).

TABLE 8–3 ■ NUMBER AND TYPE OF INDIVIDUAL SAFETY CASE REPORTS IN THE FDA ADVERSE EVENT REPORTING SYSTEM FROM 1968 TO MARCH 31, 2022

REPORT	NUMBER OF REPORTS
Total reports	24,251,919
Reports with serious adverse events[a]	13,552,221
Reports with death as outcome	2,290,629

[a] Serious adverse events do not include deaths.

Data are as of June 30, 2021, and adapted from the FDA Adverse Event Reporting public dashboard. https://fis.fda.gov/sense/app/95239e26-e0be-42d9-a960-9a5f7f1c25ee/sheet/7a47a261-d58b-4203-a8aa-6d3021737452/state/analysis, accessed May 23, 2022.

Denominator Is Unknown. Databases of spontaneous adverse event reports contain no information about the total number of patients who received a drug (the denominator). The potential number of patients exposed is often inferred from information about drug sales, but this may not reflect actual use accurately (Hazell and Shakir, 2006), and information about use in pertinent subpopulations or clinical settings is not available.

The Information in Case Reports Often Is Incomplete. Spontaneous reports are frequently missing critical information. The quality of individual case safety reports (ICSRs) in FAERS for the outcome of death, the most serious adverse event, illustrates the problem (Table 8–4). Approximately a quarter of these ICSRs recorded only the outcome of death and no other adverse events; these reports lacked important information more often than those that included additional adverse events (Marwitz et al., 2020). However, both sets of ICSRs were suboptimal; reports with death as the only adverse event and those that included additional adverse events frequently lacked information such as cause of death, past medical history, concomitant medications, and when the drug was started.

It Is Difficult to Detect a Small-to-Moderate Increased Risk for a Common Event. Because of these limitations, true event rates can almost never be calculated from spontaneous reporting systems. If such a rate is estimated, it cannot be compared to that in other medications for the same indication nor can it be adjusted for critical patient comorbidity. Consequently, spontaneous adverse event reporting systems are unable to detect small-to-moderate increases in events that are not rare; this requires properly controlled studies. For example, cardiovascular events are not unexpected in elderly patients, particularly those with diabetes or hypertension, and thus are unlikely to trigger an adverse event report. However, because many more people have myocardial infarction than liver failure, a drug that causes what may appear to be a small increase in a common event (e.g., a relative risk of 1.2 for myocardial infarction) will have a far greater effect on public health than one that causes a rare but eye-catching event such as liver failure.

Finding True Signals Is Challenging. One common use of adverse event reporting systems is to screen for potential adverse events that

TABLE 8–4 ■ INFORMATION PRESENT IN REPORTS WITH DEATH AS THE OUTCOME IN THE FDA ADVERSE EVENT REPORTING SYSTEM

REPORT INCLUDES	REPORTS WITH DEATH AS ONLY ADVERSE EVENT (N = 994)	REPORTS WITH DEATH AND OTHER ADVERSE EVENTS (N = 998)
Age	76%	84%
Sex	85%	91%
Concomitant medications	10%	41%
Cause of death	7%	72%
Past medical history	54%	74%
Causality assessment	29%	64%
All of criteria on the three lines above	2%	40%

Data are from a stratified random sample of 2000 individual case safety reports in the FDA Adverse Event Reporting System through December 31, 2017, with death reported as the outcome.

Source: Data are extracted from Marwitz K, et al. An evaluation of postmarketing reports with an outcome of death in the US FDA adverse event reporting system. *Drug Safety,* **2020**, *43*:457–465.

warrant further evaluation (signals). The large number of reports and the limitations of their data make it difficult to identify signals. Quantitative signal detection methods have evolved that do not require information about the true volume of drug use (the denominator) but rely only on the spontaneous adverse event database. These approaches often use disproportionality methods that contrast the observed number of particular adverse events reported for a particular drug with the number expected based on information in the entire database (Hazell and Shakir, 2006). However, their accuracy relies on several assumptions that are difficult to verify.

Appropriate Use of Case Reports. Although one does not need to be certain of causality to publish a case report or file an adverse event report, confidence about a causal relationship between a drug and an adverse event is essential for guiding decisions that affect public safety. Thus, signals from spontaneous reporting systems nearly always require additional evaluation. Case reports are most valuable for inferring a causal relationship between a drug and an adverse event when the event is otherwise rare, has few other risk factors, has a close temporal relation with exposure (e.g., anaphylaxis, Stevens-Johnson syndrome), and the drug confers a large excess risk (e.g., rhabdomyolysis with *cerivastatin*; Table 8–5) (Staffa et al., 2002). When these conditions can be met, no other technique can identify adverse drug effects as quickly or inexpensively.

Controlled Studies

Controlled studies can assess signals that arise from case reports and identify new potential drug safety problems. The studies can be observational pharmacoepidemiologic studies, including those from the FDA Sentinel Initiative, or postmarketing clinical trials.

Observational Pharmacoepidemiologic Studies

Pharmacoepidemiologic studies of the relationship between a drug and a potential adverse event frequently use routinely collected clinical or insurance data (real-world data) such as that from large health maintenance organizations and the Medicare and Medicaid programs. The data include records of drug exposures and other medical care encounters that can define both potential outcomes and patient comorbidity. Although much less cumbersome than clinical trials, these epidemiologic studies involve considerable time and expense and may take years to complete. They require identifying one or more suitable databases, obtaining data access (including requisite permissions and payments), cleaning the data, extracting the relevant information, checking data quality, and performing analyses.

The Sentinel System

To facilitate more timely observational studies, the FDA started the Sentinel Initiative. The Sentinel System has evolved into a partnership between the FDA and more than 30 organizations that include academic sites and managed care organizations with access to curated electronic health information on over a 100 million people (Dal Pan, 2019). Participating sites can perform analyses on their own data behind their own firewall and return the results for central analysis, although, when necessary, patient-level analytic data sets can be returned to the Sentinel Operations Center (Adimadhyam et al., 2020). All sites use a Sentinel Common Data Model and have access to a suite of reusable analytical tools that can be used or modified for particular studies (Adimadhyam et al., 2020; Dal Pan, 2019). These include propensity score matching, a statistical technique to reduce confounding in nonrandomized studies by matching individuals in different cohorts for a large number of covariates. Because sites maintain control of their own data, there are fewer concerns about loss of patient privacy or use of the data for commercial advantage; however, a disadvantage of this model is heterogeneity amongst participating sites in data quality and completeness.

The Sentinel System has provided information about drug utilization and has been employed to assess drug safety signals observed in FAERS (*olmesartan*/sprue-like enteropathy, rotavirus vaccine/intussusception, various vaccine combinations/febrile seizures) and has provided information that has obviated the need for expensive postmarketing clinical trials (Adimadhyam et al., 2020; Dal Pan, 2019). As Sentinel has grown in resources and complexity, plans have become more ambitious; in addition to signal assessment and refinement, Sentinel aims to include signal detection, use of artificial intelligence, and expansion of the system to incorporate large-scale pragmatic trials that use real-world data (the FDA-Catalyst program).

Real-World Data: Strengths and Weaknesses

Pharmacoepidemiologic studies, including those supported by the Sentinel System, address many of the limitations of clinical trials. Clinical trials are expensive, complex, may take years to complete, and lack generalizability, thereby precluding study of uncommon side effects, effects of long-term therapy, or many high-risk populations. In contrast, real-world data studies can include a long-term follow-up of large and diverse populations that represent the usual clinical practice population and are more timely and less expensive to conduct.

Two major weakness of real-world data studies are the quality of the data available and their observational nature. Real-world data generally originate from insurance or electronic health records, systems that were not designed for research and thus have limitations in accuracy, completeness, and availability. The accuracy of billing codes varies temporally, across geographic locations, and in different healthcare systems. The accuracy and completeness of the medication information can also vary, and identifying medications for patients who are hospitalized or who change insurers or healthcare systems presents problems. Databases that primarily link billing codes to medications (e.g., Medicare and Medicaid) often lack important clinical information such as weight, smoking history, and laboratory results. On the other hand, electronic health records have clinical details, but these may be incomplete or inaccessible because patients receive treatment from multiple providers and because much of the useful information resides in the text describing the medical encounter rather than in a structured database.

Even if the study data are of very high quality, a fundamental limitation of nonrandomized studies is confounding by unmeasured differences amongst the groups being compared. For example, millions of women in the U.S. received hormone replacement therapy (HRT) because large observational studies had consistently demonstrated a striking protective effect for cardiovascular disease. To the surprise of many, the large

TABLE 8–5 ■ COMPARATIVE RATES OF FATAL RHABDOMYOLYSIS PER MILLION PRESCRIPTIONS IN THE FDA ADVERSE EVENT REPORTING SYSTEM FOR VARIOUS STATINS

VARIABLE	LOVASTATIN	PRAVASTATIN	SIMVASTATIN	FLUVASTATIN	ATORVASTATIN	CERIVASTATIN
Year approved	1987	1991	1991	1993	1996	1997
Fatal cases of rhabdomyolysis	19	3	14	0	6	31
Number of prescriptions dispensed since marketing	99,197,000	81,364,000	116,145,000	37,392,000	140,360,000	9,815,000
Rate per million prescriptions	0.19	0.04	0.12	0	0.04	3.16

Cases were reported to the FDA before June 26, 2001; prescription data were from the National Prescription Audit Plus.

Source: Adapted from Staffa JA, et al. Cerivastatin and reports of fatal rhabdomyolysis. *N Engl J Med*, **2002**, *346*:539–540.

Woman's Health Initiative randomized controlled trial (RCT) demonstrated that there was a possible increase in the risk of cardiovascular disease (Manson et al., 2003) and a 24% increased risk of breast cancer (Chlebowski et al., 2003) in the HRT arm. One of the primary explanations of this discrepancy was that the women who sought HRT, who were the group exposed to HRT in observational studies, were healthier in ways that were very difficult to measure and that this unmeasured factor, rather than exposure to HRT, explained the spurious cardiovascular protective effect reported in observational studies. This and other sobering examples of observational studies that reached incorrect conclusions have led to increasing caution in the interpretation of their findings (Collins et al., 2020).

Despite these limitations, real-world data can often provide critical drug safety information that would be difficult or impossible to obtain in a clinical trial. An example is the identification of the increased risk of type 2 diabetes for children and young adults taking antipsychotics (Bobo et al., 2013). Antipsychotics, particularly the second-generation drugs, have metabolic effects, such as weight gain, increased glucose levels, and insulin resistance, that are considered precursors to diabetes. Epidemiologic studies of adults confirmed an increased risk of type 2 diabetes in antipsychotic users. The frequent use of antipsychotics in children and young adults raised the concern that the risk of type 2 diabetes was increased in this vulnerable population. Addressing this question in a clinical trial would be expensive, logistically difficult, and ethically questionable. A large observational study that compared new users of antipsychotics with matched users of alternative psychiatric medications reported a 3-fold increased risk of newly diagnosed type 2 diabetes (Figure 8–2).

Postmarketing Clinical Trials

Clinical trials performed after a drug is marketed can provide important drug safety information. The motivation for such trials is often a requirement by the FDA to resolve a potential safety signal or a desire by the manufacturer to obtain a new indication. Postmarketing trials can assess suspected safety problems and potentially identify unexpected adverse effects. For example, after the withdrawal of several antiobesity drugs from the market because of adverse cardiovascular effects (e.g., *dexfenfluramine*, *sibutramine*), the FDA required postmarketing cardiovascular trials for new drugs approved for obesity. Lorcaserin, approved for the treatment of obesity in 2012, was withdrawn from the market in 2020 when an FDA analysis of the postmarketing cardiovascular trial data showed an increased risk of cancer (but not cardiovascular disease) (Sharretts et al., 2020).

Postmarketing studies performed specifically to confirm or refute a drug safety signal can raise difficult ethical questions. If two treatments available on the market are equally effective for an illness but one may have increased risk of a serious adverse effect, a person entering an RCT of the two drugs has the expectation of increased risk and no personal benefit. Under what circumstances is that ethical? Uncertainty regarding comparative safety is not necessarily the same as true clinical equipoise. Indeed, few people would agree to be randomly assigned to a two-drug comparison when one of the drugs appears to have a downside, possibly a fatal adverse effect, and no expectation of added benefit.

The ethical problems inherent in randomized safety studies were a major concern for the postmarketing trial that the FDA required to define the cardiovascular safety of *rosiglitazone*. The trial, which compared the safety of *rosiglitazone* to that of *pioglitazone* (with no negative cardiovascular signal), came under ethical scrutiny and subsequent review by an IOM committee (Mello et al., 2012). Among other recommendations, the IOM review emphasized that in these circumstances there should be a transparent and complete evaluation of all existing premarketing studies; that a clinical trial should be mandated only if an observational study could not possibly provide the necessary information; that there must be a necessity to answer a critically important public health question; and that there were heightened obligations to ensure that participants understood the risks posed by study enrollment.

The ethical difficulties of randomizing patients to evaluate a well-established safety signal argue for performing comparative efficacy studies as early as possible in the drug life cycle (Ray and Stein, 2006). As information, even if imperfect, begins to accumulate regarding the relative merits of clinical alternatives, it will become increasingly difficult to perform randomized comparisons.

Postmarketing Actions to Improve Drug Safety

Identification of new adverse events or suboptimal drug use will not by itself improve drug safety. Translating expanded knowledge into clinical practice requires active steps to inform, educate, and change behavior. Just as detecting adverse drug events reliably is difficult, so too is improving drug safety by changing the behavior of patients and prescribers. There

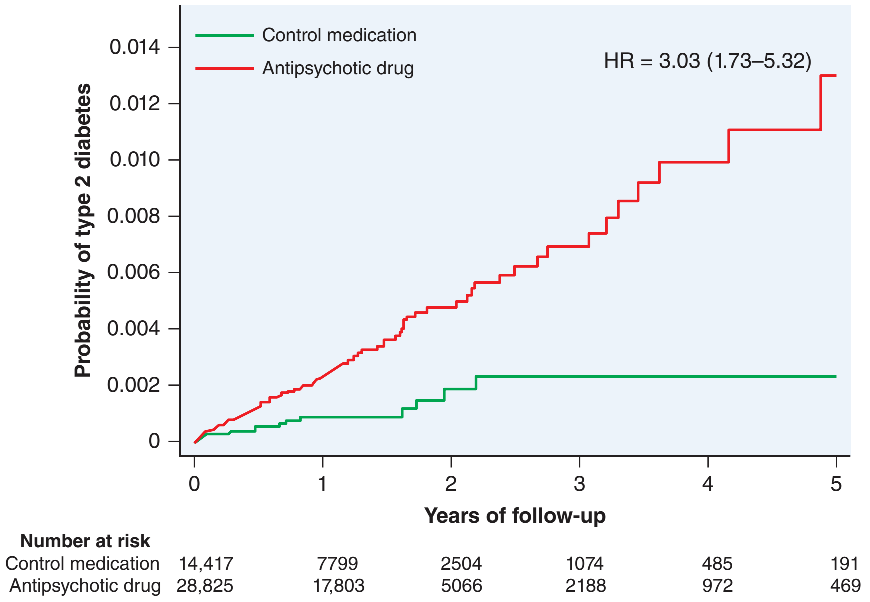

Figure 8–2 *Estimated probability of new-onset type 2 diabetes in Tennessee Medicaid enrollees.* Enrollees were between 6 and 24 years of age and began either an antipsychotic or control medication between January 1, 1996, and December 31, 2007. The study groups were matched according to 112 covariates that are potentially associated with either antipsychotic use or diabetes. The adjusted hazard ratio (HR [95% confidence interval]) estimates the relative risk of new-onset diabetes in the antipsychotic group.

are three common regulatory approaches to change behavior: (1) communication; (2) REMS; and (3) withdrawal of a drug from the market.

Communication With Prescribers and Patients

Tools for Communication

Regulatory authorities use multiple methods to communicate safety concerns, tailoring the message according to the strength of the evidence and the seriousness of the outcome. The earliest communications originate from the regular review of adverse event reports in FAERS or other case reports. Quarterly, the FDA posts information about possible safety problems on the FAERS website (FDA, 2021). These postings, although not necessarily reflecting causality, are an early alert to prescribers and the public about a potential safety issue.

Further communications ensue once the FDA determines there is a credible safety problem. One common measure is to change the drug label or even to add a boxed warning (commonly known as a "black-box warning") that draws attention to serious or potentially fatal adverse events. However, label changes *per se* may have limited efficacy (Smalley et al., 2000).

Another important tool for alerting patients and prescribers to medication risks is the drug safety communication (DSC). DSCs are posted on the FDA website, and the information is also disseminated through emails, podcasts, press releases, social media, and medical societies and associations. If a drug is contaminated or poses an immediate danger, the FDA uses other tools such as Public Health Alerts (Kesselheim et al., 2019).

How Effective Is Communication?

Communications about risk compete with a barrage of other information directed at prescribers. It is difficult to isolate the effect of the communication *per se* on patient and prescriber behavior because many other simultaneously changing factors also influence prescribing. The available evidence suggests that regulatory communications are often ineffective, but the response is so heterogeneous and unpredictable that it is impossible to generalize about communication efficacy (Dusetzina et al., 2012; Rosenberg et al., 2020). A literature review suggests several conclusions: (1) recommendations for increased monitoring (e.g., laboratory tests) have not led to large, sustained changes; (2) warnings are more likely to affect prescribing for new patients than for those already on the drug; (3) warnings are most effective when they are specific and repeated and when alternative drugs are available; and (4) warnings can have unintended consequences (Dusetzina et al., 2012).

A potentially serious unintended consequence of safety warnings is changes in drug prescribing in population groups other than those targeted. For example, warnings to avoid certain antidepressants in children (increased risk of suicide) and atypical antipsychotics in patients with dementia (increased risk of death) resulted in decreased prescribing of these drugs to other patients, with unknown clinical consequences (Dusetzina et al., 2012).

Risk Evaluation and Mitigation Strategies

The FDA can mandate specific actions both pre- and postmarketing to manage medication-associated risks. The REMS for a specific drug can require one or more of several components, including medication guides for patients, patient package inserts, patient enrollment in a registry, evidence that the patient is following guidelines for safe use, laboratory testing, communications to healthcare providers, education and training for providers, and certification of providers and pharmacies. For example, most of these requirements are present in the REMS in place for *isotretinoin* to prevent fetal exposure. Currently, 60 drugs have REMS requirements, and although the program is based on sound reasoning there is limited evaluation of its effectiveness (Boudes, 2017).

Withdrawal of a Drug From the Market

If other strategies do not appropriately manage drug risk, the FDA can require withdrawal of an approved drug from the market. This difficult decision must balance the magnitude of the drug risks, the potential to mitigate them effectively, the benefits of the medication, and the availability of alternative therapies (Sharretts et al., 2020).

Withdrawal of a medication from the market is a drastic action, often resulting from failure to identify a major drug risk earlier in the life cycle. The discovery of adverse effects that lead to withdrawal can have profound effects on future preclinical and premarketing studies. For example, prolongation of the QT interval and torsades de pointes led to the withdrawal of several drugs (*terfenadine*, *astemizole*, *grepafloxacin*, *cisapride*, and others; see Garnett, 2017). As a consequence, the FDA increased requirements to evaluate the propensity of any new drug to prolong the QT interval. Similarly, the withdrawal of antiobesity drugs that caused cardiac valve abnormalities (*fenfluramine, dexfenfluramine*) or increased the risk of myocardial infarction and stroke (*sibutramine*) led to stricter cardiovascular requirements for new antiobesity drugs.

Challenges and Future Approaches

Pharmacovigilance efforts in all countries face similar challenges; harmonization and cooperation will be important as the science evolves. Several areas will be important drivers of future improvements in drug safety.

Better Access to Data

The ability of individual researchers to readily access the data required to perform observational pharmacoepidemiologic studies remains limited. Access to resources, such as Medicare data, is expensive, and the approval process is onerous. The Sentinel System has expanded the ability of the FDA to perform large observational studies rapidly. These resources are available to other investigators but only through collaborations with individual Sentinel sites or through the Sentinel Operations Center (Adimadhyam et al., 2020). A large comprehensive data set for pharmacoepidemiologic research based on a model such as UK Biobank, which provides easy and cheap access to data and also protects individual privacy, would be a major advance. Although commercial insurance-based databases are beginning to provide some of this capability, publicly based systems that are validated and include key linkages to vital records and electronic health record data will be needed.

New Approaches to Detecting and Refining Signals

The millions of adverse event reports every year and information about drug prescriptions linked to clinical outcomes for millions of patients provide great opportunities but also new challenges for improving drug safety. The application of artificial intelligence and natural language processing to this information holds great promise (Lavertu et al., 2021); however, it is important to remember that algorithms and humans both confront the same fundamental challenge: the limitations of observational data. The idea of large simple postmarketing efficacy and safety trials with remote enrollment and outcomes determined through vital records or electronic health records is attractive, but there are many challenges that will limit the types of questions that can be addressed.

Pragmatic studies are best suited to relatively safe interventions that do not require complex consent procedures or close monitoring of patients or have endpoints that require detailed clinical evaluation. The most practical design is open label with little control over exposure to the intervention and co-therapies (i.e., usual clinical care). These trial design features can affect the trial's robustness. The disadvantages of open-label trial designs are well recognized; poor adherence to an intervention or lack of control of confounding co-therapies can make the results difficult to interpret. The ADAPTABLE pragmatic trial, which compared the efficacy of aspirin 81 mg versus 325 mg for the prevention of atherosclerotic heart disease, illustrates some of the challenges. In the ADAPTABLE study, many patients did not continue to take the dose to which they were randomized; 41.6% of patients randomized to the 325-mg dose of aspirin switched to the 81-mg dose. This dose switching may have affected the results. The intent-to-treat analysis showed no significant difference between the two doses for the primary composite endpoint of death and hospitalization for myocardial infarction or stroke (hazard ratio [HR], 1.02; 95% confidence interval [CI], 0.91–1.14). However, in a sensitivity analysis performed using the aspirin dose the patient was actually taking, the primary endpoint occurred more frequently with the 81-mg dose (HR, 1.25; 95% CI, 1.10–1.43) (Schuyler Jones et al., 2021), leaving open the possibility that poor adherence may have obscured a beneficial effect of the higher dose.

Incorporating Pharmacogenetics Into Risk Detection and Management

The FDA label for approximately 300 drugs already contains pharmacogenetic information, including some boxed warnings. Genetic information in the label often relates to increased risk of an adverse event (e.g., *abacavir* hypersensitivity/*HLA-B*5701*) or altered drug metabolism affecting drug response (e.g., *warfarin*/*CYP2C9*, *clopidogrel*/*CYP2C19*) (see Chapter 7). Defining the clinical significance of pharmacogenetic effects and deciding how they should be incorporated into communications and actions to improve drug safety will become increasingly important, particularly since it will not be possible to perform an RCT for each potential effect.

Genetic information can also be useful to predict the efficacy and adverse effects likely to be associated with a drug when naturally occurring human genetic variation reproduces the effect of a drug. A Mendelian randomization analysis, which classifies the population according to the genetic variation rather than drug exposure, is free of bias related to the multiple factors that influence medication choice, given that genetic makeup is fixed at conception (Denny et al., 2018). For example, variation in the 3-hydroxy-3-methylglutaryl-CoA reductase gene (*HMGCR*) mimics the effects of statin therapy in that some people have lower genetically determined enzyme activity and therefore lower low-density lipoprotein cholesterol levels. Thus, "randomization" at conception to different levels of HMGCR activity occurs because of natural variability in genetic makeup; by studying the consequences of genetically predicted different levels of HMGCR activity in a population, we can learn about possible effects of a drug that alters this enzyme. Indeed, Mendelian randomization analyses predicted an increased risk of diabetes with statin therapy—something that took decades of clinical use to discover (Swerdlow et al., 2015).

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Chapter 9

Principles of Clinical Toxicology

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RESOURCES FOR INFORMATION ON DRUG TOXICITY AND POISONING

Introduction

Toxicology is the study of the adverse effects of substances on living organisms. Any substance is considered a *poison* when exposure results in a damaging physiological effect (toxic effect). Among the agents that can produce toxic effects are pharmaceuticals, illicit drugs, plants and botanicals, and myriad chemicals and pollutants. Chapter 76 focuses on *environmental toxicology*. This chapter focuses on *clinical toxicology*, the discipline of toxicology that studies undesired effects of pharmaceutical therapies in humans and the effects and treatment of poisoning.

Toxicological testing is performed in preclinical studies to assess for the toxicity of a substance in animals and in in vitro models (see Chapters 1 and 76). Additional studies, including carcinogenicity, teratogenicity, and effects on fertility, are performed concurrently with the first stages of a clinical trial. More adverse effects may be discovered in postmarketing surveillance as more doses are administered and more patients are exposed to the drug (see Chapter 9).

Dose-Response

Conventional Dose-Response Curves

Dose-response relationships are *graded in an individual* and *quantal in the population* (see Figures 3–7 and 3–9). In a graded dose-response, the magnitude of an individual's response usually increases as the dose of the drug is increased. In a quantal dose relationship, the percentage of the population that responds increases as the dose of the drug increases, but the response is only judged to be either present or absent in a given individual. This quantal dose-response phenomenon is used to determine the LD_{50} (median lethal dose) of drugs, as defined in Figure 9–1A.

Both a quantal dose-response curve for LD_{50} and a quantal dose-response curve for ED_{50} (median effective dose), the concentration of drug at which 50% of the population will have the desired therapeutic response, can be determined for the same agent (Figure 9–1B). These two curves can be used to generate a therapeutic index (TI) in animal models, which quantifies the relative safety of a drug (Equation 9–1). The median toxic dose (TD_{50}) is the concentration of drug at which 50% of the population will have a toxic effect and is used instead of LD_{50} when calculating a TI in humans.

$$\text{In animal toxicity studies: TI} = LD_{50}/ED_{50} \qquad \text{(Equation 9–1A)}$$

$$\text{In human toxicity studies: TI} = TD_{50}/ED_{50} \qquad \text{(Equation 9–1B)}$$

For agents in current therapeutic use, values of TI vary widely, from 1 to 2 to more than 100. Drugs with low TIs (e.g., the cardiac glycoside *digoxin* and cancer chemotherapeutic agents) must be administered with caution and therapeutic drug monitoring. Drugs with very high TIs (e.g., *penicillin*) are considered safe in the absence of a known allergic response in a given patient. Note that the use of median doses fails to consider the slopes of the dose-response curves, and there may be considerable overlap between doses in the upper end of the therapeutic curve and the lower end of the lethal or toxic curve (see Figure 9–1). As an alternative, the ED_{99} for the therapeutic effect can be compared to the LD_1 in animals (TD_1 in humans), to yield a *margin of safety*.

$$\text{Margin of safety} = \frac{LD_1}{ED_{99}} \qquad \text{(Equation 9–2)}$$

Nonmonotonic Dose-Response Curves

Not all dose-response curves follow a typical sigmoidal shape. Consider three examples. *U-shaped dose-response curves* can be observed for endocrine disrupters and hormones and for essential metals and vitamins. (Figure 9–2A) (Vandenberg et al., 2012). Deficient or low doses produce adverse effects. As dose increases, homeostasis is achieved and there are no adverse effects. When dose surpasses the amount required to maintain homeostasis, toxicity can occur. Thus, there are adverse effects at concentrations both below and above the levels that support homeostasis.

A *"hockey stick" dose-response* (Figure 9–2B) is characterized by a region of no response at low doses, followed by an adverse response as the toxicant exceeds endogenous protective mechanisms. Some toxicants, like

Abbreviations

CYP: cytochrome P450
ED$_{50}$: median effective dose
GI: gastrointestinal
hERG: human ether-a-go-go gene
Ig: immunoglobulin, as in IgE, IgG, IgM
LD$_{50}$: median lethal dose
MDAC: multiple-dose activated charcoal
NAPQI: *N*-acetyl-*p*-benzoquinonimine
NSAID: nonsteroidal anti-inflammatory drug
PCC: poison control center
PDE5: phosphodiesterase type 5
Pgp: P-glycoprotein (MDR1, ABCB1)
SNRI: serotonin-norepinephrine reuptake inhibitor
TD$_{50}$: median toxic dose
TI: therapeutic index
WBI: whole-bowel irrigation

formaldehyde, are also metabolic by-products for which cells have detoxifying mechanisms. Formaldehyde is detoxified through metabolism by alcohol dehydrogenase. Very low doses of exogenous formaldehyde do not overwhelm endogenous alcohol dehydrogenase, and toxic effects are not observed. Adverse responses to formaldehyde are observed when exogenous formaldehyde saturates alcohol dehydrogenase (ADH5, GSNOR; Pontel et al., 2015).

An *inverted U-shaped dose-response curve* (Figure 9–2C) can be observed when receptor downregulation/desensitization occurs following exposure to a ligand or when an additional and distinct negative effect occurs at a concentration beyond that which produces the primary positive effect. For example, cannabidiol at maximal doses can produce anxiolytic effects. However, at submaximal or supramaximal doses, cannabidiol does not produce an anxiolytic effect (Zuardi et al., 2017). High levels of the hormone estrogen can achieve maximal effects; supramaximal levels produce lower effects, possible as a result of receptor downregulation. A number of endocrine-disrupting chemicals appear to have inverted U-shaped or multiphasic dose-response curves. Such curves are common in complex systems where ligands elicit multiple responses. These phenomena indicate the necessity of performimg extensive dose-response and time-course studies when evaluating the effects of potential toxicants.

Toxicokinetics and Alterations in ADME

Exposure to supratherapeutic quantities of a pharmaceutical may lead to alterations in the expected ADME. The pharmacokinetics of a drug under circumstances that produce toxicity are referred to as *toxicokinetics*. Data on toxicokinetics are generally limited to case reports and observational studies. Table 9–1 lists examples of common alterations to ADME in drug overdose.

Drug Absorption in Drug Overdose

In clinical toxicology, the time to peak drug concentration is critical to determining observation periods for asymptomatic patients. Table 9–2 lists prominent factors that may influence drug absorption in instances of overdose.

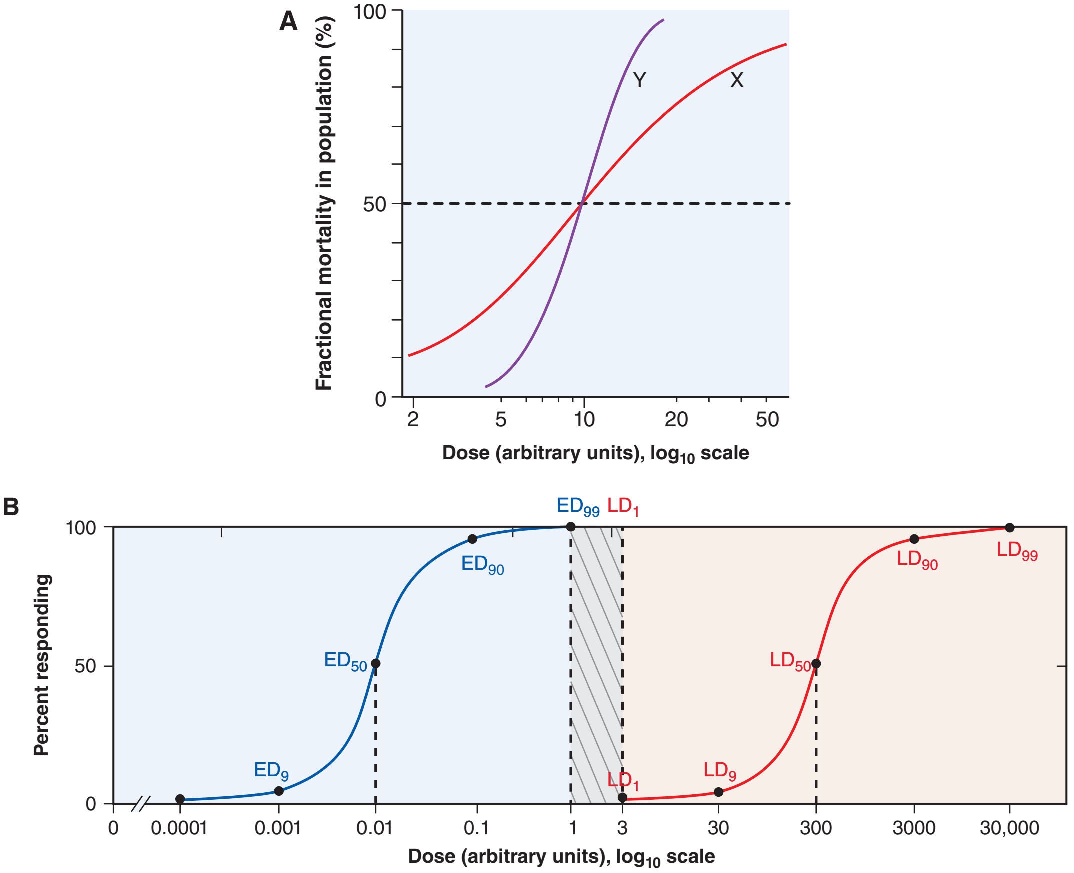


Figure 9–1 *Dose-response relationships.* **A.** The midpoint of the curve represents the LD$_{50}$, or the dose of drug that is lethal in 50% of the population. The LD$_{50}$ of a compound is determined experimentally, usually by administration of the drug to mice or rats (orally or intraperitoneally). The LD$_{50}$ values for both compounds are the same (~10 mg/kg); however, the slopes of the dose-response curves are quite different. Thus, at a dose equal to one-half the LD$_{50}$ (5 mg/kg), fewer than 5% of the animals exposed to compound Y would die, but about 25% of the animals given compound X would die. **B.** Depiction of the effective dose (ED) and lethal dose (LD). The crosshatched area between the ED$_{99}$ (1 mg/kg) and the LD$_{1}$ (3 mg/kg) gives an estimate of the margin of safety.

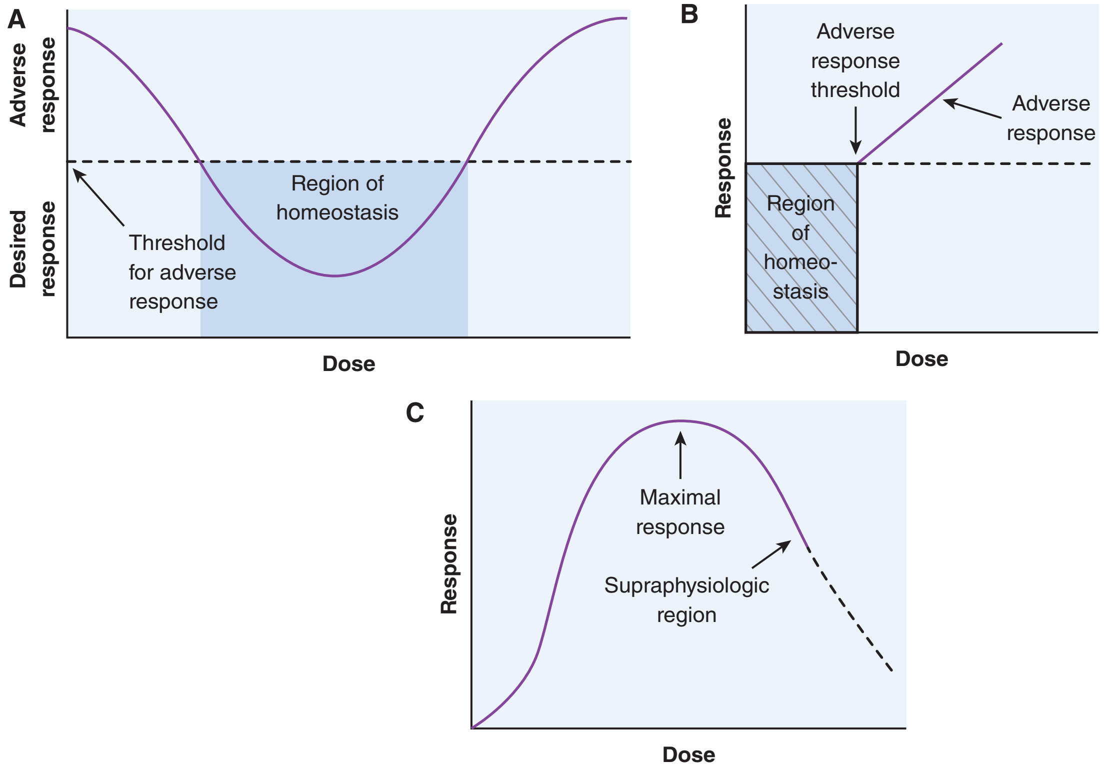


Figure 9–2 *Nonmonotonic dose-response relationships.* **A.** U-shaped dose-response curve such as may be observed for some hormones and endocrine disrupters and for essential metals. **B.** Hockey stick–shaped dose-response curve for toxicants that are removed by a saturable process such that adverse effects occur when toxin removal is saturated and becomes zero-order. **C.** Inverted U-shaped dose-response curve for ligands that downregulate their receptors or have multiple effects, both positive and negative.

Unusual Kinetics of Absorption

Drugs with modified-release forms (i.e., sustained release, controlled release, or extended release) will influence the time to the drug's peak concentration and result in delayed symptoms.

Delayed gastric emptying is typically associated with drugs that have a direct pharmacological effect on smooth muscles of the gastrointestinal (GI) tract. Two classes of medications that have this effect are opioid analgesics and anticholinergics. One prospective study demonstrated significant delays in gastric emptying half-time following ingestion of tricyclic antidepressants, opioid-acetaminophen combinations, *acetaminophen*, and *carbamazepine* as measured by gastric scintigraphy.

TABLE 9–1 ■ EXAMPLES OF COMMON ALTERATIONS TO ADME IN DRUG OVERDOSE

Acetaminophen	↑ **Metabolism** through CYP2E1 and subsequent buildup of hepatotoxic metabolite
Aspirin	Delayed and erratic **absorption**; ↑ **distribution** to CNS
Buprenorphine	Prolonged symptoms due to long **half-life**
Bupropion	Delayed **absorption** (modified-release preparations); active **metabolites**
Calcium channel blockers	Delayed **absorption** (modified-release preparations); active **metabolites**
Illicit drugs in rubber or plastic packages (body packers/body stuffers)	Delayed and erratic **absorption**
Insulin	Delayed and prolonged **absorption** due to subcutaneous depot
Monoamine oxidase inhibitors	Delayed time to enzyme inhibition/**metabolism**
Sulfonylureas	Delayed hypoglycemia, mechanism **unclear**
Valproic acid (VPA)	Delayed **absorption** (enteric coating); saturated **protein binding** ↑ free VPA

In addition to the role that pharmacological activity may play in delayed absorption, one must consider the quantity of tablets or capsules ingested (Adams et al., 2004). Bactrian pharmacokinetics (double-hump) have been described in several cases following *acetaminophen* overdose (Figure 9–3) (Hendrickson et al., 2010). This phenomenon results in two distinct peak serum concentrations separated by 12 to 42 h. The mechanism for this pattern of absorption is unknown, but it occurs following exposures to large quantities of *acetaminophen* alone (30–100 g) and in combination with drugs that slow gastric emptying, suggesting that either factor can influence the pattern of absorption. Thus, the time course of drug absorption in overdose is not always straightforward; knowing the possible pattern of drug absorption is an important aspect in the time course of managing drug overdose.

Enterohepatic Cycling

Enterohepatic recirculation occurs when drugs undergo conjugation in the liver, excretion in the bile, and reabsorption in the small intestine. Many drugs are excreted into the GI tract as polar conjugates with UDP-glucuronic acid, glutathione, or sulfate and are not sufficiently

TABLE 9–2 ■ FACTORS THAT INFLUENCE DRUG ABSORPTION

- Modified-release preparations
- Delayed/prolonged gastric emptying
- Pylorospasm
- Enterohepatic recirculation
- Pharmacobezoar

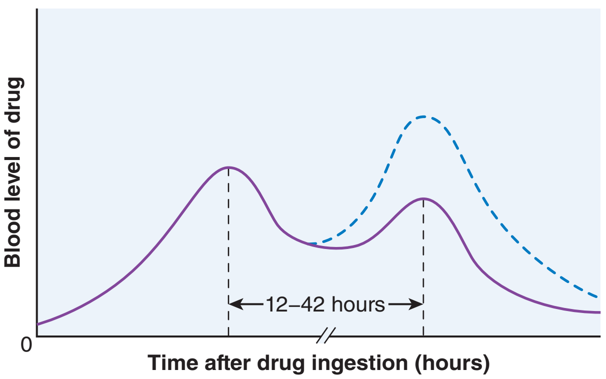


Figure 9–3 *Bactrian "double-hump" kinetics.* Bactrian kinetics are seen in some cases of drug overdose, such as those involving *acetaminophen*. Following an excessive dose of *acetaminophen* (30–100 g), the time course of drug level in the blood may show two peaks. The second peak concentration of *acetaminophen* may occur 12 to 42 h after the first peak and may be less than or greater than (blue dashed line) that of the first peak concentration. See text for details.

lipophilic to be reabsorbed. The intestinal microbiome plays a role in deconjugation, hydrolyzing glucuronides and sulfates, and rendering them more lipophilic and thus more likely to be absorbed across the GI epithelium (see Chapter 6). The more lipophilic moieties reenter the portal circulation, as they would following oral administration, and begin the cycle again. The events of this recirculation require participation of hepatic CYPs (cytochromes P450) and UGTs (see Chapter 5) as well as an assortment of membrane transporters (e.g., members of the SLC and ABC superfamilies; see Figures 4–2, 4–8, and 4–9). Enterohepatic cycling can cause secondary peaks in the C_p-time profile of a drug. Repetitive cycling ultimately prolongs the elimination half-life of the cycled xenobiotic (Ibarra et al., 2021). In the instance of drug overdose or poisoning, it can be useful to interrupt this recycling and promote excretion. Introduction of a suitable nonabsorbable binding agent like activated charcoal, can interrupt the recycling by sequestering the molecule for excretion in the feces. This is the basis for using bile acid sequestrants (e.g., *cholestyramine*; see Chapter 37) to reduce bile acid reabsorption and decrease hepatic cholesterol content and polythiol resins in treating dimethylmercury poisoning, for instance.

In addition to the many drugs that are conjugated for excretion, a number of biological molecules in addition to bile salts are subject to enterohepatic recirculation, including thyroxine and steroid hormones. Bilirubin, excreted in bile as a glucuronide, is hydrolyzed by colonic bacteria to urobilinogen, a portion of which is reabsorbed.

Miscellaneous Contributors to Altered Drug Absorption

Pharmacobezoars form when pharmaceutical preparations conglomerate into a mass in the GI tract. These conglomerates alter absorption by causing gastric obstruction, reducing the effective surface area for absorption, or prolonging drug absorption (Simpson, 2011). The incidence of pharmacobezoar formation is rare in drug overdose.

Pylorospasm results in closure of the pylorus and prevents passage of gastric contents to the small intestine. Drugs that induce pylorospasm can theoretically delay their own GI absorption. Salicylates are commonly cited as a drug class that induces pylorospasm, an effect that may contribute to the erratic absorption of salicylates (Harris, 1973).

Drug Distribution in Drug Overdose

Protein binding affects the toxicity of an agent. In overdose, the toxicity of highly protein bound drugs may be enhanced as the fraction of unbound drug is increased due to saturation of protein binding sites. Additionally, alterations in physiological pH can reduce protein binding. For example, tricyclic antidepressants become less protein bound when plasma pH become more acidic, resulting in more pronounced drug toxicity (Levitt et al., 1986).

Drugs with larger volumes of distribution are associated with more extensive distribution to and sequestration in tissues and often cannot be as easily removed through hemodialysis as drugs with smaller volumes of distribution.

Drug Metabolism and Elimination in Drug Overdose

Elimination via Metabolic Processes May Change From First Order to Zero Order as the Dose Increases

Drug elimination is dependent upon biotransformation and excretion. In the context of drug overdose, saturation of the enzymatic processes of biotransformation can greatly impact drug elimination. Ethanol is a familiar example of saturated metabolism. With the ingestion of miniscule amounts, ethanol metabolism would be first order, that is, a fixed fraction metabolized per unit of time. However, as used socially, ethanol readily achieves blood levels that saturate hepatic alcohol dehydrogenase activity as the supply of the cofactor NAD$^+$ becomes inadequate due to the limiting rate at which the cofactor NAD$^+$ can be regenerated from NADH (Alexandrovich et al., 2017). Thus, in practice, ethanol metabolism is zero order, that is, a fixed amount per unit time (~10 g/h in a typical adult); addition of more ethanol to the system increases the blood level and the deleterious effects of ethanol but does not result in a greater rate of metabolic elimination (see Chapter 27).

The antiepileptic medication *phenytoin* is a good clinical example of the potential for toxicity as blood levels rise and enzymatic biotransformation becomes saturated. *Phenytoin* is metabolized predominantly by two polymorphic CYPs, 2C9 and 2C19. At therapeutic doses, *phenytoin* elimination is largely first order with an elimination half-life between 6 and 24 h, averaging about 20 h. However, at supratherapeutic concentrations, CYPs 2C9 and 2C19 become saturated and *phenytoin* elimination becomes zero order (Chua et al., 2000), and the elimination half-life can increase to 103 h (Brandolese et al., 2001) (see Chapter 20).

Shunting From Saturated Metabolic Pathways to Alternative Pathways May Produce Toxic Metabolites

Acetaminophen overdose exemplifies the toxic consequences of saturated metabolic pathways. Under normal conditions, *acetaminophen* largely undergoes conjugation to nontoxic metabolites. A small portion of *acetaminophen* undergoes metabolism through CYP2E1 to the toxic metabolite NAPQI (*N*-acetyl-*p*-benzoquinonimine), but glutathione stores are sufficient to support detoxification of the NAPQI produced by therapeutic doses of *acetaminophen*. Following *acetaminophen* overdose, however, conjugation becomes saturated, resulting in increased metabolism through CYP2E1. Glutathione stores become depleted and insufficient to detoxify the NAPQI produced. NAPQI accumulates and is ultimately responsible for the hepatotoxic effects associated with *acetaminophen* overdose (Figure 9–4).

Drug Metabolites Can Be More Potent and Toxic Species

Drugs with active metabolites can be toxic via the metabolite. For instance, *tramadol* is a serotonin-norepinephrine reuptake inhibitor (SNRI) that binds to SERT (neuronal serotonin transporter) and NET (neuronal norepinephrine transporter) and is also a weak μ-opioid agonist that is used as an analgesic. The active metabolite of *tramadol*, *O*-desmethyl-tramadol (M1), results from the action of CYP2D6 and is a more potent μ-opioid receptor agonist by a factor of approximately 300 (Grond et al., 2004) but lacks activity as an SNRI. Thus, metabolism changes the activity profile of the parent compound. CYP2D6 is highly polymorphic; thus, CYP2D6 activity in the human population runs the gamut from ultra-rapid metabolizers to poor metabolizers. Consequently, production of M1 varies as well, and more rapid metabolizers may have increased exposure to the adverse reactions of the μ-opioid agonist metabolite (Gong et al., 2014). In other cases, the toxic metabolites of a parent drug may simply have longer half-lives, thereby adversely influencing toxicity.

Drug Elimination by Membrane Transporters

Drug transporters play roles in both the absorption and elimination of xenobiotics. The interplay of substrates and inhibitors at transport proteins can give rise to drug-drug interactions that increase the AUC (integrated area under the time-C_p curve) and C_{Pmax} of drugs into the toxic range by decreasing elimination or decrease the AUC and C_{Pmax} below

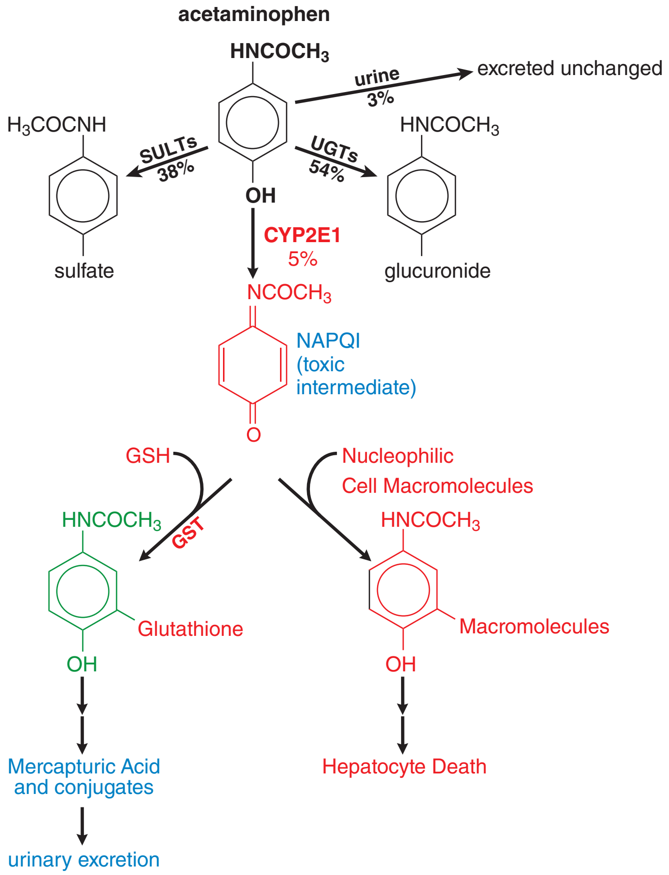


Figure 9–4 *Pathways of acetaminophen metabolism and toxicity in hepatocytes.* With normal dosing (1–3 g/day), *acetaminophen* is metabolized largely by sulfation and glucuronidation; small amounts are excreted unchanged in the urine or oxidized to a reactive and toxic metabolite, *N*-acetyl-*p*-benzoquinonimine (NAPQI); NAPQI is detoxified via conversion to a glutathione (GSH) adduct that is ultimately excreted in the urine. At a dose of 4 g/day, the sulfation pathway becomes saturated, shunting more *acetaminophen* through glucuronidation and oxidation. As the daily dose further increases (>4 g), the glucuronidation pathway also becomes saturated, and higher fractions of the drug get excreted unchanged and oxidized. This increased production of NAPQI depletes cellular GSH; the excess NAPQI interacts with nucleophilic cellular molecules, leading to hepatocyte death. Agents that act to shunt more *acetaminophen* through the oxidative pathway, such as inducers of CYP2E1 activity (e.g., *isoniazid*), will enhance NAPQI formation, GSH depletion, and hepatocyte damage and death. For further details, see Mazaleuskaya et al. (2015). GSTs, glutathione-*S*-transferases; SULTs, sulfotransferases; URTs, uridine diphosphate-glucuronosyltransferases.

an effective level by reducing GI absorption. See Table 9–3 for examples of drugs that are substrates or inhibitors of several common transporters and that can give rise to such effects. Appendix II gives a more quantified presentation of such interactions, as does Chapter 4.

Types of Drug Toxicity

Side effects of drugs are undesirable effects from the therapeutic use but often do not require discontinuation of treatment. Other undesirable effects are characterized as *toxic effects*. Toxic effects may be described as either adverse drug effects or drug toxicity. These terms are often used interchangeably; however, adverse effects occur with normal therapeutic use, whereas drug toxicity occurs as a result of supratherapeutic drug concentrations (unintentional or intentional).

Types of drug toxicity can be classified mechanistically as on-target, off-target, biologic activation, hypersensitivity, and idiosyncratic (Guengerich, 2011; Liebler et al., 2005).

On-Target

On-target toxicity is a result of the interaction of a drug and its primary pharmacological target (i.e., receptor). On-target toxicity is typically dose dependent. At therapeutic doses, barbiturates bind to $GABA_A$ receptors and prolong the opening of the $GABA_A$-mediated Cl^- channels (the primary inhibitory channels in the central nervous system [CNS]). The desired therapeutic effects are anxiolysis and sedation. At supratherapeutic doses of barbiturates, increased agonism at the $GABA_A$ receptors causes greater Cl^- influx and inhibition of neurotransmission in responding neurons, leading to somnolence, coma, and death.

On-target toxicity is not always specific to the target tissue of the drug administered. At therapeutic doses, *nifedipine*, a dihydropyridine calcium channel blocker, is selective for the L-type calcium channels in peripheral vascular smooth muscle, reducing contraction of these muscles and thereby reducing peripheral vascular resistance and blood pressure. At supratherapeutic doses, however, *nifedipine* blocks the L-type calcium channels in both its target tissue (peripheral vasculature) and the myocardium, resulting in potentially life-threatening hypotension.

Off-Target

Off-target toxicity results from the interaction of a drug with targets other than its intended pharmacological targets. Off-target toxicity is discovered in preclinical testing, the early stages of clinical trials, and in postmarketing surveillance. *Terfenadine* is a good example. *Terfenadine* is a prodrug that is metabolized by CYP3A4 to the active form, *fexofenadine*, a selective H_1 antagonist. In 1997, terfenadine was removed from the U.S. market due to reports of life-threatening dysrhythmias with its use. At therapeutic doses, the primary pharmacological target of *terfenadine* is the H_1 receptor via the metabolite, fexofenadine. At supratherapeutic doses or in the presence of inhibitors of CYP3A4, the toxicity of the parent drug becomes apparent: blockade of K^+ channels (K_v11.1; the channel's α subunit is encoded by *hERG* [human ether-a-go-go gene]) in the myocardium, leading to QTc prolongation and the development of torsades de pointes (DuBuske, 1999). While toxic effects are discovered in clinical trials, postmarketing surveillance involving much larger numbers of patients and administered doses finds the rarer adverse effects. A number of the drug withdrawals in the U.S. from 1969 to mid-2003 were in response to off-target effects and drug-drug interactions (Wysowski and Swartz, 2005).

Biological Activation

A drug can be biologically activated to a toxic metabolite that is capable of causing organ or tissue-specific toxicity. The metabolism of *acetaminophen* to NAPQI is an example of biological activation. NAPQI interacts with nucleophilic cellular macromolecules in the liver leading to cell death (see Figure 9–4). Most genotoxic carcinogens, including many cancer chemotherapeutic agents, are compounds that have been biologically activated to intermediates that directly damage DNA (see Chapters 6, 7, and 71).

Hypersensitivity Reactions

A hypersensitivity reaction, or *allergy,* is mediated by the immune system and results from previous sensitization to a particular chemical or to one that is structurally similar. (See Chapter 38 for a discussion of the immunological basis of hypersensitivity reactions.) Hypersensitivity reactions are divided into four general categories based on the mechanism of immunological involvement.

Type I: Anaphylactic Reactions

Anaphylaxis is mediated by immunoglobulin (Ig) E antibodies. The Fc portion of IgE can bind to receptors on mast cells and basophils. If the Fab portion of the IgE antibody then binds an antigen, various mediators (e.g., histamine, leukotrienes, and prostaglandins) are released and cause vasodilation, edema, and an inflammatory response. The main targets of this type of reaction are the GI tract (food allergies), the skin (urticaria and atopic dermatitis), the respiratory system (rhinitis and asthma), and the vasculature (anaphylactic shock). These responses tend to occur quickly after challenge with an antigen to which the individual has been sensitized and are termed *immediate hypersensitivity reactions*.

Many chemical compounds, including a large number of therapeutic agents, stimulate the release of histamine from mast cells directly and without prior sensitization (McNeil, 2021a). Responses of this sort are most likely to occur following intravenous injections of certain categories

TABLE 9–3 ■ SELECTED SUBSTRATES AND INHIBITORS OF TRANSPORTERS

TRANSPORTER	SUBSTRATE		INHIBITOR	
Pgp	Dabigatran		Amiodarone	Propafenone
	Digoxin		Carvedilol	Quinidine
	Fexofenadine		Clarithromycin	Verapamil
	Loperamide		Itraconazole	
OAT1B1 and OAT1B3	Atorvastatin	Paclitaxel	Atazanavir, ritonavir	Lopinavir, ritonavir
	Danoprevir[a]	Pitavastatin	Clarithromycin	Rifampin
	Docetaxel	Pravastatin	Cyclosporine	Simeprevir[a]
	Fexofenadine	Repaglinide	Erythromycin	
	Glyburide	Rosuvastatin	Gemfibrozil	
	Nateglinide	Simvastatin		
OAT1 and OAT3	Adefovir	Ganciclovir	P-aminohippuric acid	
	Cefaclor	Methotrexate	Probenecid	
	Ceftizoxime[a]	Oseltamivir	Teriflunomide	
	Famotidine	Penicillin G		
	Furosemide			

[a] Discontinued in the U.S.

Source: Data from Hachad et al., 2010.

of substances, particularly organic bases. Such secretagogues appear to elicit histamine release by interacting directly with MRGPRX2, a mast cell–specific G protein-coupled receptor, independently of the IgE pathway. The phenomenon is one of clinical concern and may account for unexpected anaphylactoid reactions and adverse drug responses (McNeil, 2021b).

Type II: Cytolytic Reactions

Type II allergies are mediated by both IgG and IgM antibodies and usually are attributed to their capacity to activate the complement system. The major target tissues for cytolytic reactions are the cells in the circulatory system. Examples of type II allergic responses include *penicillin*-induced hemolytic anemia, *quinidine*-induced thrombocytopenic purpura, and sulfonamide-induced granulocytopenia. This type of reaction usually subsides within several months after removal of the offending agent.

Type III: Arthus Reactions

Type III allergic reactions are mediated predominantly by IgG; the mechanism involves the generation of antigen-antibody complexes that subsequently fix complement. The complexes are deposited in the vascular endothelium, where a destructive inflammatory response called *serum sickness* occurs. The clinical symptoms of serum sickness include urticarial skin eruptions, arthralgia or arthritis, lymphadenopathy, and fever. Several drugs, including commonly used antibiotics, can induce serum sickness–like reactions. These reactions usually last 6 to 12 days and then subside after the offending agent is eliminated.

Type IV: Delayed Hypersensitivity Reactions

These reactions are mediated by sensitized T lymphocytes and macrophages. When sensitized cells come in contact with an antigen, an inflammatory reaction is generated by the production of lymphokines and the subsequent influx of neutrophils and macrophages. An example of type IV or delayed hypersensitivity reaction is allergic contact dermatitis caused by poison ivy.

Idiosyncratic Reactions

An *idiosyncratic reaction* is an abnormal drug reaction to a drug that is unique to an individual. These reactions are rare and often hard to identify in preclinical studies. Idiosyncratic reactions are the least well-understood type of toxicity but generally involve an immunotoxicological reaction in a patient with a pharmacogenetic predisposition. Severe cutaneous adverse reactions, such as Stevens-Johnson syndrome and toxic epidermal necrolysis, and drug-induced liver injury from drugs other than *acetaminophen* are the most commonly reported idiosyncratic reactions (Usui et al., 2017).

Drug-Drug Interactions

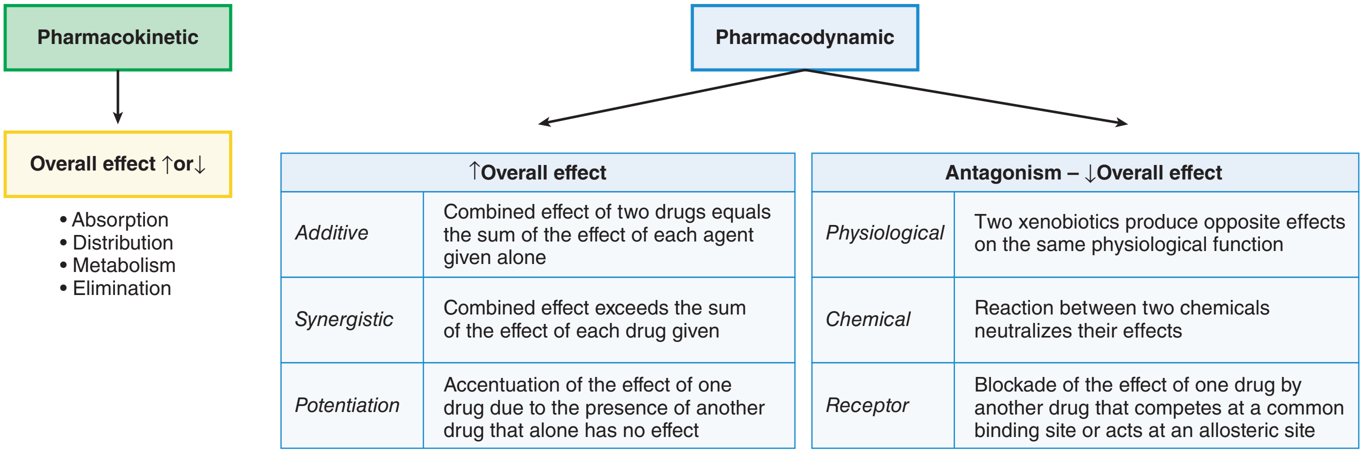
Managing a disease state or multiple disease states may require the use of more than one medication. Some patients may also elect to take additional over-the-counter medications such as vitamins, herbal supplements, and nutraceuticals. The use of multiple medications simultaneously can lead to drug-drug interactions. Two common mechanisms of drug-drug interactions include interactions of pharmacokinetics and interactions of pharmacodynamics. Drug-drug interactions can result in failure of pharmaceutical therapy or unintended toxic effects at therapeutic doses. Figure 9–5 summarizes the mechanisms and types of drug interactions.

Pharmacokinetic Interactions

Pharmacokinetic drug-drug interactions occur when components of ADME of one chemical are altered by another and result in increased or decreased drug effects.

Absorption. Interactions of absorption occur when the presence of one drug changes the absorption of another drug, generally from the intestinal lumen. These changes may occur due to an alteration of GI pH or adsorption. *Ranitidine*, an H_2 receptor antagonist, raises GI pH and may increase the absorption of basic drugs such as *triazolam* (O'Connor-Semmes et al., 2001). Conversely, coadministration of *levothyroxine* and calcium-containing antacids results in decreased absorption of *levothyroxine* due to adsorption of *levothyroxine* to calcium (Zamfirescu et al., 2011).

Involvement of Transporters. Interactions at transporters can alter drug absorption, distribution, and elimination. Common transporters implicated in clinically relevant drug interactions include P-glycoprotein (Pgp), OATs (organic anion transporters), and BCRP (breast cancer resistance protein) (Torino et al., 2019). Inhibition of intestinal Pgp, an efflux transporter, results in increased absorption and bioavailability. One study of healthy volunteers demonstrated an increase of 91% in the peak concentration and an increase of 71% in the AUC of *dabigatran* (Pgp substrate) when coadministered with *verapamil* (Pgp inhibitor). Table 9–3 lists common substrates, inhibitors, and inducers of transporters recommended by the U.S. Food and Drug Administration (FDA) for use in clinical drug-drug interaction studies. Box 9–1 illustrates how inhibition of transporters on multiple tissues can influence drug toxicity. Chapter 4 presents details of some drug-drug interactions mediated by



↑Overall effect	
Additive	Combined effect of two drugs equals the sum of the effect of each agent given alone
Synergistic	Combined effect exceeds the sum of the effect of each drug given
Potentiation	Accentuation of the effect of one drug due to the presence of another drug that alone has no effect

Antagonism – ↓Overall effect	
Physiological	Two xenobiotics produce opposite effects on the same physiological function
Chemical	Reaction between two chemicals neutralizes their effects
Receptor	Blockade of the effect of one drug by another drug that competes at a common binding site or acts at an allosteric site

Figure 9–5 *Mechanisms and classification of drug interactions.* Drugs can interact by a single mechanism or multiple mechanisms.

transport proteins; Figure 4–3 summarizes the major mechanisms by which transporters mediate adverse responses. Appendix II is a compendium of prominent drug-drug interactions, a number of which derive from interactions at membrane transporters.

Interactions at Protein Binding Sites. Many drugs, such as *aspirin*, barbiturates, *phenytoin*, sulfonamides, *valproic acid*, and *warfarin*, are highly protein bound in the plasma. Coadministration of such drugs can result in competition for binding sites and displacement of one drug by another, altering, for a time, the free concentrations of the drugs. Drugs that are highly bound to plasma proteins may have enhanced toxicity in overdose if protein binding sites become saturated, in physiological states that lead to hypoalbuminemia, or when drugs bound to plasma proteins are replaced on plasma proteins by other drugs (Guthrie et al., 1995).

Interactions via Drug Metabolism. One drug can frequently influence the metabolism of one or several other drugs, especially when hepatic CYPs are involved (see Chapter 5). *Acetaminophen* has complicated interactions with ethanol. During an overdose, normal metabolic pathways become saturated and a larger portion of *acetaminophen* is metabolized through CYP2E1 to the resultant toxic metabolite NAPQI (see Figure 9–4). Ethanol is both a substrate and potent inducer of CYP2E1. Acute coingestion of ethanol may be protective against *acetaminophen*-induced hepatotoxicity as ethanol is preferentially metabolized by CYP2E1 compared to *acetaminophen* (Waring et al., 2008). However, chronic ingestion of alcohol may increase hepatotoxicity due to induction of CYP2E1 and depletion of glutathione (Thummel et al., 2000; Zhao et al., 2002).

Interactions Affecting Elimination. In using *lithium* to treat mania, bipolar disorder, and treatment-resistant depression, maintenance of a therapeutic blood level of Li^+ (0.5–1.0 mEq/L) is crucial (see Chapter 19). *Lithium* clearance is dependent on renal excretion. Common drug classes implicated in reducing renal clearance of *lithium* include angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, nonsteroidal anti-inflammatory drugs (NSAIDs), thiazides, and loop diuretics. For example, NSAIDs inhibit prostaglandin synthesis, leading to decreased renal blood flow and clearance. In prospective studies, the mean decrease in *lithium* clearance was 12% to 66% in participants taking *ibuprofen* 400 mg four times daily (Finley, 2016); a 66% drop in clearance would likely cause an increase in C_P beyond the therapeutic range, possibly resulting in toxicity.

BOX 9–1 ■ Loperamide, Pgp, and Drug-Drug Interactions

Loperamide is a μ-opioid receptor agonist that acts on the myenteric plexus to reduce smooth muscle tone and motility in the GI tract, thereby acting as an antidiarrheal. Efflux via Pgp reduces the intestinal absorption of *loperamide* and excludes it from the CNS. Thus, *loperamide* is considered a safe antidiarrheal. However, when taken in combination with a Pgp inhibitor or substrate, *loperamide*'s bioavailability is increased and the drug can penetrate the blood-brain barrier (Kim et al., 2014; see Chapter 17 for details of the functions of the blood-brain barrier). Clinically, this drug interaction manifests with symptoms of central opioid agonism. There are reports of patients coadministering Pgp inhibitors with high doses of *loperamide* to prevent symptoms associated with opioid withdrawal (Daniulaityte et al., 2013). This drug interaction has resulted in life-threatening cardiovascular toxicity (Eggleston et al., 2016).

Pharmacodynamic Interactions

Pharmacodynamic drug-drug interactions occur due to activity at the same receptor or via physiological effect. Such drug interactions that produce an increase in effect are classified as *agonistic*, which is further differentiated as *additive*, *synergistic*, or *potentiating*. Drug interactions that produce a decrease in effect are classified as *antagonistic*, which can be further described by *physiological, chemical*, or *receptor* antagonism. Terms to describe pharmacodynamic interactions in research are not standardized, which leads to variation in their use (Roell et al., 2017). Classifications are often determined in preclinical studies in which the magnitudes of pharmacodynamic drug-drug interactions are not clearly described.

Additive pharmacodynamic interactions are desirable in some instances, such as in treating hypertension with combinations of drugs that block β_1 receptors, inhibit effects of angiotensin, and promote diuresis. On the other hand, some drug combinations produce functionally adverse pharmacodynamic interactions. For example, nitrate vasodilators produce vasodilation via nitric oxide–dependent elevation of cGMP in vascular smooth muscle. *Sildenafil*, *tadalafil*, and *vardenafil*, used for erectile dysfunction, inhibit phosphodiesterase type 5 (PDE5) that hydrolyzes cGMP to 5′GMP in the vasculature. Thus, coadministration of a nitric oxide donor (e.g., *nitroglycerin*) with a PDE5 inhibitor can cause potentially catastrophic vasodilation and severe hypotension.

Descriptive Toxicity Testing in Preclinical Studies

Two main principles or assumptions underlie all descriptive toxicity tests performed in animals.

First, those effects of chemicals produced in laboratory animals, when properly qualified, apply to human toxicity. When calculated on the basis of dose per unit of body surface, toxic effects in human beings usually are encountered in the same range of concentrations as those in experimental animals. On the basis of body weight, human beings generally are more vulnerable than experimental animals.

Second, exposure of experimental animals to toxic agents in high doses is a necessary and valid method to discover possible hazards to human beings who are exposed to much lower doses. This principle is based on the quantal dose-response concept. As a matter of practicality, the number of animals used in experiments on toxic materials usually will be small compared with the size of human populations potentially at risk. For example, 0.01% incidence of a serious toxic effect (e.g., cancer) represents 25,000 people in a population of 250 million. Such an incidence is unacceptably high. Yet, detecting an incidence of 0.01% experimentally probably would require a minimum of 30,000 animals. To estimate risk at low dosage, large doses must be given to relatively small groups instead. The validity of the necessary extrapolation is clearly a crucial question. These issues are considered in Chapters 1 and 8.

Epidemiology of Poisoning

Multiple data sources are needed to understand the scope of pharmaceutical poisoning. Death certificate and hospital discharge data are two common sources of data on drug poisoning but tend to be biased toward more severe overdoses that result in hospitalization or death. Fifty-five regional poison control centers (PCCs) in the U.S. serve as a repository for overdose data. Reporting to PCCs is voluntary and is biased toward less severe poisoning. Nevertheless, PCCs record more than two million human cases annually (Gummin et al., 2020).

Demographics of Poisoning

The 2019 National Poison Data System Annual Report describes a rate of poison exposures reported to PCCs as 643/100,000 population (Gummin et al., 2020). Children account for the majority of exposures reported to PCCs, with the highest rates of exposure in children less than 6 years of age. Adults experience the highest exposure rates between the ages of 20 and 39 years. There are also common poisoning scenarios (Table 9–4). Unintentional exposures predominate in children due to the exploratory nature of development. Intentional exposures, including self-poisoning, have been increasing in adolescents in recent years (Froberg et al., 2019). Adult exposures are mostly due to intentional exposures or adverse reactions. Table 9–5 displays the most common substances involved in human exposure by age group.

Unintentional and intentional drug poisoning were the leading causes of injury-related death in the U.S. in 2019 (70,630, 28.7%), occurring at a rate of 21.6 per 100,000 population in 2019 (Hedegaard et al., 2020). The U.S. opioid epidemic has substantially contributed to the number of drug-related deaths, with opioids being involved in the majority of overdose deaths in 2019 (49,860, 70.6%). Only 0.12% of cases reported to PCCs resulted in a fatality in 2019. The most common substances associated with fatalities reported to PCCs are listed in Table 9–6.

Prevention of Poisoning

Prevention of Poisoning in the Home

Poisoning exposures occur in a number of scenarios and most often in the home. Primary prevention and secondary prevention strategies play a role in reducing the number of poisoning exposures and their associated harm (Table 9–7). The incidence of poisoning in children has dramatically decreased over the past four decades, largely due to improved safety packaging of drugs and household chemicals and increased public awareness of potential poisons.

TABLE 9–4 ■ POTENTIAL SCENARIOS FOR THE OCCURRENCE OF POISONING

UNINTENTIONAL	INTENTIONAL
Exploratory exposure by young children	Self-harm
Environmental exposure	Recreational abuse
Occupational exposure	Misuse
Therapeutic errors	Harm to another
Iatrogenic errors	

TABLE 9–5 ■ SUBSTANCES MOST FREQUENTLY INVOLVED IN HUMAN POISONING EXPOSURES

PEDIATRIC ≤5 YEARS	ADULT ≥20 YEARS
Cosmetics/personal care products	Analgesics
Household cleaning substances	Sedative/hypnotics/antipsychotics
Analgesics	Antidepressants
Foreign bodies/toys/ miscellaneous	Cardiovascular drugs
Dietary supplements/herbals/ homeopathic	Household cleaning substances
Antihistamines	Alcohols
Topical preparations	Anticonvulsants
Vitamins	Antihistamines
Pesticides	Pesticides
Plants	Hormones and hormone antagonists

Source: Data from Gummin et al., 2020.

TABLE 9–6 ■ SUBSTANCES ASSOCIATED WITH THE GREATEST NUMBER OF HUMAN FATALITIES REPORTED TO POISON CONTROL CENTERS IN THE U.S.

Sedatives/hypnotics/ antipsychotics	Ca^{2+} channel blockers
Opioids (illicit and pharmaceutical)	β Adrenergic antagonists
Alcohols	Antidepressants
Stimulants and street drugs	Hypoglycemic agents
Acetaminophen (alone and in combinations)	Sedating antihistamines

Source: Data from Gummin et al., 2020.

TABLE 9–7 ■ POISON PREVENTION GOALS AND STRATEGIES

Primary prevention goal	*Reduce occurrence of poisoning exposures*
Primary prevention examples	Change product formulation • Removing ethanol from mouthwash Decrease amount of poison in a consumer product • Limiting number of pills in a single bottle of baby aspirin Reduce manufacture/sale of poisons • Withdrawal of medications like phenformin from U.S. pharmaceutical market Prevent access to poison • Using child-resistant packaging • Keeping poisons up and out of reach or in locked cabinets
Secondary prevention goal	*Reduce the effect of a poisoning exposure*
Secondary prevention examples	Increase awareness of poison control services • Promoting 24-h poison helpline number 1-800-222-1222

Reduction of Medication Errors

Over the past decade, considerable attention has been given to the reduction of medication errors and adverse drug events. A medication error is "any preventable event that may cause or lead to inappropriate medication use or patient harm while medication is in control of the healthcare professional, patient, or consumer" (Billstein-Leber et al., 2018). Medication errors are estimated to be 50 to 100 times more common than adverse drug events (Bates et al., 1995). In clinical practice, reducing medication errors involves careful evaluation of the systems involved in prescribing, transcribing, dispensing, administering, and monitoring. Good medication use processes focus on a system-based approach and involve adequate training of those involved and the use of mandatory and redundant checkpoints. Several practical strategies can help to reduce medication errors within healthcare settings (Table 9–8). These issues, especially issues of postmarketing surveillance, are covered extensively in Chapter 8.

TABLE 9–8 ■ BEST PRACTICE RECOMMENDATIONS TO REDUCE MEDICATION ERRORS IN THE MEDICATION MANAGEMENT SYSTEM[a]

STEPS	RECOMMENDATIONS
Planning	• Develop system for reporting and reviewing errors • Form a multidisciplinary medication safety team
Selection and procurement	• Limit the number of medications on formulary • Use of standard concentrations
Storage	• Use of barcode scanning • Designate separate areas for each dosage form • Segregate high-alert medications and look-alike, sound-alike (LASA) medications • Rotate stock to prevent expiration of medication • Store certain medications within the pharmacy ONLY (e.g., concentrated electrolyte solutions, concentrated oral opioid solutions, U-500 insulin) • Use automated dispensing cabinets (ADC) on nursing units
Patient admission	• Medication reconciliation
Ordering	• Avoid unapproved abbreviations • Limit as-needed orders • Avoid range of frequency orders • Specify dosage strengths • Use drug's generic name • Pediatric doses in both units/weight and total dose • Use a leading zero before decimal; no trailing zeros • Maximize computerized prescriber order entry (CPOE) • Maximize use of standard order sets
Transcribing	• Minimize error-prone abbreviations • Use a leading zero before a decimal • Check the medication administration record document against active orders • Implement a second check system
Reviewing	• Prospective review of medication orders by a pharmacist prior to preparation and dispensing
Preparing	• Prepare medications under proper conditions • Independent double check of prepared medications by a pharmacist • Use unit-of-use packaging and ready-to-administer packaging • Use oral syringes for oral preparations only
Dispensing	• Nonemergency medications reviewed by a pharmacist prior to dispensing
Administration	• Use barcode-assisted medication administration (BCMA) • Standardize concentrations of IV medications • Perform independent double checks of high-alert medications
Monitoring	• Develop guidelines for standard times to obtain laboratory values • Train staff to identify and report adverse reactions • Build monitoring parameters into order sets
Patient discharge	• Medication reconciliation • Patient education and counseling
Evaluation	• Root cause analysis • Medication-use evaluation • Quality improvement • Event detection

[a] See ASHP Guidelines on Preventing Medication Errors in Hospitals, 2018.

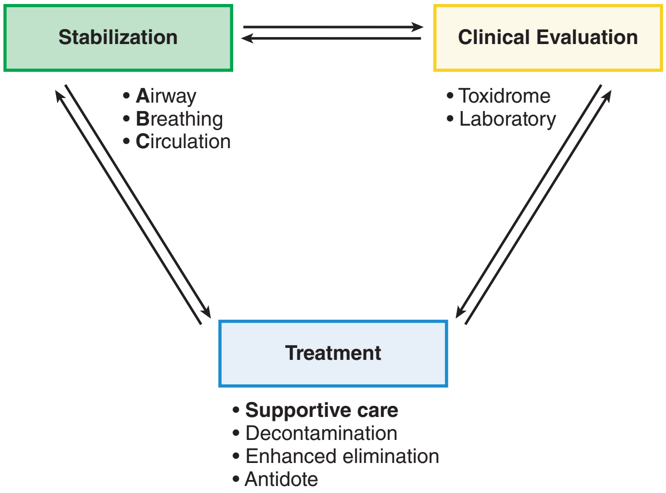


Figure 9–6 *Managing the poisoned patient.* Clinical management of the poisoned patient involves simultaneous stabilization, clinical evaluation, and treatment.

Clinical Management of Poisoned Patients

While most cases reported to U.S. PCCs are managed at home, a significant portion of patients will require medical management in a healthcare facility. Clinically managing a poisoned patient presents a number of challenges. Regional PCCs are available 24/7 to provide expert consultation on poisoned patients and can be contacted within the U.S. by calling the national Poison Help hotline: 1-800-222-1222.

Figure 9–6 illustrates a stepwise approach to managing poisoned patients in an emergency department setting.

Clinical Stabilization and Evaluation

ABCs

The utmost priority in managing a poisoned patient is ensuring their vital physiological functions are maintained. Box 9–2 outlines the “ABC” mnemonic of emergency care as it applies to the management of a poisoned patient. In severely poisoned patients, endotracheal intubation, mechanical ventilation, and pharmacological or extracorporeal circulatory support may be required to support vital physiological functions.

Toxidromes

Groups of physical signs and symptoms associated with specific poisoning syndromes are known as *toxidromes* (Table 9–9) (Erickson et al., 2007). Often, physical symptoms and signs may be the only clues to a poisoning diagnosis. Diagnosis of a specific toxidrome can help guide specific therapeutic options. It is important to recognize that patients may not demonstrate the entire constellation of symptoms in a toxidrome. Further complicating diagnosis, patients with multiple substance exposure can have features of multiple toxidromes.

Toxicology Labs

Most hospital laboratories can quickly test for common substances in serum or urine using qualitative or quantitative assays. Serum toxicology lab tests are commonly ordered for *acetaminophen* and *aspirin* due to their wide availability, quick turnaround times, and utility in guiding treatment. Many other drugs can be detected using serum testing, but results may not be available until after the patient is discharged since testing is often conducted by regional or national laboratories. Urine toxicology screens use immunoassays designed to rapidly detect common drugs of abuse, such as amphetamines, barbiturates, benzodiazepines, cannabis, cocaine, and opiates. Urine toxicology screens are ubiquitous in hospitals and have turnaround times of about 1 h. However, the results may offer little clinical utility for management and must be cautiously interpreted in the context of the patient. Immunoassay positive cross-reactivity (i.e., false positives) and long drug detection windows may misrepresent the patient’s acute toxicity. Further laboratory analysis should be tailored to the individual poisoning circumstance. For instance, an electrocardiogram is useful for detecting QRS interval widening (Na$^+$ channel blockade) and QTc prolongation (K$^+$ channel blockade) associated with specific medication classes (Table 9–10).

BOX 9–2 ■ ABC Mnemonic for Emergency Care of the Poisoned Patient

Airway	Maintain patency
Breathing	Maintain adequate oxygenation and ventilation
Circulation	Maintain perfusion of vital organs

Treatment

Throughout the process of stabilization and clinical evaluation, treatment options are simultaneously being considered. Supportive therapies are particularly important in managing a poisoned patient, especially if the causative substance(s) is not known. Common examples of supportive therapies include intravenous fluids to restore volume and maintain blood pressure, antiemetics for vomiting, and benzodiazepines for agitation or seizures. More specific therapies may be considered based on the suspected exposure; these options include decontamination, enhanced elimination, and antidotal therapies.

Decontamination of the Poisoned Patient

Poisoning exposures occur by a variety of routes (Table 9–11). Decontamination efforts are used to prevent or reduce the ongoing absorption of a poison into the body. For dermal and ocular exposures, eyes and skin should be washed copiously. Patients with inhalational exposures should be moved to fresh air. Neutralization of an acidic or basic substance is not a recommended form of decontamination.

The most common therapies for GI decontamination after an ingestion are activated charcoal and whole-bowel irrigation (WBI). *Other GI decontamination techniques like gastric emptying* and *catharsis are rarely recommended in current clinical practice* (Benson, 2013; Höjer, 2013). Minimal indications for considering GI decontamination include the following:

- The poison must be potentially dangerous.
- The poison must still be unabsorbed in the stomach or intestine.
- The procedure must be able to be performed safely and with proper technique.

Activated Charcoal

Charcoal is created through controlled pyrolysis of organic matter and is *activated* through steam or chemical treatment, which increases its internal pore structure and adsorptive surface capacity. Carbon moieties on the surface of activated charcoal are capable of adsorbing poisons via hydrogen bonding and van der Waals forces, thereby decreasing the overall absorption of the poison into the body. As a rough estimate, 10 g of activated charcoal is expected to adsorb about 1 g of the drug/poison. The recommended dose is typically 0.5 to 2 g/kg of body weight, up to a maximum tolerated dose of about 75 to 100 g. Alcohols, corrosives, hydrocarbons, and ionic salts (e.g., iron and *lithium*) are poorly adsorbed by charcoal. The most common complications of activated charcoal therapy are nausea, vomiting, and constipation. Deaths secondary to pulmonary aspiration of activated charcoal have occurred (American Academy of Clinical Toxicology, 2008). Activated charcoal is contraindicated in patients at risk of aspiration without an unprotected airway; in patients with a suspected GI perforation; and in patients who may be candidates for endoscopy. The use of activated charcoal in the treatment of poisoning has declined over the last 20 years to 1.59% of cases in 2019 (Gummin et al., 2020).

TABLE 9–9 ■ COMMON TOXIDROMES

TOXIDROME	EXAMPLES	MENTAL STATUS	HR	BP	RR	T	PUPIL SIZE	BOWEL SOUNDS	DIAPHORESIS	OTHER
Sympathomimetic	Cocaine Amphetamine	Agitation	↑	↑	↑	↑	↑	↑	↑	Tremor, seizures
Anticholinergic	Diphenhydramine Atropine	Delirium	↑	↑	±	↑	↑	↓	↓	Flushing, urinary retention, dry mucus membranes
Cholinergic	Organophosphates	Somnolence	±	±	±	nc	↓	↑	↑	DUMBBELLSS,[a] fasciculations
Opioid	Heroin Oxycodone	Somnolence	↓	↓	↓	↓	↓	↓	nc	
Sedative-hypnotic	Benzodiazepines Barbiturates	Somnolence	↓	↓	↓	↓	±	↓	nc	Ataxia, hyporeflexia
Serotonin toxicity	Sertraline Citalopram	Normal or agitation	↑	↑	↑	↑	↑	↑		Clonus, hyperreflexia, tremor, seizures
Sympatholytics	Clonidine	Somnolence	↓	↓	↓	↓	↓	↓	↓	

BP, blood pressure; HR, heart rate; RR, respiratory rate; T, temperature; ±, variable; nc; no change.

[a] DUMBBELLSS: mnemonic for muscarinic effects of diarrhea, urination, miosis, bronchorrhea, bradycardia, emesis, lacrimation, lethargy, salivation, sweating.

TABLE 9–10 ■ DIFFERENTIAL POISONING DIAGNOSIS: DRUGS COMMONLY ASSOCIATED WITH ELECTROCARDIOGRAM CHANGES (PARTIAL LISTING)[a]

BRADYCARDIA/ HEART BLOCK	QRS INTERVAL PROLONGATION	QTc INTERVAL PROLONGATION
Cholinergic agents Physostigmine Neostigmine Organophosphates, carbamates *Sympatholytic agents* β Receptor antagonists Clonidine Opioids *Other* Digoxin Ca^{2+} channel blockers Lithium	Antiarrhythmics (class 1) Bupropion Chloroquine/ hydroxychloroquine Cocaine Diphenhydramine Lamotrigine Propoxyphene[b] Propranolol Tricyclic antidepressants	Antiarrhythmics (class 1a, 1c, III) Atypical antipsychotics (ziprasidone, quetiapine) Fluoroquinolones Loperamide Macrolides Methadone Ondansetron Phenothiazines SNRIs (venlafaxine, desvenlafaxine) SSRIs (citalopram, escitalopram) Tricyclic antidepressants Typical antipsychotics (haloperidol, droperidol)

SSRI, selective serotonin reuptake inhibitor.

[a] This is not an exhaustive list. For more information, see Bruccoleri et al. (2016) and CredibleMeds®.

[b] Discontinued in the U.S.

Whole-Bowel Irrigation

Whole-bowel irrigation involves the enteral administration of large amounts of a high-molecular-weight, iso-osmotic polyethylene glycol electrolyte solution with the goal of passing poison by the rectum before it can be absorbed. Table 9–12 lists type of ingestions that may benefit from WBI.

Polyethylene glycol electrolyte solution is typically administered at a rate of 1500 to 2000 mL/h in adults until the rectal effluent is clear and no more drug is being passed. To achieve these high administration rates, a nasogastric tube may be used. Administration of WBI can be complicated by patients experiencing nausea and vomiting or by patients at risk for pulmonary aspiration. Contraindications to WBI include bowel perforation or obstruction, ileus, uncontrollable vomiting, unprotected compromised airway, and GI hemorrhage.

Gastric Emptying

Gastric emptying techniques include gastric lavage (commonly referred to as "stomach pumping") and administration of syrup of ipecac. Gastric emptying reduces drug absorption only by about one-third under optimal conditions (Tenenbein et al., 1987). *Based on the review of existing evidence, the American Academy of Pediatrics no longer recommends syrup of ipecac as part of its childhood injury prevention program, and the American Academy of Clinical Toxicology recommends avoiding its use in the poisoned patient* (American Academy of Pediatrics, 2003; Höjer, 2013). Due to their lack of benefit in published studies and limited availability, both gastric lavage and ipecac are rarely

TABLE 9–11 ■ ROUTES OF EXPOSURE

Bite/sting	Inhalation/nasal	Parenteral
Dermal	Ocular	Rectal
Ingestion*	Otic	Vaginal

*most common

TABLE 9–12 ■ TYPES OF INGESTIONS THAT MAY BENEFIT FROM WBI

Packets of illicit drugs ("body packers")
Iron
Patch pharmaceuticals
Sustained-release pharmaceutical formulations

used in current clinical practice and should only be considered with the consultation of a clinical or medical toxicologist.

Gastric Lavage. The procedure for gastric lavage involves passing an orogastric tube into the stomach with the patient in the left lateral decubitus position with head lower than feet. After withdrawing stomach contents, 10 to 15 mL/kg (up to 250 mL) of saline lavage fluid is administered and withdrawn. This process continues until the lavage fluid returns clear. Additionally, large pills may not pass through the orogastric tube. Complications of the procedure include mechanical trauma to the stomach or esophagus, pulmonary aspiration of stomach contents, and vagus nerve stimulation.

Syrup of Ipecac. Syrup of ipecac contains the alkaloids cephaeline and emetine. Both act as emetics due to their local irritant effect on the GI tract and their central effect on the chemoreceptor trigger zone in the area postrema of the medulla. Ipecac is no longer manufactured in the U.S., and its use is not recommended. As a result, ipecac was administered in only 0.003% of all human poisonings in the U.S. in 2019 (Gummin et al., 2020).

Cathartics

The two most common categories of simple cathartics are the Mg^{2+} salts, such as magnesium citrate and magnesium sulfate, and the nondigestible carbohydrates, such as sorbitol. Sorbitol is found in some activated charcoal preparations. However, the use of simple cathartics alone has been abandoned as a GI decontamination strategy.

Enhancing the Elimination of Poisons

Other therapeutic options for poisoned patients involve enhancing elimination of toxins.

Manipulating Urinary pH

Drugs subject to renal clearance are excreted into the urine by glomerular filtration and active tubular secretion; nonionized compounds may be reabsorbed far more rapidly than ionized polar molecules (see Figure 2–3). Weakly acidic drugs are susceptible to "ion trapping" in the urine. If a weakly acidic xenobiotic depends on renal excretion for the majority of its elimination, alkalizing the urine will increase ionization of the compound and thereby increase its rate of elimination. Urinary alkalinization enhances the elimination of *phenobarbital*, *chlorpropamide*, and *salicylates*. However, the American Academy of Clinical Toxicologists recommends urine alkalinization as a first-line treatment only for moderately severe salicylate poisoning that does not meet the criteria for hemodialysis (Proudfoot et al., 2004).

To achieve alkalinization of the urine, 100 to 150 mEq of sodium bicarbonate in 1 L of 5% dextrose in water (D5W) is infused intravenously and titrated to a serum pH of 7.45 to 7.55. Acidification of the urine is not recommended for basic drugs since the risks of acidification outweigh the benefits.

Multiple-Dose Activated Charcoal

Multiple-dose activated charcoal (MDAC) can increase the elimination of absorbed drug by two mechanisms: Charcoal may interrupt enterohepatic circulation of hepatically metabolized drug excreted in the bile, and charcoal may create a diffusion gradient across the GI mucosa and promote movement of drug from the bloodstream onto the charcoal in the intestinal lumen. There is not one standardized dosing regimen, but an example of a reasonable dosage regimen for an adult is 50 g of activated charcoal every 4 h. Table 9–13 lists drugs that may demonstrate an enhanced elimination half-life following MDAC administration.

TABLE 9–13 ■ DRUGS WITH ENHANCED ELIMINATION FOLLOWING MDAC THERAPY

Carbamazepine
Dapsone
Digoxin
Phenobarbital
Quinine
Salicylate
Theophylline

Sources: American Academy of Clinical Toxicology; European Association of Poisons Centres and Clinical Toxicologists, 2004.

Extracorporeal Drug Removal

The ideal drug amenable to removal by hemodialysis has a low molecular weight, a low volume of distribution, high solubility in water, and minimal protein binding. Hemodialysis is often reserved for patients with a life-threatening toxicity that does not respond to maximal supportive care measures. Other patients may necessitate hemodialysis due to impaired elimination, electrolyte abnormalities, or acid-base disturbances. Common poisonings for which hemodialysis is used include *carbamazepine*, *ethylene glycol*, *lithium*, *metformin*, *methanol*, *salicylate*, and *valproate*.

Antidotal Therapies

Antidotes are a diverse class of medications that prevent morbidity and mortality in poisoned patients when administered appropriately. There are a number of unique mechanisms by which antidotes exert their therapeutic action. Common classes of antidotes are described below.

Chemical Antidotes

Chemical antidotes bind to toxins, forming biologically inactive complexes that are subsequently excreted. Antidotes including *digoxin immune Fab* and the antivenom *Crotalidae* polyvalent immune Fab use antigen-binding fragments (Fab) to bind to the target toxin. Metal chelators, like *deferoxamine*, function similarly in that they bind to metals to form stable complexes that are biologically inactive and excreted (see Chapter 76). *Sugammadex* is a modified cyclodextrin that, administered intravenously, binds several aminosteroid muscle relaxants (e.g., *rocuronium*) that are sometimes used in surgical anesthesia, thereby permitting rapid recovery of skeletal muscle tone after surgery.

Pharmacological Antidotes

Pharmacological antidotes work by antagonizing the effect of the toxin at the receptor it innervates. *Naloxone* is a competitive antagonist at μ-opioid receptors and rapidly reverses the effects of opioid toxicity when administered.

Physiological (Functional) Antidotes

A physiological antidote may use a different cellular mechanism to overcome the effects of a poison, as in the use of glucagon to circumvent a blocked β adrenergic receptor and increase cellular cyclic AMP in the setting of an overdose of a β adrenergic antagonist. Antivenoms and chelating agents bind and directly inactivate poisons. The biotransformation of a drug can also be altered by an antidote; for example, *fomepizole* will inhibit alcohol dehydrogenase and stop the formation of toxic acid metabolites from ethylene glycol and methanol. Many drugs used in the supportive care of a poisoned patient (e.g., anticonvulsants, vasoconstricting agents) may be considered nonspecific functional antidotes.

Dispositional Antidotes

Dispositional antidotes can prevent the formation of toxic metabolites by antagonizing toxic metabolic pathways. *Fomepizole* competitively inhibits alcohol dehydrogenase to prevent the metabolism of methanol and ethylene glycol to their respective toxic metabolites. Other antidotes support endogenous detoxifying metabolic pathways. In *valproic acid* overdose, carnitine depletion shunts valproate metabolism to ω-oxidation, producing metabolites that disrupt the urea cycle and cause hepatotoxicity. Administering L-carnitine restores hepatic carnitine to support nontoxic β-oxidation.

Table 9–14 provides a list of commonly recommended antidotes (Dart et al., 2018).

Resources for Information on Drug Toxicity and Poisoning

Additional information on poisoning from drugs and chemicals can be found in many dedicated books of toxicology (Klaassen, 2019; Nelson et al., 2019; Olson et al., 2018; Shannon et al., 2007). Popular drug information databases have toxicology-specific monographs including POISINDEX® (Micromedex, Inc., Denver, CO) and Lexi-Tox™ (Lexicomp online, Lexi-Tox online, Hudson, OH: UpToDate, Inc.). The National Library of Medicine offers information on toxicology through PubChem (https://pubchem.ncbi.nlm.nih.gov/). The Consumer Product Information

TABLE 9–14 ■ ANTIDOTAL THERAPIES AND POISONING INDICATION(S)

ANTIDOTE	POISONING INDICATION(S)
N-Acetylcysteine	Acetaminophen
Atropine sulfate	Pesticide (carbamate and organophosphate types)
Antivenin, *Latrodectus mactans*	Black widow spider envenomation
Antivenin, *Micrurus fulvius*	North American coral snake envenomation
Antivenom, *Centruroides* (scorpion) immune $F(ab')_2$	*Centruroides* scorpion envenomation
Antivenom, *Crotalidae* [Polyvalent immune fab and immune $F(ab')_2$]	North American rattlesnake envenomation
Calcium	Ca^{2+} channel blockers; fluoride
Calcium disodium EDTA	Chronic lead poisoning
Cyproheptadine	Serotonin
Dantrolene	Anesthetic-induced malignant hyperthermia
Deferoxamine	Iron overload (acute and chronic)
Digoxin immune Fab	Cardiac glycosides
Dimercaprol (BAL)	Lead, mercury, arsenic
Fomepizole	Ethylene glycol, methanol
Flumazenil	Benzodiazepines
Glucagon hydrochloride	β Blockers and calcium channel blockers
Glucarpidase	Methotrexate
Hydroxocobalamin	Cyanide
Idarucizumab	Dabigatran
Leucovorin	Methotrexate
L-Carnitine	Valproic acid (off-label use)
Lipid emulsion	Local anesthetics
Methylene blue	Methemoglobinemia
Naloxone	Opioids
Octreotide	Sulfonylurea-induced hypoglycemia
Physostigmine	Anticholinergic delirium
Pralidoxime (2-PAM)	Organophosphate pesticides
Protamine	Heparin
Prothrombin complex concentrate *(3- or 4-factor, or activated)*	Vitamin K antagonist
Prussian blue	Thallium (^{201}Ti); cesium (^{137}Cs)
Pyridoxine	Hydrazine; isoniazid
Sodium bicarbonate	Salicylate; tricyclic antidepressants
Succimer (DMSA)	Lead (on label); mercury and arsenic (off label)
Vitamin K_1 (phytonadione)	Vitamin K antagonist (warfarin, coumarin, etc.)

Database stores information for the health effects of consumer products. (whatsinproducts.com). The Center for Disease Control and Prevention's Agency for Toxic Substances and Disease Registry hosts information on environmental toxins (https://www.atsdr.cdc.gov/). The Extracorporeal Treatments in Poisoning Workgroup publishes evidence-based recommendations for various substances (https://www.extrip-workgroup.org/recommendations). Regional poison control centers are available for poison information 24/7 in the U.S. by calling the Poison Help hotline: 1-800-222-1222.

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Section II

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Chapter 10

Neurotransmission: The Autonomic and Somatic Motor Nervous Systems

Rebecca Petre Sullivan, Steven R. Houser, and Walter J. Koch

ANATOMY AND GENERAL FUNCTIONS

- Differences Between Autonomic and Somatic Nerves
- Divisions of the Peripheral Autonomic System
- Comparison of Sympathetic, Parasympathetic, and Motor Nerves

NEUROCHEMICAL TRANSMISSION

- Evidence for Neurohumoral Transmission
- Steps Involved in Neurotransmission
- Cholinergic Transmission
- Adrenergic Transmission

PHARMACOLOGICAL CONSIDERATIONS

- Interference With the Synthesis or Release of the Transmitter
- Promotion of Release of the Transmitter
- Agonist and Antagonist Actions at Receptors
- Interference With the Destruction of the Transmitter

OTHER AUTONOMIC NEUROTRANSMITTERS

- Cotransmission in the Autonomic Nervous System
- Nonadrenergic, Noncholinergic Transmission by Purines
- Signal Integration and Modulation of Vascular Responses by Endothelium-Derived Factors: NO and Endothelin

Anatomy and General Functions

The autonomic nervous system, also called the *visceral, vegetative,* or *involuntary nervous system,* is distributed widely throughout the body and regulates autonomic functions that occur without conscious control. In the periphery, it consists of nerves, ganglia, and plexuses that innervate the heart, blood vessels, glands, other visceral organs, and smooth muscle in various tissues. This system enables the body to constantly monitor, analyze, and anticipate needs, and control the response to the organ systems, in order to maintain homeostasis.

Differences Between Autonomic and Somatic Nerves

- The *efferent nerves* of the autonomic nervous system supply all innervated structures of the body except skeletal muscles, which are served by *somatic nerves.*
- The most distal synaptic junctions in the autonomic reflex arc occur in *ganglia* that are entirely *outside the cerebrospinal axis.* Somatic nerves contain no peripheral *ganglia,* and the synapses are located entirely *within the cerebrospinal axis.*
- Many autonomic nerves form extensive peripheral plexuses; such networks are absent from the somatic system.
- Postganglionic autonomic nerves generally are *nonmyelinated*; motor nerves to skeletal muscles are *myelinated.*
- When the spinal efferent nerves are interrupted, smooth muscles and glands generally retain some level of spontaneous activity, whereas the *denervated* skeletal muscles are paralyzed.

Sensory Information: Afferent Fibers and Reflex Arcs

Afferent fibers from visceral structures are the first link in the reflex arcs of the autonomic system. With certain exceptions, such as local axon reflexes, most visceral reflexes are mediated through the CNS.

Visceral Afferent Fibers. Information on the status of the visceral organs is transmitted to the CNS through two main sensory systems: the *cranial nerve (parasympathetic) visceral sensory system* and the *spinal (sympathetic) visceral afferent system.* The cranial visceral sensory system carries mainly mechanoreceptor and chemosensory information, whereas the afferents of the spinal visceral system principally convey sensations related to temperature and tissue injury of mechanical, chemical, or thermal origin.

Cranial visceral sensory information enters the CNS by four cranial nerves: the trigeminal (V), facial (VII), glossopharyngeal (IX), and vagus (X) nerves. These four cranial nerves transmit visceral sensory information from the internal face and head (V); tongue (taste, VII); hard palate and upper part of the oropharynx (IX); and carotid body, lower part of the oropharynx, larynx, trachea, esophagus, and thoracic and abdominal organs (X), with the exception of the pelvic viscera. The pelvic viscera are innervated by nerves from the second through fourth sacral spinal segments. The visceral afferents from these four cranial nerves terminate topographically in the solitary tract nucleus (STN).

Sensory afferents from visceral organs also enter the CNS from the spinal nerves. Those concerned with muscle chemosensation may arise at all spinal levels, whereas sympathetic visceral sensory afferents generally arise at the thoracic levels where sympathetic preganglionic neurons are found. Pelvic sensory afferents from spinal segments S2–S4 enter at that level and are important for the regulation of sacral parasympathetic outflow. In general, visceral afferents that enter the spinal nerves convey information concerned with temperature as well as nociceptive visceral inputs related to mechanical, chemical, and thermal stimulation. The primary pathways taken by ascending spinal visceral afferents are complex. An important feature of the ascending pathways is that they provide collaterals that converge with the cranial visceral sensory pathway at virtually every level (Saper, 2002).

The neurotransmitters that mediate transmission from sensory fibers have not been characterized unequivocally. Substance P and calcitonin gene-related peptide (CGRP), present in afferent sensory fibers, dorsal root ganglia, and the dorsal horn of the spinal cord, likely communicate nociceptive stimuli from the periphery to the spinal cord and higher structures. Somatostatin (SST), vasoactive intestinal polypeptide (VIP), and cholecystokinin also occur in sensory neurons (Hökfelt et al., 2000). ATP appears to be a neurotransmitter in certain sensory neurons (e.g., the urinary bladder). Enkephalins, present in interneurons in the dorsal spinal cord (within the *substantia gelatinosa*), have antinociceptive effects both pre- and postsynaptically to inhibit the release of substance P and diminish the activity of cells that project from the spinal cord to higher centers in the CNS. The excitatory amino acids glutamate and aspartate also play major roles in transmission of sensory responses to the spinal cord. These transmitters and their signaling pathways are reviewed in Chapter 16.

Central Autonomic Connections

There probably are no purely autonomic or somatic centers of integration, and extensive overlap occurs. Somatic responses always are accompanied

Abbreviations

ACh: acetylcholine
AChE: acetylcholinesterase
BuChE: butyrylcholinesterase
CaM: calmodulin
CGRP: calcitonin gene-related peptide
CHT1: choline transporter
COMT: catechol-*O*-methyltransferase
DA: dopamine
DAT: DA transporter
DβH: dopamine β-hydroxylase
DOMA: 3,4-dihydroxymandelic acid
DOPEG: 3,4-dihydroxyphenyl glycol
DOPGAL: dihydroxyphenylglycolaldehyde
ENS: enteric nervous system
ENT: extraneuronal transporter
EPI: epinephrine
EPSP: excitatory postsynaptic potential
ICC: interstitial cells of Cajal
GABA: γ-aminobutyric acid
GI: gastrointestinal
GPCR: G protein-coupled receptor
5HT: serotonin (5-hydroxytryptamine)
ICC: interstitial cells of Cajal
IP_3: inositol 1,4,5-trisphosphate
IPSP: inhibitory postsynaptic potential
KO: knockout
mAChR: muscarinic acetylcholine receptor
MAO: monoamine oxidase
MAPK: mitogen-activated protein kinase
MOPEG: 3-methyl,4-hydroxyphenylglycol
MOPGAL: monohydroxyphenylglycolaldehyde
nAChR: nicotinic ACh receptor
NE: norepinephrine (noradrenaline)
NET: norepinephrine transporter
NMJ: neuromuscular junction (of skeletal muscle)
NO: nitric oxide
NOS: nitric oxide synthase
NPY: neuropeptide Y
NSF: *N*-ethylmaleamide sensitive factor
PACAP: pituitary adenylyl cyclase–activating peptide
PK_: protein kinase _, as in PKA
PL_: phospholipase _, as in PLA_2, PLC, etc.
PNMT: phenylethanolamine-*N*-methyltransferase
SA: sinoatrial
SLC: solute carrier
SNAP: soluble NSF attachment protein, synaptosome-associated protein
SNARE: SNAP receptor
SST: somatostatin
STN: solitary tract nucleus
TH: tyrosine hydroxylase
VIP: vasoactive intestinal polypeptide
VMA: vanillyl mandelic acid
VMAT2: vesicular uptake transporter

by visceral responses and vice versa. Autonomic reflexes can be elicited at the level of the spinal cord. They clearly are demonstrable in experimental animals or humans with spinal cord transection and are manifested by sweating, blood pressure alterations, vasomotor responses to temperature changes, and reflex emptying of the urinary bladder, rectum, and seminal vesicles. Extensive central ramifications of the autonomic nervous system exist above the level of the spinal cord. For example, integration of the control of respiration in the medulla oblongata is well known. The hypothalamus and the STN generally are regarded as principal loci of integration of autonomic nervous system functions, which include regulation of body temperature, water balance, carbohydrate and fat metabolism, blood pressure, emotions, sleep, respiration, and reproduction. Signals are received through ascending spinobulbar pathways, the limbic system, neostriatum, cortex, and to a lesser extent other higher brain centers. Stimulation of the STN and the hypothalamus activates bulbospinal pathways, which originate in the brainstem, and hormonal output to mediate autonomic and motor responses (Andresen and Kunze, 1994) (see Chapter 16). The hypothalamic nuclei that lie posteriorly and laterally are sympathetic in their main connections and are responsible for myriad responses including body temperature regulation, blood pressure, and pupillary dilation (posterior hypothalamus), and cardiovascular control, feeding, satiety, and insulin release (lateral hypothalamus). Parasympathetic functions evidently are integrated by the midline nuclei in the region of the tuber cinereum and by nuclei lying anteriorly.

Highly integrated patterns of response generally are organized at a hypothalamic level and involve autonomic, endocrine, and behavioral components. More limited patterned responses are organized at other levels of basal forebrain, brainstem, and spinal cord.

Divisions of the Peripheral Autonomic System

On the efferent side, the autonomic nervous system consists of two large divisions: (1) the *sympathetic* or *thoracolumbar outflow*, which includes T1–L2, and (2) the *parasympathetic* or *craniosacral outflow*, which includes cranial nerves III, VII, IX, and X, as well as S2–S4. Figure 10–1 schematically summarizes the arrangement of the principal parts of the peripheral autonomic nervous system.

Neurotransmitters of the Autonomic Nervous System

The neurotransmitter of all preganglionic autonomic fibers, most postganglionic parasympathetic fibers, and a few postganglionic sympathetic fibers is acetylcholine (Ach). Some postganglionic parasympathetic nerves use nitric oxide (NO) as a neurotransmitter and are termed *nitrergic* (Toda and Okamura, 2003). The majority of the postganglionic sympathetic fibers are *adrenergic,* in which the transmitter is norepinephrine (NE; also called noradrenaline). The terms *cholinergic* and *adrenergic* describe neurons that liberate ACh or NE, respectively. Not all the transmitters of the primary afferent fibers, such as those from the mechano- and chemoreceptors of the carotid body and aortic arch, have been identified conclusively. Substance P and glutamate may mediate many afferent impulses; both are present in high concentrations in the dorsal horn of the spinal cord.

Sympathetic Nervous System

The cells that give rise to the preganglionic fibers of the sympathetic nervous system division lie mainly in the intermediolateral columns of the spinal cord and extend from the first thoracic to the second or third lumbar segment. The axons from these cells are carried in the anterior (ventral) nerve roots and synapse, with neurons lying in sympathetic ganglia outside the cerebrospinal axis. Sympathetic ganglia are found in three locations: paravertebral, prevertebral, and terminal.

The 22 pairs of paravertebral sympathetic ganglia form the lateral chains on either side of the vertebral column. The ganglia are connected to each other by nerve trunks and to the spinal nerves by *rami communicantes.* The white rami are restricted to the segments of the thoracolumbar outflow; they carry the preganglionic myelinated fibers that exit the spinal cord by the anterior spinal roots. The gray rami arise from the ganglia and carry postganglionic fibers back to the spinal nerves for distribution to sweat glands and pilomotor muscles and to blood vessels of skeletal muscle and skin. The prevertebral ganglia lie in the abdomen and the pelvis near the ventral surface of the bony vertebral column and consist mainly of the celiac (solar), superior mesenteric, aorticorenal, and inferior mesenteric ganglia. The terminal ganglia are few in number, lie near the organs they innervate, and include ganglia connected with the

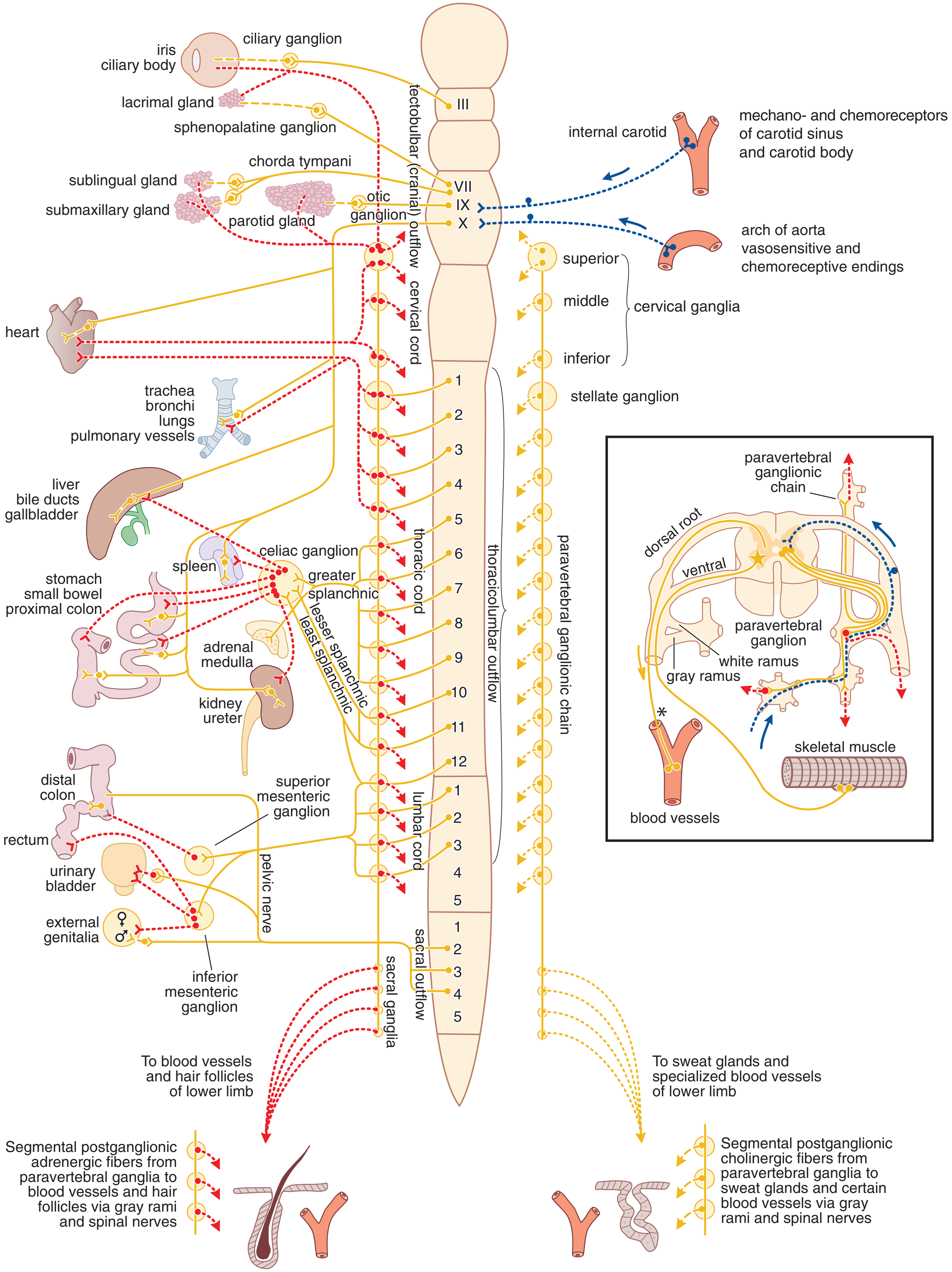
iris
ciliary body
ciliary ganglion
lacrimal gland
sphenopalatine ganglion
chorda tympani
sublingual gland
submaxillary gland
parotid gland
otic ganglion
tectobulbar (cranial) outflow
III
VII
IX
X
internal carotid
mechano- and chemoreceptors of carotid sinus and carotid body
arch of aorta vasosensitive and chemoreceptive endings
superior
middle
inferior
cervical ganglia
cervical cord
stellate ganglion
heart
trachea
bronchi
lungs
pulmonary vessels
liver
bile ducts
gallbladder
spleen
celiac ganglion
greater splanchnic
lesser splanchnic
least splanchnic
stomach
small bowel
proximal colon
adrenal medulla
kidney
ureter
thoracic cord
thoracicolumbar outflow
paravertebral ganglionic chain
1
2
3
4
5
6
7
8
9
10
11
12
distal colon
rectum
superior mesenteric ganglion
urinary bladder
external genitalia
♀
♂
inferior mesenteric ganglion
pelvic nerve
lumbar cord
1
2
3
4
5
sacral outflow
1
2
3
4
5
sacral ganglia
paravertebral ganglionic chain
dorsal root
ventral
paravertebral ganglion
white ramus
gray ramus
*
blood vessels
skeletal muscle
To blood vessels and hair follicles of lower limb
To sweat glands and specialized blood vessels of lower limb
Segmental postganglionic adrenergic fibers from paravertebral ganglia to blood vessels and hair follicles via gray rami and spinal nerves
Segmental postganglionic cholinergic fibers from paravertebral ganglia to sweat glands and certain blood vessels via gray rami and spinal nerves

Figure 10–1 *The autonomic nervous system.* Schematic representation of the autonomic nerves and effector organs based on chemical mediation of nerve impulses. Yellow (——), cholinergic; red (——), adrenergic; dotted blue (••••••), visceral afferent; solid lines, preganglionic; broken lines, postganglionic. The rectangle at right shows the finer details of the ramifications of adrenergic fibers at any one segment of the spinal cord, the path of the visceral afferent nerves, the cholinergic nature of somatic motor nerves to skeletal muscle, and the presumed cholinergic nature of the vasodilator fibers in the dorsal roots of the spinal nerves. The asterisk (*) indicates that it is not known whether these vasodilator fibers are motor or sensory or where their cell bodies are situated.

urinary bladder and rectum and the cervical ganglia in the region of the neck. In addition, small intermediate ganglia lie outside the conventional vertebral chain, especially in the thoracolumbar region. They are variable in number and location but usually are in proximity to the communicating rami and the anterior spinal nerve roots.

Preganglionic fibers issuing from the spinal cord may synapse with the neurons of more than one sympathetic ganglion. Their principal ganglia of termination need not correspond to the original level from which the preganglionic fiber exits the spinal cord. Many of the preganglionic fibers from the fifth to the last thoracic segment pass through the paravertebral ganglia to form the splanchnic nerves. Most of the splanchnic nerve fibers do not synapse until they reach the celiac ganglion; others directly innervate the adrenal medulla.

Postganglionic fibers arising from sympathetic ganglia innervate visceral structures of the thorax, abdomen, head, and neck. The trunk and the limbs are supplied by the sympathetic fibers in spinal nerves. The prevertebral ganglia contain cell bodies whose axons innervate the glands and smooth muscles of the abdominal and the pelvic viscera. Many of the upper thoracic sympathetic fibers from the vertebral ganglia form terminal plexuses, such as the cardiac, esophageal, and pulmonary plexuses. The sympathetic distribution to the head and the neck (vasomotor, pupillodilator, secretory, and pilomotor) is by means of the cervical sympathetic chain and its three ganglia. All postganglionic fibers in this chain arise from cell bodies located in these three ganglia. All preganglionic fibers arise from the upper thoracic segments of the spinal cord, there being no sympathetic fibers that leave the CNS above the first thoracic level.

Pharmacologically, anatomically, and embryologically, the chromaffin cells of the adrenal medulla resemble a collection of postganglionic sympathetic nerve cells. Typical preganglionic fibers that release ACh innervates these chromaffin cells, stimulating the release of epinephrine (EPI; also called adrenaline), in distinction to the NE released by postganglionic sympathetic fibers.

Parasympathetic Nervous System

The parasympathetic nervous system consists of preganglionic fibers that originate in the CNS and their postganglionic connections. The regions of central origin are the midbrain, the medulla oblongata, and the sacral part of the spinal cord. The midbrain, or tectal, outflow consists of fibers arising from the Edinger-Westphal nucleus (accessory oculomotor nucleus) of the third cranial nerve and going to the ciliary ganglion in the orbit. The medullary outflow consists of the parasympathetic components of cranial nerves VII, IX, and X.

The fibers in the VII (facial) cranial nerve form the chorda tympani, which innervates the ganglia lying on the submaxillary and sublingual glands. They also form the greater superficial petrosal nerve, which innervates the sphenopalatine ganglion. The autonomic components of the IX (glossopharyngeal) cranial nerve innervate the otic ganglia. Postganglionic parasympathetic fibers from these ganglia supply the sphincter of the iris (pupillary constrictor muscle), the ciliary muscle, the salivary and lacrimal glands, and the mucous glands of the nose, mouth, and pharynx. These fibers also include vasodilator nerves to these same organs. Cranial nerve X (vagus) arises in the medulla and contains preganglionic fibers, most of which do not synapse until they reach the many small ganglia lying directly on or in the viscera of the thorax and abdomen. In the intestinal wall, the vagal fibers terminate around ganglion cells in the myenteric and submucosal plexuses. *Thus, in the parasympathetic branch of the autonomic nervous system, preganglionic fibers are very long, whereas postganglionic fibers are very short.* The vagus nerve also carries a far greater number of afferent fibers from the viscera into the medulla. The parasympathetic sacral outflow consists of axons that arise from cells in the second, third, and fourth segments of the sacral cord and proceed as preganglionic fibers to form the pelvic nerves (*nervi erigentes*). They synapse in terminal ganglia lying near or within the bladder, rectum, and sexual organs. The vagal and sacral outflows provide motor and secretory fibers to thoracic, abdominal, and pelvic organs (see Figure 10–1).

Enteric Nervous System

The processes of mixing, propulsion, and absorption of nutrients in the gastrointestinal (GI) tract are controlled locally through a restricted part of the peripheral nervous system called the *enteric nervous system* (ENS). The ENS comprises components of the sympathetic and parasympathetic nervous systems and has sensory nerve connections through the spinal and nodose ganglia (see Figure 54–1 and Furness et al., 2014). The ENS is involved in sensorimotor control and thus consists of both afferent sensory neurons and a number of motor nerves and interneurons that are organized principally into two nerve plexuses: the myenteric (Auerbach) plexus and the submucosal (Meissner) plexus. The myenteric plexus, located between the longitudinal and circular muscle layers, plays an important role in the contraction and relaxation of GI smooth muscle, therefore exerting control over peristaltic movements and gastrointestinal motility. The submucosal plexus is involved with secretory and absorptive functions of the GI epithelium, local blood flow, and neuroimmune activities. The microbiota of the gut, the immune system, and their interactions with the ENS inform GI homeostasis and may play a role in the development of neurodegenerative diseases (Obata and Pachnis, 2016).

Parasympathetic preganglionic inputs are provided to the GI tract via the vagus and pelvic nerves. ACh released from *preganglionic neurons* activates nicotinic ACh receptors (nAChRs) on postganglionic neurons within the enteric ganglia. Excitatory preganglionic input activates both excitatory and inhibitory motor neurons that control processes such as muscle contraction and secretion/absorption. *Postganglionic sympathetic nerves* also synapse with intrinsic neurons and generally induce relaxation. Sympathetic input is excitatory (contractile) at some sphincters. Information from afferent and preganglionic neural inputs to the enteric ganglia is integrated and distributed by a network of interneurons. ACh is the primary neurotransmitter providing excitatory inputs between interneurons, but other substances, such as ATP (via postjunctional P2X receptors), substance P (by NK_3 receptors), and serotonin (5HT; via $5HT_3$ receptors), are also important in mediating integrative processing via interneurons.

The muscle layers of the GI tract are dually innervated by excitatory and inhibitory motor neurons, with cell bodies primarily in the myenteric ganglia. ACh is a primary excitatory motor neurotransmitter released from postganglionic neurons. ACh activates M_2 and M_3 receptors in postjunctional cells to elicit motor responses. Pharmacological blockade of muscarinic acetylcholine receptors (mAChRs) does not block all excitatory neurotransmission, however, because neurokinins (neurokinin A and substance P) are also co-released by excitatory motor neurons and contribute to postjunctional excitation. Inhibitory motor neurons in the GI tract regulate motility events such as accommodation, sphincter relaxation, and descending receptive relaxation. Inhibitory responses are elicited by NO and a purine derivative (either ATP or β-nicotinamide adenine dinucleotide) acting at postjunctional $P2Y_1$ receptors. Inhibitory neuropeptides, such as VIP and pituitary adenylyl cyclase–activating peptide, may also be released from inhibitory motor neurons under conditions of strong stimulation.

In general, motor neurons do not directly innervate smooth muscle cells in the GI tract. Nerve terminals make synaptic connections with the interstitial cells of Cajal (ICCs), and these cells make electrical connections (gap junctions) with smooth muscle cells. Thus, the ICCs are the receptive, postjunctional transducers of inputs from enteric motor neurons, and loss of these cells has been associated with conditions that appear to be neuropathies. ICCs have all of the major receptors and effectors necessary to transduce both excitatory and inhibitory neurotransmitters into postjunctional responses (Foong et al., 2020).

Comparison of Sympathetic, Parasympathetic, and Motor Nerves

Differences among somatic motor, sympathetic, and parasympathetic nerves are shown schematically in Figure 10–2. To summarize:

- The *sympathetic* system is distributed to effectors throughout the body, whereas *parasympathetic* distribution is much more limited.
- A *preganglionic sympathetic fiber* may traverse a considerable distance of the sympathetic chain and pass through several ganglia before it

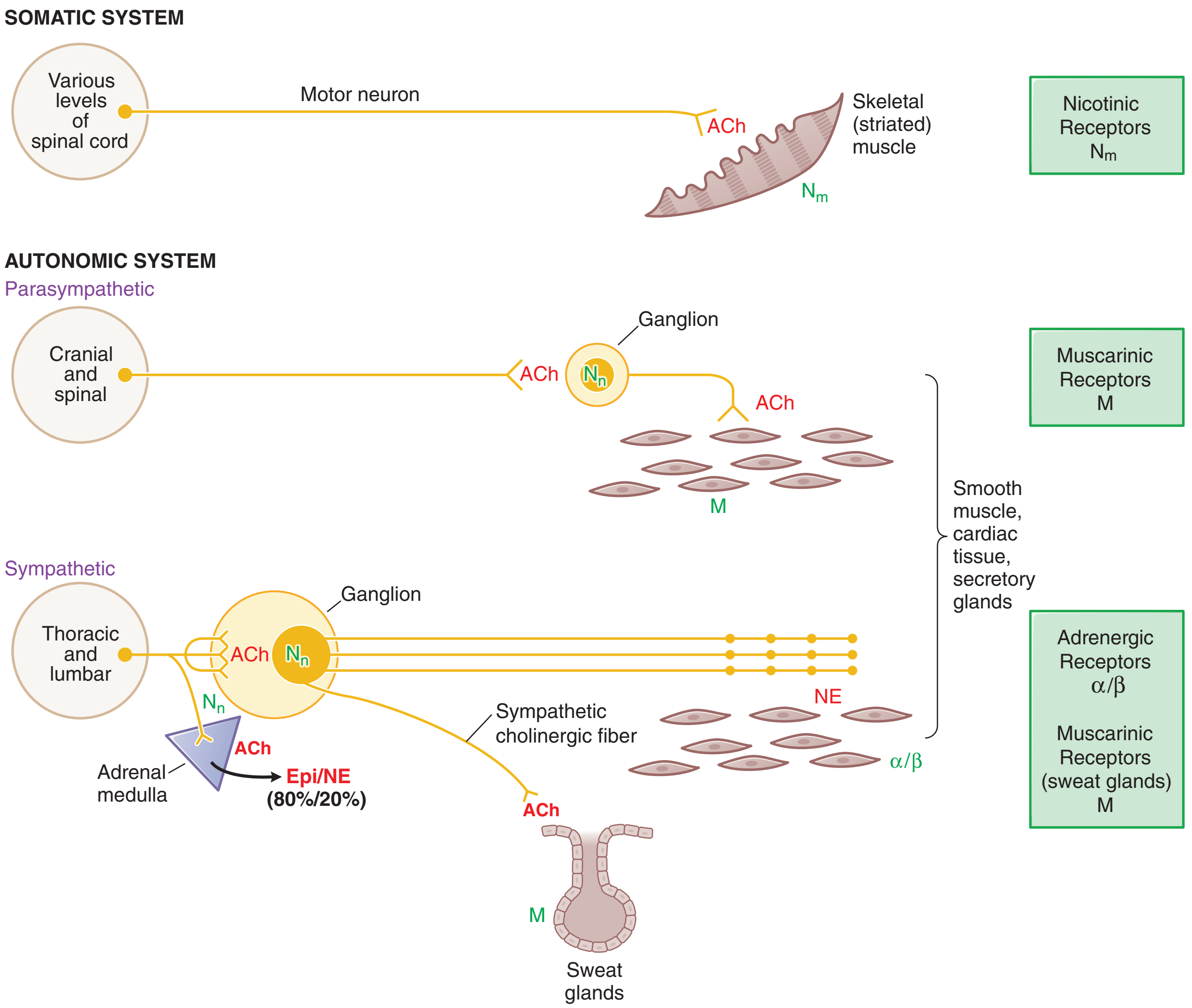
SOMATIC SYSTEM
Various levels of spinal cord
Motor neuron
ACh
Skeletal (striated) muscle
N_m
Nicotinic Receptors N_m
AUTONOMIC SYSTEM
Parasympathetic
Cranial and spinal
ACh
N_n
Ganglion
ACh
M
Muscarinic Receptors M
Smooth muscle, cardiac tissue, secretory glands
Sympathetic
Thoracic and lumbar
Ganglion
ACh
N_n
N_n
ACh
Adrenal medulla
Epi/NE
(80%/20%)
NE
α/β
Sympathetic cholinergic fiber
ACh
M
Sweat glands
Adrenergic Receptors α/β
Muscarinic Receptors (sweat glands) M

Figure 10–2 *Comparative features of somatic motor nerves and efferent nerves of the autonomic nervous system.* The principal neurotransmitters, ACh and NE, are shown in *red*. The receptors for these transmitters, nicotinic (N) and muscarinic (M) cholinergic receptors, α and β adrenergic receptors, are shown in *green*. Somatic nerves innervate skeletal muscle directly at a specialized synaptic junction, the motor end plate, where ACh activates N_m receptors. Autonomic nerves innervate smooth muscles, cardiac tissue, and glands. Both parasympathetic and sympathetic systems have ganglia, where ACh is released by the preganglionic fibers; ACh acts on N_n receptors on the postganglionic nerves. ACh is also the neurotransmitter at cells of the adrenal medulla, where it acts on N_n receptors to cause release of EPI and NE into the circulation. ACh is the dominant neurotransmitter released by postganglionic parasympathetic nerves and acts on muscarinic receptors. The ganglia in the parasympathetic system are near or within the organ being innervated, with generally a one-to-one relationship between pre- and postganglionic fibers. NE is the principal neurotransmitter of postganglionic sympathetic nerves, acting on α or β adrenergic receptors. Autonomic nerves form a diffuse pattern with multiple synaptic sites. In the sympathetic system, the ganglia are generally far from the effector cells (e.g., within the sympathetic chain ganglia). Preganglionic sympathetic fibers may make contact with a large number of postganglionic fibers.

finally synapses with a postganglionic neuron; also, its terminals make contact with a large number of postganglionic neurons. The *parasympathetic system* has terminal ganglia very near or within the organs innervated and is generally more circumscribed in its influences.

- The cell bodies of *somatic motor neurons* reside in the ventral horn of the spinal cord; the axon divides into many branches, each of which innervates a single muscle fiber; more than 100 muscle fibers may be supplied by one motor neuron to form a motor unit. At each neuromuscular junction (NMJ), the axonal terminal loses its myelin sheath and forms a terminal arborization that lies in apposition to a specialized surface of the muscle membrane, termed the *motor end plate* (see Figure 13–4). Reciprocal trophic signals between muscle and nerve regulate the development of the NMJ (Witzemann et al., 2013).
- Ganglionic organization can differ among the different types of nerves and locales. In some organs innervated by the parasympathetic branch, a 1:1 relationship between the number of preganglionic and postganglionic fibers has been suggested. In sympathetic ganglia, one ganglion cell may be supplied by several preganglionic fibers, and the ratio of preganglionic axons to ganglion cells may be 1:20 or more; this organization permits diffuse discharge of the sympathetic system. The ratio of preganglionic vagal fibers to ganglion cells in the myenteric plexus has been estimated as 1:8000.

A Few Details About Innervation

The terminations of the postganglionic autonomic fibers in smooth muscle and glands form a rich plexus, or terminal reticulum. The terminal reticulum (sometimes called the *autonomic ground plexus*) consists of the final ramifications of the postganglionic sympathetic, parasympathetic, and visceral afferent fibers, all of which are enclosed within a frequently interrupted sheath of satellite or Schwann cells. At these interruptions, varicosities packed with vesicles are seen in the efferent fibers. Such varicosities occur repeatedly but at variable distances along the course of the ramifications of the axon.

"Protoplasmic bridges" occur between the smooth muscle fibers themselves at points of contact between their plasma membranes. They are believed to permit the direct conduction of impulses from cell to cell without the need for chemical transmission. These structures have been termed *nexuses,* or *gap* junctions, and they enable the smooth muscle fibers to function as a syncytial unit.

Sympathetic ganglia are extremely complex anatomically and pharmacologically (see Chapter 13). The preganglionic fibers lose their myelin sheaths and divide repeatedly into a vast number of end fibers with diameters ranging from 0.1 to 0.3 μm; except at points of synaptic contact, they retain their satellite cell sheaths. The vast majority of synapses are axodendritic. Apparently, a given axonal terminal may synapse with multiple dendritic processes.

Responses of Effector Organs to Autonomic Nerve Impulses. *In many instances, the sympathetic and parasympathetic neurotransmitters can be viewed as physiological or functional antagonists* (Table 10–1). Most viscera are innervated by both divisions of the autonomic nervous system, and their activities on specific structures may be either discrete and independent or integrated and interdependent. The effects of sympathetic and parasympathetic stimulation of the heart and the iris show a pattern of functional antagonism in controlling heart rate and pupillary aperture, respectively, whereas their actions on male sexual organs are complementary and are integrated to promote sexual function. These physiological effects are mediated through negative, and sometimes positive, feedback mechanisms.

From the responses of the various effector organs to autonomic nerve impulses and the knowledge of the intrinsic autonomic tone, one can predict the actions of drugs that mimic or inhibit the actions of these nerves.

General Functions of the Autonomic Nervous System. *The autonomic nervous system is the primary regulator of the constancy of the internal environment of the organism, or the maintenance of homeostasis.*

The *sympathetic system* and its associated adrenal medulla are not essential to life in a controlled environment, but the lack of sympathoadrenal functions becomes evident under circumstances of stress. For example, in the absence of the sympathetic system, body temperature cannot be regulated when environmental temperature varies; the concentration of glucose in blood does not rise in response to urgent need; compensatory vascular responses to hemorrhage, oxygen deprivation, excitement, and exercise are lacking; and resistance to fatigue is lessened. Sympathetic components of instinctive reactions to the external environment are lost, and other serious deficiencies in the protective forces of the body are discernible. The sympathetic system normally is continuously active, the degree of activity varying from moment to moment and from organ to organ, adjusting to a constantly changing environment in order to maintain homeostasis. The sympathoadrenal system can discharge as a unit. Heart rate is accelerated; blood pressure rises; blood flow is shifted from the skin and splanchnic region to the skeletal and cardiac muscles; blood glucose rises; the bronchioles and pupils dilate; and the organism is better prepared for "fight or flight." Many of these effects result primarily from or are reinforced by the actions of EPI secreted by the adrenal medulla.

The *parasympathetic system* is organized mainly for discrete and localized discharge. Although it is concerned primarily with conservation of energy and maintenance of organ function during periods of minimal

HISTORICAL PERSPECTIVE

The earliest concrete proposal of a neurohumoral mechanism was made shortly after the turn of the 20th century. Lewandowsky and Langley independently noted the similarity between the effects of injection of extracts of the adrenal gland and stimulation of sympathetic nerves. In 1905, T. R. Elliott, while a student with Langley at Cambridge, postulated that sympathetic nerve impulses release minute amounts of an EPI-like substance in immediate contact with effector cells. He considered this substance to be the chemical step in the process of transmission. He also noted that long after sympathetic nerves had degenerated, the effector organs still responded characteristically to the hormone of the adrenal medulla. Langley suggested that effector cells have excitatory and inhibitory "receptive substances" and that the response to EPI depended on which type of substance was present. In 1907, Dixon, impressed by the correspondence between the effects of the alkaloid muscarine and the responses to vagal stimulation, advanced the concept that the vagus nerve liberated a muscarine-like substance that acted as a chemical transmitter of its impulses. In the same year, Reid Hunt described the actions of ACh and other choline esters. In 1914, Dale investigated the pharmacological properties of ACh and other choline esters and distinguished its nicotine-like and muscarine-like actions. Intrigued with the remarkable fidelity with which this drug reproduced the responses to stimulation of parasympathetic nerves, he introduced the term *parasympathomimetic* to characterize its effects. Dale also noted the brief duration of action of this chemical and proposed that an esterase in the tissues rapidly splits ACh to acetic acid and choline, thereby terminating its action.

The studies of Loewi, begun in 1921, provided the first direct evidence for the chemical mediation of nerve impulses by the release of specific chemical agents. Loewi stimulated the vagus nerve of a perfused (donor) frog heart and allowed the perfusion fluid to come in contact with a second (recipient) frog heart used as a test object. The recipient frog heart was found to respond, after a short lag, in the same way as the donor heart. It thus was evident that a substance was liberated from the first organ that slowed the rate of the second. Loewi referred to this chemical substance as *Vagusstoff* ("vagus substance," "parasympathin"); subsequently, Loewi and Navratil presented evidence of its identity as ACh. Loewi also discovered that an accelerator substance similar to EPI and called *Acceleranstoff* was liberated into the perfusion fluid in summer, when the action of the sympathetic fibers in the frog's vagus, a mixed nerve, predominated over that of the inhibitory fibers. Feldberg and Krayer demonstrated in 1933 that the cardiac "vagus substance" also is ACh in mammals.

In the same year as Loewi's discovery, Cannon and Uridil reported that stimulation of the sympathetic hepatic nerves resulted in the release of an EPI-like substance that increased blood pressure and heart rate. Subsequent experiments firmly established that this substance is the chemical mediator liberated by sympathetic nerve impulses at neuroeffector junctions. Cannon called this substance "sympathin." In many of its pharmacological and chemical properties, sympathin closely resembled EPI, but also differed in certain important respects. As early as 1910, Barger and Dale noted that the effects of sympathetic nerve stimulation were reproduced more closely by the injection of sympathomimetic primary amines than by that of EPI or other secondary amines. The possibility that demethylated EPI (NE) might be sympathin had been advanced repeatedly, but definitive evidence for its being the sympathetic nerve mediator was not obtained until specific assays were developed for the determination of sympathomimetic amines in extracts of tissues and body fluids. In 1946, von Euler found that the sympathomimetic substance in highly purified extracts of bovine splenic nerve resembled NE by all criteria used (von Euler, 1946).

We now know that NE is the predominant sympathomimetic substance in the postganglionic sympathetic nerves of mammals and is the adrenergic mediator liberated by their stimulation. NE, its immediate precursor dopamine (DA), and its *N*-methylated derivative EPI also are neurotransmitters in the CNS (see Chapter 16). As for ACh, in addition to its role as the transmitter of most postganglionic parasympathetic fibers and of a few postganglionic sympathetic fibers, ACh functions as a neurotransmitter in three additional classes of nerves: preganglionic fibers of both the sympathetic and the parasympathetic systems, motor nerves to skeletal muscle, and certain neurons within the CNS.

TABLE 10–1 ■ RESPONSES OF EFFECTOR ORGANS TO AUTONOMIC NERVE IMPULSES

ORGAN SYSTEM	SYMPATHETIC EFFECT^{a}	ADRENERGIC RECEPTOR SUBTYPE^{b}	PARASYMPATHETIC EFFECT^{a}	CHOLINERGIC RECEPTOR SUBTYPE^{b}
Eye				
Radial muscle, iris pupillary dilator (dilator pupillae)	Contraction (mydriasis)++	α_1		
Sphincter muscle, iris pupillary constrictor (sphincter pupillae)			Contraction (miosis)+++	M_3, M_2
Ciliary muscle	Relaxation for far vision+	β_2	Contraction for near vision+++	M_3, M_2
Lacrimal glands	Secretion+	α	Secretion+++	M_3, M_2
Heart^{c}				
Sinoatrial node	↑ heart rate++	$\beta_1 > \beta_2$	↓ heart rate+++	$M_2 >> M_3$
Atria	↑ contractility and conduction velocity++	$\beta_1 > \beta_2$	↓ contractility++ and shortened AP duration	$M_2 >> M_3$
Atrioventricular node	↑ automaticity and conduction velocity++	$\beta_1 > \beta_2$	↓ conduction velocity; AV block+++	$M_2 >> M_3$
His-Purkinje system	↑ automaticity and conduction velocity	$\beta_1 > \beta_2$	Little effect	$M_2 >> M_3$
Ventricle	↑ contractility, conduction velocity, automaticity, and rate of idioventricular pacemakers+++	$\beta_1 > \beta_2$	Slight ↓ in contractility	$M_2 >> M_3$
Blood vessels				
Arteries and arterioles^{d}				
Coronary	Constriction+; dilation^{e}++	α_1, α_2; β_2	No innervation^{h}	—
Skin and mucosa	Constriction+++	α_1, α_2	No innervation^{h}	—
Skeletal muscle	Constriction; dilation^{e,f}++	α_1; β_2	Dilation^{h} (?)	—
Cerebral	Constriction (slight)	α_1	No innervation^{h}	—
Pulmonary	Constriction+; dilation	α_1; β_2	No innervation^{h}	—
Abdominal viscera	Constriction+++; dilation+	α_1; β_2	No innervation^{h}	—
Salivary glands	Constriction+++	α_1, α_2	Dilation^{h}++	M_3
Renal	Constriction++; dilation++	α_1, α_2; β_1, β_2	No innervation^{h}	
(Veins)^{d}	Constriction; dilation	α_1, α_2; β_2		
Endothelium	—	—	↑ NO synthase^{h}	M_3
Lung				
Tracheal and bronchial smooth muscle	Relaxation	β_2	Contraction	$M_2 = M_3$
Bronchial glands	↓ secretion, ↑ secretion	α_1	Stimulation	M_2, M_3
		β_2		
Stomach				
Motility and tone	↓ (usually)^{i}+	α_1, α_2, β_1, β_2	↑^{i}+++	$M_2 = M_3$
Sphincters	Contraction (usually)+	α_1	Relaxation (usually)+	M_3, M_2
Secretion	Inhibition	α_2	Stimulation++	M_3, M_2
Intestine				
Motility and tone	Decrease^{h}+	α_1, α_2, β_1, β_2	↑^{i}+++	M_3, M_2
Sphincters	Contraction+	α_1	Relaxation (usually)+	M_3, M_2
Secretion	↓	α_2	↑++	M_3, M_2
Gallbladder and ducts kidney	Relaxation+	β_2	Contraction+	M
Renin secretion	↓+; ↑++	α_1; β_1	No innervation	—
Urinary bladder				
Detrusor	Relaxation+	β_2	Contraction+++	$M_3 > M_2$

(Continued)

TABLE 10–1 ■ RESPONSES OF EFFECTOR ORGANS TO AUTONOMIC NERVE IMPULSES (*CONTINUED*)

ORGAN SYSTEM	SYMPATHETIC EFFECT[a]	ADRENERGIC RECEPTOR SUBTYPE[b]	PARASYMPATHETIC EFFECT[a]	CHOLINERGIC RECEPTOR SUBTYPE[b]
Trigone and sphincter	Contraction++	α_1	Relaxation++	$M_3 > M_2$
Ureter				
Motility and tone	↑	α_1	↑ (?)	M
Uterus	Pregnant contraction	α_1		
	Relaxation	β_2	Variable[j]	M
	Nonpregnant relaxation	β_2		
Sex organs, male skin	Ejaculation+++	α_1	Erection+++	M_3
Pilomotor muscles	Contraction++	α_1	—	
Sweat glands	Localized secretion[k]++	α_1	—	
		—	Generalized secretion+++	M_3, M_2
Spleen capsule	Contraction+++	α_1	—	—
	Relaxation+	β_2	—	
Adrenal medulla	—		Secretion of EPI and NE	N $(\alpha_3)_2(\beta_4)_3$; M (secondarily)
Skeletal muscle	Increased contractility; glycogenolysis; K^+ uptake	β_2	—	—
Liver	Glycogenolysis and gluconeogenesis+++	α_1	—	—
		β_2		
Pancreas				
Acini	↓ secretion+	α	Secretion++	M_3, M_2
Islets (β cells)	↓ secretion+++	α_2	—	
	↑ secretion+	β_2		
Fat cells[l]	Lipolysis+++; thermogenesis	α_1, β_1, β_2, β_3	—	—
	Inhibition of lipolysis	α_2		
Salivary glands	K^+ and water secretion+	α_1	K^+ and water secretion+++	M_3, M_2
Nasopharyngeal glands	—		Secretion++	M_3, M_2
Pineal glands	Melatonin synthesis	β	—	
Posterior pituitary	ADH secretion	β_1	—	
Autonomic nerve endings				
Sympathetic terminal				
Autoreceptor	Inhibition of NE release	$\alpha_{2A} > \alpha_{2C}(\alpha_{2B})$		
Heteroreceptor	—		Inhibition of NE release	M_2, M_4
Parasympathetic terminal				
Autoreceptor	—	—	Inhibition of ACh release	M_2, M_4
Heteroreceptor	Inhibition ACh release	$\alpha_{2A} > \alpha_{2C}$	—	—

[a] Responses are designated + to +++ to provide an approximate indication of the importance of sympathetic and parasympathetic nerve activity in the control of the various organs and functions listed.

[b] Adrenergic receptors: α_1, α_2 and subtypes thereof; β_1, β_2, β_3. Cholinergic receptors: nicotinic (N); muscarinic (M), with subtypes 1–4. The receptor subtypes are described more fully in Chapters 11 and 14 and in Tables 10–2, 10–3, 10–6, and 10–7. When a designation of subtype is not provided, the nature of the subtype has not been determined unequivocally. Only the principal receptor subtypes are shown. Transmitters other than ACh and NE contribute to many of the responses.

[c] In the human heart, the ratio of β_1 to β_2 is about 3:2 in atria and 4:1 in ventricles. While M_2 receptors predominate, M_3 receptors are also present.

[d] The predominant α_1 receptor subtype in most blood vessels (both arteries and veins) is α_{1A}, although other α_1 subtypes are present in specific blood vessels. The α_{1D} is the predominant subtype in the aorta.

[e] Dilation predominates *in situ* owing to metabolic autoregulatory mechanisms.

[f] Over the usual concentration range of physiologically released circulating EPI, the β receptor response (vasodilation) predominates in blood vessels of skeletal muscle and liver; β receptor response (vasoconstriction) predominates in blood vessels of other abdominal viscera. The renal and mesenteric vessels also contain specific dopaminergic receptors whose activation causes dilation.

[g] Sympathetic cholinergic neurons cause vasodilation in skeletal muscle beds, but this is not involved in most physiological responses.

[h] The endothelium of most blood vessels releases NO, which causes vasodilation in response to muscarinic stimuli. However, unlike the receptors innervated by sympathetic cholinergic fibers in skeletal muscle blood vessels, these muscarinic receptors are not innervated and respond only to exogenously added muscarinic agonists in the circulation.

[i] While adrenergic fibers terminate at inhibitory β receptors on smooth muscle fibers and at inhibitory β receptors on parasympathetic (cholinergic) excitatory ganglion cells of the myenteric plexus, the primary inhibitory response is mediated via enteric neurons through NO, P2Y receptors, and peptide receptors.

[j] Uterine responses depend on stages of menstrual cycle, amount of circulating estrogen and progesterone, and other factors.

[k] Palms of hands and some other sites ("adrenergic sweating").

[l] There is significant variation among species in the receptor types that mediate certain metabolic responses. All three β adrenergic receptors have been found in human fat cells. Activation of β_3 receptors produces a vigorous thermogenic response as well as lipolysis. The significance is unclear. Activation of β receptors also inhibits leptin release from adipose tissue.

activity, its elimination is not compatible with life. The parasympathetic system slows the heart rate, lowers the blood pressure, stimulates GI movements and secretions, aids absorption of nutrients, protects the retina from excessive light, and empties the urinary bladder and rectum.

The balance of the activity of the sympathetic and parasympathetic systems establishes the basal, homeostatic state of the organism.

Neurochemical Transmission

Nerve impulses elicit responses in smooth, cardiac, and skeletal muscles; exocrine glands; and postsynaptic neurons by liberating specific chemical neurotransmitters.

Evidence for Neurohumoral Transmission

The concept of neurohumoral transmission or chemical neurotransmission was developed primarily to explain observations relating to the transmission of impulses from postganglionic autonomic fibers to effector cells. Evidence supporting this concept includes the following:

- Demonstration of the presence of a physiologically active compound and its biosynthetic enzymes at appropriate sites
- Recovery of the compound from the perfusate of an innervated structure during periods of nerve stimulation but not (or in greatly reduced amounts) in the absence of stimulation
- Demonstration that the compound is capable of producing responses identical to responses to nerve stimulation
- Demonstration that the responses to nerve stimulation and to the administered compound are modified in the same manner by various drugs, usually competitive antagonists

While these criteria are applicable for most neurotransmitters, including NE and ACh, there are now exceptions to these general rules. For instance, NO has been found to be a neurotransmitter in a few postganglionic parasympathetic nerves; in nonadrenergic, noncholinergic neurons in the periphery; in the ENS; and in the CNS. However, NO is not stored in neurons and released by exocytosis. Rather, it is synthesized when needed and readily diffuses across membranes.

Neurotransmission in the peripheral nervous system and CNS once was believed to conform to the hypothesis that each neuron contains only one transmitter substance. However, we now know that synaptic transmission can be mediated by the release of more than one neurotransmitter. Additional peptides, such as enkephalin, substance P, neuropeptide Y (NPY), VIP, and SST; purines such as ATP and adenosine; eicosanoids and endocannabinoids; and small molecules such as NO have been found in nerve endings along with the "classical" biogenic amine neurotransmitters. These additional substances can depolarize or hyperpolarize various nerve terminals and postsynaptic cells. For example, enkephalins are found in postganglionic sympathetic neurons and adrenal medullary chromaffin cells. VIP is localized selectively in peripheral cholinergic neurons that innervate exocrine glands, and NPY is found in sympathetic nerve endings and released along with NE and ATP. These observations suggest that synaptic transmission in many instances may be mediated by the release of more than one neurotransmitter (see the next section).

Steps Involved in Neurotransmission

The sequence of events involved in neurotransmission is of particular importance because pharmacologically active agents modulate the individual steps.

Axonal Conduction

Conduction refers to the passage of an electrical impulse along an axon or muscle fiber. At rest, the interior of the typical mammalian axon is about 70 mV negative to the exterior. In response to depolarization to a threshold level, an action potential is initiated at a local region of the membrane. The action potential consists of two phases. Following depolarization that induces an open conformation of the channel, the *initial phase* is caused by a rapid increase in the permeability and inward movement of Na^+ through voltage-sensitive Na^+ channels, and a rapid depolarization from the resting potential continues to a positive overshoot. The *second phase* results from the rapid inactivation of the Na^+ channel and the delayed opening of a K^+ channel, which permits outward movement of K^+ to terminate the depolarization. Although not important in axonal conduction, Ca^{2+} channels in other tissues (e.g., L-type Ca^{2+} channels in heart) contribute to the action potential by prolonging depolarization by an inward movement of Ca^{2+}. This influx of Ca^{2+} also serves as a stimulus to initiate intracellular events (Catterall, 2000), and Ca^{2+} influx is important in excitation-exocytosis coupling (transmitter release).

The transmembrane ionic currents produce local circuit currents such that adjacent resting channels in the axon are activated, and excitation of an adjacent portion of the axonal membrane occurs, leading to propagation of the action potential without decrement along the axon. The region that has undergone depolarization remains momentarily in a refractory state to ensure that depolarization proceeds in the appropriate (forward) direction.

With the exception of the local anesthetics, few drugs modify axonal conduction in the doses employed therapeutically. The puffer fish poison, *tetrodotoxin*, and a close congener found in some shellfish, *saxitoxin*, selectively block axonal conduction by blocking the voltage-sensitive Na^+ channel and preventing the increase in Na^+ permeability associated with the rising phase of the action potential. In contrast, *batrachotoxin*, an extremely potent steroidal alkaloid secreted by a South American frog, produces paralysis through a selective increase in permeability of the Na^+ channel, which induces a persistent depolarization. Scorpion toxins are peptides that also cause persistent depolarization by inhibiting the inactivation process (Catterall, 2000). Na^+ and Ca^{2+} channels are discussed in more detail in Chapters 13, 16, and 25.

Junctional Transmission

The term *transmission* refers to the passage of an impulse across a synaptic or neuroeffector junction. The arrival of the action potential at the axonal terminals initiates a series of events that trigger transmission of an excitatory or inhibitory biochemical message across the synapse or neuroeffector junction. These events, diagrammed in Figures 10–3, 10–4, and 10–5, are the following:

1. *Storage and release of transmitter*. The nonpeptide (small-molecule) neurotransmitters, such as biogenic amines, are largely synthesized in the region of the axonal terminals and stored there in synaptic vesicles. Neurotransmitter transport into storage vesicles is driven by an electrochemical gradient generated by the vesicular proton pump (vesicular ATPase) (Figures 10–5 and 10–6). Synaptic vesicles cluster in discrete areas underlying the presynaptic plasma membrane, termed *active zones*, often aligning with the tips of postsynaptic folds. Proteins in the vesicular membrane (e.g., synapsin, synaptophysin, synaptogyrin) are involved in development and trafficking of the storage vesicle to the active zone. The processes of priming, docking, fusion, and exocytosis involve the interactions of proteins in the vesicular and plasma membranes and the rapid entry of extracellular Ca^{2+} and its binding to synaptotagmins (Figure 10–4).

Life Cycle of a Storage Vesicle; Molecular Mechanism of Exocytosis. Fusion of the storage vesicle and plasma membrane involves formation of a multiprotein complex that includes proteins in the membrane of the synaptic vesicle, proteins embedded in the inner surface of the plasma membrane, and several cytosolic components. These proteins are referred to as soluble NSF attachment protein (SNAP) receptor (SNARE) proteins. Through the assembly of these proteins, vesicles draw near the membrane (priming, docking), spatially prepared for the next step, which the entry of Ca^{2+} initiates. When Ca^{2+} enters with the action potential, fusion and exocytosis occur rapidly. After fusion, the chaperone ATPase *N*-ethylmaleamide sensitive factor (NSF) and its SNAP adapters catalyze dissociation of the SNARE complex. Figures 10–4 and 10–5 depict this life cycle. Figure 10–4 shows some details of the assembly of the SNARE protein complex leading to fusion and exocytosis of neurotransmitter. The isoforms of the participating proteins may

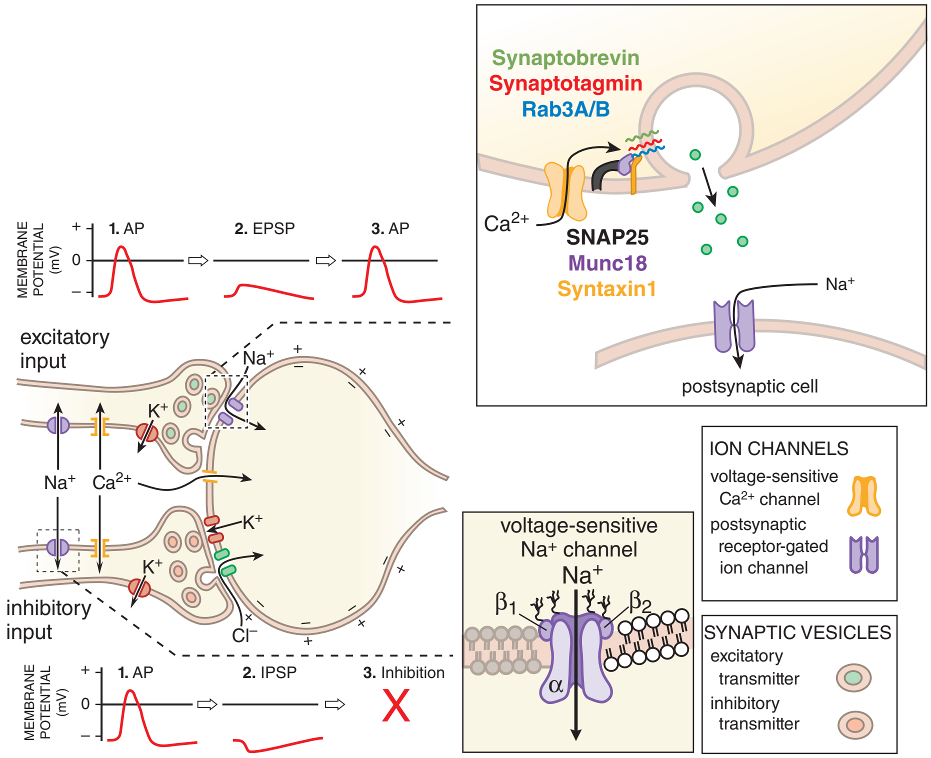

Figure 10–3 *Steps involved in excitatory and inhibitory neurotransmission.* ***1.*** The nerve action potential (AP) consists of a transient self-propagated reversal of charge on the axonal membrane. The membrane potential E_i goes from a negative value, through zero potential, to a slightly positive value, primarily through increases in Na^+ permeability, and then returns to resting values by an increase in K^+ permeability. When the AP arrives at the presynaptic terminal, it initiates release of the excitatory or inhibitory transmitter. Depolarization at the nerve ending and entry of Ca^{2+} initiate docking and then fusion of the synaptic vesicle with the membrane of the nerve ending. Some of the SNARE proteins involved in docking and fusion are shown. Figures 10–4 and 10–5 show additional details of the life cycle of neurotransmitter storage vesicle and exocytosis. ***2.*** Interaction of the excitatory transmitter with postsynaptic receptors produces a localized depolarization, the EPSP, through an increase in permeability to cations, most notably Na^+. The inhibitory transmitter causes a selective increase in permeability to K^+ or Cl^-, resulting in a localized hyperpolarization, the IPSP. ***3.*** The EPSP initiates a conducted AP in the postsynaptic neuron; this can be prevented, however, by the hyperpolarization induced by a concurrent IPSP. The transmitter is dissipated by enzymatic destruction, by reuptake into the presynaptic terminal or adjacent glial cells, or by diffusion. Depolarization of the postsynaptic membrane can permit Ca^{2+} entry if voltage-gated Ca^{2+} channels are present.

differ in different neurotransmitter systems, but the general mechanism seems to be conserved.

During the resting state, there is continual slow release of isolated quanta of the transmitter; this produces electrical responses (*miniature end-plate potentials*) at the postjunctional membrane that are associated with the maintenance of the physiological responsiveness of the effector organ. A low level of spontaneous activity within the motor units of skeletal muscle is particularly important because skeletal muscle lacks inherent tone.

The action potential causes the synchronous release of several hundred quanta of neurotransmitter. In the fusion/exocytosis process, the contents of the vesicles, including enzymes and other proteins, are discharged to the synaptic space. Synaptic vesicles may either fully exocytose with complete fusion or form a transient, nanometer-size pore that closes after transmitter has escaped, "kiss-and-run" exocytosis. In full-fusion exocytosis, the pit formed by the vesicle's fusing with the plasma membrane is clathrin-coated and retrieved from the membrane via endocytosis and transported to an endosome for full recycling. During kiss-and-run exocytosis, the pore closes, and the vesicle is immediately and locally recycled for reuse in neurotransmitter repackaging (Alabi and Tsien, 2013; Südhof, 2014).

Modulation of Transmitter Release. A number of autocrine and paracrine factors may influence the exocytotic process, including the released neurotransmitter itself. Adenosine, DA, glutamate, γ-aminobutyric acid (GABA), prostaglandins, and enkephalins influence neurally mediated release of neurotransmitters. Receptors for these factors exist in the membranes of the soma, dendrites, and axons of neurons (Miller, 1998). *Soma-dendritic receptors*, when activated, primarily modify functions of the soma-dendritic region, such as protein synthesis and generation of action potentials. *Presynaptic receptors*, when activated, modify functions of the terminal region, such as synthesis and release of transmitters.

Two main classes of presynaptic receptors have been identified on most neurons: *Heteroreceptors* are presynaptic receptors that respond to neurotransmitters, neuromodulators, or neurohormones released from adjacent neurons or cells. For example, NE can influence the release of ACh from parasympathetic neurons by acting on α_{2A}, α_{2B}, and α_{2C} receptors, whereas ACh can influence the release of NE from sympathetic neurons by acting on M_2 and M_4 receptors. *Autoreceptors* are receptors located on or close to axon terminals of a neuron through which the neuron's own transmitter can modify transmitter synthesis and release (see Figures 10–6 and 10–8). For example, NE released from sympathetic neurons may interact with α_{2A} and α_{2C} receptors to inhibit neurally released NE. Similarly, ACh released from parasympathetic neurons may interact with M_2 and M_4 receptors to inhibit neurally released ACh.

2. *Interaction of the transmitter with postjunctional receptors and production of a postjunctional potential.* The transmitter diffuses across the synaptic or junctional cleft and combines with specialized receptors on the postjunctional membrane; this often results in a localized increase in the ionic permeability, or conductance, of the membrane. With certain exceptions (noted in the following discussion), one of three types of permeability change can occur:

- Generalized increase in the permeability to cations (notably Na^+ but occasionally Ca^{2+}), resulting in a localized depolarization of the membrane, that is, an excitatory postsynaptic potential (EPSP).
- Selective increase in permeability to anions, usually Cl^-, resulting in stabilization or actual hyperpolarization of the membrane, which constitutes an inhibitory postsynaptic potential (IPSP).

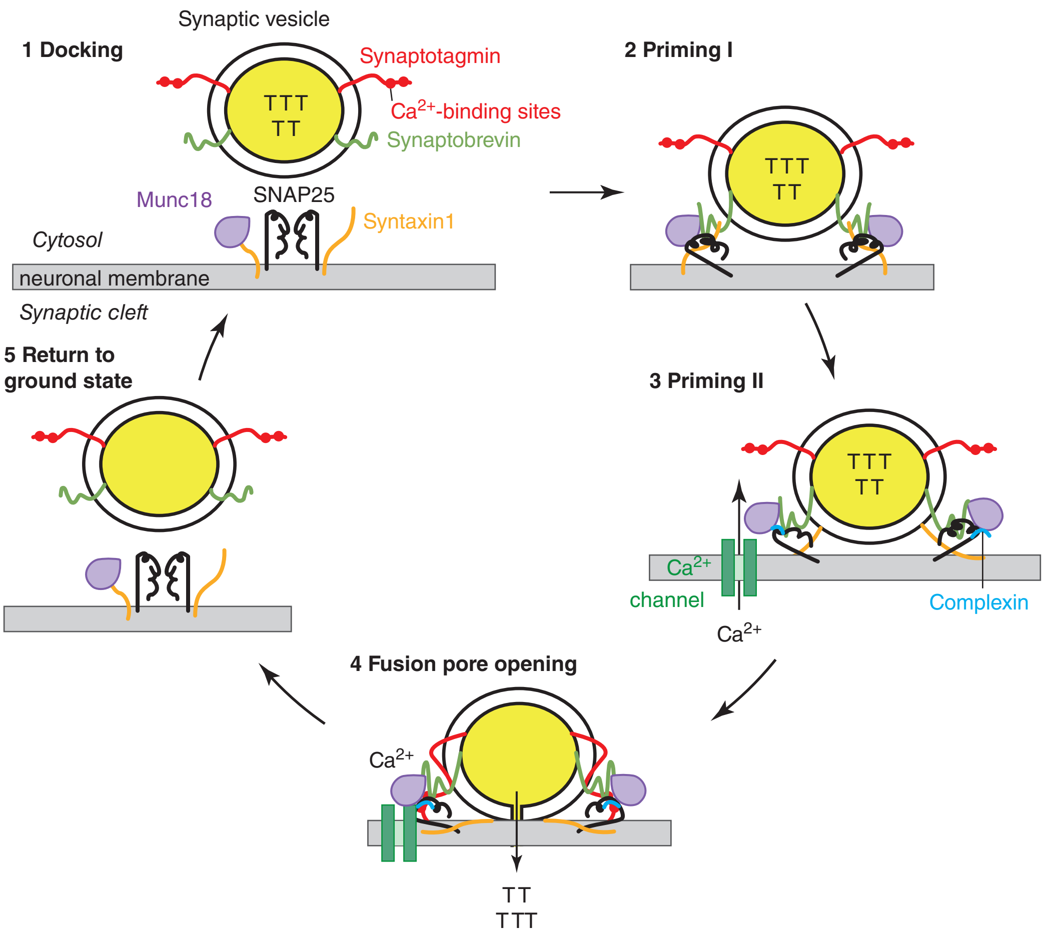

Figure 10–4 *Molecular basis of exocytosis: docking and fusion of synaptic vesicles with neuronal membranes.* **1.** *Vesicular docking in the active zone*: Munc18 binds to syntaxin 1, stabilizing the neuronal membrane SNARE proteins. **2.** *Priming I*: Syntaxin assembles with SNAP25, allowing for the vesicle SNARE protein synaptobrevin to bind to the complex. **3.** *Priming II*: Complexin binds to the SNARE complex and allows for the vesicular synaptotagmin to bind Ca^{2+} that drives the full fusion process. **4.** *Fusion pore opening*: Synaptotagmin interacts with the SNARE complex and binds Ca^{2+}, permitting pore fusion and exocytosis of neurotransmitter. Other components, not shown, are the vesicular GTP-binding Rab3/27; the linking proteins Munc13, RIM, and RIM-BP; and tethering to the Ca^{2+} channel. **5.** *Return to ground state*: After fusion, the chaperone ATPase NSF and its SNAP adapters catalyze dissociation of the SNARE complex. For a more detailed view of this process, see Südhof (2014).

- Increased permeability to K$^+$. Because the K$^+$ gradient is directed out of the cell, hyperpolarization and stabilization of the membrane potential occur (an IPSP).

Electric potential changes associated with the EPSP and IPSP at most sites are the results of passive fluxes of ions down their concentration gradients. The changes in channel permeability that cause these potential changes are specifically regulated by the specialized postjunctional receptors for the neurotransmitter that initiates the response (see Figures 10–4, 10–6, and 10–8 and Chapter 16). These receptors may be clustered on the effector cell surface, as seen at the NMJs of skeletal muscle and other discrete synapses, or distributed more uniformly, as observed in smooth muscle. These *high-conductance, ligand-gated ion channels* usually permit passage of Na$^+$ or Cl$^-$; K$^+$ and Ca^{2+} are involved less frequently. In the presence of an appropriate neurotransmitter, the channel opens rapidly to a high-conductance state, remains open for about a millisecond, and then closes. A short square-wave pulse of current is observed as a result of the channel's opening and closing. The summation of these microscopic events gives rise to the EPSP.

The ligand-gated channels belong to a superfamily of ionotropic receptor proteins that includes the nicotinic, glutamate, and certain 5HT$_3$ and purine receptors, which conduct primarily Na$^+$, cause depolarization, and are excitatory; and GABA acid and glycine receptors, which conduct Cl$^-$, cause hyperpolarization, and are inhibitory. Neurotransmitters also can modulate the permeability of K$^+$ and Ca^{2+} channels indirectly. In these cases, the receptor and channel are separate proteins, and information is conveyed between them by G proteins (see Chapter 3).

The nicotinic, GABA, glycine, and 5HT$_3$ receptors are closely related, whereas the glutamate and purinergic ionotropic receptors have distinct structures (see Figure 13–1 and Chapter 16). Neurotransmitters also can modulate the permeability of K$^+$ and Ca^{2+} channels indirectly. In these cases, the receptor and channel are separate proteins, and information is conveyed between them by G proteins. Other receptors for neurotransmitters act by influencing the synthesis of intracellular second messengers and do not necessarily cause a change in membrane potential. The most widely documented examples of receptor regulation of second-messenger systems are the activation or inhibition of adenylyl cyclase to modulate cellular cAMP concentrations and the increase in cytosolic concentrations of Ca^{2+} that results from release of the ion from internal stores by inositol trisphosphate (see Chapter 3).

3. *Initiation of postjunctional activity*. If an EPSP exceeds a certain threshold value, it initiates a propagated action potential in a postsynaptic neuron or a muscle action potential in skeletal or cardiac muscle by activating voltage-sensitive channels in the immediate vicinity. In certain smooth muscle types in which propagated impulses are minimal, an EPSP may increase the rate of spontaneous depolarization, cause Ca^{2+} release, and enhance muscle tone; in gland cells, the EPSP initiates secretion through Ca^{2+} mobilization. An IPSP, which is found

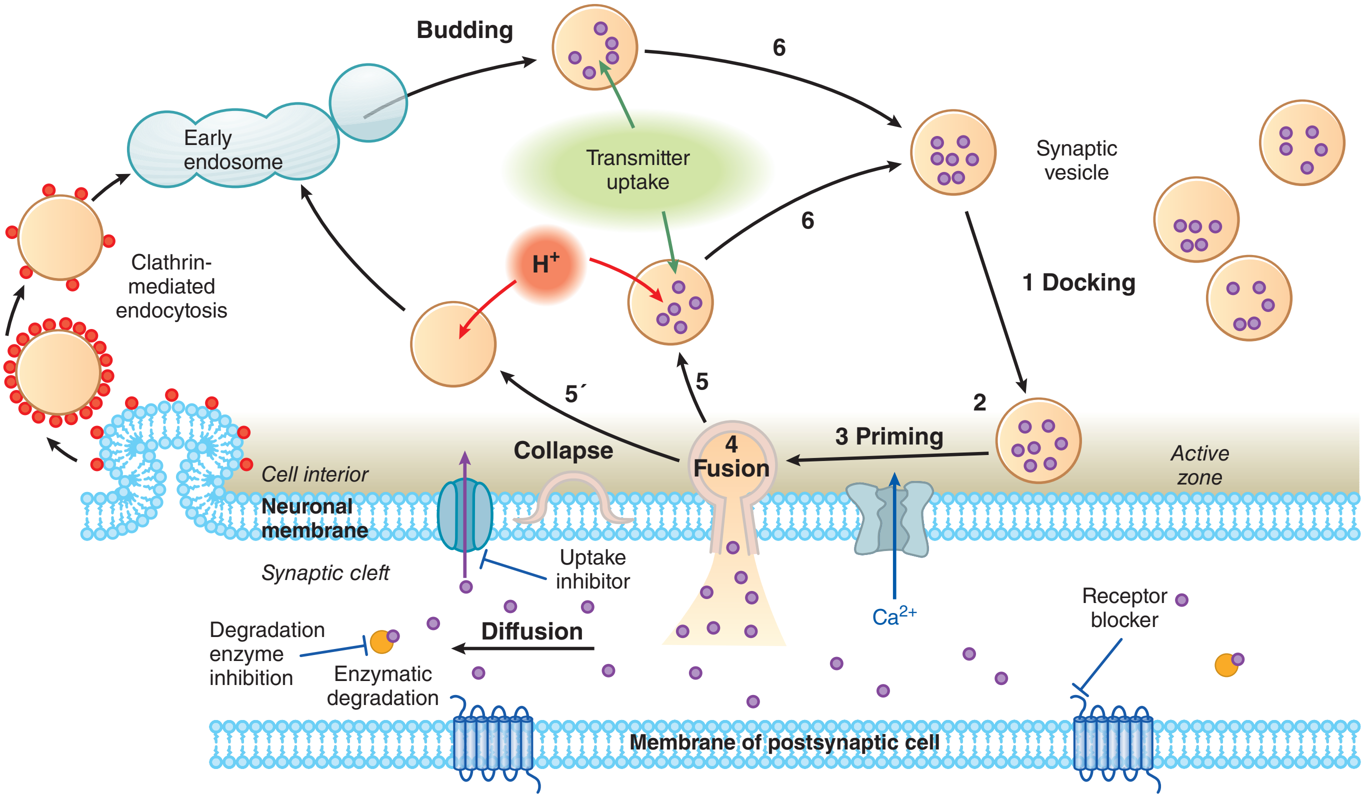

Figure 10–5 *Life cycle of a synaptic vesicle.* A mature storage vesicle, replete with transmitter, is translocated to the perimembrane space (active zone) (1). Once in the active zone (2), the vesicle undergoes docking and priming (see Figure 10–4), as proteins from the cytosol and the vesicular and plasma membranes (SNARE proteins) interact to tether the vesicle in a prefusion stage. The rapid entry of Ca^{2+} via voltage-sensitive channels located in the active zone (3) activates the calcium sensor synaptotagmin and initiates the process of fusion and exocytosis of the vesicular contents into the synaptic space (4). After transmitter release, the vesicle is endocytosed, the SNARE protein complex is disassembled by the action of the chaperone ATPase NSF and its SNAP adapters, and the empty vesicle is recycled, either trafficked directly back into use (5) or routed through an early endosomal pathway (5′). In either event, the vesicular ATPase is at work, promoting H+ uptake to establish the gradient that drives transmitter uptake and repletion of the vesicle (6). Alternatively, full collapse and subsequent endocytosis via a clathrin-mediated process may occur. For a more detailed view of the exocytotic process, see Südhof (2014). Secreted neuropeptides are stored in larger, dense core vesicles (see text). Their secretory process is similar; however, there are no uptake transporters for peptide neurotransmitters; rather, vesicles containing releasable peptides are formed in the trans-Golgi network of the nerve cell body and transported to the release site by molecular motors (kinesins, F-actin, etc.); nonsecreted vesicle components are recycled. Heaslip et al. (2014), Salogiannis and Reck-Peterson (2017), and Milosevic (2018) have reviewed aspects of the transport of such vesicles.

in neurons and smooth muscle but not in skeletal muscle, will tend to oppose excitatory potentials simultaneously initiated by other neuronal sources. Whether a propagated impulse or other response ensues depends on the summation of all the potentials.

4. *Destruction or dissipation of the transmitter*. When impulses can be transmitted across junctions at frequencies up to several hundred per second, there must be an efficient means of disposing of the transmitter following each impulse. At cholinergic synapses involved in rapid neurotransmission, high and localized concentrations of acetylcholinesterase (AChE) are available for this purpose. When AChE activity is inhibited, removal of the transmitter is accomplished principally by diffusion. Under these circumstances, the effects of released ACh are potentiated and prolonged (see Chapter 12).

   Rapid termination of NE occurs by a combination of simple diffusion and reuptake by the axonal terminals of most of the released NE. Termination of the action of amino acid transmitters results from their active transport into neurons and surrounding glia. Peptide neurotransmitters are hydrolyzed by various peptidases and dissipated by diffusion.

5. *Nonelectrogenic functions*. The activity and turnover of enzymes involved in the synthesis and inactivation of neurotransmitters, the density of presynaptic and postsynaptic receptors, and other characteristics of synapses are controlled by trophic actions of neurotransmitters and other trophic factors released by the neuron or target cells. The actions of such factors contribute to the plasticity observed in physiological and pathophysiological states.

Cholinergic Transmission

The neurochemical events that underlie cholinergic neurotransmission are summarized in Figure 10–6.

Synthesis and Storage of ACh

Two enzymes, choline acetyltransferase and AChE, are involved in ACh synthesis and degradation, respectively.

Choline Acetyltransferase. *Choline acetyltransferase* catalyzes the synthesis of ACh—the acetylation of choline with acetyl CoA. ChAT is synthesized within the perikaryon and then is transported along the length of the axon to its terminal. Axonal terminals contain a large number of mitochondria, where acetyl CoA is synthesized. Choline is taken up from the extracellular fluid into the axoplasm by active transport. The final step in the synthesis occurs within the cytoplasm, following which most of the ACh is sequestered within synaptic vesicles. Although moderately potent inhibitors of ChAT exist, they have no therapeutic utility, in part because the rate-limiting step in ACh biosynthesis is the uptake of choline. However, there is a potential for using choline acetyl transferase as an *in vivo* target for biomarkers to enable early diagnosis of Alzheimer's disease and related dementias (Kumar et al., 2017).

Choline and Choline Transport. The availability of choline is critical to the synthesis of ACh. Choline must be derived primarily from the diet (there is little *de novo* synthesis of choline in cholinergic neurons) or, secondarily, from recycling of choline. Once ACh is released from cholinergic

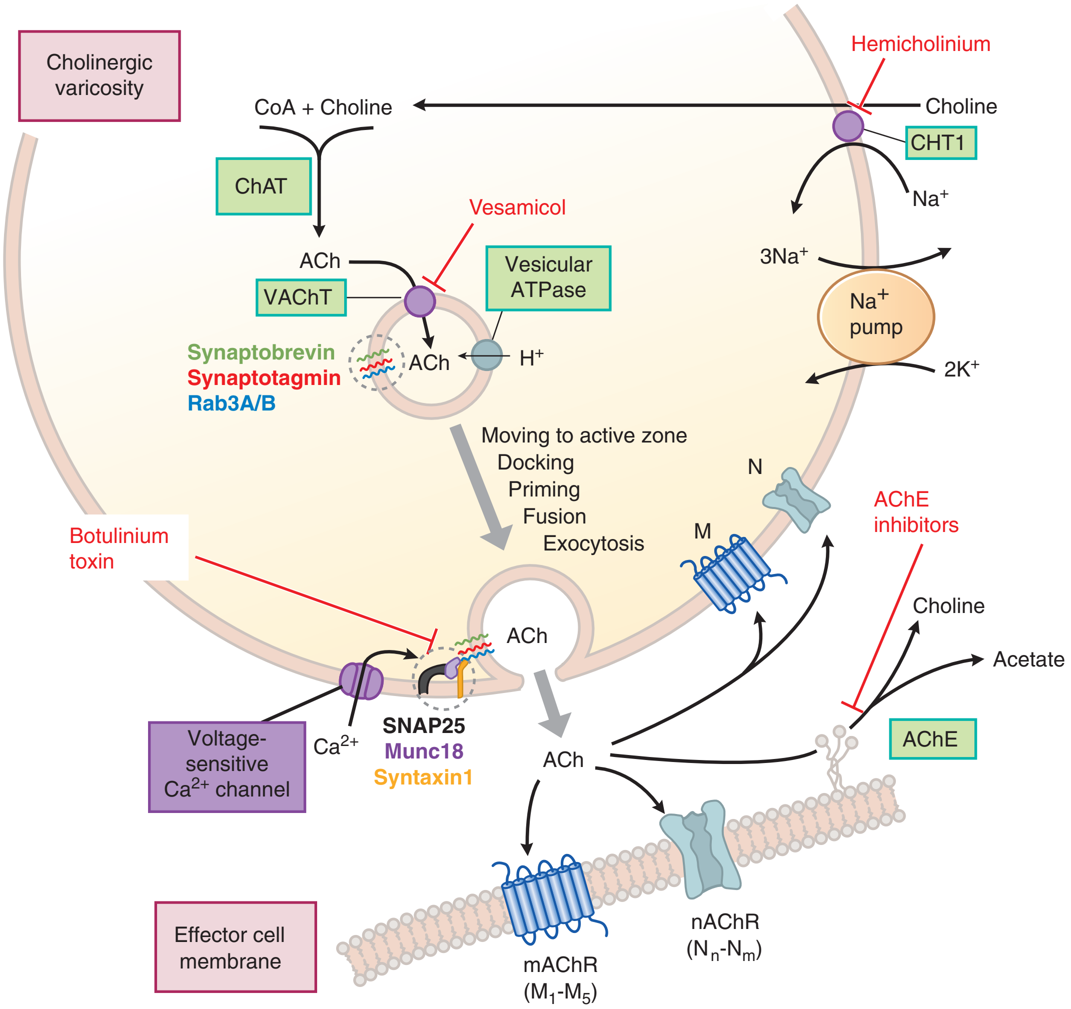

Figure 10–6 *A typical cholinergic neuroeffector junction.* The synthesis of ACh in the varicosity depends on the uptake of choline via a Na$^+$-dependent carrier, CHT1, that hemicholinium can block. The enzyme choline acetyl transferase catalyzes the synthesis of ACh from choline and the acetyl moiety of acetyl CoA. ACh is transported into the storage vesicle by vesicular ACh transporter (VAChT), which can be inhibited by *vesamicol*. ACh is stored in vesicles (along with other potential cotransmitters, such as ATP and VIP, at certain neuroeffector junctions). Release of ACh and any cotransmitters occurs via exocytosis (the stages are itemized along the gray arrow), triggered by Ca^{2+} entry via a voltage-sensitive Ca^{2+} channel in response to membrane depolarization, as described in Figures 10–3, 10–4, and 10–5. Exocytotic release of ACh at the NMJ can be blocked by botulinum toxins, the active fragments of which are endopeptidases that cleave synaptobrevin, an essential member of the SNARE proteins that mediate docking/priming/exocytosis. Once released, ACh can interact with the muscarinic receptors (M), which are GPCRs, or nicotinic receptors (N), which are ligand-gated ion channels, to produce the characteristic response of the postsynaptic cell. ACh also can act on presynaptic mAChRs or nAChRs to modify its own release. The action of ACh is terminated by extracellular metabolism to choline and acetate by AChE, which is associated with synaptic membranes.

neurons in response to an action potential, ACh is hydrolyzed by AChE to acetate and choline. Much of the choline is taken up at cholinergic nerve terminals and reused for ACh synthesis. Under many circumstances, this reuptake and availability of choline appear to be rate-limiting in ACh synthesis. There are three mammalian transport systems for choline; all three are transmembrane proteins with multiple transmembrane segments; all are inhibited by hemicholinium but at distinct concentrations in the same order as their affinities for choline (Haga, 2014):

- The high-affinity (4 μM) choline transporter CHT1 (SLC5A7) present on presynaptic membranes of cholinergic neurons. This transporter is a member of the SLC5 family of solute carrier proteins that includes Na$^+$-glucose cotransporters and shares about 25% homology with those transporters (Haga, 2014). Choline transport by CHT1 is Na$^+$ and Cl$^-$ dependent. This system provides choline for ACh synthesis and is the high-affinity hemicholinium-binding protein (K_i = 0.05 μM).
- A low-affinity (40 μM), Na$^+$-independent transporter, CTL1 (SLC44A), which is widely distributed and appears to supply choline for phospholipid synthesis (e.g., phosphatidyl choline, sphingomyelin).
- A lower-affinity (100 μM), Na$^+$-independent transporter, OCT2 (SLC22A2), a nonspecific organic cation secretory transporter found in renal proximal tubules (see Figures 4–8 and 4–9), hepatocytes, the choroid plexus, the luminal membrane of brain endothelium, and synaptic vesicles from cholinergic neurons. Its role in neurons remains to be clarified.

In model systems, CHT1 localizes mainly to intracellular organelles, including transmitter storage vesicles; neural activity increases the fraction of CHT1 in the plasma membrane, and phosphorylation by protein kinase C (PKC) enhances internalization (Haga, 2014).

Storage of ACh. ACh is transported into synaptic vesicles by the vesicular ACh transporter (a solute carrier protein, SLC18A3) using the potential energy of a proton electrochemical gradient that a vacuolar ATPase establishes, such that the transport of protons out of the vesicle is coupled to uptake of ACh into the vesicle and against a concentration gradient. The process is inhibited by the noncompetitive and reversible inhibitor *vesamicol*, which does not affect the vesicular ATPase (Figure 10–6). The gene for choline acetyltransferase and the vesicular transporter are found at the same locus, with the transporter gene positioned in the first intron of the transferase gene. Hence, a common promoter regulates the expression of both genes.

There appear to be two types of vesicles in cholinergic terminals: electron-lucent vesicles (40–50 nm in diameter) and dense-cored vesicles (80–150 nm). The core of the vesicles contains both ACh and ATP, at a ratio of about 11:1, which are dissolved in the fluid phase with metal ions (Ca^{2+} and Mg^{2+}) and a proteoglycan called vesiculin. Vesiculin, negatively charged and thought to sequester the Ca^{2+} or ACh, is bound within the vesicle, with the protein moiety anchoring it to the vesicular membrane. In some cholinergic terminals, there are peptides, such as VIP, that act as *cotransmitters*. The peptides usually are located in the dense-cored vesicles.

Estimates of the ACh content of synaptic vesicles range from 1000 to over 50,000 molecules per vesicle, with a single motor nerve terminal containing 300,000 or more vesicles. In addition, an uncertain but possibly significant amount of ACh is present in the extravesicular cytoplasm. Recording the electrical events associated with the opening of single channels at the motor end plate during continuous application of ACh has permitted estimation of the potential change induced by a single molecule of ACh (3×10^{-7} V); from such calculations, it is evident that even the lower estimate of the ACh content per vesicle (1000 molecules) is sufficient to account for the magnitude of the miniature end-plate potentials.

Release of ACh. Exocytotic release of ACh and cotransmitters (e.g., ATP, VIP) occurs on depolarization of the nerve terminals. Depolarization of the terminals allows the entry of Ca^{2+} through voltage-gated Ca^{2+} channels and promotes fusion of the vesicular membrane with the plasma membrane, allowing exocytosis to occur, as described previously and shown in Figure 10–6.

Two pools of ACh appear to exist. One pool, the "depot" or "readily releasable" pool, consists of vesicles located near the plasma membrane of the nerve terminals; these vesicles contain newly synthesized transmitter. Depolarization of the terminals causes these vesicles to release ACh rapidly or readily. The other pool, the "reserve pool," seems to replenish the readily releasable pool and may be required to sustain ACh release during periods of prolonged or intense nerve stimulation.

Botulinum toxin blocks ACh release by interfering with the machinery of transmitter release. The active fragments of botulinum toxins are endopeptidases; the SNARE proteins are their substrates. There are eight isotypes of botulinum toxin, each cleaving a specific site on SNARE proteins. Tetanus toxins act similarly, but in the CNS. The active fragments of these toxins cleave synaptobrevin and block exocytosis in specific sets of neurons (inhibitory neurons in the CNS for tetanus, the NMJ for botulinum).

Acetylcholinesterase. At the NMJ, immediate hydrolysis of ACh by AChE reduces lateral diffusion of the transmitter and activation of adjacent receptors. Rapid release of ACh onto the nAChRs of the motor end plate, followed by rapid hydrolysis of the neurotransmitter, spatially limits receptor activation and facilitates rapid control of responses. The time required for hydrolysis of ACh at the NMJ is less than a millisecond. Chapter 12 presents details of the structure, mechanism, and inhibition of AChE.

AChE is found in cholinergic neurons and is highly concentrated at the postsynaptic end plate of the NMJ. Butyrylcholinesterase (BuChE; also called pseudocholinesterase) is virtually absent in neuronal elements of the central and peripheral nervous systems. BuChE is synthesized primarily in the liver and is found in liver and plasma; its likely physiological function is the hydrolysis of ingested esters from plant sources. AChE and BuChE typically are distinguished by the relative rates of ACh and butyrylcholine hydrolysis and by effects of selective inhibitors (see Chapter 12).

Almost all pharmacological effects of the anti-cholinesterase agents are due to the inhibition of AChE, with the consequent accumulation of endogenous ACh in the vicinity of the nerve terminal. Distinct but single genes encode AChE and BuChE in mammals; the diversity of molecular structure of AChE arises from alternative mRNA processing.

Numerous reports suggest that AChE plays roles in addition to its classical function in terminating impulse transmission at cholinergic synapses. Nonclassical functions of AChE might include hydrolysis of ACh in a nonsynaptic context, action as an adhesion protein involved in synaptic development and maintenance or as a bone matrix protein, involvement in neurite outgrowth, and acceleration of the assembly of Aβ peptide into amyloid fibrils.

Characteristics of Cholinergic Transmission at Various Sites

There are marked differences among various sites of cholinergic transmission with respect to architecture and fine structure, the distributions of AChE and receptors, and the temporal factors involved in normal function. In skeletal muscle, for example, the junctional sites occupy a small, discrete portion of the surface of the individual fibers and are relatively isolated from those of adjacent fibers; in the superior cervical ganglion, about 100,000 ganglion cells are packed within a volume of a few cubic millimeters, and both the presynaptic and the postsynaptic neuronal processes form complex networks.

Skeletal Muscle. At the NMJ, ACh stimulates the nicotinic receptor's intrinsic channel, which opens for about 1 msec, admitting about 50,000 Na^+ ions. The channel-opening process is the basis for the localized depolarizing end-plate potential within the end plate, which triggers the muscle action potential and leads to contraction. The amount of ACh (10^{-17} mol) required to elicit an end-plate potential following its microiontophoretic application to the motor end plate of a rat diaphragm muscle fiber is equivalent to that recovered from each fiber following stimulation of the phrenic nerve.

Following sectioning and degeneration of the motor nerve to skeletal muscle or of the postganglionic fibers to autonomic effectors, there is a marked reduction in the threshold doses of the transmitters and of certain other drugs required to elicit a response; that is, denervation supersensitivity occurs. In skeletal muscle, this change is accompanied by a spread of the receptor molecules from the end-plate region to the adjacent portions of the sarcoplasmic membrane, which eventually involves the entire muscle surface. Embryonic muscle also exhibits this uniform sensitivity to ACh prior to innervation. Hence, innervation represses the expression of the receptor gene by the nuclei that lie in extrajunctional regions of the muscle fiber and directs the subsynaptic nuclei to express the structural and functional proteins of the synapse.

Autonomic Effector Cells. Stimulation or inhibition of autonomic effector cells occurs on activation of muscarinic ACh receptors (discussed below). In this case, the effector is coupled to the receptor by a G protein (see Chapter 3). In contrast to skeletal muscle and neurons, smooth muscle and the cardiac conduction system (sinoatrial [SA] node, atrium, atrioventricular node, and the His-Purkinje system) normally exhibit intrinsic activity, both electrical and mechanical, that is modulated but not initiated by nerve impulses.

In the basal condition, unitary smooth muscle exhibits waves of depolarization or spikes that are propagated from cell to cell at rates considerably slower than the action potential of axons or skeletal muscle. The spikes apparently are initiated by rhythmic fluctuations in the membrane resting potential. Application of ACh (0.1–1 μM) to isolated intestinal muscle causes the membrane potential to become less negative and increases the frequency of spike production, accompanied by a rise in tension. A primary action of ACh in initiating these effects through muscarinic receptors is probably partial depolarization of the cell membrane brought about by an increase in Na^+ and, in some instances, Ca^{2+} conductance. ACh also can produce contraction of some smooth muscles when the membrane has been depolarized completely by high concentrations of K^+, provided that Ca^{2+} is present. Hence, ACh stimulates ion fluxes across membranes or mobilizes intracellular Ca^{2+} to cause contraction.

In the heart, spontaneous depolarizations normally arise from the SA node, enabling it to act as a pacemaker. In the cardiac conduction system, particularly in the SA and atrioventricular nodes, stimulation of the cholinergic innervation or the direct application of ACh causes inhibition, associated with hyperpolarization of the membrane and a marked decrease in the rate of depolarization. These effects are due, at least in part, to a selective increase in permeability to K^+.

Autonomic Ganglia. The primary pathway of cholinergic transmission in autonomic ganglia is similar to that at the NMJ of skeletal muscle. The initial depolarization is the result of activation of nAChRs, which are ligand-gated cation channels with properties similar to those found at the NMJ. Several secondary transmitters or modulators either enhance or diminish the sensitivity of the postganglionic cell to ACh (see Figure 13–5).

Prejunctional Sites. ACh release is subject to complex regulation by mediators, including ACh itself acting on M_2 and M_4 *autoreceptors*, and activation of *heteroreceptors* (e.g., NE acting on α_{2A} and α_{2C} adrenergic receptors) or substances produced locally in tissues (e.g., NO) (Philipp and Hein, 2004). ACh-mediated inhibition of ACh release following activation of M_2 and M_4 autoreceptors is a physiological negative-feedback control mechanism. At some neuroeffector junctions (e.g., the myenteric plexus in the GI tract or the cardiac SA node), sympathetic and parasympathetic nerve terminals often lie juxtaposed to each other. There, opposing effects of NE and ACh result not only from the opposite effects of the two neurotransmitters on the smooth muscle or cardiac cells but also from the inhibition of ACh release by NE or inhibition of NE release by ACh acting on heteroreceptors on parasympathetic or sympathetic terminals.

Inhibitory heteroreceptors on parasympathetic terminals include adenosine A_1 receptors, histamine H_3 receptors, opioid receptors, and α_{2A} and α_{2C} adrenergic receptors. The parasympathetic nerve terminal varicosities also may contain additional heteroreceptors that could respond by inhibition or enhancement of ACh release by locally formed autacoids, hormones, or administered drugs.

Extraneuronal Sites. All elements of the cholinergic system are functionally expressed independently of cholinergic innervation in numerous nonneuronal cells. These *nonneuronal cholinergic* systems can both modify and control phenotypic cell functions such as proliferation, differentiation, formation of physical barriers, migration, and ion and water movements.

The widespread synthesis of ACh in nonneuronal cells has changed the thinking that ACh acts only as a neurotransmitter. Each component of the cholinergic system in nonneuronal cells can be affected by pathophysiological conditions. Dysfunctions of nonneuronal cholinergic systems may be involved in the pathogenesis of diseases (e.g., inflammatory processes) (Wessler and Kirkpatrick, 2008).

HISTORICAL PERSPECTIVE

Sir Henry Dale noted that the various esters of choline elicited responses that were similar to those of either nicotine or muscarine depending on the pharmacological preparation. A similarity in response also was noted between muscarine and nerve stimulation in those organs innervated by the craniosacral divisions of the autonomic nervous system. Thus, Dale suggested that ACh or another ester of choline was a neurotransmitter in the autonomic nervous system; he also stated that the compound had dual actions, which he termed a "nicotine action" (*nicotinic*) and a "muscarine action" (*muscarinic*).

The capacities of tubocurarine and atropine to block nicotinic and muscarinic effects of ACh, respectively, provided further support for the proposal of two distinct types of cholinergic receptors. Although Dale had access only to crude plant alkaloids of then-unknown structure from *Amanita muscaria* and *Nicotiana tabacum,* this classification remains the primary subdivision of cholinergic receptors. Its utility has survived the discovery of several distinct subtypes of nicotinic and muscarinic receptors.

Cholinergic Receptors and Signal Transduction

Nicotinic receptors are ligand-gated ion channels whose activation always causes a rapid (millisecond) increase in cellular permeability to Na^+ and Ca^{2+}, depolarization, and excitation. *Muscarinic receptors* are G protein-coupled receptors (GPCRs). Responses to muscarinic agonists are slower; they may be either excitatory or inhibitory, and they are not necessarily linked to changes in ion permeability. The muscarinic and nicotinic receptors for ACh belong to two different families whose features are described in Chapters 11 and 13, respectively.

Subtypes of nAChRs. The *nAChRs* exist at the skeletal NMJ, autonomic ganglia, adrenal medulla, and CNS and in nonneuronal tissues. The nAChRs are composed of five homologous subunits organized around a central pore (see Table 10–2 and Figure 13–1). In general, the nAChRs are further divided into two groups:

- *Muscle type* (N_m), found in the vertebrate skeletal muscle, where they mediate transmission at the NMJ
- *Neuronal type* (N_n), found mainly throughout the peripheral nervous system, CNS, and nonneuronal tissues

Neuronal nAChRs are widely distributed in the CNS and are found at presynaptic, perisynaptic, and postsynaptic sites. At pre- and perisynaptic sites, nAChRs appear to act as autoreceptors or heteroreceptors to regulate the release of several neurotransmitters (ACh, DA, NE, glutamate, and 5HT) at diverse sites in the brain (Albuquerque et al., 2009).

Muscle-Type nAChRs. In fetal muscle prior to innervation, in adult muscle after denervation, and in fish electric organs, the nAChR subunit stoichiometry is $(\alpha1)_2\beta1\gamma\delta$, whereas in adult muscle, the γ subunit is replaced by ε to give the $(\alpha1)_2\beta1\varepsilon\delta$ stoichiometry (Table 10–2). The γ/ε and δ subunits are involved together with the α1 subunits in forming the ligand-binding sites and in the maintenance of cooperative interactions between the α1 subunit. Different affinities to the two binding sites are conferred by the presence of different non-α subunits. Binding of ACh to the αγ and αδ sites is thought to induce a conformational change predominantly in the α1 subunits that interacts with the transmembrane region to cause channel opening.

Neuronal-Type nAChRs. Neuronal nAChRs are widely expressed in peripheral ganglia, the adrenal medulla, numerous areas of the brain, and nonneuronal cells, such as epithelial cells and cells of the immune system. To date, nine α (α2–α10) and three β (β2–β4) subunit genes have been cloned. The α7–α10 subunits are found either as homopentamers (of five α7, α8, and α9 subunits) or as heteropentamers of α7, α8, and α9/α10. By contrast, the α2–α6 and β2–β4 subunits form heteropentamers usually with $(\alpha x)_2(\beta y)_3$ stoichiometry. The α5 and β3 subunits do not appear to be able to form functional receptors when expressed alone or in paired combinations with α or β subunits, respectively (Zoli et al., 2018).

The precise function of many of the neuronal nAChRs in the brain is not known; they appear to act more as synaptic modulators, the molecular diversity of the subunits putatively resulting in numerous nAChR subtypes with different physiological properties. Neuronal nAChRs are widely distributed in the CNS and are found at presynaptic, perisynaptic, and postsynaptic sites. At pre- and perisynaptic sites, nAChRs appear to act as autoreceptors or heteroreceptors to regulate the release of several neurotransmitters (ACh, DA, NE, glutamate, and 5HT) at sites throughout the brain (Exley and Cragg, 2008). The synaptic release of a particular neurotransmitter can be regulated by different neuronal-type nAChR subtypes in different CNS regions. For instance, DA release from striatal and thalamic DA neurons can be controlled by the α4β2 subtype or both α4β2 and α6β2β3 subtypes, respectively. In contrast, glutametergic neurotransmission is regulated everywhere by α7 nAChRs.

Subtypes of Muscarinic Receptors. In mammals, there are five distinct subtypes of mAChRs, each produced by a different gene. These variants have distinct anatomic locations in the periphery and CNS and differing chemical specificities. The mAChRs are GPCRs

TABLE 10–2 ■ CHARACTERISTICS OF SUBTYPES OF NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs)

RECEPTOR (PRIMARY RECEPTOR SUBTYPE)[a]	MAIN SYNAPTIC LOCATION	MEMBRANE RESPONSE	MOLECULAR MECHANISM	AGONISTS	ANTAGONISTS
Skeletal Muscle (N_m) $(\alpha1)_2\beta1\varepsilon\delta$ adult $(\alpha1)_2$ $\beta1\gamma\delta$ fetal	Skeletal neuromuscular junction (postjunctional)	Excitatory; end-plate depolarization; skeletal muscle contraction	Increased cation permeability (Na^+; K^+)	ACh Nicotine Succinylcholine	Atracurium Vecuronium *d*-Tubocurarine Pancuronium α-Conotoxin α-Bungarotoxin
Peripheral neuronal **(N_n)**$(\alpha3)_2(\beta4)_3$	Autonomic ganglia; adrenal medulla	Excitatory; depolarization; firing of postganglion neuron; depolarization and secretion of catecholamines	Increased cation permeability (Na^+; K^+)	ACh Nicotine Epibatidine Dimethylphenylpiperazinium	Trimethaphan Mecamylamine
CNS neuronal $(\alpha4)_2(\beta4)_3$ *(α-BTX-insensitive)*	CNS; pre- and postjunctional	Pre- and postsynaptic excitation; prejunctional control of transmitter release	Increased cationpermeability (Na^+; K^+)	Cytosine, epibatidine Anatoxin A	Mecamylamine DHbE Erysodine Lophotoxin
$(\alpha7)_5$ *(α-BTX-sensitive)*	CNS; pre- and postsynaptic	Pre- and postsynaptic excitation; prejunctional control of transmitter release	Increased permeability (Ca^{2+})	Anatoxin A	Methyllycaconitine α-Bungarotoxin α-Conotoxin ImI

[a] Nine α (α2–α10) and three β (β2–β4) subunits have been identified and cloned in human brain, which combine in various conformations to form individual receptor subtypes. The structure of individual receptors and the subtype composition are incompletely understood. Only a finite number of naturally occurring functional nAChR constructs have been identified. DHbE, dihydro-β-erythroidine.

(see Table 10–3 and Chapter 11), present in virtually all organs, tissues, and cell types (Table 10–3 and Chapter 11). Most cell types have multiple mAChR subtypes, but certain subtypes often predominate in specific sites. For example, the M_2 receptor is the predominant subtype in the heart and in CNS neurons is mostly located presynaptically, whereas the M_3 receptor is the predominant subtype in the detrusor muscle of the bladder.

In the periphery, mAChRs mediate the classical muscarinic actions of ACh in organs and tissues innervated by parasympathetic nerves, although receptors may be present at sites that lack parasympathetic innervation (e.g., most blood vessels). In the CNS, mAChRs are involved in regulating a large number of cognitive, behavioral, sensory, motor, and autonomic functions. Owing to the lack of specific muscarinic agonists and antagonists that demonstrate selectivity for individual mAChRs and the fact that most organs and tissues express multiple mAChRs, it has been a challenge to assign specific pharmacological functions to distinct mAChRs. The development of gene-targeting techniques in mice has been helpful in defining specific functions (see Table 10–3).

The functions of mAChRs are mediated by interactions with G proteins. The M_1, M_3, and M_5 subtypes couple through $G_{q/11}$ to stimulate the phospholipase (PL) C–inositol 1,4,5-trisphosphate (IP_3)/diacylglycerol-Ca^{2+} pathway, leading to activation of PKC and Ca^{2+}-sensitive enzymes. Activation of M_1, M_3, and M_5 receptors can also cause the activation of PLA_2, leading to the release of arachidonic acid and consequent eicosanoid synthesis; these effects of M_1, M_3, and M_5 mAChRs are generally secondary to elevation of intracellular Ca^{2+}. Stimulated M_2 and M_4 cholinergic receptors couple to G_i and G_o, with resulting inhibition of adenylyl cyclase, leading to a decrease in cellular cAMP, activation of inwardly rectifying K^+ channels, and inhibition of voltage-gated Ca^{2+} channels. The functional consequences of these effects are hyperpolarization and inhibition of excitable membranes. In the myocardium, inhibition of adenylyl cyclase and activation of K^+ conductances account for the negative inotropic and chronotropic effects of ACh. In addition, heterologous systems may produce different receptor-transducer-effector interactions (Zenko and Hislop, 2018).

Following activation by classical or allosteric agonists, mAChRs can be phosphorylated by a variety of receptor kinases and second-messenger regulated kinases; the phosphorylated mAChR subtypes then can interact with β-arrestin and possibly other adapter proteins. As a result, mAChR signaling pathways may be differentially altered. Agonist activation of mAChRs also may induce receptor internalization and downregulation. Muscarinic AChRs can also regulate other signal transduction pathways that have diverse effects on cell growth, survival, and physiology, such as mitogen-activated protein kinase (MAPK), phosphoinositide-3-kinase, RhoA, and Rac1.

Changes in mAChR levels and activity have been implicated in the pathophysiology of numerous major diseases in the CNS and in the autonomic nervous system (see Table 10–3). Phenotypic analysis of mAChR-mutant mice as well as the development of selective agonists and antagonists has led to a wealth of new information regarding the physiological and potential pathophysiological roles of the individual mAChR subtype (Langmead et al., 2008).

Adrenergic Transmission

Norepinephrine (NE) is the principal transmitter of most sympathetic postganglionic fibers and of certain tracts in the CNS; DA is the

TABLE 10–3 ■ CHARACTERISTICS OF MUSCARINIC ACETYLCHOLINE RECEPTOR (mAChRs) SUBTYPES

RECEPTOR	CELLULAR AND TISSUE LOCATION[a]	CELLULAR RESPONSE[b]	FUNCTIONAL RESPONSE[c]	DISEASE RELEVANCE
M_1	CNS; most abundant in cerebral cortex, hippocampus, striatum, and thalamus Autonomic ganglia Glands (gastric and salivary) Enteric nerves	Couples by $G_{q/11}$ to activate PLC-IP_3-Ca^{2+}-PKC pathway Depolarization and excitation (↑ sEPSP) Activation of PLD_2, PLA_2; ↑AA	Increased cognitive function (learning and memory) Increased seizure activity Decrease in dopamine release and locomotion Increase in depolarization of autonomic ganglia Increase in secretions	Alzheimer's disease Cognitive dysfunction Schizophrenia
M_2	Widely expressed in CNS, hindbrain, thalamus, cerebral cortex, hippocampus, striatum, heart, smooth muscle, autonomic nerve terminals	Couples by G_i/G_o (PTX sensitive) Inhibition of AC, ↓ cAMP Activation of inwardly rectifying K^+ channels Inhibition of voltage-gated Ca^{2+} channels Hyperpolarization and inhibition	*Heart:* SA node: slowed spontaneous depolarization; hyperpolarization, ↓ HR AV node: decrease in conduction velocity Atrium: ↓ refractory period, ↓ contraction Ventricle: slight ↓ contraction *Smooth muscle:* ↑ Contraction *Peripheral nerves:* Neural inhibition via autoreceptors and heteroreceptor ↓ Ganglionic transmission. *CNS:* Neural inhibition ↑ Tremors; hypothermia; analgesia	Alzheimer's disease Cognitive dysfunction Pain
M_3	Widely expressed in CNS (<other mAChRs), cerebral cortex, hippocampus Abundant in smooth muscle and glands Heart	Couples by $G_{q/11}$ to activate PLC-IP_3/DAG-Ca^{2+}-PKC pathway Depolarization and excitation (↑ sEPSP) Activation of PLD_2, PLA_2; ↑AA	*Smooth muscle:* ↑ Contraction (predominant in some, e.g., bladder) *Glands:* ↑ Secretion (predominant in salivary gland) Increases food intake, body weight, fat deposits Inhibition of DA release Synthesis of NO	Chronic obstructive pulmonary disease (COPD) Asthma Urinary incontinence Irritable bowel disease
M_4	Preferentially expressed in CNS, particularly forebrain, also striatum, cerebral cortex, hippocampus	Couples by G_i/G_0 (PTX sensitive) Inhibition of AC, ↓ cAMP Activation of inwardly rectifying K^+ channels Inhibition of voltage-gated Ca^{2+} channels Hyperpolarization and inhibition	Autoreceptor- and heteroreceptor-mediated inhibition of transmitter release in CNS and periphery Analgesia; cataleptic activity Facilitation of DA release	Parkinson's disease Schizophrenia Neuropathic pain Alzheimer's disease
M_5	Substantia nigra Expressed in low levels in CNS and periphery Predominant mAchR in neurons in VTA and substantia nigra	Couples by $G_{q/11}$ to activate PLC-IP_3-Ca^{2+}-PKC pathway Depolarization and excitation (↑ sEPSP) Activation of PLD_2, PLA_2; ↑AA	Mediator of dilation in cerebral arteries and arterioles (?) Facilitates DA release Augmentation of drug-seeking behavior and reward (e.g., opiates, cocaine)	Drug dependence Parkinson's disease Schizophrenia

[a] Most organs, tissues, and cells express multiple mAChRs.

[b] M_1, M_3, and M_5 mAChRs appear to couple to the same G proteins and signal through similar pathways. Likewise, M_2 and M_4 mAChRs couple through similar G proteins and signal through similar pathways.

[c] Despite the fact that in many tissues, organs, and cells multiple subtypes of mAChRs coexist, one subtype may predominate in producing a particular function; in others, there may be equal predominance.

VTA, ventral tegmentum area.

predominant transmitter of the mammalian extrapyramidal system and of several mesocortical and mesolimbic neuronal pathways; and EPI is the major hormone of the adrenal medulla. Collectively, these three amines are called *catecholamines*. Drugs affecting these endogenous amines and their actions are used in the treatment of hypertension, mental disorders, and a variety of other conditions. The details of these interactions and of the pharmacology of the sympathomimetic amines themselves can be found in subsequent chapters. The basic physiological, biochemical, and pharmacological features are presented here.

Synthesis of Catecholamines

The steps in the synthesis of catecholamines and the characteristics of the enzymes involved are shown in Figure 10–7 and Table 10–4. Tyrosine is sequentially 3-hydroxylated and decarboxylated to form DA. DA is β-hydroxylated to yield NE, which is *N*-methylated in chromaffin tissue to give EPI. The enzymes involved have been identified, cloned, and characterized. Table 10–4 summarizes some of the important characteristics of the four enzymes. These enzymes are not completely specific; consequently, other endogenous substances, as well as certain drugs, are also substrates. For example, 5HT can be produced from 5-hydroxy-*L*-tryptophan by aromatic *L*-amino acid decarboxylase (dopa decarboxylase). Dopa decarboxylase also converts dopa into DA (see Chapter 15) and *methyldopa* to α-methyldopamine, which dopamine β-hydroxylase (DβH) converts to methylnorepinephrine.

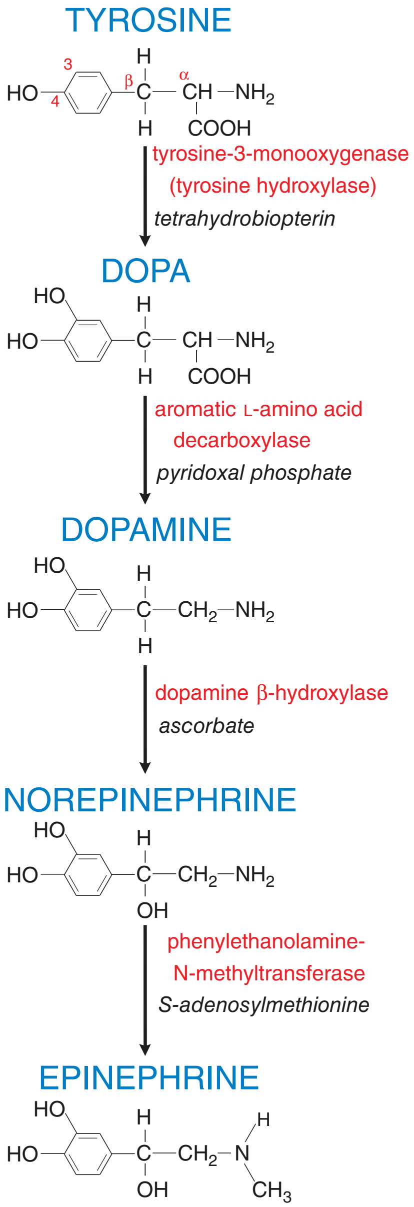

Figure 10–7 *Steps in the enzymatic synthesis of dopamine, norepinephrine, and epinephrine.* The enzymes involved are shown in red; essential cofactors in italics. The final step occurs only in the adrenal medulla and in a few epinephrine-containing neuronal pathways in the brainstem.

The hydroxylation of tyrosine by tyrosine hydroxylase (TH) is the rate-limiting step in the biosynthesis of catecholamines. This enzyme is activated following stimulation of sympathetic nerves or the adrenal medulla. The enzyme is a substrate for PKA, PKC, and calmodulin (CaM) kinase; phosphorylation is associated with increased hydroxylase activity. In addition, there is a delayed increase in TH gene expression after nerve stimulation. These mechanisms serve to maintain the content of catecholamines in response to increased transmitter release. TH also is subject to feedback inhibition by catechol compounds.

Deficiency of TH has been reported in humans and is characterized by generalized rigidity, hypokinesia, and low cerebrospinal fluid levels of NE and DA metabolites homovanillic acid and 3-methoxy-4-hydroxyphenylethylene glycol. TH knockout is embryonically lethal in mice, presumably because the loss of catecholamines results in altered cardiac function. Interestingly, residual levels of DA are present in these mice. Tyrosinase may be an alternate source for catecholamines, although tyrosinase-derived catecholamines are clearly not sufficient for survival (Carson and Robertson, 2002).

Deficiency of DβH in humans is characterized by orthostatic hypotension, ptosis of the eyelids, retrograde ejaculation, and elevated plasma levels of DA. In the case of DβH-deficient mice, there is about 90% embryonic mortality (Carson and Robertson, 2002).

Our understanding of the cellular sites and mechanisms of synthesis, storage, and release of catecholamines derives from studies of sympathetically innervated organs and the adrenal medulla. Nearly all the NE content of innervated organs is confined to the postganglionic sympathetic fibers; it disappears within a few days after section of the nerves. In the adrenal medulla, catecholamines are stored in chromaffin granules. These vesicles contain extremely high concentrations of catecholamines (~21% dry weight), ascorbic acid, and ATP, as well as specific proteins, such as chromogranins, DβH, and peptides, including enkephalin and neuropeptide Y. Chromogranin A fragments reportedly have antibacterial and antifungal activity (Mercer and O'Neil, 2020). Two types of storage vesicles are found in sympathetic nerve terminals: large dense-core vesicles corresponding to chromaffin granules and small dense-core vesicles containing NE, ATP, and membrane-bound DβH.

The main features of the mechanisms of synthesis, storage, and release of catecholamines at an adrenergic neuroeffector junction and their modifications by drugs are summarized in Figure 10–8 and its legend. The *adrenal medulla* has two distinct catecholamine-containing cell types: those with NE and those with primarily EPI. The latter cell population contains the enzyme phenylethanolamine-*N*-methyltransferase (PNMT). In these cells, the NE formed in the granules leaves these structures and is methylated in the cytoplasm to EPI. EPI then reenters the chromaffin granules, where it is stored until released. EPI accounts for about 80% of the catecholamines of the adrenal medulla and NE about 20%.

A major factor that controls the rate of synthesis of EPI, and hence the size of the store available for release from the adrenal medulla, is the level of glucocorticoids secreted by the adrenal cortex. The intra-adrenal portal vascular system carries the corticosteroids directly to the adrenal medullary chromaffin cells, where they induce the synthesis of PNMT (see Figure 10–7). The activities of both TH and DβH also increase in the adrenal medulla when the secretion of glucocorticoids is stimulated. Thus, any stress that persists sufficiently to evoke an enhanced secretion of corticotropin mobilizes the appropriate hormones of both the adrenal cortex (predominantly cortisol in humans) and medulla (EPI). This remarkable relationship is present only in certain mammals, including humans, in which the adrenal chromaffin cells are enveloped entirely by steroid-secreting cortical cells. PMNT is expressed in mammalian tissues such as brain, heart, and lung, leading to extra-adrenal EPI synthesis (Ziegler et al., 2002).

TABLE 10–4 ■ ENZYMES FOR SYNTHESIS OF CATECHOLAMINES

ENZYME	OCCURRENCE	SUBCELLULAR DISTRIBUTION	COFACTORS	SUBSTRATE SPECIFICITY	COMMENTS
TH	Widespread	Cytoplasm	tetrahydrobiopterin (BH_4), O_2, Fe^{2+}	Specific for L-tyrosine	Rate-limiting step. Inhibition can deplete NE.
AAADC	Widespread	Cytoplasm	Pyridoxal PO_4	Nonspecific	Inhibition does not alter tissue NE and EPI appreciably.
DβH	Widespread	Synaptic vesicles	Ascorbate, O_2 (DβH contains Cu)	Nonspecific	Inhibition can ↓ NE and EPI levels.
PNMT	Largely in adrenal gland	Cytoplasm	*S*-adenosyl methionine (SAM) as (CH_3 donor)	Nonspecific	Inhibition can ↓ adrenal EPI/NE; regulated by glucocorticoids.

In addition to *de novo* synthesis, NE stores in the terminal portions of the adrenergic fibers are replenished by reuptake and restorage of NE following its release (see discussion in the following material).

Storage, Release, and Reuptake of Catecholamines; Termination of Action

Storage. NE, ATP, and NPY are stored frequently in the same nerve endings.

Catecholamines. Catecholamines are stored in vesicles, thereby ensuring their regulated release, protecting them from metabolism by cellular enzymes, and preventing their leakage out of the neuron. The vesicular monoamine transporter (VMAT) 2, a vesicular membrane protein, moves NE and other catecholamines from the cytosol into neuronal storage vesicles. VMAT2 is driven by a pH gradient established by an ATP-dependent proton translocase in the vesicular membrane; for each molecule of amine taken up, two H^+ ions are extruded. VMAT2 is a member of the solute carrier (SLC) protein superfamily and is designated SLC18A. Monoamine transporters in the SLC18 family are relatively promiscuous and transport DA, NE, EPI, and 5HT, as well as metaiodobenzylguanidine, which can be used to image chromaffin cell tumors. *Reserpine* and *tetrabenazine* inhibit monoamine transport into storage vesicles; by this action, catecholamines remain in the cytosol where they are vulnerable to degradation, mainly by monoamine oxidase (MAO); ultimately, inhibition of VMAT2 leads to depletion of catecholamine from sympathetic nerve endings and in the brain. *Reserpine*, an irreversible agent, inhibits both VMAT2 and the peripheral isoform, VMAT1. *Tetrabenazine* is a reversible inhibitor with greater specificity for VMAT2.

ATP. ATP is an essential component of catecholamine storage; the capacity of ATP and catecholamines to form relatively stable complexes apparently facilitates accumulation of high concentrations of neurotransmitter within the adrenergic storage granule. The granule accumulates ATP via another vesicular nucleotide carrier, VNUT, a member of the SLC superfamily. VNUT is a Na^+/anion cotransporter, designated as SLC17A9 (see Chapter 4). The frequency and quantal size of exocytotic release mirror VNUT activity (Estévez-Herrera et al., 2016). Thus, vesicular ATP has multiple actions beyond its role as a cellular energy source and energy storage molecule: Vesicular ATP facilitates vesicular storage of high concentrations of catecholamines and, when released with the vesicular contents, acts as a transmitter at purinergic receptors (Burnstock et al., 2015; see Table 16–7).

Neuropeptide Y. NPY, a peptide with 36 amino acids, is synthesized in the endoplasmic reticulum, first as a 97-amino-acid precursor, prepro-NPY, that is processed by three steps of proteolysis and a final C-terminal amidation; the resultant $NPY_{1–36}$ is stored in large, dense-core vesicles that may also contain NE. NE and ATP are more generally stored in smaller dense-core vesicles, but NPY, ATP, and NE are often co-released following nerve stimulation, albeit in proportions that change with the pattern and intensity of stimulation (Westfall, 2004). NPY is abundant in the brain and is a powerful orexigenic. In the peripheral nervous system, NPY occurs in sympathetic nerves and adrenal chromaffin cells; it can also be found in platelets, endothelium, and the GI tract and is inducible in the immune system.

Release. Details of excitation-secretion coupling in sympathetic neurons and adrenal medulla are summarized in Figures 10–3 and 10–8. The triggering event is the entry of Ca^{2+}, which results in the exocytosis of the granular contents, including the catecholamine, ATP, some neuroactive peptides (e.g., NPY) or their precursors, chromogranins, and DβH. The various SNARE proteins (e.g., SNAP-25, syntaxin, and synaptobrevin) described for exocytosis of ACh are also involved here (see Figures 10–3 through 10–6).

Reuptake and Termination of Action. Following its release from a sympathetic nerve varicosity, NE interacts with presynaptic and postsynaptic membrane receptors. Adrenergic fibers can sustain the output of NE during prolonged periods of stimulation without exhausting their supply, provided that synthesis and reuptake of the transmitter are unimpaired. Acute regulation of transmitter synthesis involving activation of TH and DβH has been described previously in this chapter. Recycling of transmitter is also essential, and this is provided by reuptake, restorage, and reuse of transmitter. *The actions of catecholamines are terminated by reuptake into the nerve and postjunctional cells and to a smaller extent by diffusion out of the synaptic cleft.* Two distinct carrier-mediated transport systems are involved in reuptake (see Figure 10–8; Table 10–5):

- NE transporter (NET): This transporter, previously called *uptake 1*, moves NE across the neuronal membrane from the extracellular fluid to the cytoplasm. NET has a higher affinity for NE than for EPI (see Table 10–5). NET is a member of an SLC family of similar transporters and is designated as SLC6A2. This family of proteins transports amino acids and their derivatives into cells using cotransport of extracellular Na^+ as a driving force for substrate translocation against chemical gradients (see Chapter 4). The SLC6A monoamine transporters include NET, DA transporter (DAT) (SLC6A3), and serotonin transporter (SLC6A4).
- Extraneuronal transporter (ENT): This transporter, previously called *uptake 2,* is an organic cation transporter, OCT3, designated as SLC22A3. OCT3 facilitates passive transmembrane movement of organic anions down their electrochemical gradients, including the movement of catecholamines into nonneuronal cells. Compared to NET, it has a lower affinity for catecholamines, favors EPI over NE and DA, has a higher maximum uptake rate for catecholamines, is not Na^+ dependent, and has a different profile for pharmacological inhibition. The synthetic β adrenergic receptor agonist *isoproterenol* is not a substrate for this system. OCT3 activity is altered by MAPK and Ca^{2+}-CaM signaling (Roth et al., 2012). In addition to catecholamines, OCT3 can transport a wide variety of other organic cations, including 5HT, histamine, choline, spermine, guanidine, and creatinine, as can the closely related OCT1 and OCT2. The characteristics and locations of the nonneuronal transporters are summarized in Table 10–5.

For NE released by neurons, uptake by NET is more important than uptake by ENT. Sympathetic nerves as a whole remove about 87% of

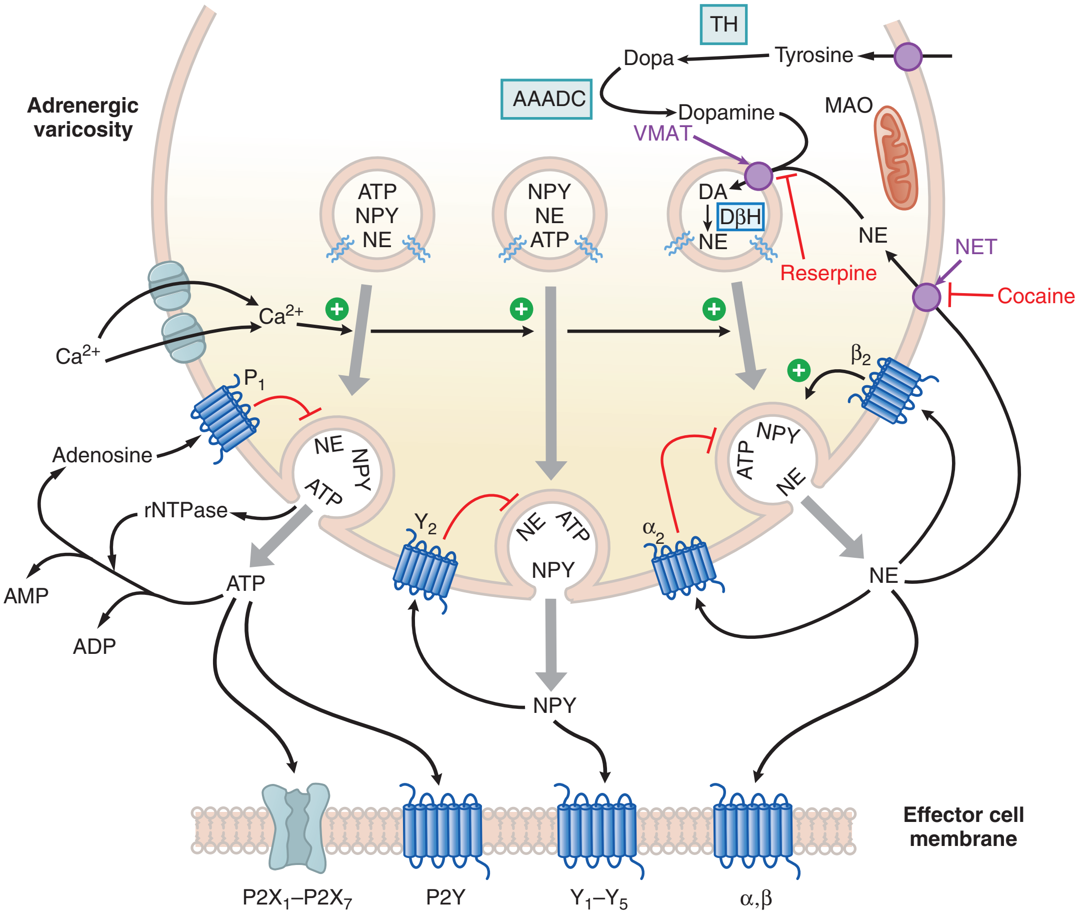

Figure 10–8 *A typical adrenergic neuroeffector junction.* Tyrosine is transported into the varicosity and is converted to DOPA by TH and DOPA to DA by the action of aromatic L-amino acid decarboxylase (AAADC). DA is taken up into the vesicles of the varicosity by a transporter, VMAT2, that can be blocked by *reserpine*. Cytoplasmic NE also can be taken up by this transporter. DA is converted to NE within the vesicle via the action of DβH. NE is stored in vesicles along with other cotransmitters, NPY and ATP, depending on the particular neuroeffector junction. Release of the transmitters occurs via exocytosis, a process activated by depolarization of the varicosity, which allows entry of Ca^{2+} through voltage-dependent Ca^{2+} channels and the interaction of numerous docking and fusion proteins located in the vesicle and the neuronal cell membrane, as described in Figures 10–3, 10–4, and 10–5. In this schematic representation, NE, NPY, and ATP are stored in the same vesicles. Different populations of vesicles, however, may preferentially store different proportions of the cotransmitters. Once in the synapse, NE can interact with α and β adrenergic receptors (GPCRs) to produce the responses characteristic of the particular postsynaptic cell. The α and β receptors also can be located presynaptically, via which NE can either diminish (α$_2$) or facilitate (β) its own release and that of the cotransmitters. The principal mechanism by which NE is cleared from the synapse is via a *cocaine*-sensitive neuronal uptake transporter, NET. Once transported into the cytosol, NE can be re-stored in the vesicle or metabolized by MAO. NPY produces its effects by activating NPY receptors (also GCPRs), of which there are at least five types (Y$_1$ through Y$_2$). NPY can modify its own release and that of the other transmitters via presynaptic Y$_2$ receptors. NPY action is terminated by the actions of peptidases. ATP produces its effects by activating P2X receptors (ligand-gated ion channels) or P2Y receptors (GPCRs). There are multiple subtypes of both P2X and P2Y receptors. As with other cotransmitters, ATP can act prejunctionally to modify its own release via receptors for ATP or via its metabolic breakdown to adenosine that acts on P1 (adenosine) receptors. ATP is cleared from the synapse primarily by releasable nucleotidases (rNTPases) and by cell-fixed ectonucleotidases.

released NE by NET, compared with 5% by extraneuronal uptake (ENT) and 8% by diffusion to the circulation. In contrast, clearance of circulating catecholamines, such as those released from the adrenal medulla, is primarily by nonneuronal mechanisms, with liver and kidney accounting for over 60% of the clearance of circulating catecholamines. Because VMAT2 has a much higher affinity for NE than does MAO, over 70% of recaptured NE is resequestered into storage vesicles (Eisenhofer, 2001).

The NET is also present in the adrenal medulla, the liver, and the placenta, whereas DAT is present in the stomach, pancreas, and kidney (Eisenhofer, 2001). These plasma membrane transporters appear to have greater substrate specificity than does VMAT2. NET and DAT are targets for inhibitors such as cocaine and tricyclic antidepressants (e.g., *imipramine*); selective 5HT reuptake inhibitors such as *fluoxetine* inhibit serotonin transporter. Inhibitors of OCT3 include normetanephrine (an *O*-methylated metabolite of NE; see Figure 10–9). Pharmacological probes of OCT3 include *corticosterone* (an inhibitor) and the substrates *metformin* and *cimetidine*; the interaction of substrates and inhibitors at renal OCT3 can lead to adverse drug effects (see Chapter 4).

The use of selective inhibitors of NET in animal and human studies and data from analysis of mice with targeted deletions (knockout [KO]) of the NET and DAT genes reveal the impact of these uptake systems. The NET-KO and DAT-KO animals exhibit increased extracellular levels and decreased intracellular levels of NE despite increased or unaltered neurotransmitter synthesis. A loss or reduction of NET activity has been implicated in behavior, hemodynamics (e.g., excessive tachycardia and increased blood pressure) during sympathetic activation due to activity, increased central sympatho-inhibition, orthostatic intolerance, posttraumatic stress, and depression.

Certain sympathomimetic drugs (e.g., *ephedrine* and *tyramine*) produce some of their effects indirectly by displacing NE from the nerve terminals to the extracellular fluid, where it then acts at receptor sites of the effector cells. The mechanisms by which these drugs release NE

TABLE 10–5 ■ CHARACTERISTICS OF PLASMA MEMBRANE TRANSPORTERS FOR ENDOGENOUS CATECHOLAMINES

TYPE OF TRANSPORTER	SUBSTRATE SPECIFICITY	TISSUE	REGION/CELL TYPE	INHIBITORS
Neuronal				
NET	DA > NE > EPI	All sympathetically innervated tissue	Sympathetic nerves	Desipramine Cocaine Nisoxetine
		Adrenal medulla	Chromaffin cells	
		Liver	Capillary endothelial cells	
		Placenta	Syncytiotrophoblast	
DAT	DA > NE > EPI	Kidney	Endothelium	Cocaine Imazindol
		Stomach	Parietal and endothelial cells	
		Pancreas	Pancreatic duct	
Nonneuronal				
OCT1	DA > EPI >> NE	Liver	Hepatocytes	Isocyanines Corticosterone
		Intestine	Epithelial cells	
		Kidney (not human)	Distal tubule	
OCT2	DA >> NE > EPI	Kidney	Medullary proximal and distal tubules	Isocyanines Corticosterone
		Brain	Glial cells of DA-rich regions, some nonadrenergic neurons	
ENT (OCT3)	EPI >> NE > DA	Liver	Hepatocytes	Isocyanines Corticosterone *O*-methyl-isoproterenol
		Brain	Glial cells, others	
		Heart	Myocytes	
		Blood vessels	Endothelial cells	
		Kidney	Cortex, proximal and distal tubules	
		Placenta	Syncytiotrophoblasts (basal membrane)	
		Retina	Photoreceptors, ganglion amacrine cells	

from nerve endings are complex. All such agents are substrates for NET. As a result of their uptake by NET, they make carrier proteins available at the inner surface of the membrane for the outward transport of NE ("facilitated exchange diffusion"). In addition, these amines are able to mobilize NE stored in the vesicles by competing for the vesicular uptake process (VMAT2).

The actions of indirect-acting sympathomimetic amines are subject to *tachyphylaxis*. For example, repeated administration of tyramine results in rapidly decreasing effectiveness, whereas repeated administration of NE does not reduce effectiveness and, in fact, reverses the tachyphylaxis to tyramine. These phenomena have not been explained fully. One hypothesis is that the pool of neurotransmitter available for displacement by these drugs is small relative to the total amount stored in the sympathetic nerve ending. This pool is presumed to reside close to the plasma membrane, and the NE of such vesicles may be replaced by the less-potent amine following repeated administration of the latter substance. In any case, neurotransmitter release by displacement is not associated with the release of DβH and does not require extracellular Ca^{2+}; thus, it is presumed not to involve exocytosis.

Prejunctional Regulation of NE Release. The release of the three sympathetic cotransmitters can be modulated by prejunctional autoreceptors and heteroreceptors. Following their release from sympathetic terminals, all three cotransmitters—NE, NPY, and ATP—can feed back on prejunctional receptors to inhibit the release of each other (Westfall, 2004; Westfall et al., 2002). The most thoroughly studied have been prejunctional α_2 adrenergic receptors. The α_{2A} and α_{2C} adrenergic receptors are the principal prejunctional receptors that inhibit sympathetic neurotransmitter release, whereas the α_{2B} adrenergic receptors also may inhibit transmitter release at selected sites. Antagonists of this receptor, in turn, can enhance the electrically evoked release of sympathetic neurotransmitter. NPY, acting on Y_2 receptors, and ATP-derived adenosine, acting on P1 receptors, also can inhibit sympathetic neurotransmitter release. Activation of numerous heteroreceptors on sympathetic nerve varicosities can inhibit the release of sympathetic neurotransmitters; these include M_2 and M_4 muscarinic, 5HT, prostaglandin E_2, histamine, enkephalin, and DA receptors. Enhancement of sympathetic neurotransmitter release can be produced by activation of β_2 adrenergic receptors, angiotensin AT_2 receptors, and nAChRs. All of these receptors can be targets for agonists and antagonists.

Metabolism of Catecholamines. Uptake of released catecholamine terminates the neurotransmitter's effects at the synaptic junction. Following uptake, catecholamines can be metabolized (in neuronal and nonneuronal cells) or re-stored in vesicles (in neurons). Two enzymes are important in the initial steps of metabolic transformation of catecholamines—MAO and catechol-*O*-methyltransferase (COMT).

MAO and COMT. MAO metabolizes transmitter that is released within the nerve terminal or that is in the cytosol as a result of reuptake and has not yet reached the safety of the storage vesicle. COMT, particularly in the liver, plays a major role in the metabolism of endogenous circulating and administered catecholamines. The importance of neuronal reuptake of catecholamines is shown by observations that inhibitors of uptake (e.g., *cocaine* and *imipramine*) potentiate the effects of the neurotransmitter; inhibitors of MAO and COMT have less effect.

Both MAO and COMT are distributed widely throughout the body, including the brain; their highest concentrations are in the liver and the kidney. However, little or no COMT is found in sympathetic neurons. In the brain, there is no significant COMT in presynaptic terminals, but it is found in some postsynaptic neurons and glial cells. In the kidney, COMT is localized in proximal tubular epithelial cells, where DA is synthesized and is thought to exert local diuretic and natriuretic effects.

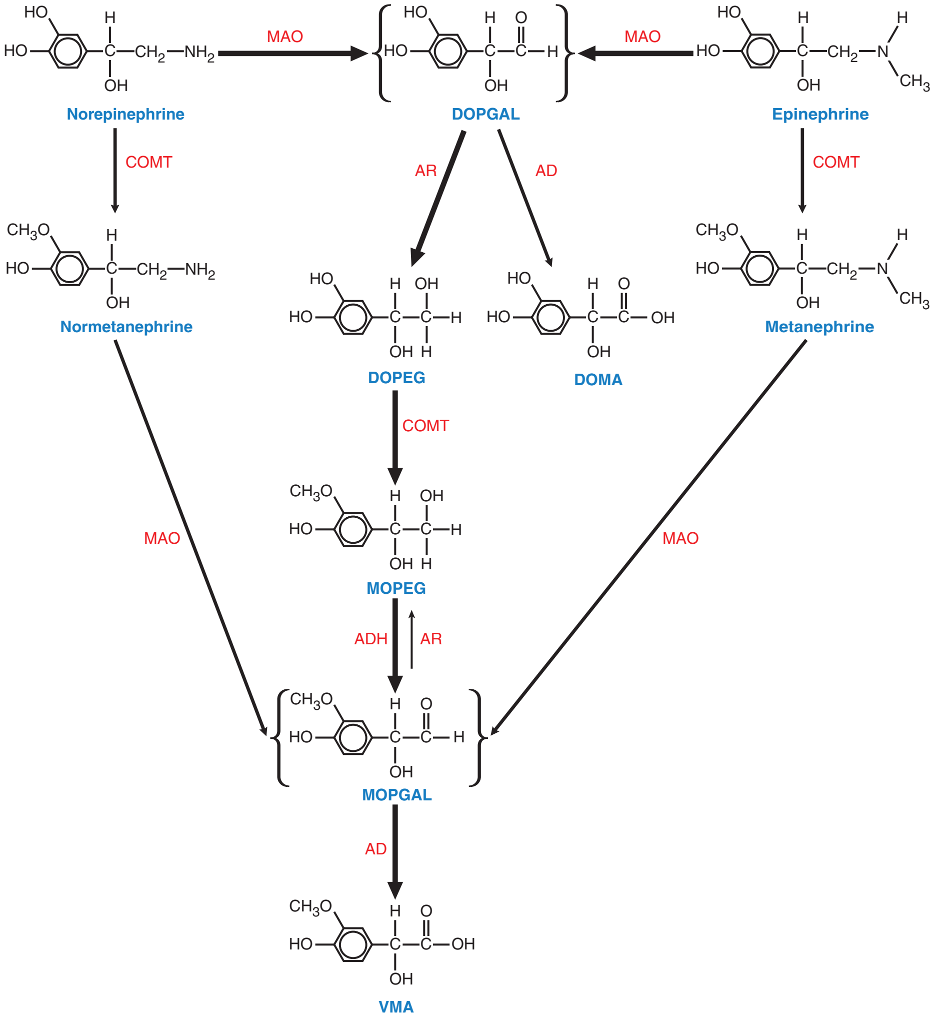

Figure 10–9 *Metabolism of catecholamines.* NE and EPI are first oxidatively deaminated to a short-lived intermediate (DOPGAL) by MAO. DOPGAL then undergoes further metabolism to more stable alcohol- or acid-deaminated metabolites. AD metabolizes DOPGAL to DOMA, while AR metabolizes DOPGAL to DOPEG. Under normal circumstances, DOMA is a minor metabolite, with DOPEG being the major metabolite produced from NE and EPI. Once DOPEG leaves the major sites of its formation (sympathetic nerves; adrenal medulla), it is converted to MOPEG by COMT. MOPEG is then converted to the unstable aldehyde (MOPGAL) by ADH and finally to VMA by AD. VMA is the major end product. Another route for the formation of VMA is conversion of NE or EPI into normetanephrine or metanephrine by COMT in either the adrenal medulla or extraneuronal sites, with subsequent metabolism to MOPGAL and thence to VMA. Catecholamines are also metabolized by *sulfotransferases*.

There are distinct differences in the localizations of the two enzymes; MAO is associated chiefly with the outer surface of mitochondria, including those within the terminals of sympathetic or central noradrenergic neuronal fibers, whereas COMT is largely cytosolic, except in the chromaffin cells of the adrenal medulla, where COMT is membrane bound. These factors are of importance both in determining the primary metabolic pathways followed by catecholamines in various circumstances and in explaining the effects of certain drugs. The physiological substrates for COMT include L-dopa, all three endogenous catecholamines (DA, NE, and EPI), their hydroxylated metabolites, catecholestrogens, ascorbic acid, and dihydroxyindolic intermediates of melanin (Männistö and Kaakkola, 1999).

Two different isozymes of MAO (MAO-A and MAO-B) are found in widely varying proportions in different cells in the CNS and in peripheral tissues. In the periphery, MAO-A is located in the syncytiotrophoblast layer of term placenta and liver, whereas MAO-B is located in platelets, lymphocytes, and liver. In the brain, MAO-A is located in all regions containing catecholamines, with the highest abundance in the locus ceruleus. MAO-B, on the other hand, is found primarily in regions that are known to synthesize and store 5HT. MAO-B is most prominent not only in the nucleus raphe dorsalis but also in the posterior hypothalamus and in glial cells in regions known to contain nerve terminals. MAO-B is also present in osteocytes around blood vessels.

Many MAO inhibitors are not selective for MAO-A or MAO-B, and these nonselective agents (e.g., *phenelzine*, *tranylcypromine*, and *isocarboxazid*) enhance the bioavailability of tyramine contained in many foods; tyramine-induced NE release from sympathetic neurons may lead to markedly increased blood pressure (hypertensive crisis). Drugs with selectivity for MAO-B (e.g., *selegiline*, *rasagiline*, *pargyline*) or reversible inhibitors of MAO-A (e.g., *moclobemide*) are less likely to cause this potential interaction.

Inhibitors of MAO activity can cause an increase in the concentration of NE, DA, and 5HT in the brain and other tissues accompanied by a variety of pharmacological effects. No striking pharmacological action in the periphery can be attributed to the inhibition of COMT. COMT inhibitors that are efficacious in the therapy of Parkinson's disease are discussed in Chapter 21.

The Metabolic Pathway (Figure 10–9). There is ongoing passive leakage of catecholamines from vesicular storage granules of sympathetic neurons and adrenal medullary chromaffin cells. As a consequence, most metabolism of catecholamines takes place in the same cells where the amines are synthesized and stored. VMAT2 effectively sequesters about 90% of the amines leaking into the cytoplasm back into storage vesicles; about 10% escapes sequestration and is metabolized (Eisenhofer et al., 2004).

Sympathetic nerves contain MAO but not COMT, and this MAO catalyzes only the first step of a two-step reaction. MAO converts NE or EPI into a short-lived intermediate, dihydroxyphenylglycolaldehyde (DOPGAL), which undergoes further metabolism in a second step catalyzed by another group of enzymes forming more stable alcohol- or acid-deaminated metabolites. Aldehyde dehydrogenase metabolizes DOPGAL to 3,4-dihydroxymandelic acid (DOMA), while aldehyde reductase metabolizes DOPGAL to 3,4-dihydroxyphenyl glycol (DOPEG). In addition to aldehyde reductase, a related enzyme, aldose reductase, can reduce a catecholamine to its corresponding alcohol. This latter enzyme is present in sympathetic neurons and adrenal chromaffin cells. Under normal circumstances, DOMA is an insignificant metabolite of NE and EPI, with DOPEG being the main metabolite produced by deamination in sympathetic neurons and adrenal medullary chromaffin cells.

Once it leaves the sites of formation (sympathetic neurons, adrenal medulla), DOPEG is converted to 3-methyl,4-hydroxyphenylglycol (MOPEG) by COMT. Thus, most MOPEG comes from extraneuronal *O*-methylation of DOPEG produced in and diffusing rapidly from sympathetic neurons into the extracellular fluid. MOPEG is then converted to vanillyl mandelic acid (VMA) by the sequential actions of alcohol and aldehyde dehydrogenases. MOPEG is first converted to the unstable aldehyde metabolite monohydroxyphenylglycolaldehyde (MOPGAL) and then to VMA, with VMA being the major end product of NE and EPI metabolism. Another route for the formation of VMA is conversion by COMT of NE and EPI into normetanephrine and metanephrine, respectively, followed by deamination to MOPGAL and thence to VMA. This is now thought to be only a minor pathway, as indicated by the size of the arrows on Figure 10–9.

In contrast to sympathetic neurons, adrenal medullary chromaffin cells contain both MAO and COMT, the COMT mainly as the membrane-bound form. This isoform of COMT has a higher affinity for catecholamines than does the soluble form found in most other tissues (e.g., liver and kidney). In adrenal medullary chromaffin cells, leakage of NE and EPI from storage vesicles leads to substantial intracellular production of the *O*-methylated metabolites normetanephrine and metanephrine. In humans, over 90% of circulating metanephrine and 25% to 40% of circulating normetanephrine are derived from catecholamines metabolized within adrenal chromaffin cells.

The sequence of cellular uptake and metabolism of catecholamines in extraneuronal tissues contributes only modestly (~25%) to the total metabolism of endogenously produced NE in sympathetic neurons or the adrenal medulla. However, extraneuronal metabolism is an important mechanism for the clearance of circulating and exogenously administered catecholamines.

Classification of Adrenergic Receptors

Adrenergic receptors are broadly classified as either α or β, with subtypes within each group (Table 10–6). The original subclassification was based on the rank order of agonist potency:

- EPI ≥ NE >> *isoproterenol* for α adrenergic receptors.
- *Isoproterenol* > EPI ≥ NE for β adrenergic receptors.

Elucidation of the characteristics of these receptors and the biochemical and physiological pathways they regulate has increased our understanding of the seemingly contradictory and variable effects of catecholamines on various organ systems. Although structurally related (discussed further in the chapter), different receptors regulate distinct physiological processes by controlling the synthesis or mobilization of a variety of second messengers.

Raymond Ahlquist and the Functional Definition of α and β Receptors. Based on studies of the capacities of EPI, NE, and related agonists to regulate various physiological processes, Ahlquist (1948) proposed the existence of more than one adrenergic receptor. It was known that adrenergic agents could cause either contraction or relaxation of smooth muscle depending on the site, the dose, and the agent chosen. For example, NE was known to have potent excitatory effects on smooth muscle and correspondingly low activity as an inhibitor; *isoproterenol* displayed the opposite pattern of activity. EPI could both excite and inhibit smooth muscle. Thus, Ahlquist proposed the designations α and β for receptors on smooth muscle where catecholamines produce excitatory and inhibitory responses, respectively (an exception was the gut, which generally is relaxed by activation of either α or β receptors). He developed the rank orders of potency that define α and β receptor–mediated responses, as noted above. This initial classification was corroborated by the finding that certain antagonists produced selective blockade of the effects of adrenergic nerve impulses and sympathomimetic agents at α receptors (e.g., *phenoxybenzamine*), whereas others produced selective β receptor blockade (e.g., *propranolol*).

α and β Receptor Subtypes. Subsequent to Ahlquist's functional description of α and β receptors, adrenergic pharmacologists used increasingly sophisticated probes, tools, and methods to elucidate subtypes of α and β receptors. The β receptors were subclassified as β_1 (e.g., those in the myocardium) and β_2 (smooth muscle and most other sites), reflecting the finding that EPI and NE essentially are equipotent at β_1 sites, whereas EPI is 10 to 50 times more potent than NE at β_2 sites. Antagonists that discriminate between β_1 and β_2 receptors were subsequently developed (see Chapter 14). Cloning confirmed that these β subtypes are products of different genes, and a human gene that encodes a third β receptor (designated β_3) was isolated (Ahles and Engelhardt, 2014). The β_3 receptor is about 10-fold more sensitive to NE than to EPI and is relatively resistant to blockade by antagonists such as *propranolol*. The β_3 receptor may mediate responses to catecholamine at sites with "atypical" pharmacological characteristics (e.g., adipose tissue, which expresses all three β receptor subtypes). Animals treated with β_3 receptor agonists exhibit a vigorous thermogenic response as well as lipolysis. Polymorphisms in the β_3 receptor gene may be related to risk of obesity or type 2 diabetes in some populations (Ahles and Engelhardt, 2014).

There is also heterogeneity among α adrenergic receptors. The initial distinction was based on functional and anatomic considerations: NE and other α adrenergic agonists profoundly inhibit the release of NE from neurons (see Figure 10–8); conversely, certain α receptor antagonists markedly increase NE release when sympathetic nerves are stimulated. This feedback-inhibitory effect of NE on its release from nerve terminals is mediated by α receptors that are pharmacologically distinct from the classical postsynaptic α receptors. Accordingly, these presynaptic α adrenergic receptors were designated α_2, whereas the postsynaptic "excitatory" α receptors were designated α_1. Compounds such as *clonidine* are more potent agonists at α_2 than at α_1 receptors; by contrast, *phenylephrine* and *methoxamine* selectively activate postsynaptic α_1 adrenergic receptors.

Although there is little evidence to suggest that α_1 adrenergic receptors function presynaptically in the autonomic nervous system, α_2 receptors are present at postjunctional or nonjunctional sites in several tissues. For example, stimulation of postjunctional α_2 receptors in the brain is associated with reduced sympathetic outflow from the CNS and appears to be responsible for a significant component of the antihypertensive effect of drugs such as clonidine (see Chapter 14). Thus, the anatomic concept of prejunctional α_2 and postjunctional α_1 adrenergic receptors has been abandoned in favor of a pharmacological and functional classification (Tables 10–6 and 10–7).

Cloning revealed additional heterogeneity of both α_1 and α_2 adrenergic receptors. There are three pharmacologically defined α_1 receptors (α_{1A}, α_{1B}, and α_{1D}) with distinct sequences and tissue distributions and three cloned subtypes of α_2 receptors (α_{2A}, α_{2B}, and α_{2C}) (see Table 10–6). A fourth type of α_1 receptor, α_{1L}, has been defined on the basis of a low affinity for the selective antagonists *prazosin* and *5-methyl urapidil* but a high affinity for *tamsulosin* and *silodosin*. This phenotype could be of physiological significance; the α_{1L} profile has been identified in myriad tissues across a number of species, where it appears to regulate smooth muscle contractility in the vasculature and lower urinary tract. Despite intense efforts, the α_{1L} adrenergic receptor has not been cloned. Currently, it is viewed as a second phenotype originating from the α_{1A} receptor gene. Distinct pharmacological phenotypes of the α_{1B} receptor have also been described (Yoshiki et al., 2014).

Owing to the lack of sufficiently subtype-selective ligands, the precise physiological functions and therapeutic potential of the subtypes of

TABLE 10–6 ■ CHARACTERISTICS FOR ADRENERGIC RECEPTOR SUBTYPES[a]

	G PROTEIN COUPLING	PRINCIPLE EFFECTORS	TISSUE LOCALIZATION	DOMINANT EFFECTS[b]
α_{1A}	$G\alpha_q$ $(\alpha_{11}/\alpha_{14}/\alpha_{16})$	↑ PLC, ↑ PLA_2 ↑ Ca^{2+} channels ↑ Na^+/H^+ exchanger Modulation of K^+ channels ↑ MAPK Signaling	Heart, lung Liver Smooth muscle Blood vessels Vas deferens, prostate Cerebellum, cortex Hippocampus	• Dominant receptor for contraction of vascular smooth muscle • Promotes cardiac growth and structure • Vasoconstriction of large resistant arterioles in skeletal muscle
α_{1B}	$G\alpha_q$ $(\alpha_{11}/\alpha_{14}/\alpha_{16})$	↑ PLC, ↑ PLA_2 ↑ Ca^{2+} channels ↑ Na^+/H^+ exchanger Modulation of K^+ channels ↑ MAPK signaling	Kidney, lung Spleen Blood vessels Cortex Brainstem	• Most abundant subtype in heart • Promotes cardiac growth and structure
α_{1D}	$G\alpha_q$ $(\alpha_{11}/\alpha_{14}/\alpha_{16})$	↑ PLC, ↑ PLA_2 ↑ Ca^{2+} channels ↑ Na^+/H^+ exchanger Modulation of K^+ channels ↑ MAPK signaling	Platelets, aorta Coronary artery Prostate Cortex Hippocampus	• Dominant receptor for vasoconstriction in aorta and coronaries
α_{2A}	$G\alpha_i$ $G\alpha_o$ $(\alpha_{o1}/\alpha_{o2})$	↓ AC-cAMP-PKA pathway	Platelets Sympathetic neurons Autonomic ganglia Pancreas Coronary/CNS vessels Locus ceruleus Brainstem, spinal cord	• Dominant inhibitory receptor on sympathetic neurons • Vasoconstriction of precapillary vessels in skeletal muscle
α_{2B}	$G\alpha_i$ $G\alpha_o$ $(\alpha_{o1}/\alpha_{o2})$	↓ AC-cAMP-PKA pathway	Liver, kidney Blood vessels Coronary/CNS vessels Diencephalon Pancreas, platelets	• Dominant mediator of α_2 vasoconstriction
α_{2C}	$G\alpha_i$ $(\alpha_{11}/\alpha_{12}/\alpha_{13})$ $G\alpha_o$ $(\alpha_{o1}/\alpha_{o2})$	↓ AC-cAMP-PKA pathway	Basal ganglia Cortex, cerebellum Hippocampus	• Dominant receptor modulating DA neurotransmission • Dominant receptor inhibiting hormone release from adrenal medulla
β_1	$G\alpha_s$	↑ AC-cAMP-PKA pathway ↑ L-type Ca^{2+} channels	Heart, kidney Adipocytes Skeletal muscle Olfactory nucleus Cortex, brainstem Cerebellar nuclei Spinal cord	• Dominant mediator of positive inotropic and chronotropic effects in heart
β_2[c]	$G\alpha_s$	↑ AC-cAMP-PKA pathway ↑ Ca^{2+} channels	Heart, lung, kidney Blood vessels Bronchial smooth muscle GI smooth muscle Skeletal muscle Olfactory bulb Cortex, hippocampus	• Smooth muscle relaxation • Skeletal muscle hypertrophy
β_3[c,d]	$G\alpha_s$	↑ AC-cAMP-PKA pathway ↑ Ca^{2+} channels	Adipose tissue GI tract, heart	• Metabolic effects

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[a] At least three subtypes each of α_1 and α_2 adrenergic receptors are known, but distinctions in their mechanisms of action have not been clearly defined.

[b] In some species (e.g., rat), metabolic responses in the liver are mediated by α_1 adrenergic receptors, whereas in others (e.g., dog) β_2 adrenergic receptors are predominantly involved. Both types of receptors appear to contribute to responses in human beings.

[c] β Receptor coupling to cell signaling can be more complex. In addition to coupling to G_s to stimulate AC, β_2 receptors can activate signaling via a GRK/β-arrestin pathway. β_2 and β_3 receptors can couple to both G_s and G_i in a manner that may reflect agonist stereochemistry. See also Chapter 14.

[d] Metabolic responses in tissues with atypical pharmacological characteristics (e.g., adipocytes) may be mediated by β_3 receptors. Most β receptor antagonists (including propranolol) do not block these responses.

TABLE 10–7 ■ REPRESENTATIVE AGENTS ACTING AT PERIPHERAL CHOLINERGIC AND ADRENERGIC NEUROEFFECTOR JUNCTIONS

MECHANISM OF ACTION	SYSTEM	AGENTS	EFFECT
1. Interference with synthesis of transmitter	Cholinergic	Choline acetyl transferase inhibitors	Minimal depletion of ACh
	Adrenergic	α-Methyltyrosine (inhibition of tyrosine hydroxylase)	Depletion of NE
2. Metabolic transformation by same pathway as precursor of transmitter	Adrenergic	Methyldopa	Displacement of NE by α-methyl-NE, which is an α_2 agonist, similar to clonidine, that reduces sympathetic outflow from CNS
3. Blockade of transport system at nerve terminal membrane	Cholinergic	Hemicholinium	Block of choline uptake with consequent depletion of ACh
	Adrenergic	Cocaine, imipramine	Accumulation of NE at receptors
4. Blockade of transport system of storage vesicle	Cholinergic	Vesamicol	Block of ACh storage
	Adrenergic	Reserpine	Destruction of NE by mitochondrial MAO and depletion from adrenergic terminals
5. Promotion of exocytosis or displacement of transmitter from storage sites	Cholinergic	Latrotoxins	Cholinomimetic followed by anticholinergic
	Adrenergic	Amphetamine, tyramine	Sympathomimetic
6. Prevention of release of transmitter	Cholinergic	Botulinum toxin (BTX, endopeptidase, acts on synaptobrevin)	Anticholinergic (prevents skeletal muscle contraction)
	Adrenergic	Bretylium, guanadrel	Antiadrenergic
7. Mimicry of transmitter at postjunctional sites	Cholinergic		
	Muscarinic[a]	Methacholine, bethanachol	Cholinomimetic
	Nicotinic[b]	Nicotine, epibatidine, cytisine	Cholinomimetic
	Adrenergic		
	α_1	Phenylephrine	Selective α_1 agonist
	α_2	Clonidine	Sympathomimetic (periphery); reduced sympathetic outflow (CNS)
	α_1, α_2	Oxymetazoline	Nonselective α adrenomimetic
	β_1	Dobutamine	Selective cardiac stimulation (also activates α_1 receptors)
	β_2	Terbutaline, albuterol metaproterenol	Selective β_2 receptor agonist (selective inhibition of smooth muscle contraction)
	β_1, β_2	Isoproterenol	Nonselective β agonist
8. Blockade of postsynaptic receptor	Cholinergic		
	Muscarinic[a]	Atropine	Muscarinic blockade
	Nicotinic (N_m)[b]	*d*-Tubucurarine, atracurium	Neuromuscular blockade
	Nicotinic (N_n)[b]	Trimethaphan	Ganglionic blockade
	Adrenergic		
	α_1, α_2	Phenoxybenzamine	Nonselective α receptor blockade (irreversible)
	α_1, α_2	Phentolamine	Nonselective α receptor blockade (reversible)
	α_1	Prazosin, terazosin, doxasozin	Selective α_1 receptor blockade (reversible)
	α_2	Yohimbine	Selective α_2 receptor blockade
	β_1, β_2	Propranolol	Nonselective β receptor blockade
	β_1	Metoprolol, atenolol	Selective β_1 receptor blockade (cardiomyocytes; renal j-g cells)
	β_2	—	Selective β_2 receptor blockade (smooth muscle)

(*Continued*)

TABLE 10–7 ■ REPRESENTATIVE AGENTS ACTING AT PERIPHERAL CHOLINERGIC AND ADRENERGIC NEUROEFFECTOR JUNCTIONS *(CONTINUED)*

MECHANISM OF ACTION	SYSTEM	AGENTS	EFFECT
9. Inhibition of enzymatic breakdown of transmitter	Cholinergic	AChE inhibitors edrophonium, neostigmine, pyridostigmine	Cholinomimetic (muscarinic sites) Depolarization blockade (nicotinic sites)
	Adrenergic	Nonselective MAO inhibitors: pargyline, nialamide	Little direct effect on NE or sympathetic response; potentiation of tyramine
		Selective MAO-B inhibitor: selegeline	Adjunct in Parkinson's disease
		Peripheral COMT inhibitor: Entacapone	Adjunct in Parkinson's disease
		COMT inhibitor:Tolcapone	

The j-g cells are renin-secreting cells in the juxtaglomerular complex of the kidney.

[a] At least five subtypes of muscarinic receptors exist (see Table 10–3). Agonists show little subtype selectivity; several antagonists show partial subtype selectivity (see Chapter 11).

[b] Two subtypes of muscle acetylcholine nicotinic receptors and several subtypes of neuronal receptors have been identified (see Table 10–2).

adrenergic receptors have not been elucidated fully. Genetic approaches using transgenic and receptor knockout experiments in mice (discussed further in the chapter) have advanced our understanding. These mouse models have been used to identify and localize particular receptor subtypes and to describe the pathophysiological relevance of individual adrenergic receptor subtypes (Ahles and Engelhardt, 2014; Philipp and Hein, 2004; Xiao et al., 2006).

Molecular Basis of Adrenergic Receptor Function

Structural Features. All adrenergic receptors are GPCRs that link to heterotrimeric G proteins. Structurally, there are similarities in the regions for ligand binding and modulation by intracellular protein kinases (Figure 10–10). The coding region of each of the three β adrenergic receptor genes and the three α_2 adrenergic receptor genes is contained in a single exon, whereas each of the three α_1 adrenergic receptor genes has a single large intron separating regions that encode the body of the receptor from those that encode the seventh transmembrane domain and carboxy terminus (Dorn, 2010). Each major receptor type shows preference for a particular class of G proteins, that is, α_1 to G_q, α_2 to G_i, and β to G_s (see Table 10–6). The responses that follow receptor activation result from G protein-mediated effects on the generation of second messengers and on the activity of ion channels (see Chapter 3). The signaling pathways overlap broadly with those discussed for muscarinic ACh receptors.

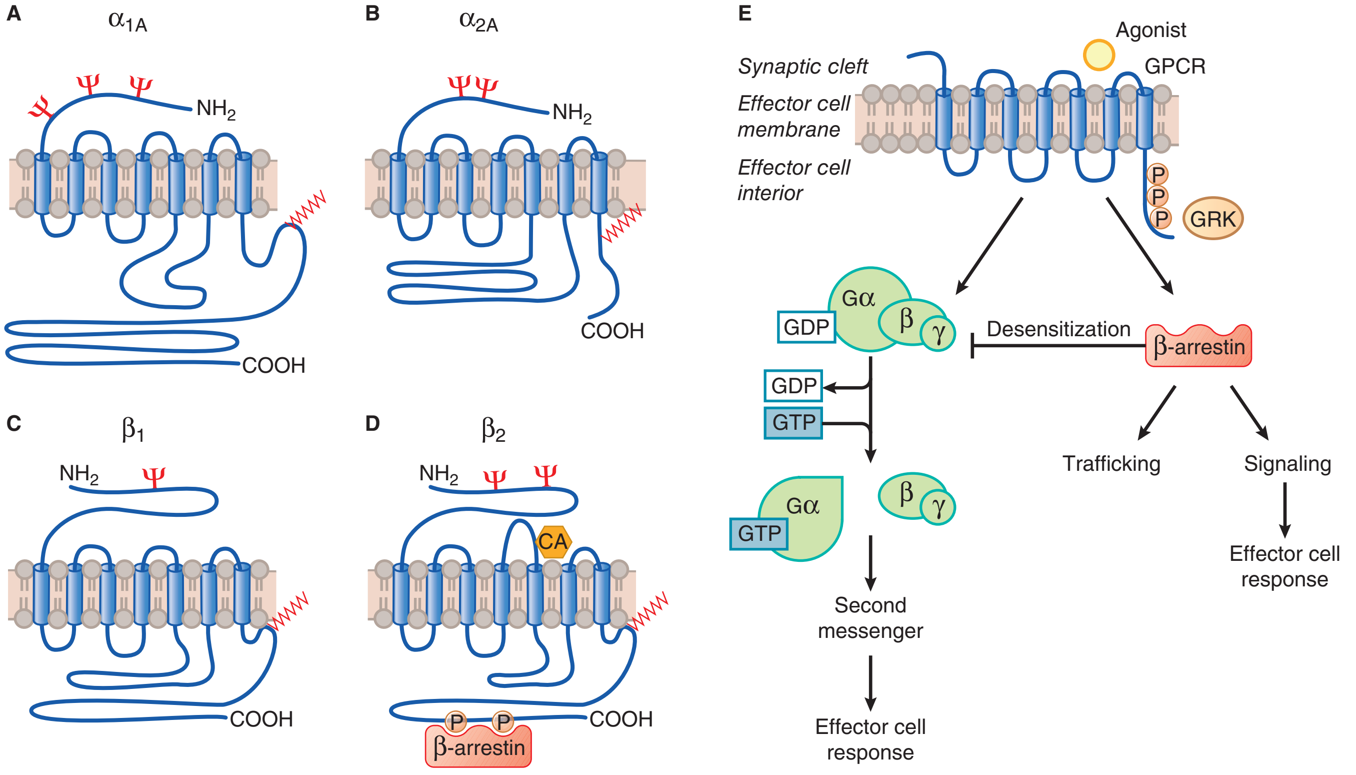

Figure 10–10 *Structural features and functional signaling of adrenergic receptors and subtypes.* All of the adrenergic receptors are hepta-spanning GPCRs. **A–D.** Representatives of each type are shown; each type has three subtypes: α1A, α1B, and α1D; α2A, α2B, and α2C; and β1, β2, and β3. (**E**) shows binding of catecholamine (CA) and β-arrestin. The principal effector systems affected by α1, α2, and β receptors are depicted in Table 10–6. ψ indicates a site for N-glycosylation. **E.** The ligand-receptor complex activates G protein-mediated and β-arrestin–mediated signaling pathways. β-arrestin can act to regulate G protein signaling, desensitization, and trafficking (internalization and translocation), but can also initiate signaling. Biased agonists can act preferentially toward the G protein signaling or β-arrestin–mediated signaling pathways, depending on which arm is favored.

α Adrenergic Receptors. The α_1 receptors (α_{1A}, α_{1B}, and α_{1D}) and the α_2 receptors (α_{2A}, α_{2B}, and α_{2C}) are heptahelical proteins that couple differentially to a variety of G proteins to regulate smooth muscle contraction, secretory pathways, and cell growth (see Table 10–6). Within the membrane-spanning domains, the three α_1 adrenergic receptors share about 75% identity in amino acid residues, as do the three α_2 receptors, but the α_1 and α_2 subtypes are no more similar than are the α and β subtypes (~30%–40%).

α_1 Adrenergic Receptors. Stimulation of α_1 receptors activates the G_q-PLC_β-IP_3/diacylglycerol-Ca^{2+} pathway and results in the activation of PKC and other Ca^{2+} and CaM-sensitive pathways, such as CaM kinases, with sequelae depending on cell differentiation (e.g., contraction of vascular smooth muscle, stimulation of growth in smooth muscles and hypertrophy in cardiac myocytes, and activation of endothelial nitric oxide synthase [NOS] in vascular endothelium) (see Chapter 3). PKC phosphorylates many substrates, including membrane proteins such as channels, pumps, and ion exchange proteins (e.g., Ca^{2+}-transport ATPase). α_1 Receptor stimulation of PLA_2 leads to the release of free arachidonate, which is then metabolized by cyclooxygenase (yielding prostaglandins) and lipoxygenase (yielding leukotrienes) (see Chapter 41); PLD hydrolyzes phosphatidylcholine to yield phosphatidic acid, which can yield diacylglycerol, a cofactor for PKC activation. PLD is an effector for ADP-ribosylating factor, suggesting that PLD may play a role in membrane trafficking. In most smooth muscles, the increased concentration of intracellular Ca^{2+} causes contraction (see Figure 3–26). In contrast, the increased concentration of intracellular Ca^{2+} following α_1 stimulation of GI smooth muscle causes hyperpolarization and relaxation by activation of Ca^{2+}-dependent K^+ channels. Stimulation of α_1 receptors can activate p38/p42/p44, PI3K, JNK, and others to affect cell growth and proliferation, albeit in receptor subtype-specific and tissue-specific manners.

The α_{1A} receptor is the predominant receptor causing vasoconstriction in many vascular beds, including the following arteries: mammary, mesenteric, splenic, hepatic, omental, renal, pulmonary, and epicardial coronary. It is also the predominant subtype in the vena cava and the saphenous and pulmonary veins. Together with the α_{1B} receptor subtype, it promotes cardiac growth and structure. The α_{1B} receptor subtype is the most abundant subtype in the heart, whereas the α_{1D} receptor subtype is the predominant receptor causing vasoconstriction in the aorta. There is evidence to support the idea that α_{1B} receptors mediate behaviors such as reaction to novelty and exploration and are involved in behavioral sensitizations and in the vulnerability to addiction (see Chapter 28).

In addition to their traditional localization in the plasma membrane, α_1 receptors have nuclear localization signals (as do β receptors and receptors for endothelin and angiotensin) and have been found on the nuclear membrane of adult mouse cardiac myocytes, where they activate intranuclear signaling and appear to play a cardioprotective role (Wu and O'Connell, 2015).

α_2 Adrenergic Receptors. The α_2 adrenergic receptors couple to a variety of effectors. Inhibition of adenylyl cyclase activity was the first effect observed, but in some systems, the enzyme actually is stimulated by α_2 adrenergic receptors, either by G_i βγ subunits or by weak direct stimulation of G_s. The physiological significance of these last processes is unclear. The α_2 receptors activate G protein-gated K^+ channels, resulting in membrane hyperpolarization. In some cases (e.g., cholinergic neurons in the myenteric plexus), this may be Ca^{2+} dependent, whereas in others (e.g., muscarinic ACh receptors in atrial myocytes), it results from direct interaction of βγ subunits with K^+ channels. The α_2 receptors also can inhibit voltage-gated Ca^{2+} channels; this is mediated by G_o. Other second-messenger systems linked to α_2 receptor activation include acceleration of Na^+/H^+ exchange, stimulation of $PLC\beta_2$ activity and arachidonic acid mobilization, increased phosphoinositide hydrolysis, and increased intracellular availability of Ca^{2+}. The last is involved in the smooth muscle–contracting effect of α_2 adrenergic receptor agonists. In addition, the α_2 receptors activate MAPKs via mechanisms dependent on both the α and βγ components of G_i, with involvement of protein tyrosine kinases and small GTPases (Goldsmith and Dhanasekaran, 2007). These pathways are reminiscent of pathways activated by tyrosine kinase activities of growth factor receptors. The α_{2A} and α_{2C} receptors play a major role in inhibiting NE release from sympathetic nerve endings and suppressing sympathetic outflow from the brain, leading to hypotension (Kable et al., 2000).

Thus, depending on subtype, the major biological effects of α_2 adrenergic receptors can be on platelet aggregation, regulation of sympathetic outflow from the CNS, reuptake of NE from within peripheral sympathetic nerve synapses, insulin secretion and lipolysis, or, to a limited extent, vasoconstriction (Gyires et al., 2009). Similar studies with knockout mice have been carried out as was done with α_1 adrenergic receptors.

In the CNS, α_{2A} receptors, which appear to be the dominant adrenergic receptor, probably mediate the antinociceptive effects, sedation, hypothermia, hypotension, and behavioral actions of α_2 agonists. The α_{2C} receptor occurs in the ventral and dorsal striatum and hippocampus. It appears to modulate DA neurotransmission and various behavioral responses. The α_{2B} receptor is the main receptor mediating α_2-induced vasoconstriction, whereas the α_{2C} receptor is the predominant receptor inhibiting the release of catecholamines from the adrenal medulla and modulating DA neurotransmission in the brain.

β Adrenergic Receptors

Subtypes. The three β receptor subtypes share about 60% amino acid sequence identity within the putative membrane-spanning domains where the ligand-binding pockets for EPI and NE are found. Based on results of site-directed mutagenesis, individual amino acids in the β_2 receptor that interact with each of the functional groups on the catecholamine agonist molecule have been identified. Figure 10–10 depicts the general hepta-spanning structure of adrenergic receptors and notes some differences in the sizes of the third and fourth intracellular loops.

The β receptors regulate numerous functional responses, including heart rate and contractility, smooth muscle relaxation, and myriad metabolic events in numerous tissues, including skeletal muscle, liver, and adipose tissue (see Table 10–1).

β Receptor Signaling. All three of the β receptor subtypes (β_1, β_2, and β_3) couple to G_s and activate adenylyl cyclase (see Table 10–7). Stimulation of β adrenergic receptors leads to the accumulation of cAMP, activation of the PKA, and altered function of numerous cellular proteins as a result of their phosphorylation (see Chapter 3). In addition, G_s subunits can enhance directly the activation of voltage-sensitive Ca^{2+} channels in the plasma membrane of skeletal and cardiac muscle cells.

The β_1, β_2, and β_3 receptors can differ in their intracellular signaling pathways and subcellular location (Brodde et al., 2006; Violin and Lefkowitz, 2007). While the positive chronotropic effects of β_1 receptor activation are clearly mediated by G_s in myocytes, dual coupling of β_2 receptors to G_s and G_i occurs in myocytes from newborn mice. Stimulation of β_2 receptors causes a transient increase in heart rate that is followed by a prolonged decrease. Following pretreatment with pertussis toxin, which prevents activation of G_i, the negative chronotropic effect of β_2 activation is abolished. These specific signaling properties of β adrenergic receptor subtypes likely result from subtype-selective association with intracellular scaffolding and signaling proteins (Baillie and Houslay, 2005). The β_2 receptors normally are confined to caveolae in cardiac myocyte membranes. The activation of PKA by cAMP and the importance of compartmentation of components of the cAMP pathway are discussed in Chapter 3.

Refractoriness to Catecholamines. Exposure of catecholamine-sensitive cells and tissues to adrenergic agonists causes a progressive diminution in their capacity to respond to such agents. This phenomenon, variously termed *refractoriness, desensitization,* or *tachyphylaxis,* can limit the therapeutic efficacy and duration of action of catecholamines and other agents and is due to phosphorylation of the active receptor by G protein-coupled receptor kinases (GRKs) followed by arrestin binding to receptors, blocking further G protein activation (Chapter 3). An understanding of the mechanisms involved in regulation of G protein-coupled receptor (GPCR) desensitization and the roles of GRKs and β-arrestins has developed over the past two decades due to the efforts of Lefkowitz and colleagues (Violin and Lefkowitz, 2007) and Houslay and colleagues

(Baillie and Houslay, 2005), among others. For a perspective on refractoriness and on the roles of GRKs and β-arrestins in biased agonism, see the discussion that follows.

Desensitization has functional correlates in human health. Long-term exposure to catecholamines can cause cardiac dysfunction and contribute to the course of deterioration in heart failure. Data support the idea that the β_1 receptor is the primary mediator of catecholamine cardiotoxicity (Dorn, 2010). Studies in genetically manipulated mice indicate that β_1 receptor signaling has greater potential than β_2 receptor signaling to contribute to heart failure.

Desensitization, Downregulation, Sustained Signaling. Catecholamines promote β receptor feedback regulation, that is, desensitization, receptor downregulation, and internalization into endosomes. The β receptors differ in the extent to which they undergo such regulation, with the β_2 receptor being the most susceptible, as described in Chapter 3. Poststimulatory interactions of the agonist-liganded β_2 receptor with a GPCR kinase produces a phosphorylated receptor that readily interacts with β-arrestin, which blocks receptor access to the G protein and directs the receptor toward an endocytotic pathway, thereby reducing the number of receptors available at the cell surface. As a scaffolding protein, β-arrestin can also anchor proteins such as phosphodiesterase 4, which can modulate cAMP accumulation. The β receptor–β-arrestin complexes localize to coated pits and are subsequently internalized reversibly into endosomes (where the receptors may be dephosphorylated; such receptors can reenter the plasma membrane to aid resensitization), with some complexes reaching lysosomes, where they are degraded (see Chapter 3). β-Arrestin also serves as an organizing center for the formation of a complex of a phospho-GPCR, a G protein, and β-arrestin, and this complex may provide sustained intracellular signaling from the internalized GPCR (Thomsen et al., 2016).

Biased Agonism and Selective Responsiveness. The original idea that a β adrenergic agonist activates just the G_s–adenylyl cyclase–cAMP-PKA pathway and all else follows is incomplete. What follows the elevation of cAMP depends on the differentiated state of the cell type in question; cells have specialized responses just as they have specialized functions and functional compartmentation of signaling (Steinberg and Brunton, 2001). There are also notable differences in downstream signals activated by the three β receptor subtypes and differences when different ligands activate a single receptor subtype. Ligands can induce receptor conformations that may not activate G protein/adenylyl cyclase signaling or that activate G protein pathways but do not elicit GPCR kinase–β-arrestin actions, generating "biased" responses (see Chapters 3, 14, and 23). This is demonstrated by four findings:

- Signaling resulting from GPCR activation can be complex and involve a host of pathways.
- Ligand-activated GPCRs can adopt a multiplicity of conformations.
- GRKs and β-arrestins are also signal transducers, independently of G proteins.
- Distinct GRKs are recruited to and phosphorylate receptors based on specific ligand-induced receptor conformations, leading to specific signaling mediated by β-arrestin.

A biased agonist stabilizes one or a subset of possible GPCR conformations and thereby activates only a subset of all possible responses (such as G protein only or β-arrestin only); these responses may involve signaling mechanisms mediated by β-arrestins through its myriad scaffolding partners. In work leading to the Nobel Prize in 2012, Lefkowitz and colleagues described this "pluridimensionality of β-arrestin–dependent signaling" at GPCRs (Reiter et al., 2012). This idea raises the possibility that one may design biased agonists that have unusually precise specificity. Advantages of biased agonism include targeting of specific response pathways, improving efficacy while reducing or avoiding side effects, and permitting exploration of signaling pathways previously deemed unattractive targets due to overbroad effects. Biased agonism is discussed at greater length in Chapter 3, in Chapter 14 for adrenergic receptor ligands, and in Chapter 23 with regard to mu opioid agonists.

Adrenergic Receptor Polymorphism

Numerous polymorphisms and splice variants of adrenergic receptors continue to be identified. Such polymorphisms in adrenergic receptors could result in altered physiological responses to activation of the sympathetic nervous system, contribute to disease states, and alter the responses to adrenergic agonists or antagonists (Ahles, 2014; Brodde, 2008). Knowledge of the functional consequences of specific polymorphisms holds the potential for the individualization of drug therapy based on a patient's genetic makeup and could, in part, contribute to the understanding of interindividual variability within the human population, in conjunction with other environmental and societal (nongenetic) factors.

α_1 Adrenergic Receptor Polymorphisms. The α_1 adrenergic receptor is abundant in vascular smooth muscle and is implicated in regulating arterial resistance and blood pressure. The α_1 adrenergic receptor polymorphism most often studied in human hypertension is α_{1A} Arg347Cys; the accumulated data so far suggest only a marginal effect of this polymorphism in cardiovascular responses to sympathetic stimulation or human hypertension. Recent studies suggest adaptive and cardioprotective roles of α_{1A} adrenergic receptor stimulation, including positive inotropy in failing myocytes, protection of the myocardium from ischemia, and physiologic hypertrophy. In addition, it is thought that the pathways that confer these cardioprotective benefits may be selective and operate without affecting blood pressure (Zhang et al., 2021).

α_{2A} Adrenergic Receptor Polymorphisms. As with the α_{1A} adrenergic receptor, there is insufficient evidence supporting a major effect of α_2 receptor polymorphisms in essential hypertension (Dorn, 2010). Although studies suggest an association between α_{2A}, α_{2BA}, and α_{2C} polymorphisms and coronary heart disease, heart failure, and sudden death, these linkages are not yet conclusive and require further study. In contrast, a convincing role for α_{2A} adrenergic receptor polymorphisms in human type 2 diabetes has been elucidated. Moreover, in mice, deletion of the α_{2A} adrenergic receptor results in enhanced insulin secretion, and β-cell–specific overexpression of α_{2A}R mimics diabetes (Lin et al., 2021).

β_1 Adrenergic Receptor Polymorphisms. Evidence supports the notion that increased cardiomyocyte β_1 receptor signaling by chronic agonist stimulation, increased receptor expression, or enhanced receptor signaling can ultimately result in cardiac toxicity and contribute to heart dysfunction and failure (Dorn, 2010). On the other hand, β_1 adrenergic receptor polymorphisms do not seem to be major risk factors in human hypertension.

Biochemical, functional, and structural studies in cultured cell expression systems and genetic mouse models indicate that the Gly389Arg β_1 adrenergic receptor exhibits a gain-of-signaling function that can initially improve cardiac contractility but ultimately predisposes to cardiomyopathic decompensation. This abnormally active Arg389 receptor is more sensitive to pharmacological blockade and exhibits distinctive responses to various β blockers. This polymorphism may affect heart failure risk or progression, but the β blockers currently in use are sufficient to overcome the subtle differences that polymorphic receptor function may have on heart failure survival (Dorn, 2010).

β_2 Adrenergic Receptor Polymorphisms. Data supporting an interaction between β_2 adrenergic receptor polymorphisms and hypertension are inconclusive and suggest that effects of β_2 adrenergic receptor polymorphisms on blood pressure are modest. Similarly, there is no consensus about β_2 adrenergic receptor polymorphisms and heart disease (Dorn, 2010).

β_3 Adrenergic Receptor Polymorphisms. Polymorphisms of the β_3 adrenergic receptor appear to be associated with diabetes phenotypes, but there have been few clinical cardiac studies (Dorn, 2010).

Localization of Adrenergic Receptors

Presynaptic α_2 and β_2 receptors regulate neurotransmitter release from sympathetic nerve endings. Presynaptic α_2 receptors also may mediate inhibition of release of neurotransmitters other than NE in the central and peripheral nervous systems. Both α_2 and β_2 receptors are located at postsynaptic sites (see Table 10–6), such as on many types of neurons in the brain. In peripheral tissues, postsynaptic α_2 receptors are found in vascular and other smooth muscle cells (where they mediate contraction), adipocytes, and various secretory epithelial cells (intestinal, renal, endocrine). Postsynaptic β_2 receptors can be found in the myocardium (where they mediate contraction) as well as on vascular and other smooth muscle cells (where they mediate relaxation) and skeletal muscle (where they can mediate hypertrophy). Indeed, most normal human cell types express β_2 receptors. Both α_2 and β_2 receptors may be situated at sites that are relatively remote from nerve terminals that release NE. Such extrajunctional receptors typically are found on vascular smooth muscle cells and blood elements (platelets and leukocytes) and may be activated preferentially by circulating catecholamines, particularly EPI.

In contrast, α_1 and β_1 receptors appear to be located mainly in the immediate vicinity of sympathetic adrenergic nerve terminals in peripheral target organs, strategically placed to be activated during stimulation of these nerves. These receptors also are distributed widely in the mammalian brain (see Table 10–6).

The cellular distributions of the three α_1 and three α_2 receptor subtypes still are incompletely understood. Studies using *in situ* hybridization with receptor mRNA and receptor subtype-specific antibodies have allowed subtype identification in some tissues and organs. For example, α_2 receptors in the brain may be both pre- and postsynaptic, and this receptor subtype may function as a presynaptic autoreceptor in central noradrenergic neurons. A similar approach led to the discovery that α_{1A} mRNA is the dominant subtype in prostatic smooth muscle, leading to the development of α blockers as an effective therapeutic approach for treating prostate dysfunction (Akinaga et al., 2019; Gyires et al., 2009).

Pharmacological Considerations

Each step involved in neurotransmission is a potential point of pharmacological intervention. The diagrams of the cholinergic and adrenergic terminals and their postjunctional sites (see Figures 10–6 and 10–8) show these points of intervention. Drugs that affect processes involved in the steps of transmission at both cholinergic and adrenergic junctions are summarized in Table 10–7, which lists representative agents that act through the mechanisms below.

Interference With the Synthesis or Release of the Transmitter

Cholinergic

Hemicholinium, a synthetic compound, blocks the transport system by which choline accumulates in the terminals of cholinergic fibers, thus limiting the synthesis of ACh. *Vesamicol* blocks the transport of ACh into its storage vesicles, thereby preventing repletion of ACh stores following transmitter release and thus reducing ACh available for subsequent release. The site on the presynaptic nerve terminal for block of ACh release by botulinum toxin was discussed previously; death usually results from respiratory paralysis unless patients with respiratory failure receive artificial ventilation. Injected locally, botulinum toxin type A is used in the treatment of certain ophthalmic conditions associated with spasms of ocular muscles (e.g., strabismus and blepharospasm) (see Chapter 74) and for a wide variety of unlabeled uses, ranging from treatment of muscle dystonias and palsy (see Chapter 13) to cosmetic erasure of facial lines and wrinkles (a modern medical testament to the vanity of human wishes; see Chapter 75).

Adrenergic

α-Methyltyrosine (*metyrosine*) blocks the synthesis of NE by inhibiting TH, the enzyme that catalyzes the rate-limiting step in catecholamine synthesis. This drug occasionally may be useful in treating selected patients with pheochromocytoma. On the other hand, *methyldopa*, an inhibitor of aromatic *L*-amino acid decarboxylase, is—like dopa itself—successively decarboxylated and hydroxylated in its side chain to form the putative “false neurotransmitter” α-methylnorepinephrine. The use of *methyldopa* in the treatment of hypertension is discussed in Chapter 32. *Bretylium*, *guanadrel*, and *guanethidine* act by preventing the release of NE by the nerve impulse. However, such agents can transiently stimulate the release of NE because of their capacity to displace the amine from storage sites.

Promotion of Release of the Transmitter

Cholinergic

The capacity of pharmacological agents to promote the release of ACh is limited. The latrotoxins from black widow spider venom and stonefish are known to promote neuroexocytosis by binding to receptors on the neuronal membrane.

Adrenergic

Several drugs that promote the release of NE already have been discussed. Based on the rate and duration of the drug-induced release of NE from adrenergic terminals, one of two opposing effects can predominate. *Tyramine*, *ephedrine*, *amphetamine*, and related drugs cause a relatively rapid, brief liberation of the transmitter and produce a sympathomimetic effect.

On the other hand, *reserpine*, by irreversibly blocking the uptake of amines by both VMAT2 and VMAT1, produces a slow, prolonged depletion of the adrenergic transmitter from adrenergic storage vesicles, where the transmitters, trapped in the cytosol, are largely metabolized by intraneuronal MAO. The resulting depletion of transmitter produces the equivalent of adrenergic blockade. *Reserpine* also causes the depletion of 5HT, DA, and possibly other, unidentified, amines from central and peripheral sites; many of its effects may be consequences of the depletion of transmitters other than NE. Another VMAT inhibitor, *tetrabenazine*, acts reversibly and more specifically on VMAT2, avoiding some of the effects of inhibition of VMAT1 that are components of *reserpine*’s actions.

As discussed previously, deficiencies of TH in humans cause a neurologic disorder (Carson and Robertson, 2002) that can be treated by supplementation with the DA precursor *levodopa*.

A syndrome caused by congenital DβH deficiency is characterized by the absence of NE and EPI, elevated concentrations of DA, intact baroreceptor reflex afferent fibers and cholinergic innervation, and undetectable concentrations of plasma DβH activity (Carson and Robertson, 2002). Patients with this syndrome have severe orthostatic hypotension, ptosis of the eyelids, and retrograde ejaculations. *Dihydroxyphenylserine* (L-DOPS) improves postural hypotension in this rare disorder. This therapeutic approach takes advantage of the nonspecificity of aromatic *L*-amino acid decarboxylase, which synthesizes NE directly from this drug in the absence of DβH. Despite the restoration of plasma NE in humans with L-DOPS, EPI levels are not restored, leading to speculation that PNMT may require DβH for appropriate functioning (Carson and Robertson, 2002).

Agonist and Antagonist Actions at Receptors

Cholinergic

The nicotinic receptors of autonomic ganglia and skeletal muscle are not identical; they respond differently to certain stimulating and blocking agents, and their pentameric structures contain different combinations of homologous subunits (see Table 10–2). *Dimethylphenylpiperazinium* (DMPP) and *phenyltrimethylammonium* (PTMA) show some selectivity for stimulation of autonomic ganglion cells and muscle motor end plates. *Trimethaphan* and *hexamethonium* are relatively selective competitive and noncompetitive ganglionic blocking agents, respectively. Although *tubocurarine* effectively blocks transmission at both motor end plates and autonomic ganglia, its action at the former site predominates. *Succinylcholine*, a depolarizing agent, produces selective neuromuscular blockade. Transmission at autonomic ganglia and the adrenal medulla is complicated further by the presence of muscarinic receptors in addition to the principal nicotinic receptors (see Chapter 13).

Various toxins in snake venoms exhibit a high degree of specificity toward cholinergic receptors. The α-neurotoxins from the *Elapidae* family interact with the agonist-binding site on the nicotinic receptor. α-Bungarotoxin is selective for the muscle receptor and interacts with

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only certain neuronal receptors, such as those containing α7 through α9 subunits. Neuronal bungarotoxin shows a wider range of inhibition of neuronal receptors. A second group of toxins, called the *fasciculins,* inhibits AChE. A third group of toxins, termed the *muscarinic toxins* (MT_1 through MT_4), includes partial agonists and antagonists for muscarinic receptors. Venoms from the *Viperidae* family of snakes and the fish-hunting cone snails also have relatively selective toxins for nicotinic receptors.

Muscarinic ACh receptors, which mediate the effects of ACh at autonomic effector cells, now can be divided into five subclasses. *Atropine* blocks all the muscarinic responses to injected ACh and related cholinomimetic drugs whether they are excitatory, as in the intestine, or inhibitory, as in the heart. Several newer compounds show selectivity as muscarinic-blocking agents: *pirenzepine* for M_1, *tripitramine* for M_2, and *darifenacin* for M_3 receptors. These muscarinic antagonists show sufficient selectivity in the clinical setting to minimize the bothersome side effects seen with the nonselective agents at therapeutic doses (see Chapter 11).

Adrenergic

A vast number of synthetic compounds that bear structural resemblance to the naturally occurring catecholamines can interact with α and β adrenergic receptors to produce sympathomimetic effects (see Chapter 14). *Phenylephrine* acts selectively at α_1 receptors, whereas *clonidine* is a selective α_2 adrenergic agonist. *Isoproterenol* exhibits agonist activity at both β_1 and β_2 receptors. Preferential stimulation of cardiac β_1 receptors follows the administration of *dobutamine*. *Terbutaline* exerts relatively selective action on β_2 receptors; it produces effective bronchodilation with minimal effects on the heart. The main features of adrenergic blockade, including the selectivity of various blocking agents for α and β adrenergic receptors, are considered in detail in Chapter 14. Partial dissociation of effects at β_1 and β_2 receptors has been achieved by subtype-selective antagonists, as exemplified by the β_1 receptor antagonists *metoprolol* and *atenolol*, which antagonize the cardiac actions of catecholamines while causing less antagonism at the β_2 receptors of the bronchioles. *Prazosin* and *yohimbine* are representative of α_1 and α_2 receptor antagonists, respectively; *prazosin* has a relatively high affinity at α_{2B} and α_{2C} subtypes compared with α_{2A} receptors. Several important drugs that promote the release of NE (e.g., *tyramine*) or deplete the transmitter (e.g., *reserpine*) resemble, in their effects, activators or blockers of postjunctional receptors.

Interference With the Destruction of the Transmitter

Cholinergic

The anti-cholinesterase agents (see Chapter 12) constitute a chemically diverse group of compounds, the primary action of which is inhibition of AChE, with the consequent accumulation of endogenous ACh. At the NMJ, accumulation of ACh produces depolarization of end plates and flaccid paralysis. At postganglionic muscarinic effector sites, the response is either excessive stimulation resulting in contraction and secretion or an inhibitory response mediated by hyperpolarization. At ganglia, depolarization and enhanced transmission are observed.

Adrenergic

The reuptake of NE by the adrenergic nerve terminals by means of NET is the major mechanism for terminating NE's transmitter action. Interference with this process is the basis of the potentiating effect of *cocaine* on responses to adrenergic impulses and injected catecholamines. The antidepressant actions and some of the adverse effects of *imipramine* and related drugs may be due to a similar action at adrenergic synapses in the CNS (see Chapter 18).

Entacapone and *tolcapone* are nitro catechol-type COMT inhibitors. *Entacapone* is a peripherally acting COMT inhibitor, whereas *tolcapone* also inhibits COMT activity in the brain. COMT inhibition can attenuate *levodopa* toxicity on dopaminergic neurons and enhance DA's action in the brain of patients with Parkinson's disease (see Chapter 21). On the other hand, nonselective MAO inhibitors, such as *tranylcypromine*, potentiate the effects of *tyramine* and may potentiate effects of neurotransmitters. While most MAO inhibitors used as antidepressants inhibit both MAO-A and MAO-B, selective MAO-A and MAO-B inhibitors are available. *Selegiline* is a selective and irreversible MAO-B inhibitor that also has been used as an adjunct in the treatment of Parkinson's disease.

Other Autonomic Neurotransmitters

ATP and ACh coexist in cholinergic vesicles (Dowdall et al., 1974), and ATP, NPY, and catecholamines are found within storage granules in nerves and the adrenal medulla (see previous discussion). ATP is released along with the transmitters, and it and its metabolites can play significant roles in synaptic transmission in some circumstances (see further discussion). Recently, attention has focused on the growing list of peptides that are found in the adrenal medulla, nerve fibers, or ganglia of the autonomic nervous system or in the structures that are innervated by the autonomic nervous system. This list includes enkephalins, substance P and other tachykinins, SST, gonadotropin-releasing hormone, cholecystokinin, CGRP, galanin, pituitary adenylyl cyclase–activating peptide, VIP, chromogranins, and NPY (Hökfelt et al., 2000). Some of the orphan GPCRs discovered in the course of genome-sequencing projects may represent receptors for undiscovered peptides or other cotransmitters.

Cotransmission in the Autonomic Nervous System

There is a large body of literature on cotransmission in the autonomic nervous system. Much of the research in this area has focused on corelease of ATP by adrenergic and cholinergic nerves. Co-release of NPY, VIP, CGRP, substance P, and NO has also been studied. Whether these co-released factors act as neurotransmitters, neuromodulators, or trophic factors remains a topic of debate (Burnstock, 2013; Burnstock et al., 2015; Mutafova-Yambolieva and Durnin, 2014).

The evidence is substantial that ATP plays a role in sympathetic nerves as a cotransmitter with NE (Westfall et al., 2002). For example, the rodent vas deferens is supplied with dense sympathetic innervation, and stimulation of the nerves results in a biphasic mechanical response that consists of an initial rapid twitch followed by a sustained contraction. The first phase of the response is mediated by ATP acting on postjunctional P2X receptors, whereas the second phase is mediated mainly by NE acting on α_1 receptors (Sneddon and Westfall, 1984). The cotransmitters apparently are released from the same types of nerves because pretreatment with 6-hydroxydopamine, an agent that specifically destroys adrenergic nerves, abolishes both phases of the neurogenically induced biphasic contraction. Whether ATP and NE originate from the same populations of vesicles within a nerve ending is still open to debate and experimentation (Burnstock et al., 2015; Mutafova-Yambolieva and Durnin, 2014).

Once ATP is released into the neuroeffector junction, some of it is metabolized by extracellularly directed membrane-bound nucleotidases to ADP, AMP, and adenosine (Gordon, 1986). However, the majority of its metabolism occurs by the actions of releasable nucleotidases. There is also evidence that ATP and its metabolites exert presynaptic modulatory effects on transmitter release via P2 receptors and receptors for adenosine. In addition to evidence showing that ATP is a cotransmitter with NE, there is evidence that ATP may be a cotransmitter with ACh in certain postganglionic parasympathetic nerves, such as those in the urinary bladder.

The NPY family of peptides is distributed widely in the central and peripheral nervous systems and consists of three members: NPY, pancreatic polypeptide, and peptide YY. NPY is colocalized and co-released with NE and ATP in most sympathetic nerves in the peripheral nervous system, especially those innervating blood vessels (Westfall, 2004). There is also convincing evidence that NPY exerts prejunctional modulatory effects on transmitter release and synthesis. Moreover, there are numerous examples of postjunctional interactions that are consistent with a cotransmitter role for NPY at various sympathetic neuroeffector junctions. Thus, NPY, together with NE and ATP, qualifies as the third sympathetic cotransmitter of the sympathetic branch of the autonomic nervous system. Functions of NPY include:

- Direct postjunctional contractile effects
- Potentiation of the contractile effects of the other sympathetic cotransmitters
- Inhibitory modulation of the nerve stimulation–induced release of all three sympathetic cotransmitters, including actions on autoreceptors to inhibit its own release

Studies with selective NPY-Y_1 antagonists provided evidence that the principal postjunctional receptor is of the Y_1 subtype, although other receptors are also present at some sites and may exert physiological actions. Studies with selective NPY-Y_2 antagonists suggested that the principal prejunctional receptor is of the Y_2 subtype both in the periphery and in the CNS. There is evidence for a role for other NPY receptors, and clarification awaits the further development of selective antagonists. NPY also can act prejunctionally to inhibit the release of ACh, CGRP, and substance P. In the CNS, NPY exists as a cotransmitter with catecholamine in some neurons and with peptides and mediators in others. A prominent action of NPY is the presynaptic inhibition of the release of various neurotransmitters, including NE, DA, GABA, glutamate, and 5HT, as well as inhibition or stimulation of the release of neurohormones such as gonadotropin-releasing hormone, vasopressin, and oxytocin. Evidence also exists for stimulation of NE and DA release by NPY.

The NPY may use several mechanisms to produce its presynaptic effects, including inhibition of Ca^{2+} channels, activation of K^+ channels, and regulation of the vesicle release complex at some point distal to Ca^{2+} entry. NPY also may play a role in several pathophysiological conditions. The further development of selective NPY agonists and antagonists should enhance understanding about the physiological and pathophysiological roles of NPY.

The pioneering studies of Hökfelt and coworkers, which demonstrated the existence of VIP and ACh in peripheral autonomic neurons, initiated interest in the possibility of peptidergic cotransmission in the autonomic nervous system. Subsequent work has confirmed the frequent association of these two substances in autonomic fibers, including parasympathetic fibers that innervate smooth muscle and exocrine glands and cholinergic sympathetic neurons that innervate sweat glands (Hökfelt et al., 2000).

The role of VIP in parasympathetic transmission has been studied most extensively in the regulation of salivary secretion. The evidence for cotransmission includes the release of VIP following stimulation of the chorda lingual nerve and the incomplete blockade by *atropine* of vasodilation when the frequency of stimulation is raised; the last observation may indicate independent release of the two substances, which is consistent with histochemical evidence for storage of ACh and VIP in separate populations of vesicles. Synergism between ACh and VIP in stimulating vasodilation and secretion also has been described. VIP may be involved in parasympathetic responses in the trachea and in the GI tract, where it may facilitate sphincter relaxation.

Nonadrenergic, Noncholinergic Transmission by Purines

The smooth muscle of many tissues that are innervated by the autonomic nervous system shows inhibitory junction potentials following stimulation by field electrodes. Because such responses frequently are undiminished in the presence of adrenergic and muscarinic cholinergic antagonists, these observations have been taken as evidence for the existence of nonadrenergic, noncholinergic transmission in the autonomic nervous system.

Burnstock and colleagues have compiled compelling evidence for the existence of purinergic neurotransmission in the GI tract, genitourinary tract, and certain blood vessels; ATP fulfills all the criteria for a neurotransmitter. In at least some circumstances, primary sensory axons may be an important source of ATP (Burnstock et al., 2015). Although adenosine is generated from the released ATP by ectoenzymes and releasable nucleotidases, its primary function appears to be modulatory by causing feedback inhibition of transmitter release.

Adenosine can be transported from the cell cytoplasm to activate extracellular receptors on adjacent cells. The efficient uptake of adenosine by cellular transporters and its rapid metabolism to inosine or to adenine nucleotides contribute to its rapid turnover. Several inhibitors of adenosine transport and metabolism can influence concentrations of extracellular adenosine and ATP.

The purinergic receptors found on the cell surface may be divided into the adenosine (P1) receptors and the receptors for ATP (P2X and P2Y receptors). Both P1 and P2 receptors have various subtypes. There are four adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3) and multiple subtypes of P2X and P2Y receptors throughout the body. The adenosine receptors and the P2Y receptors mediate their responses via G proteins, whereas the P2X receptors are a subfamily of ligand-gated ion channels (see Table 16–7) (Burnstock et al., 2015). Methylxanthines such as caffeine and *theophylline* preferentially block P1 adenosine receptors (see Chapter 44).

Signal Integration and Modulation of Vascular Responses by Endothelium-Derived Factors: NO and Endothelin

The contents of adrenergic storage vesicles are not alone in regulating vascular tone. Many other factors modulate vascular contractility, including kinins, angiotensin, natriuretic peptides, substance P, VIP, CGRP, and eicosanoids, all described elsewhere in this volume. There are additional factors generated by the vascular endothelium that influence vascular reactivity: NO and endothelin.

Furchgott and colleagues demonstrated that an intact endothelium is necessary to achieve vascular relaxation in response to ACh (Furchgott, 1999). This inner cellular layer of the blood vessel now is known to modulate autonomic and hormonal effects on the contractility of blood vessels. In response to a variety of vasoactive agents and physical stimuli, endothelial cells release a short-lived vasodilator termed endothelium-derived relaxing factor, now identified as NO. Less commonly, an endothelium-derived hyperpolarizing factor and endothelium-derived contracting factor are released (Vanhoutte, 1996). Formation of endothelium-derived contracting factor depends on cyclooxygenase activity.

Products of inflammation and platelet aggregation (e.g., 5HT, histamine, bradykinin, purines, and thrombin) exert all or part of their action by stimulating the production of NO. Endothelium-dependent mechanisms of relaxation are important in a variety of vascular beds, including the coronary circulation. Activation of specific GPCRs linking to G_q and the mobilization of Ca^{2+} within endothelial cells promotes NO production. NO diffuses readily to the underlying smooth muscle and induces relaxation of vascular smooth muscle by activating the soluble form of guanylyl cyclase, which increases cyclic GMP concentrations (see Figures 3–21 and 3–26). Nitrovasodilating drugs used to lower blood pressure or to treat ischemic heart disease probably act through conversion to or release of NO (see Chapter 31). Certain nerves (termed *nitrergic*) innervating blood vessels and smooth muscles of the GI tract also release NO. NO has a negative inotropic action on the heart.

Alterations in the release or action of NO may affect a number of major clinical situations, such as atherosclerosis (Ignarro et al., 1999; Münzel et al., 2003). Furthermore, there is evidence suggesting that the hypotension of endotoxemia or that induced by cytokines is mediated by induction of NOS2 (the inducible form of NOS) and the enhanced production NO; consequently, increased NO production may have pathological significance in septic shock.

Full contractile responses of cerebral arteries also require an intact endothelium. A family of peptides, termed *endothelins,* is stored in vascular endothelial cells. Endothelin contributes to the maintenance of vascular homeostasis by acting via multiple endothelin receptors that are GPCRs. The release of endothelin-1 (21 amino acids) onto smooth muscle promotes contraction by stimulation of the ET_A receptor. Endothelin antagonists are now employed in treating pulmonary artery hypertension (see Chapter 35).

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Chapter 11

Muscarinic Receptor Agonists and Antagonists

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ACETYLCHOLINE AND ITS MUSCARINIC RECEPTOR TARGET

- Properties and Subtypes of Muscarinic Receptors
- Pharmacological Effects of Acetylcholine

MUSCARINIC RECEPTOR AGONISTS

- ADME
- Therapeutic Uses of Muscarinic Receptor Agonists
- Contraindications, Precautions, and Adverse Effects
- Toxicology

MUSCARINIC RECEPTOR ANTAGONISTS

- Structure-Activity Relationships
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- Toxicology of Drugs With Antimuscarinic Properties

Acetylcholine and Its Muscarinic Receptor Target

Muscarinic acetylcholine (ACh) receptors in the peripheral nervous system are found primarily on autonomic effector cells innervated by postganglionic parasympathetic nerves. The actions of ACh and related drugs at autonomic effector sites are referred to as *muscarinic*, based on the observation that the alkaloid muscarine acts selectively at those sites and produces the same qualitative effects as ACh. The muscarinic, or parasympathomimetic, actions of the drugs considered in this chapter are practically equivalent to the parasympathetic effects of ACh listed in Table 10–1.

Muscarinic receptors are also present in autonomic ganglia and on some cells (e.g., vascular endothelial cells) that, paradoxically, receive little or no cholinergic innervation. Muscarinic receptors in autonomic ganglia and the adrenal medulla primarily function to modulate the nicotinic actions of ACh at these sites (Chapter 13). In the CNS, muscarinic receptors are widely distributed and have a role in mediating many important responses. Within the CNS, the hippocampus, cortex, and striatum have particularly high densities of muscarinic receptors.

ACh, the naturally occurring neurotransmitter for these receptors, has virtually no systemic therapeutic applications because its actions are diffuse, and its hydrolysis, catalyzed by both acetylcholinesterase (AChE) and plasma butyrylcholinesterase, is rapid. In addition, its penetration to the CNS is limited, and the amount of ACh that reaches peripheral areas with low blood flow is negligible due to its hydrolysis.

Muscarinic agonists mimic the muscarinic receptor–mediated effects of ACh. These agonists typically are longer-acting congeners of ACh or natural alkaloids, some of which stimulate nicotinic as well as muscarinic receptors. The differences between the actions of ACh and other muscarinic agonists at muscarinic receptors are largely quantitative, with limited selectivity for one organ system over another. All of the muscarinic actions of ACh and its congeners can be competitively inhibited by *atropine*.

Properties and Subtypes of Muscarinic Receptors

Muscarinic receptors were characterized initially by analysis of the responses of cells and organ systems in the periphery and the CNS. Differential effects of two muscarinic agonists, *bethanechol* and McN-A-343, on the tone of the lower esophageal sphincter led to the initial designation of muscarinic receptors as M_1 (ganglionic) and M_2 (effector cell) (Goyal and Rattan, 1978). This concept was supplanted by information obtained through molecular cloning of muscarinic receptors, which identified five distinct gene products (Bonner et al., 1987), now designated as M_1 through M_5 muscarinic receptors (Chapter 8). All of the known muscarinic receptors are coupled to heterotrimeric G proteins, which in turn couple to various cellular effectors (Chapter 3). Although selectivity is not absolute, stimulation of M_1, M_3, and M_5 receptors causes activation of G_q and phospholipase C (PLC), hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2), and mobilization of intracellular Ca^{2+} as well as activation of protein kinase C (Figure 11–1), resulting in a variety of cellular responses. In contrast, M_2 and M_4 muscarinic receptors inhibit adenylyl cyclase and regulate specific ion channels via their coupling to the pertussis toxin–sensitive G proteins, G_i and G_o. Recent cryo-electron microscopy studies have revealed the structural basis underlying the G protein-coupling preferences of the individual muscarinic receptor subtypes (Maeda et al., 2019).

Over the past decade, high-resolution X-ray structures have been reported for all five muscarinic receptors (Haga et al., 2012; Kruse et al., 2012, 2013; Thal et al., 2016; Vuckovic et al., 2019), revealing that the structures of the five receptors are very similar (Figure 11–2, A–C). The classical (*orthosteric*) binding site for muscarinic agonists and antagonists is highly conserved among all receptor subtypes (Kruse et al., 2014; Maeda et al., 2019; Thal et al., 2016) (Figure 11–2A). The orthosteric binding site consists of a cleft deeply buried within the membrane, formed by conserved amino acid chains located on several of the receptor's seven transmembrane (TM) helices (TM1–TM7). A key feature shared by other receptors for biogenic amine ligands is the presence of a charge-charge interaction between the tertiary or quaternary nitrogen of the orthosteric ligands and a conserved TM3 aspartic acid side chain. A feature unique to muscarinic receptors is hydrogen bond interactions between the orthosteric ligand and a TM6 asparagine residue. Agonist binding to the receptor leads to considerable contraction of the ligand-binding pocket, reflecting the relatively small size of muscarinic agonists, as compared to muscarinic antagonists. Because the residues that line the orthosteric binding site are highly conserved among all muscarinic receptors (Figure 11–2A), developing orthosteric muscarinic ligands endowed with a high degree of receptor subtype selectivity has proven challenging.

The five muscarinic receptor subtypes are widely distributed in both the CNS and peripheral tissues; most cells express at least two subtypes (Abrams et al., 2006; Lebois et al., 2018; Wess et al., 2007). Identifying the role of a specific subtype in mediating a particular muscarinic response to ACh has been difficult due to the lack of subtype-specific agonists and antagonists. Recent studies with M_1–M_5 receptor knockout mice

Abbreviations

ACh: acetylcholine
AChE: acetylcholinesterase
AV: atrioventricular
eNOS: endothelial NO synthase
GI: gastrointestinal
GIRK channel: G protein-coupled inwardly-rectifying K^+ channel
GRP: gastrin-releasing peptide
HCN: hyperpolarization-activated, cyclic nucleotide–gated channels
5HT: serotonin
I_{Ca-L}: L-type Ca^{2+} current
I_f: cardiac pacemaker current
I_{K-ACh}: ACh-activated K^+ current
IP_3: inositol 1,4,5-trisphosphate
NAM: negative allosteric modulator
NO: nitric oxide
PAM: positive allosteric modulator
PIP_2: phosphatidylinositol 4,5-bisphosphate
PLC: phospholipase C
SA: sinoatrial
TM: transmembrane

(Kruse et al., 2014; Wess et al., 2007) have, however, yielded novel information about the physiological roles of the individual muscarinic receptor subtypes (see Table 10–3) and demonstrated that multiple receptor subtypes are typically involved in mediating specific muscarinic responses. For example, abolishing cholinergic bronchoconstriction, salivation, pupillary constriction, and bladder contraction generally requires deletion of more than one receptor subtype.

Various lines of evidence suggest that muscarinic receptors possess one or more topographically distinct allosteric binding sites formed by amino acid side chains located within the extracellular loops or the outer segments of different TM helices (Birdsall and Lazareno, 2005; May et al., 2007). These regions show a considerable degree of sequence variation among the M_1–M_5 receptors; thus, considerable effort has been directed at developing so-called allosteric modulators that show high selectivity for distinct muscarinic receptor subtypes (Bock et al., 2018; Conn et al., 2014; Gentry et al., 2015; Moran et al., 2019). These agents exert their pharmacological actions by altering the affinity or efficacy of the orthosteric muscarinic ligand (ACh). Positive allosteric modulators (PAMs) enhance orthosteric activity, while negative allosteric modulators (NAMs) inhibit it. Allosteric agents that can directly activate muscarinic receptors are termed *allosteric agonists*. However, these designations are not absolute; they depend on the nature of the orthosteric ligand, receptor subtype under investigation, and assay system used. The recent progress in identifying subtype-selective muscarinic allosteric agents may lead to the development of new therapeutic agents with increased efficacy and reduced side effects. Currently, much research focuses on the potential

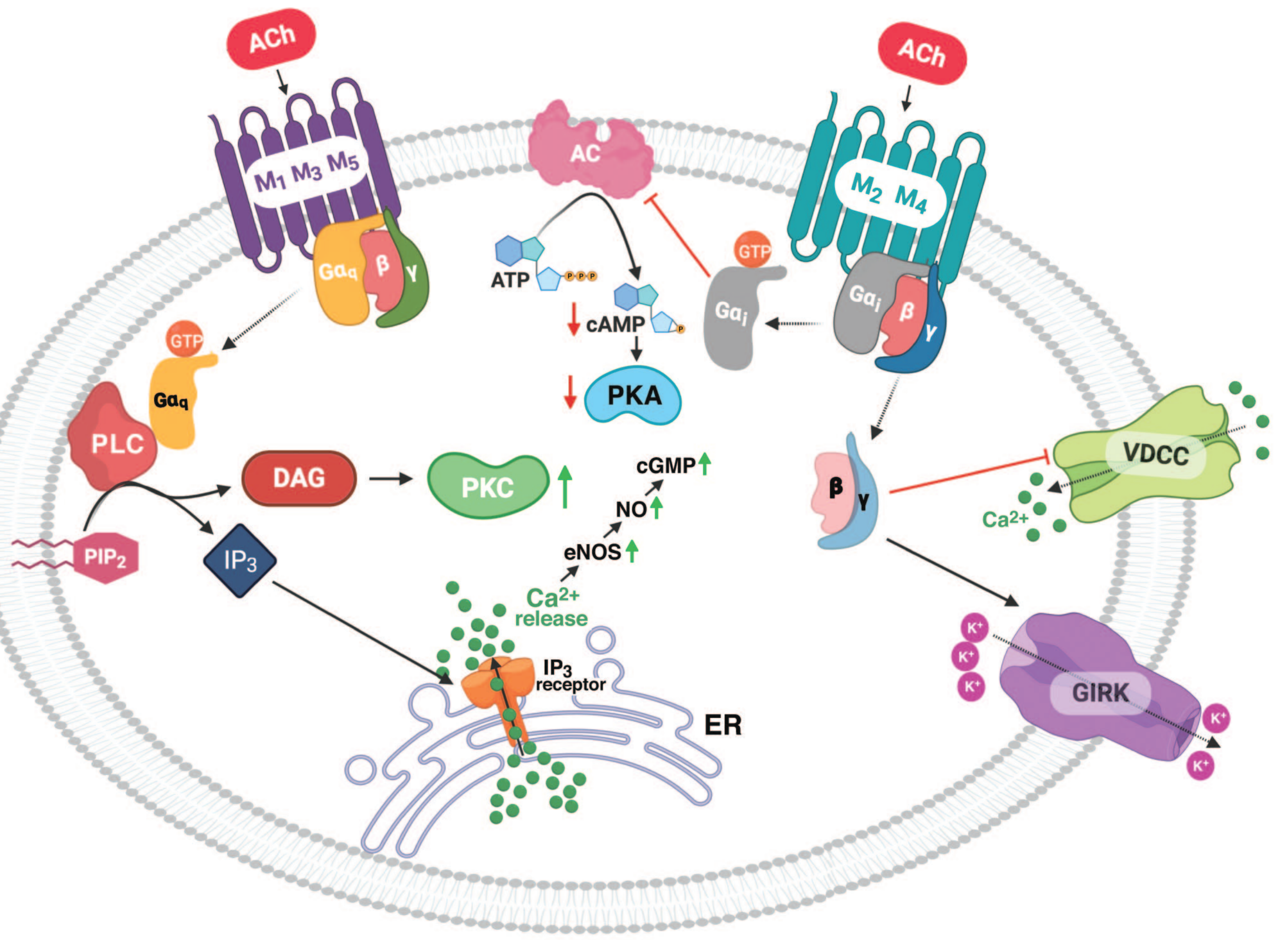

Figure 11–1 *G protein-coupling properties of the M_1–M_5 muscarinic receptors.* Note that the odd-numbered receptors (M_1, M_3, and M_5) selectively couple to G proteins of the G_q family, while the even-numbered receptors (M_2 and M_4) preferentially activate G_i-type G proteins. Some of the major downstream signaling pathways are shown. AC, adenylyl cyclase; DAG, diacylglycerol; IP_3, inositol 1,4,5-trisphosphate; PKA, protein kinase A (cyclic AMP-dependent protein kinase); GIRK channel; G protein-coupled inwardly rectifying K^+ channel; PLC, phospholipase C; PIP_2, phosphatidylinositol 4,5-bisphosphate; VDCC, voltage-dependent Ca^{2+} channel.

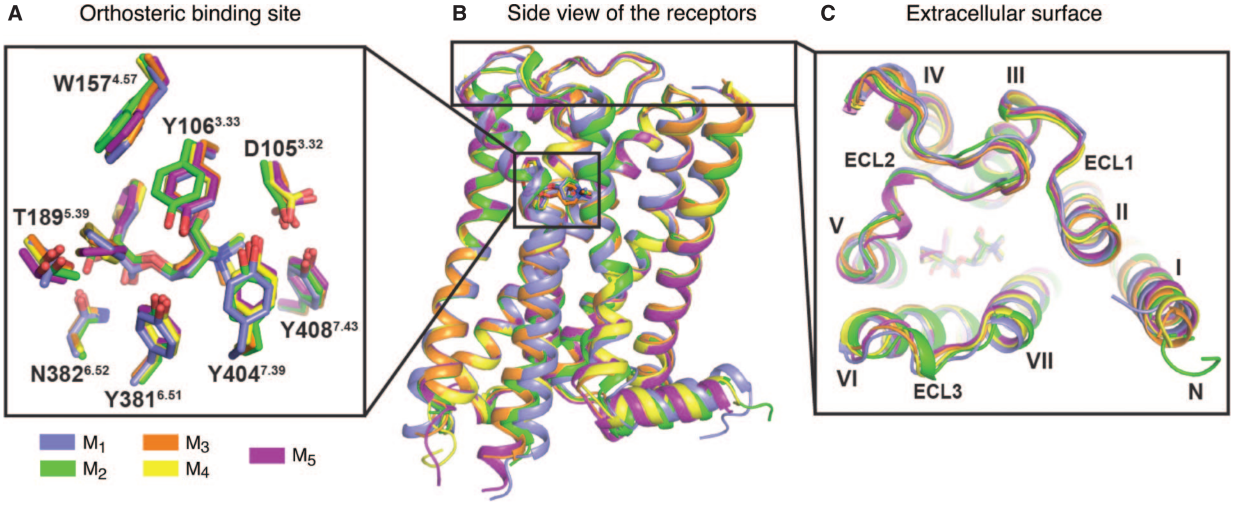

Figure 11–2 *Comparison of the crystal structures of the M1-M5 muscarinic receptors bound to the antagonists tiotropium or N-methylscopolamine (Thal et al., 2016).* **A.** The key amino acids that form the orthosteric binding site. Residues are numbered according to the M1 receptor sequence and the Ballesteros-Weinstein numbering system (superscripts). **B.** Superimposition of all five receptor structures. The orthosteric binding site is located near the middle of the receptors' transmembrane core. **C.** A view of the extracellular surface of the superimposed structures (I -VII, transmembrane helices; N, N-terminal region; ECL, extracellular loop). Note that the positions ECL2 and ECL3 differ slightly from each other among the different receptor subtypes. Additional references: Ballesteros and Weinstein, 1995; Isberg et al., 2015.

of such agents for the treatment of several severe disorders of the CNS, including Alzheimer's disease and schizophrenia (Moran et al., 2019).

An X-ray structure revealed the molecular details of the complex of a positive allosteric modulator (PAM) and the muscarinic receptor; the binding pocket for muscarinic PAMs is located just above the orthosteric binding crevice (Kruse et al., 2013). This structure also illustrates that the bound PAM interferes with the dissociation of the bound orthosteric agonist from the receptor, explaining why the PAM enhances the actions of the orthosteric ligand. Another potential strategy for achieving receptor subtype selectivity is the development of hybrid, bitopic orthosteric/allosteric ligands that interact with both the orthosteric binding cavity and an allosteric site (Bock et al., 2018; Lane et al., 2013). By targeting orthosteric and allosteric sites simultaneously, bitopic ligands achieve both high affinity and receptor subtype selectivity.

Pharmacological Effects of Acetylcholine

The influence of ACh and parasympathetic innervation on various organs and tissues was introduced in Chapter 10. The more detailed description of the effects of ACh presented below provides background for understanding the physiological basis for the therapeutic uses of the muscarinic receptor agonists and antagonists (also see Table 11–1).

Cardiovascular System

ACh has four primary effects on the cardiovascular system:

- Vasodilation
- Decrease in heart rate (negative chronotropic effect)
- Decrease in the conduction velocity in the atrioventricular (AV) node (negative dromotropic effect)
- Decrease in the force of cardiac contraction (negative inotropic effect)

The negative inotropic effect is of lesser significance in the ventricles than in the atria. In addition, some of these effects can be obscured by baroreceptor and other reflexes that dampen the direct responses to ACh.

Cardiac actions of ACh are important because the effects of cardiac glycosides, antiarrhythmic agents, and many other drugs are at least partly due to changes in parasympathetic (vagal) stimulation of the heart; in addition, afferent stimulation of the viscera during surgical interventions can reflexly increase the vagal stimulation of the heart.

The intravenous injection of a small dose of ACh produces a transient fall in blood pressure owing to generalized vasodilation mediated by vascular endothelial nitric oxide (NO). This is usually accompanied by reflex tachycardia. The generalized vasodilation produced by exogenously administered ACh is due to the stimulation of muscarinic receptors, primarily of the M_3 subtype, located on vascular endothelial cells. Occupation of these receptors activates the G_q-PLC-IP_3 pathway, leading to Ca^{2+}-calmodulin–dependent activation of endothelial eNOS (NOS3) and production of NO (endothelium-derived relaxing factor) that diffuses to adjacent vascular smooth muscle cells where it stimulates soluble guanylyl cyclase, thereby promoting relaxation via a cyclic GMP–dependent mechanism (see Figure 3–11; Farah et al., 2018; Harvey, 2012). Baroreceptor or chemoreceptor reflexes or direct stimulation of the vagus can also elicit parasympathetic coronary vasodilation mediated by ACh and the consequent production of NO by the endothelium (Feigl, 1998). If the endothelium is damaged, however, as occurs under various pathophysiological conditions, ACh acts predominantly on M_3 receptors located on the underlying vascular smooth muscle cells, causing vasoconstriction. This capacity to both relax and constrict vessels is shared by many hormones that act via the G_q-PLC-IP_3-Ca^{2+} pathway and for which receptors are present on both endothelial cells and vascular smooth muscle cells. If the agonist can reach both cell types, each cell type will respond in its distinct way to an elevation of intracellular Ca^{2+}—endothelium with a stimulation of NO synthase and smooth muscle with contraction. NO generation in the heart has a multitude of additional effects, and its presence in cholinergic vagal nerves may contribute to vagal dominance (Farah et al., 2018).

ACh has direct effects on cardiac function at doses higher than those required for NO-mediated vasodilation. The cardiac effects of ACh are mediated primarily by M_2 muscarinic receptors (Fisher et al., 2004). Direct effects of ACh include an increase in the ACh-activated K^+ current ($I_{K\text{-}ACh}$) due to activation of K-ACh channels (heterotetrameric proteins consisting of GIRK1 and GIRK4 subunits), a decrease in the L-type Ca^{2+} current ($I_{Ca\text{-}L}$) and L-type Ca^{2+} channel activity due to diminished cyclic AMP generation, and a decrease in the cardiac pacemaker current (I_f) caused by reduced activity of hyperpolarization-activated, cyclic nucleotide–gated (HCN; pacemaker) channels (DiFrancesco and Tromba, 1987; Harvey, 2012). The actions of ACh on M_2 receptors lead to a G_i-mediated decrease in cyclic AMP, which opposes and counteracts the β_1 adrenergic/G_s–mediated increase in cyclic AMP. ACh released from parasympathetic postganglionic nerve terminals also acts on presynaptic M_2 and M_3 receptors on adjacent sympathetic nerve terminals to inhibit the release of norepinephrine (Trendelenburg et al., 2005).

TABLE 11–1 ■ MUSCARINIC RECEPTOR–MEDIATED EFFECTS ON PERIPHERAL EFFECTOR TISSUES

ORGAN	EFFECTOR TISSUE	PREDOMINANT RECEPTOR SUBTYPE	INTRACELLULAR SIGNALING	RESPONSE TO RECEPTOR STIMULATION
GI tract	Gastrointestinal SM SM (internal) sphincters	M_3	↑IP_3 → ↑$[Ca^{2+}]_i$ → SM contraction	Contraction → ↑GI motility Relaxation
Urinary tract	Bladder SM SM (internal) sphincter	M_3		↑Bladder tone, urination
Lungs	Bronchiolar SM	M_3		Bronchoconstriction
Eye	Iris SM: Pupillary sphincter Ciliary muscle	M_3		Pupillary constriction (miosis) Accommodation for near vision
Secretory glands	Salivary glands	M_3	↑IP_3 → ↑$[Ca^{2+}]_i$ → ↑secretion	Salivation
	Bronchial glands			Bronchial secretion
	Lacrimal glands			Lacrimation
	Sweat glands			Sweating
Heart	Sinoatrial (SA) node Atrioventricular node	M_2 M_2	↓cAMP → ↓activity of HCN channels → ↓SA node automaticity ↓cAMP → ↓activity of PKA → ↓phosphorylation of L-type Ca^{2+} channels → ↓iCa $G_{\beta\gamma}$ → ↑activity of K-ACh channels → ↑iK-ACh → hyperpolarization	↓Heart rate (negative chronotropic effect) ↓Conduction velocity (negative dromotropic effect)
Blood vessels	Vascular endothelial cells	M_3	IP_3 → ↑$[Ca^{2+}]_i$ → NO→↑ cGMP	Vasodilation

GI, gastrointestinal; SM, smooth muscle.

In addition, there are presynaptic M_2 receptors that inhibit ACh release from parasympathetic postganglionic nerve terminals in the human heart (Oberhauser et al., 2001).

In the sinoatrial (SA) node, each normal cardiac impulse is initiated by the spontaneous depolarization of the pacemaker cells (Chapter 34). At a critical level (the threshold potential), this depolarization initiates an action potential. ACh slows the heart rate primarily by decreasing the rate of spontaneous depolarization; attainment of the threshold potential and the succeeding events in the cardiac cycle are therefore delayed. Until recently, it was widely accepted that β_1 adrenergic and muscarinic cholinergic effects on heart rate resulted from regulation of the cardiac pacemaker current mentioned earlier (I_f). Unexpected findings made through genetic deletion of HCN4 and pharmacological inhibition of I_f have generated an alternative theory involving a pace-making function for an intracellular Ca^{2+} "clock" (Lakatta and DiFrancesco, 2009) that might mediate effects of ACh on heart rate (Lyashkov et al., 2009).

In the atria, ACh causes hyperpolarization and decreased action potential duration by increasing $I_{K\text{-}ACh}$. ACh also inhibits cyclic AMP formation and norepinephrine release, as described above, decreasing atrial contractility. In the AV node, ACh slows conduction and increases the refractory period by inhibiting $I_{Ca\text{-}L}$; the decrement in AV conduction is responsible for the complete heart block that may be observed when large quantities of cholinergic agonists are administered systemically. When parasympathetic (vagal) tone to the resting heart is increased (e.g., by *digoxin*), the prolonged refractory period of the AV node can reduce the frequency with which aberrant atrial impulses are transmitted to the ventricles and thereby decrease the ventricular rate during atrial flutter or fibrillation.

The ventricular myocardium and His-Purkinje system receive only sparse cholinergic (parasympathetic vagal) innervation (Levy and Schwartz, 1994), and the effects of ACh are smaller than those observed in the atria and nodal tissues. The modest negative inotropic effect of ACh in the ventricle is most apparent when there is concomitant adrenergic stimulation or underlying sympathetic tone (Brodde and Michel, 1999; Levy and Schwartz, 1994; Lewis et al., 2001). Automaticity of Purkinje fibers is suppressed, and the threshold for ventricular fibrillation is increased.

Respiratory Tract

The parasympathetic nervous system plays a major role in regulating bronchomotor tone. A diverse set of stimuli can cause reflexes that increase parasympathetic activity mediating bronchoconstriction. The effects of ACh on the respiratory system include bronchoconstriction, increased tracheobronchial secretion, and stimulation of the chemoreceptors of the carotid and aortic bodies. These effects are mediated primarily by M_3 muscarinic receptors located on bronchial and tracheal smooth muscle (Buels and Fryer, 2012; Fisher et al., 2004) and are most obvious in the case of toxicity from cholinesterase inhibition.

Urinary Tract

Parasympathetic sacral innervation causes detrusor muscle contraction, increased voiding pressure, and ureteral peristalsis. These responses are difficult to observe with administered ACh because poor perfusion of visceral organs and rapid hydrolysis by plasma butyrylcholinesterase limit access of systemically administered ACh to visceral muscarinic receptors. Muscarinic stimulation of bladder contraction is mediated primarily by M_3 receptors expressed by detrusor smooth muscle cells. Smooth muscle M_2 receptors also seem to make a small contribution to this response by reversing β receptor–cyclic AMP–mediated relaxation of the detrusor muscle and via other indirect effects (Hegde, 2006; Matsui et al., 2002).

GI Tract

Although stimulation of vagal input to the gastrointestinal (GI) tract increases tone, amplitude of contractions, and secretory activity of the stomach and intestine, such responses are inconsistently seen with administered ACh for the same reasons that urinary tract responses are difficult to observe. As in the urinary tract, M_3 receptors appear to be primarily responsible for mediating cholinergic control of GI motility, but M_2 receptors also contribute (Matsui et al., 2002).

Secretory Effects

In addition to its stimulatory effects on the tracheobronchial and GI secretions, ACh stimulates secretion from other glands that receive parasympathetic or sympathetic cholinergic innervation, including the lacrimal, nasopharyngeal, salivary, and sweat glands. All of these effects are mediated primarily by M_3 muscarinic receptors (Caulfield and Birdsall, 1998); M_1 receptors also contribute to the cholinergic stimulation of salivary secretion (Gautam et al., 2004).

Eye

When instilled into the eye, ACh produces miosis by contracting the pupillary sphincter muscle and accommodation for near vision by contracting the ciliary muscle; both of these effects are mediated primarily by M_3 muscarinic receptors, but other subtypes may contribute to the ocular effects of cholinergic stimulation (Mitchelson, 2012).

CNS Effects

All five muscarinic receptor subtypes are expressed in the brain (Lebois et al., 2018), and recent studies suggest that muscarinic receptor–regulated pathways may have an important role in cognitive function, motor control, appetite regulation, nociception, and other processes (Thomsen et al., 2018; Wess et al., 2007). While systemically administered ACh has limited CNS penetration, muscarinic agonists that can cross the blood-brain barrier evoke a characteristic cortical arousal or activation response similar to that produced by injection of cholinesterase inhibitors or by electrical stimulation of the brainstem reticular formation.

Muscarinic Receptor Agonists

Muscarinic cholinergic receptor agonists can be divided into two groups:

- Choline esters, including ACh and several synthetic esters
- The naturally occurring cholinomimetic alkaloids (particularly *pilocarpine, muscarine*, and *arecoline*) and their synthetic congeners

Of several hundred synthetic choline derivatives investigated, only *methacholine*, *carbachol*, and *bethanechol* (Figure 11–3) are in clinical use.

Methacholine (acetyl-β-methylcholine), the β-methyl analogue of ACh, is a synthetic choline ester that differs from ACh chiefly in its greater duration and selectivity of action. Its action is more prolonged because the added methyl group increases its resistance to hydrolysis by cholinesterases. Its selectivity is reflected in a predominance of muscarinic actions manifest most clearly in the cardiovascular system; *methacholine*'s nicotinic actions are minor (Table 11–2).

Carbachol, and its β-methyl analogue, *bethanechol*, are unsubstituted carbamoyl esters that are almost completely resistant to hydrolysis by cholinesterases; their $t_{1/2}$ values are thus sufficiently long that they become distributed to areas of low blood flow. *Carbachol* retains substantial nicotinic activity, particularly on autonomic ganglia. *Bethanechol* has mainly muscarinic actions, with prominent effects on motility of the GI tract and urinary bladder.

The major natural alkaloid muscarinic agonists—*muscarine*, *pilocarpine*, and *arecoline*—have the same principal sites of action as the choline esters. *Muscarine* acts almost exclusively at muscarinic receptors. *Pilocarpine* has a dominant muscarinic action but is a partial rather than full agonist; the sweat glands are particularly sensitive to *pilocarpine*. *Arecoline* acts on both muscarinic and nicotinic receptors. Although these naturally occurring alkaloids are important pharmacological tools and *muscarine* has toxicological significance (discussed further in the chapter), present clinical use is restricted largely to the employment of *pilocarpine* as a sialagogue and miotic agent (Chapter 74).

HISTORY AND BOTANICAL ORIGINS

The alkaloid *muscarine* was isolated from the mushroom *Amanita muscaria* by Schmiedeberg in 1869. *Pilocarpine* is the chief alkaloid obtained from the leaflets of South American shrubs of the genus *Pilocarpus*. Although the natives had long known that the chewing of leaves of *Pilocarpus* plants caused salivation, the active compound, *pilocarpine*, was isolated only in 1875 and shown to affect the pupil and sweat and salivary glands. *Arecoline* is the main alkaloid of areca or betel nuts, which are consumed as a euphoretic masticatory mixture by the natives of the Indian subcontinent and East Indies. Hunt and Taveau synthesized and studied *methacholine* as early as 1911. *Carbachol* and *bethanechol* were synthesized and investigated in the 1930s.

ADME

The absorption and distribution of these compounds can be predicted from their structures. *Muscarine* and the choline esters are quaternary amines; *pilocarpine* and *arecoline* are tertiary amines (see examples in Figure 11–3). The choline esters, as quaternary amines, are poorly absorbed following oral administration and have limited ability to cross the blood-brain barrier. Even though these drugs resist hydrolysis, the choline esters are short-acting agents due to rapid renal elimination. *Pilocarpine* and *arecoline*, as tertiary amines, are readily absorbed and can cross the blood-brain barrier. While *muscarine* is a quaternary amine and is poorly absorbed, it can still be toxic when ingested and can even have CNS effects. The natural alkaloids are primarily eliminated by the kidneys; excretion of the tertiary amines can be accelerated by acidification of the urine to trap the cationic form in the urine.

Therapeutic Uses of Muscarinic Receptor Agonists

Muscarinic agonists are currently used in the treatment of urinary bladder disorders and xerostomia (dry mouth) and in the diagnosis of bronchial hyperreactivity through a bronchoprovocation test. They are also

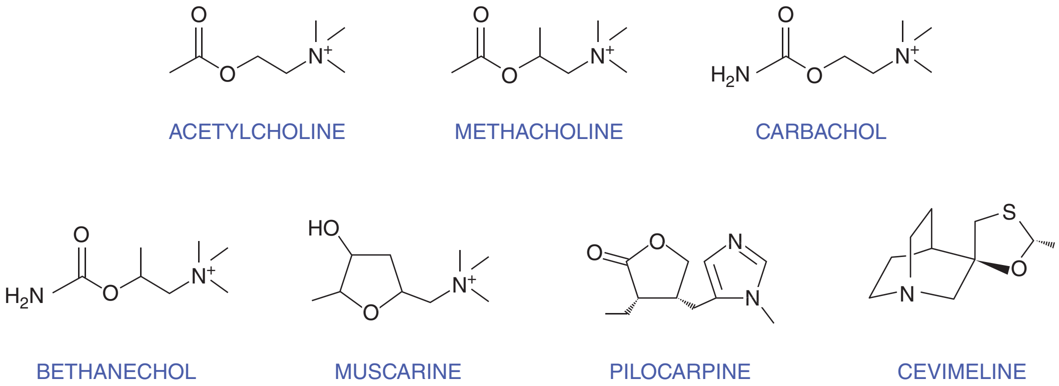

Figure 11–3 *Structural formulas of ACh, choline esters, and natural alkaloids that stimulate muscarinic receptors.*

TABLE 11–2 ■ PHARMACOLOGICAL PROPERTIES OF CHOLINE ESTERS AND NATURAL ALKALOIDS

	HYDROLYSIS BY AChE	NICOTINIC ACTIVITY
Acetylcholine	+++	++
Methacholine	+	+
Carbachol	–	+++
Bethanechol	–	–
Muscarine	–	–
Pilocarpine	–	–

used in ophthalmology as miotic agents and for the treatment of glaucoma. There is growing interest in the use of M_1 agonists in treating the cognitive impairments associated with Alzheimer's disease. Other receptor subtypes, including M_2 and M_5, also appear to be involved in the regulation of cognitive function, at least in animal models (Wess et al., 2007).

Acetylcholine

Although rarely given systemically, ACh is used topically for the induction of miosis during ophthalmologic surgery, instilled into the eye as a 1% solution (Chapter 74).

Methacholine

Methacholine is administered by inhalation for the diagnosis of bronchial airway hyperreactivity in patients who do not have clinically apparent asthma (Crapo et al., 2000). It is available as a powder that is diluted with 0.9% NaCl and administered via a nebulizer. While muscarinic agonists can cause bronchoconstriction and increased tracheobronchial secretions in all individuals, asthmatic patients respond with intense bronchoconstriction and a reduction in vital capacity. The response to *methacholine* may be exaggerated or prolonged in patients taking β adrenergic receptor antagonists. Contraindications to *methacholine* testing include severe airflow limitation, recent myocardial infarction or stroke, uncontrolled hypertension, or pregnancy. Emergency resuscitation equipment, oxygen, and medications to treat severe bronchospasm (e.g., β_2 adrenergic receptor agonists for inhalation) should be available during testing.

Bethanechol

Bethanechol primarily affects the urinary and GI tracts. In the urinary tract, *bethanechol* has utility in treating urinary retention and inadequate emptying of the bladder when organic obstruction is absent, as in postoperative urinary retention, diabetic autonomic neuropathy, and certain cases of chronic hypotonic, myogenic, or neurogenic bladder; catheterization can thus be avoided. When used chronically, 10 to 50 mg of the drug are given orally three or four times daily; the drug should be administered on an empty stomach (i.e., 1 h before or 2 h after a meal) to minimize nausea and vomiting.

In the GI tract, *bethanechol* stimulates peristalsis, increases motility, and increases resting lower esophageal sphincter pressure. *Bethanechol* formerly was used to treat postoperative abdominal distention, gastric atony, gastroparesis, adynamic ileus, and gastroesophageal reflux; more efficacious therapies for these disorders are now available (Chapters 53 and 54).

Carbachol

Carbachol is used topically in ophthalmology for the treatment of glaucoma and the induction of miosis during surgery; it is instilled into the eye as a 0.01% to 3% solution (Chapter 74).

Pilocarpine

Pilocarpine hydrochloride is used for the treatment of xerostomia that follows head and neck radiation treatments or that is associated with Sjögren syndrome (Porter et al., 2004; Ramos-Casals et al., 2010), an autoimmune disorder occurring primarily in women in whom secretions, particularly salivary and lacrimal, are compromised. Treatment can enhance salivary secretion, ease of swallowing, and subjective improvement in hydration of the oral cavity, provided salivary parenchyma maintains residual function. Side effects typify cholinergic stimulation, with sweating the most common complaint. The usual dose is 5 to 10 mg three times daily; the dose should be lowered in patients with hepatic impairment.

Pilocarpine is used topically in ophthalmology for the treatment of glaucoma and as a miotic agent; it is instilled in the eye as a 1% to 4% solution (Chapter 74).

Cevimeline

Cevimeline is a muscarinic agonist that has a long-lasting sialogogic action on lacrimal and salivary glands and may have fewer side effects and better patient compliance than *pilocarpine* (Noaiseh et al., 2014). *Cevimeline* preferentially activates M_1 and M_3 receptors (Heinrich et al., 2009). The usual dose is 30 mg three times daily.

Contraindications, Precautions, and Adverse Effects

Most contraindications, precautions, and adverse effects are predictable consequences of muscarinic receptor stimulation. Thus, important contraindications to the use of muscarinic agonists include asthma, chronic obstructive pulmonary disease, urinary or GI tract obstruction, acid-peptic disease, cardiovascular disease accompanied by bradycardia, hypotension, and hyperthyroidism (muscarinic agonists may precipitate atrial fibrillation in hyperthyroid patients). Common adverse effects include excessive sweating; diarrhea, abdominal cramps, nausea/vomiting, and other GI side effects; a sensation of tightness in the urinary bladder; visual disturbances; and hypotension, which can severely reduce coronary blood flow, especially if it is already compromised. These contraindications and adverse effects are generally of limited concern with topical administration for ophthalmic use.

Toxicology

Poisoning from the ingestion of plants containing *pilocarpine, muscarine*, or *arecoline* is characterized chiefly by exaggeration of their various parasympathomimetic effects. Treatment consists of the parenteral administration of *atropine* in doses sufficient to cross the blood-brain barrier (see Table 11–3) and measures to support the respiratory and cardiovascular systems and to counteract pulmonary edema.

TABLE 11–3 ■ EFFECTS OF ATROPINE IN RELATION TO DOSE

DOSE (mg)	EFFECTS
0.5	Slight cardiac slowing; some dryness of mouth; inhibition of sweating
1	Definite dryness of mouth; thirst; acceleration of heart, sometimes preceded by slowing; mild dilation of pupils
2	Rapid heart rate; palpitation; marked dryness of mouth; dilated pupils; some blurring of near vision
5	Previous symptoms marked; difficulty in speaking and swallowing; restlessness and fatigue; headache; dry, hot skin; difficulty in micturition; reduced intestinal peristalsis
≥10	Previous symptoms more marked; pulse rapid and weak; iris practically obliterated; vision very blurred; skin flushed, hot, dry, and scarlet; ataxia, restlessness, and excitement; hallucinations and delirium; coma

The clinical picture of a high (toxic) dose of atropine may be remembered by an old mnemonic device that summarizes the symptoms: *Red as a beet, Dry as a bone, Blind as a bat, Hot as firestone, and Mad as a hatter.*

Muscarinic Receptor Antagonists

The muscarinic receptor antagonists include:

- The naturally occurring alkaloids *atropine* and *scopolamine*
- Semisynthetic derivatives of these alkaloids, which primarily differ from the parent compounds in their disposition in the body or their duration of action
- Synthetic derivatives, some of which show a limited degree of selectivity for certain muscarinic receptor subtypes

Noteworthy agents in the last two categories are *homatropine* and *tropicamide*, which have shorter durations of action than *atropine*, and *methscopolamine, ipratropium, tiotropium, aclidinium*, and *umeclidinium*, which are quaternary amines that do not cross the blood-brain barrier or readily cross membranes. The synthetic derivatives possessing some degree of receptor subtype selectivity include *pirenzepine*, an M_1 receptor–preferring antagonist, and *darifenacin* and *solifenacin*, two M_3 receptor–preferring agents.

Muscarinic antagonists prevent the effects of ACh by blocking its binding to muscarinic receptors on effector cells at parasympathetic (and sympathetic cholinergic) neuroeffector junctions in peripheral ganglia and the CNS. In general, muscarinic antagonists cause little blockade of nicotinic receptors. However, the quaternary ammonium antagonists generally exhibit a greater degree of nicotinic-blocking activity and therefore are more likely to interfere with ganglionic or neuromuscular transmission.

While many effects of muscarinic antagonists can be predicted from an understanding of the physiological responses mediated by muscarinic receptors at parasympathetic and sympathetic cholinergic neuroeffector junctions, paradoxical responses can occur. For example, presynaptic muscarinic receptors of variable subtype are present on postganglionic parasympathetic nerve terminals. Because blockade of presynaptic receptors generally augments neurotransmitter release, the presynaptic effects of muscarinic antagonists may counteract their postsynaptic receptor blockade. Blockade of the modulatory muscarinic receptors in peripheral ganglia represents an additional mechanism for paradoxical responses.

BELLADONNA

The naturally occurring muscarinic receptor antagonists *atropine* and *scopolamine* are alkaloids of the belladonna (*Solanaceae*) plants. Preparations of belladonna were known to the ancient Hindus and have long been used by physicians. During the time of the Roman Empire and in the Middle Ages, the deadly nightshade shrub was frequently used to produce an obscure and often-prolonged poisoning, prompting Linnaeus to name the shrub *Atropa belladonna*, after Atropos, the oldest of the three Fates, who cuts the thread of life. The name *belladonna* derives from the alleged use of this preparation by Italian women to dilate their pupils; modern-day fashion models are known to use this same device for visual appeal. *Atropine* (D,L-hyoscyamine) also is found in *Datura stramonium* (Jamestown or jimson weed). *Scopolamine* (L-hyoscine) is found chiefly in *Hyoscyamus niger* (henbane). In India, the root and leaves of jimson weed were burned and the smoke inhaled to treat asthma. British colonists observed this ritual and introduced the belladonna alkaloids into Western medicine in the early 1800s. *Atropine* was isolated in pure form in 1831. In literature, belladonna sometimes plays the MacGuffin.

An important consideration in the therapeutic use of muscarinic antagonists is the fact that physiological functions in different organs vary in their sensitivity to muscarinic receptor blockade (Table 11–3). Small doses of *atropine* depress salivary and bronchial secretion and sweating. With larger doses, the pupil dilates, accommodation of the lens to near vision is inhibited, and vagal effects on the heart are blocked so that the heart rate increases. Larger doses antagonize parasympathetic control of the urinary bladder and GI tract, thereby inhibiting urination and decreasing intestinal tone and motility. Still higher doses are required to inhibit gastric motility and particularly secretion. Thus, doses of *atropine* and most related muscarinic antagonists that depress gastric secretion also almost invariably affect salivary secretion, ocular accommodation, urination, and GI motility. This hierarchy of relative sensitivities is not a consequence of differences in the affinity of *atropine* for the muscarinic receptors at these sites because *atropine* lacks selectivity toward different muscarinic receptor subtypes. More likely determinants include the degree to which the functions of various end organs are regulated by parasympathetic tone, the extent of receptor reserve, the involvement of intramural neurons and reflexes, and the presence of other regulatory mechanisms.

Most clinically available muscarinic antagonists lack receptor subtype selectivity, and their actions differ little from those of *atropine*, the prototype of the group. Notably, the clinical efficacy of some agents may actually depend on antagonistic actions at two or more receptor subtypes.

Structure-Activity Relationships

An intact ester of tropine and tropic acid (Figure 11–4) is essential for antimuscarinic action because neither the free acid nor the basic alcohol exhibits significant antimuscarinic activity. The presence of a free OH group in the acyl portion of the ester also is important for activity. Quaternary ammonium derivatives of *atropine* and *scopolamine* are generally more potent than their parent compounds in both muscarinic- and ganglionic- (nicotinic-) blocking activities when given parenterally. These derivatives are poorly and unreliably absorbed when given orally.

Mechanism of Action

Atropine and related compounds compete with ACh and other muscarinic agonists for the orthosteric ACh binding site on the muscarinic receptor. The antagonism by *atropine* is competitive; thus, it is surmountable by ACh if the concentration of ACh at muscarinic receptors is increased sufficiently. Muscarinic receptor antagonists inhibit responses to postganglionic cholinergic nerve stimulation less effectively than they inhibit responses to injected choline esters. The difference may be explained by the fact that release of ACh by cholinergic nerve terminals occurs in close proximity to the receptors, resulting in very high concentrations of the transmitter at the receptors.

Pharmacological Effects of Muscarinic Antagonists

The pharmacological effects of *atropine*, the prototypical muscarinic antagonist, provide a good background for understanding the therapeutic uses of the various muscarinic antagonists. The effects of other muscarinic antagonists will be mentioned only when they differ significantly from those of *atropine*. The major pharmacological effects of increasing doses of *atropine*, summarized in Table 11–3, offer a general guide to the problems associated with administration of this class of agents.

Cardiovascular System

Heart. The main effect of *atropine* on the heart is to alter the rate. Although the dominant response is tachycardia, there is often a transient bradycardia with average clinical doses (0.4–0.6 mg; Table 11–3). The slowing is modest (4–8 beats/min), occurs with no accompanying changes in blood pressure or cardiac output, and is usually absent after rapid intravenous injection. This unexpected effect has been attributed to the block of presynaptic M_1 muscarinic receptors on parasympathetic postganglionic nerve terminals in the SA node, which normally inhibit ACh release (Wellstein and Pitschner, 1988).

Larger doses of *atropine* cause progressive tachycardia by blocking M_2 receptors on the SA nodal pacemaker cells, thereby antagonizing parasympathetic (vagal) tone to the heart. The resting heart rate is increased by about 35 to 40 beats/min in young men given 2 mg of *atropine* intramuscularly. The maximal heart rate (e.g., in response to exercise) is not altered by *atropine*. The influence of *atropine* is most noticeable in healthy

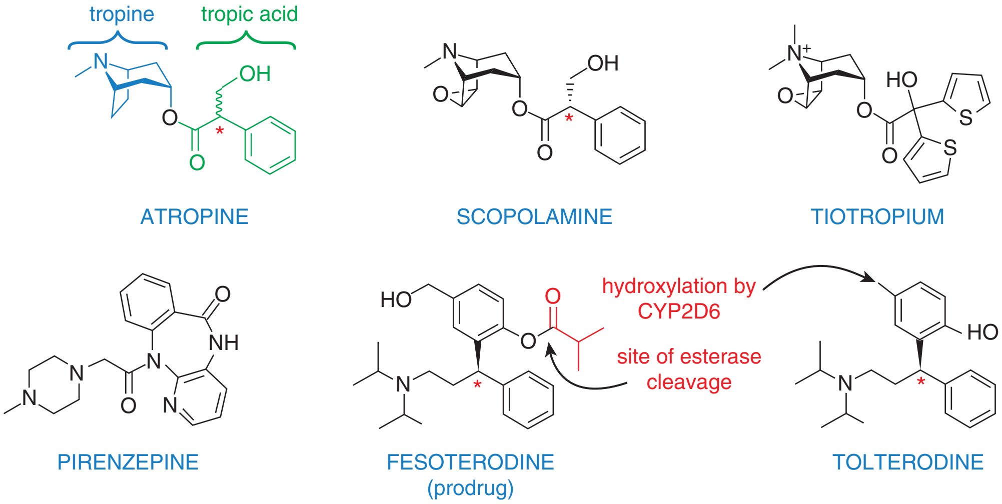

Figure 11–4 *Structural formulas of the belladonna alkaloids and semisynthetic and synthetic analogues.* *Fesoterodine* is converted to an active 5-hydroxymeythyl metabolite by esterase activity. CYP2D6 converts *tolterodine* to the same metabolite. Note that *atropine, scopolamine, tolterodine*, and *fesoterodine* each contain an asymmetric carbon atom (indicated by red asterisk); these compounds therefore exist as racemic mixtures. Clinically, only the (R)-enantiomers of *tolterodine* and *fesoterodine* are used.

young adults, in whom vagal tone is considerable. In infants, the elderly, and patients with heart failure, even large doses of *atropine* may fail to accelerate the heart.

Atropine can abolish many types of reflex vagal cardiac slowing or asystole, such as that occurring from inhalation of irritant vapors, stimulation of the carotid sinus, pressure on the eyeballs, peritoneal stimulation, or injection of contrast dye during cardiac catheterization. *Atropine* also prevents or abruptly abolishes bradycardia or asystole caused by choline esters, AChE inhibitors, or other parasympathomimetic drugs, as well as cardiac arrest from electrical stimulation of the vagus.

The removal of vagal tone to the heart by *atropine* may facilitate AV conduction. *Atropine* shortens the functional refractory period of the AV node and can increase the ventricular rate in patients who have atrial fibrillation or flutter. In certain cases of second-degree AV block (e.g., Wenckebach AV block) in which vagal activity is an etiological factor (as with *digoxin* toxicity), *atropine* may lessen the degree of block. In some patients with complete AV block, the idioventricular rate may be accelerated by *atropine*; in others, it is stabilized. *Atropine* may improve the clinical condition of patients with inferior or posterior wall myocardial infarction by relieving severe sinus or nodal bradycardia or AV block.

Circulation. *Atropine* alone has little effect on blood pressure because most vessels lack significant cholinergic innervation. However, in clinical doses, *atropine* completely counteracts the peripheral vasodilation and sharp fall in blood pressure caused by choline esters. In toxic and occasionally in therapeutic doses, *atropine* can indirectly dilate cutaneous blood vessels, especially those in the blush area (*atropine* flush). This may be a compensatory reaction permitting the radiation of heat to offset the *atropine*-induced rise in temperature that can accompany inhibition of sweating.

Respiratory System

Although *atropine* can cause some bronchodilation and decrease in tracheobronchial secretion in normal individuals by blocking parasympathetic (vagal) tone to the lungs, its effects on the respiratory system are most significant in patients with respiratory disease. *Atropine* can inhibit the bronchoconstriction caused by histamine, bradykinin, and the eicosanoids, which presumably reflects the participation of reflex parasympathetic (vagal) activity in the bronchoconstriction elicited by these agents. The ability to block the indirect bronchoconstrictive effects of these mediators forms the basis for the use of muscarinic receptor antagonists, along with β adrenergic receptor agonists, in the treatment of asthma. Muscarinic antagonists also have an important role in the treatment of chronic obstructive pulmonary disease (Chapter 40).

Atropine inhibits the secretions of the nose, mouth, pharynx, and bronchi and thus dries the mucous membranes of the respiratory tract. This action is especially marked if secretion is excessive and formed the basis for the use of *atropine* and other muscarinic antagonists to prevent irritating inhalational anesthetics such as diethyl ether from increasing bronchial secretion; newer inhalational anesthetics are less irritating. Muscarinic antagonists are used to decrease the rhinorrhea ("runny nose") associated with the common cold or with allergic and nonallergic rhinitis. Reduction of mucous secretion and mucociliary clearance can, however, result in mucus plugs, a potentially undesirable side effect of muscarinic antagonists in patients with airway disease.

The quaternary ammonium compounds *ipratropium*, *tiotropium*, *aclidinium*, and *umeclidinium* are used exclusively for their effects on the respiratory tract. Dry mouth is the only frequently reported side effect, as the absorption of these drugs from the lungs or the GI tract is inefficient. In addition, *aclidinium* has been shown to undergo rapid hydrolysis in plasma to inactive metabolites, thus reducing systemic exposure to the drug (Gavalda et al., 2009). The degree of bronchodilation achieved by these agents is thought to reflect the level of basal parasympathetic tone, supplemented by reflex activation of cholinergic pathways brought about by various stimuli. A therapeutically important property of *ipratropium* and *tiotropium* is their minimal inhibitory effect on mucociliary clearance relative to *atropine*. Hence, the choice of these agents for use in patients with airway disease minimizes the increased accumulation of lower airway secretions encountered with *atropine*.

Eye

Muscarinic receptor antagonists block the cholinergic responses of the pupillary sphincter muscle of the iris and the ciliary muscle controlling lens curvature (Chapter 74). Thus, these agents dilate the pupil (mydriasis) and paralyze accommodation (cycloplegia). The wide pupillary dilation results in photophobia; the lens is fixed for far vision, near objects are blurred, and objects may appear smaller than they are. The normal pupillary reflex constriction to light or on convergence of the eyes is abolished. These effects are most evident when the agent is instilled into the eye but can also occur after systemic administration.

Conventional systemic doses of *atropine* have little ocular effect, in contrast to equal doses of *scopolamine*, which cause evident mydriasis and loss of accommodation. Locally applied *atropine* produces ocular effects of considerable duration; accommodation and pupillary reflexes may not fully recover for 7 to 12 days. Other muscarinic receptor antagonists with shorter durations of action are therefore preferred as mydriatics in ophthalmologic practice. *Pilocarpine* and choline esters (e.g., *carbachol*) in sufficient concentrations can reverse the ocular effects of *atropine*.

Muscarinic receptor antagonists administered systemically have little effect on intraocular pressure except in patients predisposed to angle-closure glaucoma, in whom the pressure may occasionally rise dangerously. The rise in pressure occurs when the anterior chamber is narrow and the iris obstructs outflow of aqueous humor into the trabeculae. Muscarinic antagonists may precipitate a first attack in unrecognized cases of this relatively rare condition. In patients with open-angle glaucoma, an acute rise in pressure is unusual. *Atropine*-like drugs generally can be used safely in the latter condition, particularly if the glaucoma is being treated appropriately.

GI Tract

Knowledge of the actions of muscarinic receptor agonists on the stomach and intestine led to the use of muscarinic receptor antagonists as antispasmodic agents for GI disorders and to reduce gastric acid secretion in the treatment of peptic ulcer disease.

Motility. Parasympathetic nerves enhance GI tone and motility and relax sphincters, thereby favoring the passage of gastrointestinal contents. In normal subjects and in patients with GI disease, muscarinic antagonists produce prolonged inhibitory effects on the motor activity of the stomach, duodenum, jejunum, ileum, and colon, characterized by a reduction in tone and in amplitude and frequency of peristaltic contractions. Relatively large doses are needed to produce such inhibition, probably because the enteric nervous system can regulate motility independently of parasympathetic control; parasympathetic nerves serve only to modulate the effects of the enteric nervous system. Although *atropine* can completely abolish the effects of exogenous muscarinic agonists on GI motility and secretion, *atropine* does not completely inhibit the GI responses to vagal stimulation. This difference, particularly striking in the effects of *atropine* on gut motility, can be attributed to the fact that preganglionic vagal fibers innervating the GI tract synapse not only with postganglionic cholinergic fibers but also with a network of noncholinergic intramural neurons that form the plexuses of the enteric nervous system and utilize neurotransmitters (e.g., serotonin (5HT), dopamine, and various peptides) whose effects *atropine* does not block.

Gastric Acid Secretion. Similarly, *atropine* only partially inhibits the gastric acid secretory responses to vagal activity because vagal stimulation of gastrin secretion is mediated not by ACh but by peptidergic neurons in the vagal trunk that release gastrin-releasing peptide (GRP). GRP stimulates gastrin release from G cells; gastrin can act directly to promote acid secretion by parietal cells and to stimulate histamine release from enterochromaffin-like (ECL) cells (see Figure 53–1). Parietal cells (acid secretors) respond to at least three agonists: gastrin, histamine, and ACh. *Atropine* will inhibit only the components of acid secretion that result from muscarinic stimulation of parietal cells and from muscarinic stimulation of ECL cells that secrete histamine.

Secretions. Salivary secretion is particularly sensitive to inhibition by muscarinic receptor antagonists, which can completely abolish the copious, watery secretion induced by parasympathetic stimulation. The mouth becomes dry, and swallowing and talking may become difficult. The gastric cells that secrete mucin and proteolytic enzymes are more directly under vagal influence than are the acid-secreting cells, and *atropine* selectively decreases their secretory function. Although *atropine* can reduce gastric secretion, the doses required also affect salivary secretion, ocular accommodation, urination, and GI motility (Table 11–3).

In contrast to most muscarinic receptor antagonists, *pirenzepine*, which shows some degree of selectivity for M_1 receptors, inhibits gastric acid secretion at doses that have little effect on salivation or heart rate. Because parietal cells primarily express M_3 receptors, M_1 receptors in intramural ganglia may be the primary target of *pirenzepine* (Eglen et al., 1996). However, this concept has been brought into question by the observation that *pirenzepine* is still able to inhibit *carbachol*-stimulated gastric acid secretion in M_1 receptor–deficient mice (Aihara et al., 2005). In general, histamine H_2 receptor antagonists and proton pump inhibitors have replaced muscarinic antagonists as inhibitors of acid secretion (Chapter 53).

Other Smooth Muscle

Urinary Tract. Muscarinic antagonists decrease the normal tone and amplitude of contractions of the ureter and bladder and often eliminate drug-induced enhancement of ureteral tone. However, this effect is usually accompanied by reduced salivation and lacrimation and blurred vision (Table 11–3).

Biliary Tract. *Atropine* exerts mild antispasmodic action on the gallbladder and bile ducts in humans. However, this effect usually is not sufficient to overcome or prevent the marked spasm and increase in biliary duct pressure induced by opioids.

Sweat Glands and Temperature

Small doses of *atropine* inhibit the activity of sweat glands innervated by sympathetic cholinergic fibers, and the skin becomes hot and dry. Sweating may be depressed enough to raise the body temperature, but only notably so after large doses or at high environmental temperatures.

CNS

Atropine has minimal effects on the CNS at therapeutic doses, although mild stimulation of the parasympathetic medullary centers may occur. With toxic doses of *atropine*, central excitation becomes more prominent, leading to restlessness, irritability, disorientation, hallucinations, or delirium (see the discussion of *atropine* poisoning further in the chapter). With still larger doses, stimulation is followed by depression, leading to circulatory collapse and respiratory failure after a period of paralysis and coma.

In contrast to *atropine*, *scopolamine* has prominent central effects at low therapeutic doses; *atropine* therefore is preferred over *scopolamine* for most purposes. The basis for this difference is probably the greater permeation of *scopolamine* across the blood-brain barrier. *Scopolamine* in therapeutic doses normally causes CNS depression, manifest as drowsiness, amnesia, fatigue, and dreamless sleep, with a reduction in REM (rapid eye movement) sleep. It also causes euphoria and can therefore be subject to abuse. The depressant and amnesic effects formerly were sought when *scopolamine* was used as an adjunct to anesthetic agents or for preanesthetic medication. However, in the presence of severe pain, the same doses of *scopolamine* can occasionally cause excitement, restlessness, hallucinations, or delirium. These excitatory effects resemble those of toxic doses of *atropine*. *Scopolamine* also is effective in preventing motion sickness, probably by blocking neural pathways from the vestibular apparatus in the inner ear to the emetic center in the brainstem.

ADME

The belladonna alkaloids and the *tertiary* synthetic and semisynthetic derivatives are absorbed rapidly from the GI tract. They also enter the circulation when applied locally to the mucosal surfaces of the body. Absorption from intact skin is limited, although efficient absorption does occur in the postauricular region for some agents (e.g., *scopolamine*, allowing delivery by transdermal patch). Systemic absorption of inhaled or orally ingested *quaternary* muscarinic receptor antagonists is limited. The quaternary ammonium derivatives of the belladonna alkaloids also penetrate the conjunctiva of the eye less readily, and central effects are lacking because the quaternary agents do not cross the blood-brain barrier. *Atropine* has a $t_{1/2}$ of about 4 h; hepatic metabolism accounts for the elimination of about half of the dose, and the remainder is excreted unchanged in the urine.

Ipratropium is administered as an aerosol or solution for inhalation, whereas *tiotropium* is administered as a dry powder. As with most drugs administered by inhalation, about 90% of the dose is swallowed. When inhaled, their action is confined almost completely to the mouth and airways. Most of the swallowed drug appears in the feces. After inhalation, maximal responses usually develop over 30 to 90 min, with *tiotropium* having the slower onset. The effects of *ipratropium* last for 4 to 6 h; *tiotropium's* effects persist for 24 h, and the drug is amenable to once-daily dosing.

Therapeutic Uses of Muscarinic Receptor Antagonists

Muscarinic receptor antagonists have been used predominantly to inhibit effects of parasympathetic activity in the respiratory tract, urinary tract, GI tract, eye, and heart. Their CNS effects have resulted in their use in the treatment of Parkinson's disease, the management of extrapyramidal side effects of antipsychotic drugs, and the prevention of motion sickness. The major limitation in the use of drugs that are not receptor subtype selective is often failure to obtain desired therapeutic responses without concomitant side effects. While these usually are not serious, they can be sufficiently disturbing to decrease patient compliance, particularly during long-term administration. To date, selectivity is mainly achieved by local administration (e.g., by pulmonary inhalation or instillation in the eye). The development of allosteric modulators that recognize sites unique to particular muscarinic receptor subtypes is currently considered an important approach to developing receptor subtype–selective drugs for the treatment of specific clinical conditions (Moran et al., 2019).

Respiratory Tract

Ipratropium, tiotropium, aclidinium, and *umeclidinium* are important agents in the treatment of chronic obstructive pulmonary disease (COPD); they are less effective in most patients with asthma (see Chapter 44). These agents often are used with inhaled long-acting β_2 adrenergic receptor agonists, although there is limited evidence of true synergism.

Ipratropium blocks all muscarinic receptor subtypes and accordingly also antagonizes the inhibition of ACh release by presynaptic M_2 receptors on parasympathetic postganglionic nerve terminals in the lung; the resulting increase in ACh release may counteract the drug's blockade of M_3 receptor–mediated bronchoconstriction. In contrast, *tiotropium* shows some selectivity for M_1 and M_3 receptors. In addition, *tiotropium* and *aclidinium* have lower affinities for M_2 receptors and dissociate more slowly from M_3 than from M_2 receptors, thus minimizing the presynaptic effect on ACh release (Alagha et al., 2014).

Ipratropium is administered four times daily via a metered-dose inhaler or nebulizer; *aclidinium* is used twice daily via a dry powder inhaler. *Tiotropium* and *umeclidinium* are once-daily medications that can be used for maintenance therapy via a dry powder inhaler in patients with moderate-to-severe disease.

In normal individuals, inhalation of antimuscarinic drugs can provide virtually complete protection against the bronchoconstriction produced by the subsequent inhalation of such irritants as SO_2, O_3, or cigarette smoke. However, patients with atopic asthma or demonstrable bronchial hyperresponsiveness are less well protected. Although these drugs cause a marked reduction in sensitivity to *methacholine* in asthmatic subjects, inhibition of responses to challenge with histamine, bradykinin, or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is more modest, and protection against bronchoconstriction induced by 5HT or leukotrienes is slight. The therapeutic uses of *ipratropium* and *tiotropium* are discussed further in Chapter 40.

Ipratropium also is FDA approved for use in nasal inhalers for the treatment of the rhinorrhea associated with the common cold and with allergic or nonallergic perennial rhinitis. The ability of muscarinic antagonists to reduce nasopharyngeal secretions may provide some symptomatic relief. In contrast to *pseudoephedrine* or *phenylephrine*, muscarinic antagonists do not constrict blood vessels and hence they are less efficacious as nasal decongestants. It is probable that the contribution of first-generation antihistamines employed in nonprescription cold medications is due primarily to their antimuscarinic properties, except in conditions with an allergic basis (see Chapters 38 and 43).

Genitourinary Tract

Overactive urinary bladder can be successfully treated with muscarinic receptor antagonists. These agents can lower intravesicular pressure, increase capacity, and reduce the frequency of contractions by antagonizing parasympathetic control of the bladder; they also may alter bladder sensation during filling (Chapple et al., 2008). Muscarinic antagonists can be used to treat enuresis in children, particularly when a progressive increase in bladder capacity is the objective, and to reduce urinary frequency and increase bladder capacity in spastic paraplegia.

The muscarinic receptor antagonists indicated for overactive bladder are *oxybutynin, tolterodine, trospium chloride, darifenacin, solifenacin*, and *fesoterodine*. Although some comparison trials demonstrate small but statistically significant differences in efficacy between these agents (Chapple et al., 2008), the clinical relevance of these differences remains uncertain.

The most important adverse reactions are consequences of muscarinic receptor blockade and include xerostomia, blurred vision, and GI side effects such as constipation and gastrointestinal discomfort. CNS-related antimuscarinic effects including drowsiness, dizziness, and confusion can occur and are particularly problematic in elderly patients. CNS effects appear to be less likely with *trospium*, a quaternary amine, and with *darifenacin* and *solifenacin*; the latter two agents show some preference for M_3 receptors and therefore seem to have minimal effects on M_1 receptors in the CNS are thought to play an important role in memory and cognition (Kay et al., 2006). Adverse effects can limit the tolerability of these drugs with continued use, and patient acceptance declines. Xerostomia is the most common reason for discontinuation.

Oxybutynin, the oldest muscarinic antagonist currently used to treat overactive bladder disorders, is associated with a high incidence of antimuscarinic side effects, particularly xerostomia. In an attempt to increase patient acceptance, *oxybutynin* is marketed as a transdermal system that is associated with a lower incidence of side effects than the oral immediate- and extended-release formulations; a topical gel formulation of *oxybutynin* also appears to offer a more favorable side effect profile. *Oxybutynin* is metabolized by enteric and hepatic CYP3A4; thus, its serum concentration may be increased by concurrent administration of CYP3A4 inhibitors (e.g., grapefruit juice, *ritonavir*, conazoles, *clarithromycin, ciprofloxacin, erythromycin, aprepitant*; see Chapter 5). Because of the extensive metabolism of oral *oxybutynin* by enteric and hepatic CYP3A4, higher doses are used in oral than transdermal administration; the dose may need to be reduced in patients taking drugs that inhibit CYP3A4.

Tolterodine shows some degree of selectivity for the urinary bladder in animal models and in clinical studies, resulting in greater patient acceptance. *Tolterodine* is metabolized by CYP2D6 to 5-hydroxymethyltolterodine, a metabolite that possesses similar activity as the parent drug but differs pharmacokinetically. As CYP2D6 is a polymorphic enzyme, with significant variability of expression, the serum concentration of *tolterodine* and the 5-hydroxymethyl metabolite can vary. Lower initial doses are recommended for patients with low CYP26D activity. *Fesoterodine* is a prodrug that is rapidly hydrolyzed to the active metabolite of *tolterodine* by esterases (Figure 11–4) rather than CYP2D6, thereby providing a less variable source of the 5-hydroxymethyl metabolite of *tolterodine*.

Trospium, a quaternary amine, is as effective as *oxybutynin* but is better tolerated. It is the only antimuscarinic agent used for overactive bladder that is eliminated primarily by the kidneys; 60% of the absorbed *trospium* dose is excreted unchanged in the urine, and dosage adjustment is necessary for patients with impaired renal function. *Trospium* has the potential for pharmacokinetic interaction with other agents that are eliminated by active tubular excretion (see Chapter 4).

Solifenacin shows some preference for M_3 receptors, giving it a favorable efficacy-to-side-effect ratio (Chapple et al., 2004). *Solifenacin* is significantly metabolized by CYP3A4; thus, patients taking drugs that inhibit CYP3A4 should receive lower doses.

Like *solifenacin*, *darifenacin* shows some degree of selectivity for M_3 receptors (Caulfield and Birdsall, 1998). It is metabolized by CYP2D6 and CYP3A4; *darifenacin* doses may need to be reduced in patients taking drugs that inhibit either of these CYPs.

GI Tract

Muscarinic receptor antagonists were once widely used for the management of peptic ulcer. Although they can reduce gastric motility and the secretion of gastric acid, antisecretory doses produce pronounced side effects, such as xerostomia, loss of visual accommodation, photophobia, and difficulty in urination (Table 11–3). As a consequence, patient compliance in the long-term management of symptoms of acid-peptic disease with these drugs is poor. H_2 receptor antagonists and proton pump inhibitors are the current drugs of choice to reduce gastric acid secretion (Chapter 53).

Pirenzepine, a tricyclic drug similar in structure to *imipramine*, displays a limited degree of selectivity for M_1 receptors (Caulfield and Birdsall, 1998). *Telenzepine*, an analogue of *pirenzepine*, has higher potency and similar selectivity for M_1 receptors. Both drugs are used in the treatment of acid-peptic disease in Europe, Japan, and Canada, but not currently in the U.S. At therapeutic doses of *pirenzepine*, the incidence of xerostomia, blurred vision, and central muscarinic disturbances is relatively low. Central effects are not seen because of the drug's limited penetration into the CNS. Most studies indicate that *pirenzepine* (100–150 mg/day) produces about the same rate of healing of duodenal and gastric ulcers as the H_2 receptor antagonists *cimetidine* or *ranitidine*; *pirenzepine* also may be effective in preventing the recurrence of ulcers (Tryba and Cook, 1997). Side effects necessitate drug withdrawal in less than 1% of patients.

Myriad conditions known or supposed to involve increased tone (spasticity) or motility of the GI tract are treated with belladonna alkaloids (e.g., *atropine, hyoscyamine sulfate*, and *scopolamine*) alone or in combination with sedatives (e.g., *phenobarbital*) or antianxiety agents (e.g., *chlordiazepoxide*). The belladonna alkaloids and their synthetic substitutes can reduce tone and motility when administered in maximally tolerated doses. M_3 receptor–preferring antagonists would theoretically be better tolerated because cardiac and central side effects are mostly avoided. On the other hand, M_3 receptors regulate salivation, bronchial secretion and contraction, and bladder motility; thus, peripheral side effects due to muscarinic antagonism at these sites are to be expected. *Glycopyrrolate*, a muscarinic antagonist that is structurally unrelated to the belladonna alkaloids, is used to reduce GI tone and motility; as a quaternary amine, it is less likely to cause adverse CNS effects than *atropine*, *scopolamine*, and other tertiary amines. Alternative agents for treatment of increased GI motility and its associated symptoms are discussed in Chapter 54.

Diarrhea associated with irritation of the lower bowel, such as mild dysenteries and diverticulitis, may respond to *atropine*-like drugs, an effect that likely involves actions on ion transport as well as motility. However, more severe conditions such as *Salmonella* dysentery, ulcerative colitis, and Crohn's disease respond little, if at all, to muscarinic antagonists.

Dicyclomine hydrochloride is a weak muscarinic receptor antagonist that also has nonspecific direct spasmolytic effects on smooth muscle of the GI tract. It is occasionally used in the treatment of diarrhea-predominant irritable bowel syndrome.

Salivary Secretions

The belladonna alkaloids and synthetic substitutes are effective in reducing excessive salivation, such as drug-induced salivation and that associated with heavy-metal poisoning and Parkinson's disease. *Glycopyrrolate* is a quaternary amine and, as mentioned earlier, is less likely than others to penetrate the CNS. An oral solution of *glycopyrrolate* is indicated to reduce drooling (e.g., in patients with Parkinson's disease).

Eye

Effects limited to the eye are obtained by topical administration of muscarinic receptor antagonists to produce mydriasis and cycloplegia (paralysis of the ciliary muscle resulting in the inhibition of accommodation). Cycloplegia requires higher concentrations or more prolonged application of antagonist and thus cannot be achieved without concomitant mydriasis. Mydriasis often is necessary for thorough examination of the retina and optic disc and in the therapy of iridocyclitis and keratitis. *Homatropine hydrobromide*, a semisynthetic derivative of *atropine* (Figure 11–4), *cyclopentolate hydrochloride*, and *tropicamide* are agents used in ophthalmological practice. These agents are preferred to topical *atropine* or *scopolamine* because of their shorter duration of action. Chapter 74 presents additional information on the ophthalmological properties and preparations of these and other drugs.

Cardiovascular System

The cardiovascular effects of muscarinic receptor antagonists are of limited clinical utility. Generally, these agents are used only in coronary care units for short-term interventions or in surgical settings. They are also sometimes used as an adjunct to stress testing to increase heart rate in the setting of chronotropic incompetence.

Atropine may be considered in the initial treatment of patients with acute myocardial infarction in whom excessive vagal tone causes sinus bradycardia or AV nodal block. Sinus bradycardia is the most common arrhythmia seen during acute myocardial infarction of the inferior or posterior wall. *Atropine* may prevent further clinical deterioration in cases of high vagal tone or AV block by restoring heart rate to a level sufficient to maintain adequate hemodynamic status and to eliminate AV nodal block. Dosing must be judicious; doses that are too low can cause a paradoxical bradycardia (described earlier), while excessive doses will cause tachycardia that may extend the infarct by increasing the demand for O_2.

Atropine occasionally is useful in reducing the severe bradycardia and syncope associated with a hyperactive carotid sinus reflex. It has little effect on most ventricular rhythms. In some patients, *atropine* may eliminate premature ventricular contractions associated with a very slow atrial rate. It also may reduce the degree of AV block when increased vagal tone is a major factor in the conduction defect, such as the second-degree AV block that can be produced by *digoxin*. Selective M_2 receptor antagonists would be of potential utility in blocking ACh-mediated bradycardia or AV block; however, no such agents are currently available for clinical use.

Autonomic control of the heart is known to be abnormal in patients with cardiovascular disease, especially in heart failure. Patients with heart failure typically exhibit increased sympathetic tone accompanied by vagal withdrawal, both of which may contribute to the progression of disease. While β blockers have now emerged as standard of care in heart failure, less is known about whether augmentation of vagal tone may be beneficial. Studies in animals suggested that augmenting vagal tone chronically decreases the inflammatory response and prevents adverse cardiac remodeling associated with heart failure (DeMazumder et al., 2015; Dunlap et al., 2015; Schwartz and De Ferrari, 2011); clinical trials of such therapy have, however, shown limited success (McMurry and Køber, 2016).

CNS

The belladonna alkaloids were among the first drugs to be used in the prevention of motion sickness. *Scopolamine* is the most effective of these agents for short (4- to 6-h) exposures to severe motion and probably for exposures of up to several days. All agents used to combat motion sickness should be given prophylactically; they are much less effective after severe nausea or vomiting has developed. A transdermal preparation of *scopolamine* has been shown to be highly effective when used prophylactically for the prevention of motion sickness. The drug, incorporated into a multilayer adhesive unit, is applied to the postauricular mastoid region, an area where transdermal drug absorption is especially efficient, resulting in the delivery of about 0.5 mg of *scopolamine* over 72 h. Xerostomia is common, drowsiness is not infrequent, and blurred vision occurs in some individuals using the *scopolamine* patch. Mydriasis and cycloplegia can occur by inadvertent transfer of the drug to the eye from the fingers after handling the patch. Rare but severe psychotic episodes have been reported, as have various symptoms following abrupt withdrawal after prolonged use.

Muscarinic receptor antagonists have long been used in the treatment of Parkinson's disease, which is characterized by reduced dopaminergic input into the striatum, resulting in an imbalance between striatal muscarinic cholinergic and dopaminergic neurotransmission (see Chapter 21). The striatum, the major input area of the basal ganglia, contains multiple cell types, including cholinergic interneurons, all of which express one or more muscarinic receptor subtype (Goldberg et al., 2012). Studies with muscarinic receptor mutant mice suggested that the beneficial effects of muscarinic antagonists in the treatment of Parkinson's disease are primarily due to the blockade of M_1 and M_4 receptors, resulting in the activation or inhibition, respectively, of specific striatal neuronal subpopulations (Wess et al., 2007).

Muscarinic antagonists can be effective in the early stages of Parkinson's disease if tremor is predominant, particularly in young patients. Muscarinic receptor antagonists also are used to treat the extrapyramidal symptoms that commonly occur as side effects of conventional antipsychotic drug therapy (Chapter 19). Certain antipsychotic drugs are relatively potent muscarinic receptor antagonists (Roth et al., 2004) and, perhaps for this reason, cause fewer extrapyramidal side effects.

The muscarinic antagonists used for Parkinson's disease and drug-induced extrapyramidal symptoms include *benztropine mesylate, trihexyphenidyl hydrochloride*, and *biperiden* (not marketed in the U.S.); all are tertiary amines that readily gain access to the CNS.

Anesthesia

Atropine is commonly given to block responses to vagal reflexes induced by surgical manipulation of visceral organs. *Atropine* and *glycopyrrolate* are also used to block the parasympathomimetic effects of *neostigmine* when it is administered to reverse skeletal muscle relaxation after surgery. Serious cardiac arrhythmias have occasionally occurred, perhaps because of the initial bradycardia produced by *atropine* combined with the cholinomimetic effects of *neostigmine*.

Anticholinesterase Poisoning

The use of *atropine* in large doses for the treatment of poisoning by anticholinesterase organophosphorus insecticides is discussed in Chapter 12. *Atropine* also may be used to antagonize the parasympathomimetic effects of *pyridostigmine* or other anticholinesterases administered in the treatment of myasthenia gravis. It does not interfere with the salutary effects at the skeletal neuromuscular junction. It is most useful early in therapy, before tolerance to muscarinic side effects of anticholinesterases has developed.

Other Therapeutic Uses

Methscopolamine bromide is a quaternary ammonium derivative of *scopolamine* and therefore lacks the central actions of *scopolamine*. Although formerly used to treat peptic ulcer disease, at present, it is primarily used in certain combination products for the temporary relief of symptoms of allergic rhinitis, sinusitis, and the common cold.

Homatropine methylbromide, the methyl derivative of *homatropine*, is less potent than *atropine* in antimuscarinic activity but four times more potent as a ganglionic blocking agent. Formerly used for the treatment of irritable bowel syndrome and peptic ulcer disease, at present, it is sometimes used with *hydrocodone* as an antitussive combination administered as an oral syrup. This combination must be used with caution and only in adults (>18 years), with full knowledge by patient and physician of the risks associated with opioid use (respiratory depression; necessity to avoid other CNS depressants including ethanol and benzodiazepines; adverse interactions with agents altering CYP3A4 activity), and with refills only after reevaluation of the need for treatment.

Sofpironium and its structural analogue *glycopyrrolate* are both topically used for the treatment of primary axillary hyperhidrosis (excessive sweating). Both drugs reduce sweating locally by inhibiting M_3 receptors at eccrine sweat glands. *Sofpironium* is approved in Japan as a gel; *glycopyrrolate* is available in the U.S. for topical application using a single-use, premoistened, medicated cloth. *Glycopyrrolate* is also used in treating obstructive airway disease (see Chapter 44).

Contraindications and Adverse Effects

Most contraindications, precautions, and adverse effects are predictable consequences of muscarinic receptor blockade: dry mouth, constipation, blurred vision, gastrointestinal discomfort, and cognitive impairment. Important contraindications to the use of muscarinic antagonists include urinary tract obstruction, GI obstruction, and uncontrolled (or susceptibility to attacks of) angle-closure glaucoma. Muscarinic receptor antagonists also are contraindicated (or should be used with extreme caution) in patients with benign prostatic hyperplasia. These adverse effects and contraindications generally are of more limited concern with muscarinic antagonists that are administered by inhalation or used topically in ophthalmology.

Toxicology of Drugs With Antimuscarinic Properties

The deliberate or accidental ingestion of natural belladonna alkaloids is a major cause of poisonings. Moreover, many histamine H_1 receptor antagonists, phenothiazines, and tricyclic antidepressants also block muscarinic receptors and, in sufficient dosage, produce syndromes that include features of *atropine* intoxication. Among the tricyclic antidepressants, *protriptyline* and *amitriptyline* are the most potent muscarinic receptor antagonists, with affinities for muscarinic receptors only an order of magnitude less than that of *atropine*. Because these drugs are administered in therapeutic doses considerably higher than the effective dose of *atropine*, antimuscarinic effects are often observed clinically (Chapter 18). In addition, overdose with suicidal intent is a danger in the population using antidepressants. Fortunately, most of the newer antidepressants and selective serotonin reuptake inhibitors have more limited anticholinergic properties.

Like the tricyclic antidepressants, many of the older antipsychotic drugs have antimuscarinic effects. These effects are most likely to be observed with the less potent drugs (e.g., *chlorpromazine* and *thioridazine*), which must be given in higher doses. The newer antipsychotic drugs, classified as "atypical" and characterized by their low propensity for inducing extrapyramidal side effects, also include agents that are potent muscarinic receptor antagonists. In particular, *clozapine* binds to human brain muscarinic receptors with high affinity (10 nM, compared to 1–2 nM for *atropine*); *olanzapine* also is a potent muscarinic receptor antagonist (Roth et al., 2004). Accordingly, xerostomia is a prominent side effect of these drugs. A paradoxical side effect of *clozapine* is increased salivation and drooling, possibly resulting from the blockade of other biogenic amine receptors.

Infants and young children are especially susceptible to the toxic effects of muscarinic antagonists. Indeed, cases of intoxication in children have resulted from conjunctival instillation for ophthalmic refraction and use for other ocular indications. Systemic absorption occurs either from the nasal mucosa after the drug has traversed the nasolacrimal duct or from the GI tract if the drug is swallowed. Poisoning with *diphenoxylate-atropine*, used to treat diarrhea, has been extensively reported in the pediatric literature. Transdermal preparations of *scopolamine* used for motion sickness have been noted to cause toxic psychoses, especially in children and in the elderly. Serious intoxication may occur in children who ingest berries or seeds containing belladonna alkaloids. Poisoning from ingestion and smoking of jimson weed is seen with some frequency today.

Table 11–3 shows the oral doses of *atropine* causing undesirable responses or symptoms of overdosage. These symptoms are predictable results of blockade of parasympathetic innervation. In cases of full-blown *atropine* poisoning, the syndrome may last 48 h or longer. Intravenous injection of the anticholinesterase agent *physostigmine* may be used for confirmation. If *physostigmine* does not elicit the expected salivation, sweating, bradycardia, and intestinal hyperactivity, intoxication with *atropine* or a related agent is almost certain. Depression and circulatory collapse are evident only in cases of severe intoxication; the blood pressure declines, convulsions may ensue, respiration becomes inadequate, and death due to respiratory failure may follow after a period of paralysis and coma.

If the poison has been taken orally, begin measures to limit intestinal absorption without delay. For symptomatic treatment, slow intravenous injection of *physostigmine* will rapidly abolish the delirium and coma caused by large doses of *atropine* but carries some risk of overdose in mild *atropine* intoxication. Because *physostigmine* is metabolized rapidly, the patient may again lapse into coma within 1 to 2 h, and repeated doses may be needed (Chapter 12). If marked excitement is present and more specific treatment is not available, a benzodiazepine is the most suitable agent for sedation and for control of convulsions. Phenothiazines or agents with antimuscarinic activity should *not* be used because their antimuscarinic action is likely to intensify toxicity. Support of respiration and control of hyperthermia may be necessary. Ice bags and alcohol sponges can help to reduce fever, especially in children.

Drug Facts for Your Personal Formulary: *Muscarinic Receptor Agonists and Antagonists*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Muscarinic Receptor Agonists		
Methacholine	• Diagnosis of bronchial airway hyperreactivity	• Muscarinic effects: GI cramps, diarrhea, nausea, vomiting; lacrimation, salivation, sweating; urinary urgency; vision problems; bronchospasm • Do not use in patients with GI obstruction, urinary retention, asthma/COPD
Carbachol	• Glaucoma (topical administration)	• Systemic muscarinic effects minimal with proper topical application, otherwise similar to methacholine
Bethanechol	• Ileus (postoperative, neurogenic) • Urinary retention	• Similar to methacholine • Take on empty stomach to minimize nausea/vomiting

Pilocarpine	• Glaucoma (topical administration)
	• Xerostomia due to
	  • Sjögren syndrome
	  • Head and neck irradiation
		• Systemic muscarinic effects minimal with proper topical application, otherwise similar to methacholine

Cevimeline	• Xerostomia due to • Sjögren syndrome	• Similar to methacholine
Muscarinic Receptor Antagonists		
Atropine	• Acute symptomatic bradycardia (e.g., AV block) • Cholinesterase inhibitor intoxication • Aspiration prophylaxis	• Antimuscarinic adverse effects: xerostomia, constipation, blurred vision, dyspepsia, and cognitive impairment • Contraindicated in patients with urinary tract obstruction (especially in benign prostatic hyperplasia), GI obstruction, and angle-closure glaucoma
Scopolamine	• Motion sickness	• CNS effects (drowsiness, amnesia, fatigue)
Homatropine, cyclopentolate, tropicamide	• Ophthalmological examination (cycloplegia and mydriasis induction)	• Antimuscarinic adverse effects are minimal with proper topical application
Ipratropium, tiotropium, aclidinium, umeclidinium	• COPD • Rhinorrhea (ipratropium)	• Minimal absorption as quaternary amine ⇒ fewer antimuscarinic adverse effects, otherwise similar to atropine
Pirenzepine, telenzepine	• Peptic ulcer disease (not in the U.S.)	• Antimuscarinic adverse effects and contraindications similar to atropine
Oxybutynin, trospium, darifenacin, solifenacin, tolterodine, fesoterodine	• Overactive bladder, enuresis, neurogenic bladder	• Antimuscarinic adverse effects and contraindications similar to atropine • CNS-related antimuscarinic effects less likely with trospium (quaternary amine), darifenacin and solifenacin (some selectivity for M_3 receptors), fesoterodine (prodrug of tolterodine), and tolterodine (preference for muscarinic receptors in the bladder)
Glycopyrrolate	• Duodenal ulcer • Sialorrhea • Primary axillary hyperhidrosis	• Antimuscarinic adverse effects and contraindications similar to atropine • Fewer CNS effects as glycopyrrolate is a quaternary amine and therefore unable to cross the blood-brain barrier
Dicyclomine, hyoscyamine	• Diarrhea-predominant irritable bowel syndrome (IBS)	• Antimuscarinic adverse effects and contraindications similar to atropine (including constipation-dominant IBS) • Evidence for efficacy is limited
Trihexyphenidyl, benztropine	• Parkinson's disease	• Antimuscarinic adverse effects and contraindications similar to atropine • Mainly used to treat the tremor in Parkinson's disease • Not recommended for elderly or demented patients

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Chapter 12

Anticholinesterase Inhibitors and Reactivators

Palmer Taylor

ACETYLCHOLINESTERASE

- Structure of Acetylcholinesterase

ACETYLCHOLINESTERASE INHIBITORS

- Molecular Mechanism of Action of AChE Inhibitors
- Chemistry and Structure-Activity Relationships
- Basis for the Pharmacological Effects of ChE Inhibitors
- Effects on Physiological Systems
- ADME
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THERAPEUTIC USES OF AChE INHIBITORS

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- Glaucoma and Other Ophthalmologic Indications
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- Alzheimer's Disease
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Acetylcholinesterase

The hydrolytic activity of acetylcholinesterase (AChE) terminates the action of acetylcholine (ACh) at the junctions of the various cholinergic nerve endings with their effector organs or postsynaptic sites (see Chapter 10). Drugs that inhibit AChE are properly termed anti-ChEs or cholinesterase (ChE) inhibitors, since they inhibit both AChE and BChE (butyrylcholinesterase); however, such agents are also referred to as AChE inhibitors. BChE is not abundant in nerve ending synapses but is found in liver and plasma, where it metabolizes circulating esters.

AChE inhibitors cause ACh to accumulate in the vicinity of cholinergic nerve terminals and thus are potentially capable of producing effects equivalent to excessive stimulation of cholinergic receptors throughout the central and peripheral nervous systems. In view of the widespread distribution of cholinergic neurons across animal species and prominence of ACh among neurotransmitters, it is not surprising that the anti-ChE agents have received extensive application as toxic agents, in the form of agricultural insecticides, pesticides, and potential chemical warfare nerve agents or gases. AChE reactivators are particularly useful in treating poisoning by pesticides or agents of terrorism in controlled ventilation facilities. Several anti-ChEs are used therapeutically; others that cross the blood-brain barrier have been approved or are in clinical trials for the treatment of Alzheimer's disease.

Prior to World War II, only the "reversible" anti-ChE agents were generally known, of which *physostigmine* is the prototype (Box 12–1). Shortly before and during World War II, a new class of highly toxic chemicals, the organophosphates, was developed, first as agricultural insecticides and later as potential chemical warfare agents (Everts, 2016). The extreme toxicity of these compounds was found to be due to their "irreversible" inactivation of AChE, which resulted in prolonged enzyme inhibition. Because the pharmacological actions of both the reversible and irreversible anti-ChE agents are qualitatively similar, they are discussed here as a group. Interactions of anti-ChE agents with other drugs acting at peripheral autonomic synapses and the neuromuscular junction are described in Chapters 11 and 13.

Structure of Acetylcholinesterase

Acetylcholinesterase exists in two general classes of molecular forms: simple homomeric oligomers of catalytic subunits (monomers, dimers, and tetramers) and heteromeric associations of catalytic subunits with structural subunits (Massoulié, 2000; Taylor, 2021; Taylor et al., 2000).

BOX 12–1 ■ HISTORY AND PERSPECTIVE

Physostigmine, also called *eserine,* is an alkaloid obtained from the Calabar or ordeal bean, the dried, ripe seed of *Physostigma venenosum,* a perennial plant found in tropical West Africa. The Calabar bean once was used by native tribes of West Africa as an "ordeal poison" in trials for witchcraft, in which guilt was judged by death from the poison and innocence by survival after ingestion of a bean. A pure alkaloid was isolated by Jobst and Hesse in 1864 and named *physostigmine*. The first therapeutic use of the drug was in 1877 by Laqueur in the treatment of glaucoma, one of its clinical uses today. Karczmar (1970) and Holmstedt (2000) have presented accounts of the history of *physostigmine*.

After basic research elucidated the chemical basis of the activity of *physostigmine*, scientists began systematic investigations of a series of substituted aromatic esters of alkyl carbamates. *Neostigmine* was introduced in 1931 for its stimulant action on the gastrointestinal (GI) tract and subsequently was reported to be effective in the symptomatic management of myasthenia gravis.

Following the synthesis of about 2000 compounds, Schrader defined the structural requirements for insecticidal activity (and, as learned subsequently, for anti-ChE activity). One compound in this early series, parathion (a phosphorothioate), later became the most widely used insecticide of this class. Malathion also contains the thionophosphorus bond found in parathion. Prior to and during World War II, the efforts of Schrader's group were directed toward the development of chemical warfare agents. The synthesis of several compounds of much greater toxicity than parathion, such as sarin, soman, and tabun, was kept secret by the German government (Everts, 2016). Investigators in the Allied countries also followed Lange and Krueger's lead in 1932 in the search for potentially toxic compounds. DFP (diisopropyl fluorophosphate), synthesized by McCombie and Saunders, was studied extensively by British and American scientists (Giacobini, 2000).

The environmental impact from agricultural and nonagricultural uses of AChE inhibitors has come under scrutiny. For instance, approximately 10^6 pounds of malathion are used annually in the U.S. The U.S. Fish and Wildlife Service has recently concluded that malathion threatens the existence of 78 endangered plant and animal species and adversely affects 23 critical habitats (Environmental Protection Agency, 2021).

Abbreviations

ACh: acetylcholine
AChE: acetylcholinesterase
anti-ChE: anticholinesterase
BChE: butyrylcholinesterase
ChE: cholinesterase
CYPs: cytochrome P450s
DFP: diisopropyl fluorophosphate (diisopropyl phosphorofluoridate)
EPA: Environmental Protection Agency
GI: gastrointestinal
2-PAM: pralidoxime

The homomeric forms are found as soluble species in the cell, presumably destined for export or for association with the outer membrane of the cell, typically through an attached glycophospholipid. One heteromeric form, found mainly in neuronal synapses, is a tetramer of catalytic subunits disulfide-linked to a 20-kDa lipid-linked subunit and localized to the outer surface of the cell membrane. The other heteromeric form consists of tetramers of catalytic subunits, linked by disulfide bonds to each of three strands of a collagen-like structural subunit. This molecular species, whose molecular mass approaches 10^6 Da, is associated with the basal lamina of neuromuscular junctional areas of skeletal muscle.

Molecular cloning revealed that a single gene encodes vertebrate AChEs (Schumacher et al., 1986; Taylor, 2021; Taylor et al., 2000). However, multiple gene products arise from alternative processing of the mRNA that differ only in their carboxyl termini; the portion of the gene encoding the catalytic core of the enzyme is invariant. Hence, the individual AChE species, found in various tissues, can be expected to show identical substrate and inhibitor specificities.

A separate, structurally related gene encodes BChE, which is synthesized in the liver and is found primarily in plasma (Lockridge, 2015; Lockridge et al., 1987). The cholinesterases define a superfamily of proteins that share a common structural motif, the α,β-hydrolase fold (Cygler et al., 1993). The family includes several esterases, other hydrolases not found in the nervous system, and surprisingly, proteins without hydrolase activity, such as thyroglobulin and members of the tactin and neuroligin families (Taylor et al., 2000).

The three-dimensional structures of AChEs show the active center to be nearly centrosymmetric to each subunit, residing at the base of a confining, narrow gorge about 20 Å in depth (Bourne et al., 1995; Sussman et al., 1991). At the base of the gorge lie the residues of the catalytic triad: Ser^{203}, His^{447}, and Glu^{334} in mammals (Figure 12–1). The catalytic mechanism resembles that of other hydrolases; the serine hydroxyl group is rendered highly nucleophilic through a charge-relay system involving the carboxylate anion from the glutamate, the imidazole of histidine, and the hydroxyl of serine (Figure 12–2A).

During enzymatic catalysis of ACh, an ester with trigonal geometry, a tetrahedral intermediate between enzyme and substrate is formed (Figure 12–2A) that collapses to an acetyl enzyme conjugate with the concomitant release of choline. The acetyl enzyme is very labile to hydrolysis, which results in the formation of acetate and active enzyme (Froede and Wilson, 1971; Rosenberry, 1975). AChE is one of the most efficient enzymes known: One molecule of AChE can hydrolyze 6×10^5 ACh molecules per minute; this yields a turnover time of 100 μsec.

Knockout mice lacking the gene encoding AChE can survive under highly supportive conditions and with a special diet, but they exhibit continuous tremors and are stunted in growth (Xie et al., 2000). Mice that selectively lack AChE expression in skeletal muscle but have normal or near-normal expression in brain and organs innervated by the autonomic nervous system can reproduce but have tremors and severely compromised skeletal muscle strength. By contrast, mice with selective reductions of AChE in the CNS by elimination of the exons encoding alternative spliced regions or expression of the structural subunits

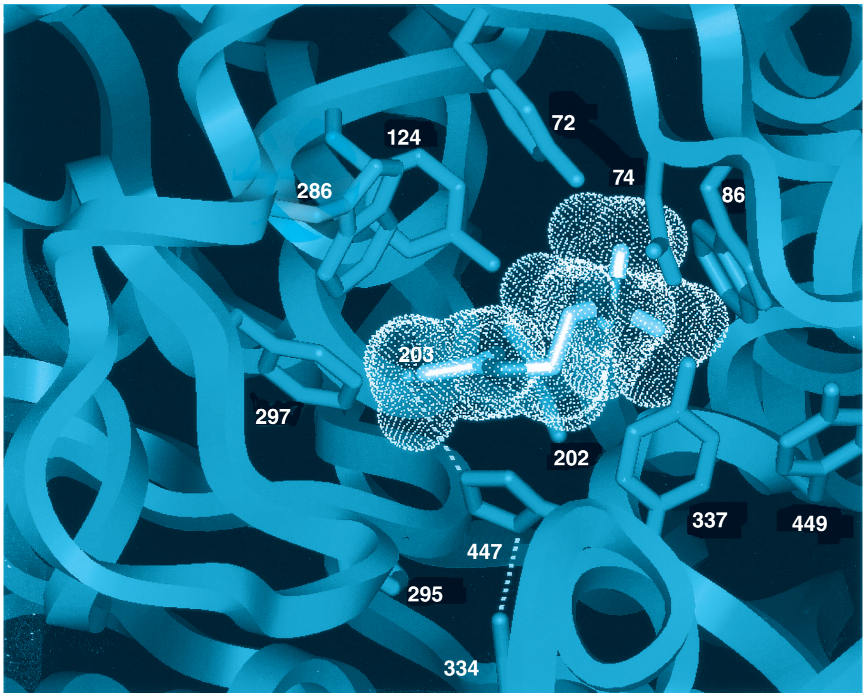


Figure 12–1 *The active center gorge of mammalian AChE, viewed from the portal of substrate entry.* Bound ACh is shown by the dotted structure depicting its van der Waals radii. The crystal structure of mouse cholinesterase active center, which is virtually identical to human AChE, is shown (Bourne et al., 1995). Included are the side chains of (1) the catalytic triad: Glu^{334}, His^{447}, Ser^{203} (hydrogen bonds are denoted by the dotted lines); (2) acyl pocket: Phe^{295} and Phe^{297}; (3) choline subsite: Trp^{86}, Glu^{202}, and Tyr^{337}; and (4) the peripheral site: Trp^{286}, Tyr^{72}, Tyr^{124}, and Asp^{74}. Tyrosines 337 and 449 are further removed from the active center but likely contribute to stabilization of certain ligands. The catalytic triad, choline subsite, and acyl pocket are located at the base of the gorge, while the peripheral site is at the rim of the gorge. The gorge is 18- to 20-Å deep, with its base centrosymmetric to the subunit.

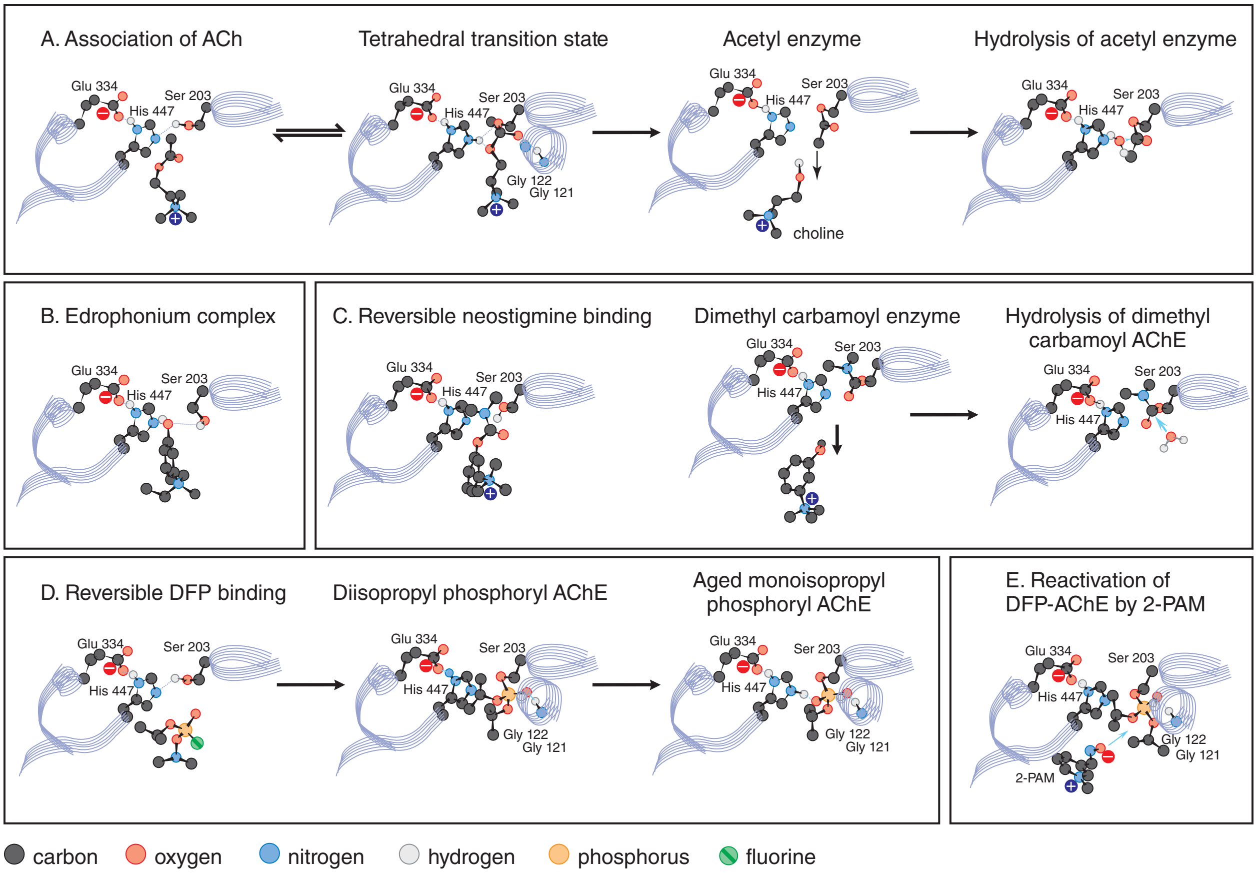

A. Association of ACh
Tetrahedral transition state
Acetyl enzyme
Hydrolysis of acetyl enzyme
Glu 334
Ser 203
His 447
Gly 122
Gly 121
choline
B. Edrophonium complex
C. Reversible neostigmine binding
Dimethyl carbamoyl enzyme
Hydrolysis of dimethyl carbamoyl AChE
D. Reversible DFP binding
Diisopropyl phosphoryl AChE
Aged monoisopropyl phosphoryl AChE
E. Reactivation of DFP-AChE by 2-PAM
2-PAM
carbon
oxygen
nitrogen
hydrogen
phosphorus
fluorine

Figure 12–2 *Steps involved in the hydrolysis of ACh by AChE and in the inhibition and reactivation of the enzyme.* Only the three residues of the catalytic triad shown in Figure 12–1 are depicted. Net charge in a region is represented by red and blue circles containing – or + signs, respectively. The associations and reactions shown are as follows: **A.** ACh catalysis: binding of ACh, formation of a tetrahedral transition state, formation of the acetyl enzyme with liberation of choline, rapid hydrolysis of the acetyl enzyme with return to the original state. **B.** Reversible binding and inhibition by *edrophonium*. **C.** *Neostigmine* reaction with and inhibition of AChE: reversible binding of *neostigmine*, formation of the dimethyl carbamoyl enzyme, slow hydrolysis of the dimethyl carbamoyl enzyme. **D.** DFP reaction and inhibition of AChE: reversible binding of DFP, formation of the diisopropyl phosphoryl enzyme, formation of the aged monoisopropyl phosphoryl enzyme. Hydrolysis of the diisopropyl enzyme is very slow and is not shown. The aged monoisopropyl phosphoryl enzyme is virtually resistant to hydrolysis and reactivation. The proposed tetrahedral transition state of ACh hydrolysis resembles the conjugates formed by the tetrahedral phosphate inhibitors and accounts for their potency. Amide bond hydrogens from Gly121 and Gly122 stabilize the carbonyl and phosphoryl oxygens. **E.** Reactivation of the diisopropyl phosphoryl enzyme by 2-PAM. 2-PAM attack of the phosphorus on the phosphorylated enzyme will form a phospho-oxime with regeneration of active enzyme. The individual steps of phosphorylation reaction and oxime reaction have been characterized by mass spectrometry. (Data from Jennings et al., 2003.)

influencing expression in brain yield no obvious phenotype. Presumably, this arises from significant adaptive responses and compensatory reductions of ACh synthesis and storage and receptor responses (Camp et al., 2008; Dobbertin et al., 2009).

Acetylcholinesterase Inhibitors

Molecular Mechanism of Action of AChE Inhibitors

The mechanisms of action of compounds that typify the three classes of anti-ChE agents are also shown in Figure 12–2.

Three distinct domains on AChE constitute binding sites for inhibitory ligands and form the basis for specificity differences between AChE and BChE:

- The acyl pocket of the active center
- The choline subsite of the active center
- The peripheral anionic site (Reiner and Radić, 2000; Taylor and Radić, 1994).

Reversible inhibitors, such as *edrophonium* and *tacrine*, bearing a quaternary cation, bind to the choline subsite in the vicinity of Trp[86] and Glu[202] (Silman and Sussman, 2000) (Figure 12–2B). *Edrophonium* has a brief duration of action because its quaternary structure facilitates rapid renal elimination and it binds reversibly to the AChE active center. Additional reversible inhibitors, such as *donepezil*, bind with higher affinity to the active center gorge. Other reversible inhibitors, such as *propidium* and the snake peptidic toxin *fasciculin*, bind to the peripheral anionic site on AChE. This site resides at the rim of the gorge and is defined by Trp[286], Tyr[72], and Tyr[124] (see Figure 12–1).

Drugs that have a carbamoyl ester linkage, such as *physostigmine* and *neostigmine*, are hydrolyzed by AChE, but much more slowly than is ACh ester. The quaternary amine in *neostigmine* and the tertiary amine in *physostigmine* exist as cations at physiological pH. By serving as alternate substrates to ACh (Figure 12–2C), their reaction with the active center serine progressively generates the carbamoylated enzyme. The conjugated carbamoyl moiety resides in the acyl pocket outlined by Phe[295] and Phe[297]. In contrast to the acetyl enzyme, methylcarbamoyl AChE and dimethylcarbamoyl AChE are far more stable (the $t_{1/2}$ for hydrolysis of the dimethylcarbamoyl enzyme is 15–30 min). Sequestration of the enzyme in its carbamoylated form thus precludes the enzyme-catalyzed hydrolysis of ACh for extended periods of time. When administered systemically, the duration of inhibition by the carbamoylating agents is 3 to 4 h.

The organophosphate inhibitors, such as DFP, serve as true hemisubstrates; the resultant conjugate with the active center serine phosphorylated or phosphonylated is extremely stable (Figure 12–2D). Organophosphorus inhibitors are tetrahedral in configuration, a configuration that resembles the transition state formed in carboxyl ester hydrolysis. Similar to the carboxyl esters, the phosphoryl oxygen binds within the oxyanion hole of the active center. If the alkyl groups in the phosphorylated enzyme are ethyl or methyl, spontaneous regeneration of active enzyme requires several hours. Secondary (as in DFP) or tertiary alkyl groups further enhance the stability of the phosphorylated enzyme, and significant regeneration of active enzyme usually is not observed. The stability of the phosphorylated enzyme is enhanced through "aging," which results from the loss of one of the alkyl groups. Hence, the return of AChE activity in the aged enzyme conjugate depends largely on biosynthesis of new AChE protein.

Accordingly, the terms *reversible* and *irreversible* as applied to the carbamoyl and organophosphorus anti-ChE agents reflect only quantitative differences in rates of decarbamoylation or dephosphorylation of the conjugated enzyme. Both chemical classes react covalently with the active center serine in essentially the same manner as does ACh in forming the transient, short-lived acetyl enzyme.

Chemistry and Structure-Activity Relationships

Structure-activity relationships of anti-ChE agents have been extensively reviewed in the scientific literature. Only agents of general therapeutic or toxicological interest are considered here.

Noncovalent Inhibitors

While these agents interact by reversible and noncovalent association with the active site in AChE, they differ in their disposition in the body and their affinity for the enzyme. *Edrophonium*, a quaternary drug whose activity is limited to peripheral nervous system synapses, has a moderate affinity for AChE. Its volume of distribution is limited and renal elimination is rapid, accounting for its short duration of action. By contrast, *tacrine* and *donepezil* (Figure 12–3) have higher affinities for AChE, are more hydrophobic, and readily cross the blood-brain barrier to inhibit AChE in the CNS. Partitioning into lipid and higher affinities for AChE may also account for their longer durations of action.

"Reversible" Carbamate Inhibitors

Drugs of this class that are of therapeutic interest are shown in Figure 12–3. Early studies showed that the essential moiety of the *physostigmine* molecule was the methylcarbamate of an amine-substituted phenol. The quaternary ammonium derivative *neostigmine* is a compound of equal or greater potency. *Pyridostigmine*, a close congener, is also used in myasthenia gravis patients.

Carbamoylating inhibitors with high lipid solubility (*rivastigmine*) have longer durations of action, cross the blood-brain barrier, and are used as an alternative in the treatment of Alzheimer's disease (Cummings, 2004) (see Chapter 21). The carbamate insecticides carbaryl, propoxur, and aldicarb, used extensively as garden insecticides, inhibit AChE with a mechanism identical to other carbamoylating agents. While more reversible and less toxic, symptoms parallel those of organophosphates (Eddleston and Clark, 2011; King and Aaron, 2015).

Organophosphorus Compounds

The general formula for the organophosphorus class of ChE inhibitors is shown at the top of Table 12–1. A great variety of substituents is possible:

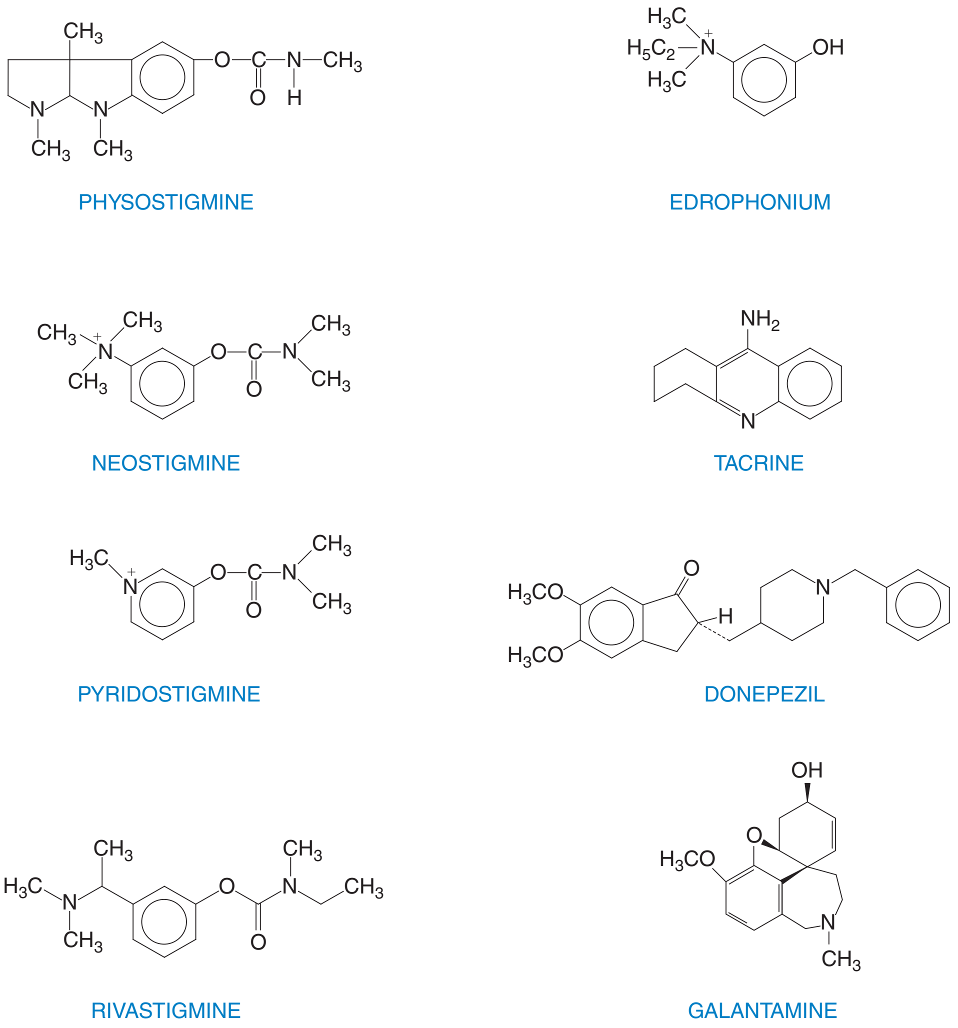
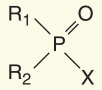
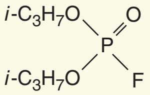
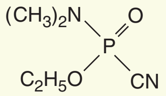
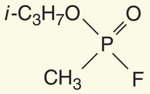
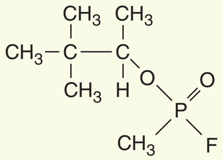
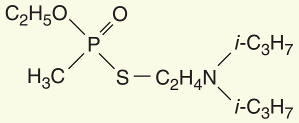
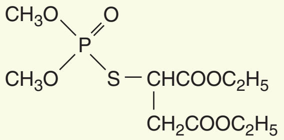
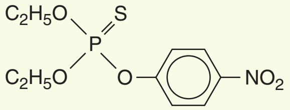
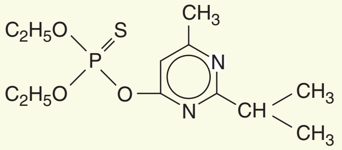
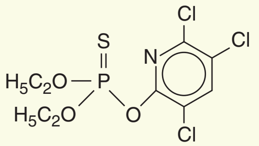
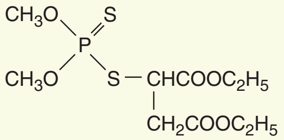
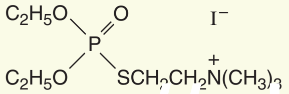


Figure 12–3 *"Reversible" carbamate and noncovalent AChE inhibitors used clinically.*

TABLE 12–1 ■ CHEMICAL CLASSIFICATION OF REPRESENTATIVE ORGANOPHOSPHORUS AChE INHIBITORS

General formula 

Group **A**, X = halogen, cyanide, or thiocyanate leaving group; group **B**, X = alkylthio, arylthio, alkoxy, or aryloxy leaving group; group **C**, thionophosphorus or thio-thionophosphorus compounds; group **D**, quaternary ammonium leaving group. R_1 can be an alkyl (phosphonates), alkoxy (phosphorates), or an alkylamino (phosphoramidates) group.

GROUP	STRUCTURAL FORMULA	COMMON, CHEMICAL, AND OTHER NAMES	COMMENTS
A		DFP; Isoflurophate; diisopropyl fluorophosphate	Potent, irreversible inactivator
		Tabun Ethyl N-dimethylphosphoramidocyanidate	Extremely toxic "nerve gas"
		Sarin (GB) Isopropyl methylphosphonofluoridate	Extremely toxic "nerve gas"
		Soman (GD) Pinacolyl methylphosphonofluoridate	Extremely toxic "nerve gas"; greatest potential for irreversible action/rapid aging
B		VX *O*-ethyl S [2-(diisopropylamino)ethyl] methyl phosponothioate	Potent, slower onset, skin-penetrating nerve agent
		Malaoxon *O,O*-Dimethyl S-(1,2-dicarboxyethyl)-phosphorothioate	Active metabolite of malathion
C		Parathion *O,O*-Diethyl *O*-(4-nitrophenyl)-phosphorothioate	Agricultural insecticide, resulting in numerous cases of accidental poisoning; phased out in 2003
		Diazinon, Dimpylate *O,O*-Diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate	Insecticide; use limited to non-residential agricultural settings
		Chlorpyrifos *O,O*-Diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate	Insecticide; use limited to non-residential agricultural settings
		Malathion *O,O*-Dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate	Widely employed insecticide of greater safety than parathion or other agents because of rapid detoxification by higher organisms
D		Echothiophate (PHOSPHOLINE IODIDE), MI-217 Diethoxyphosphinylthiocholine iodide	Extremely potent choline derivative; administered locally in treatment of glaucoma; relatively stable in aqueous solution

R_1 and R_2 may be alkyl, alkoxy, aryloxy, amido, mercaptan, or other groups; and X, the leaving group, typically a conjugate base of a weak acid, is a halide, cyanide, thiocyanate, phenoxy, thiophenoxy, phosphate, thiocholine, or carboxylate group. For a compilation of the organophosphorus compounds and their toxicity, see Gallo and Lawryk (1991).

Diisopropyl fluorophosphate produces virtually irreversible inactivation of AChE and other esterases by alkylphosphorylation. Its high lipid solubility, low molecular weight, and volatility facilitate inhalation toxicity, transdermal absorption, and penetration into the CNS. After desulfuration, the insecticides in current use form the dimethoxy or diethoxyphosphoryl conjugate of AChE.

The "nerve gases"—tabun, sarin, soman, and VX—are among the most potent synthetic toxic agents known; they are lethal to laboratory animals in nanogram doses. Insidious employment of these agents occurred in the Matsumoto incident and Tokyo subway terrorism attacks in Japan and against civilians by despotic regimes in the Middle East (Council on Foreign Relations, 2013; Dolgin, 2013; King and Aaron, 2015; Nozaki and Aikawa, 1995). While estimates of lethality in Japan amounted to 8 and 10 people killed, in Syria estimates vary, ranging up to 1000 individuals, with over 3000 showing symptoms of organophosphate toxicity. Attacks continued into 2017 with release of sarin vapor from explosive devices. Toxicity results from inhalation and rapid distribution of sarin to the central and peripheral nervous systems. The slower dermal absorption of VX was used for political assassination in Malaysia in 2017.

Parathion and methylparathion were widely used as insecticides because of their favorable properties of low volatility and stability in aqueous solution. Acute and chronic toxicity has limited their use, and potentially less-hazardous compounds have replaced them for home and garden use throughout the world. These compounds are inactive in inhibiting AChE *in vitro*; they act via the active metabolite, paraoxon. The phosphoryl oxygen for sulfur substitution is carried out predominantly by hepatic cytochrome P450s (CYPs). This reaction also occurs in the insect, typically with more efficiency. Other insecticides possessing the phosphorothioate structure have been widely employed for agricultural use. These include *diazinon* and *chlorpyrifos.* Use of these agents is restricted because of evidence of chronic toxicity in the newborn animal. They have been banned from indoor and outdoor residential use since 2005.

Malathion also requires conversion of a sulfur atom to oxygen *in vivo,* conferring resistance to mammalian species. Also, this insecticide can be detoxified by hydrolysis of the carboxyl ester linkage by plasma carboxylesterases. Plasma carboxylesterase activity dictates species resistance to malathion: The detoxification reaction is much more rapid in mammals and birds than in insects (Costa et al., 2013). In recent years, malathion has been employed in aerial spraying of relatively populous areas for control of citrus orchard–destructive Mediterranean fruit flies and mosquitoes that harbor and transmit viruses harmful to human beings, such as the West Nile encephalitis virus.

Evidence of acute toxicity from malathion arises primarily with suicide attempts or deliberate poisoning. The lethal dose in mammals is about 1 g/kg. Exposure to the skin results in a small fraction (<10%) systemically absorbed. Malathion is used topically in the treatment of pediculosis (lice) infestations in cases of *permethrin* resistance (Centers for Disease Control and Prevention, 2015).

Among the quaternary ammonium organophosphorus compounds (group D in Table 12–1), only *echothiophate* is useful clinically, and its use is limited to ophthalmic administration. As a positively charged quaternary amine, *echothiophate* is not volatile and does not readily penetrate the skin.

Basis for the Pharmacological Effects of ChE Inhibitors

The characteristic pharmacological effects of the anti-ChE agents are due primarily to the prevention of catalytic hydrolysis of ACh by AChE at sites of cholinergic transmission. Transmitter thus accumulates, enhancing the response to ACh that is liberated by cholinergic impulses or that is spontaneously released from nerve endings. Virtually all acute effects of moderate doses of organophosphates are attributable to this action. For example, the characteristic miosis that follows local application of DFP to the eye is not observed after chronic postganglionic denervation of the eye because there is no source from which to release endogenous ACh. The consequences of enhanced concentrations of ACh at motor end plates of skeletal muscle are unique to these sites and are discussed below.

Generally, the pharmacological properties of anti-ChE agents can be predicted by knowing those loci where ACh is released physiologically by nerve impulses, the degree of nerve impulse activity, and the responses of the corresponding effector organs to ACh (see Chapter 10). The anti-ChE agents potentially can produce all the following effects:

- Stimulation of muscarinic receptor responses at autonomic effector organs
- Stimulation, followed by depression or paralysis, of all autonomic ganglia and skeletal muscle (nicotinic actions)
- Stimulation, with occasional subsequent depression, of pre- and postsynaptic cholinergic receptor sites in the CNS

At therapeutic doses, several factors modify the actions of anti-ChE agents. Compounds containing a quaternary ammonium group do not penetrate cell membranes readily; hence, anti-ChE agents in this category are absorbed poorly from the GI tract or across the skin and are excluded from the CNS by the blood-brain barrier after moderate doses. On the other hand, such compounds act preferentially at the neuromuscular junctions of skeletal muscle, exerting their action both as anti-ChE agents and as direct agonists (see example of effects of *neostigmine* on denervated skeletal muscle, below, under Neuromuscular Junction). They have comparatively lower effects at autonomic effector sites and ganglia. In contrast, the more lipid-soluble agents are well absorbed after oral administration, have ubiquitous effects at both peripheral and central cholinergic sites, and may be sequestered in lipids for long periods of time. Lipid-soluble organophosphorus agents, such as the chemical warfare agents, are well absorbed through the skin, whereas the volatile agents are transferred readily across the alveolar membranes in the lung (King and Aaron, 2015; Storm et al., 2000).

The actions of anti-ChE agents on autonomic effector cells and on cortical and subcortical sites in the CNS, where ACh receptors are largely of the muscarinic type, are blocked by *atropine*. Likewise, *atropine* blocks some of the excitatory actions of anti-ChE agents on autonomic ganglia, because both nicotinic and muscarinic receptors are involved in ganglionic neurotransmission (see Chapter 13).

Effects on Physiological Systems

The sites of action of anti-ChE agents of therapeutic importance are the CNS, eye, intestine, and neuromuscular junction of skeletal muscle; other actions are of toxicological consequence.

Eye

When applied locally to the conjunctiva, anti-ChE agents cause conjunctival hyperemia and constriction of the pupillary sphincter muscle around the pupillary margin of the iris (miosis) and the ciliary muscle (block of accommodation reflex with resultant focusing to near vision). Miosis is apparent in a few minutes and can last several hours to days. Although the pupil may be "pinpoint" in size, it generally contracts further when exposed to light. The block of the accommodation reflex is more transient and generally disappears before termination of miosis. Intraocular pressure, when elevated, usually falls as the result of facilitation of outflow of the aqueous humor (see Chapter 69).

GI Tract

In humans, *neostigmine* enhances gastric contractions and increases the secretion of gastric acid. After bilateral vagotomy, the effects of *neostigmine* on gastric motility are greatly reduced. The lower portion of the esophagus is stimulated by *neostigmine*; in patients with marked achalasia and dilation of the esophagus, the drug can cause a salutary increase in tone and peristalsis.

Neostigmine also augments locomotor activity of the small and large bowel; the colon is particularly stimulated. Atony produced by muscarinic receptor antagonists or prior surgical intervention may be overcome, propulsive waves are increased in amplitude and frequency, and movement of intestinal contents is thus promoted. The total effect of anti-ChE agents on intestinal motility probably represents a combination of actions at the ganglion cells of the Auerbach plexus and at the smooth muscle fibers as a result of the preservation of ACh released by the cholinergic preganglionic and postganglionic fibers, respectively (see Chapter 54).

Neuromuscular Junction

Most of the effects of potent anti-ChE drugs on skeletal muscle can be explained on the basis of their inhibition of AChE at neuromuscular junctions. However, there is good evidence for an accessory direct action of *neostigmine* and other quaternary ammonium anti-ChE agents on skeletal muscle. For example, the intra-arterial injection of *neostigmine* into chronically denervated muscle, or muscle in which AChE has been inactivated by prior administration of DFP, evokes an immediate contraction, whereas *physostigmine* does not.

Normally, a single nerve impulse in a terminal motor-axon branch liberates enough ACh to produce a localized depolarization (end-plate potential) of sufficient magnitude to initiate a propagated muscle action potential. The ACh released is rapidly hydrolyzed by AChE, such that the lifetime of free ACh within the nerve-muscle synapse (~200 μsec) is shorter than the decay of the end-plate potential or the refractory period of the muscle. Therefore, each nerve impulse gives rise to a single wave of depolarization. After inhibition of AChE, the residence time of ACh in the synapse increases, allowing for lateral diffusion and cycles of association-dissociation of the released transmitter to multiple receptors. Successive stimulation of neighboring receptors to the release site in the end plate prolongs the decay time of the end-plate potential. Quanta released by individual nerve impulses are no longer isolated. This action destroys the synchrony between end-plate depolarizations and the development of the muscle action potentials. Consequently, asynchronous excitation and fasciculations of muscle fibers occur. With sufficient inhibition of AChE, depolarization of the endplate predominates, and blockade due to depolarization ensues (see Chapter 13). When ACh persists in the synapse, it also may depolarize the axon terminal, resulting in antidromic firing of the motor neuron; this stimulation contributes to fasciculations that involve the entire motor unit.

Anti-ChE agents will reverse the antagonism caused by competitive neuromuscular blocking agents. By contrast, *neostigmine* is not effective against the skeletal muscle paralysis caused by *succinylcholine*, which produces neuromuscular blockade by depolarization; *neostigmine* will enhance depolarization and the resultant blockade.

Cardiopulmonary System

The cardiovascular actions of anti-ChE agents are complex because they reflect both ganglionic and postganglionic effects of accumulated ACh on the heart and blood vessels as well as actions in the CNS. The predominant effect on the heart from the peripheral action of accumulated ACh is bradycardia, resulting in a fall in cardiac output. Higher doses usually enhance the fall in blood pressure, as a consequence of effects of anti-ChE agents on the medullary vasomotor centers of the CNS.

Anti-ChE agents augment vagal influences on the heart. This shortens the effective refractory period of atrial muscle fibers and increases the refractory period and conduction time at the sinoatrial and atrioventricular nodes. At the ganglionic level, accumulating ACh initially is excitatory on nicotinic receptors, but at higher concentrations, ganglionic blockade ensues as a result of persistent depolarization of the postsynaptic nerve. The excitatory action on the parasympathetic ganglion cells would tend to reinforce the diminished cardiac output, whereas the opposite sequence results from the action of ACh on sympathetic ganglion cells. Excitation followed by inhibition also is elicited by ACh at the central medullary vasomotor and cardiac centers in the brainstem. These effects are complicated further by the hypoxemia resulting from the bronchoconstrictor and secretory actions of increased ACh on the respiratory system. Hypoxemia, in turn, can reinforce both sympathetic tone and ACh-induced discharge of epinephrine from the adrenal medulla. Hence, it is not surprising that an increase in heart rate is seen with severe ChE inhibitor poisoning. Hypoxemia probably is a major factor in the CNS depression that appears after large doses of anti-ChE agents.

Actions at Other Sites

Secretory glands that are innervated by postganglionic cholinergic fibers include the bronchial, lacrimal, sweat, salivary, gastric (antral G cells and parietal cells), intestinal, and pancreatic acinar glands. Low doses of anti-ChE agents augment secretory responses to nerve stimulation, and higher doses actually produce an increase in the resting rate of secretion.

Anti-ChE agents increase contraction of smooth muscle fibers of the bronchioles and ureters, and the ureters may show increased peristaltic activity.

ADME

Physostigmine is absorbed readily from the GI tract, subcutaneous tissues, and mucous membranes. The conjunctival instillation of solutions of the drug may result in systemic effects if measures (e.g., pressure on the inner canthus) are not taken to prevent absorption from the nasal mucosa. Parenterally administered *physostigmine* is largely destroyed within 2 to 3 h, mainly by hydrolytic cleavage by plasma esterases.

Neostigmine and *pyridostigmine* are absorbed poorly after oral administration, such that much larger oral doses are needed than by the parenteral route. Whereas the effective parenteral dose of *neostigmine* is 0.5 to 2 mg, the equivalent oral dose may be 15 to 30 mg or more. *Neostigmine* and *pyridostigmine* are also destroyed by plasma esterases; the half-lives of these drugs are about 1 to 2 h (Cohan et al., 1976).

Organophosphate anti-ChE agents with the highest risk of toxicity are highly lipid-soluble liquids; others, such as sarin, have high vapor pressures, augmenting their dispersal and pulmonary absorption. The less volatile agents that are commonly used as agricultural insecticides (e.g., diazinon, chlorpyrifos, malathion) generally are dispersed as aerosols or as dusts adsorbed to an inert, finely particulate material. Consequently, the compounds are absorbed rapidly through the skin and mucous membranes following contact with moisture, by the lungs after inhalation, and by the GI tract after ingestion (Storm et al., 2000).

Following their absorption, most organophosphates are excreted almost entirely as hydrolysis products in the urine. Plasma and liver esterases are responsible for hydrolysis to the corresponding phosphoric and phosphonic acids. However, CYPs are responsible for converting the inactive phosphorothioates containing a phosphorus-sulfur (thiono) bond to phosphates with a phosphorus-oxygen bond, resulting in their activation. These enzymes also play a role in the inactivation of certain organophosphorus agents, and allelic differences are known to affect rates of metabolism (Furlong, 2007).

The organophosphate anti-ChE agents are hydrolyzed by two families of hepatic enzymes: the carboxylesterases and the paraoxonases (A-esterases). These enzymes are secreted into plasma and scavenge or hydrolyze a large number of organophosphates by cleaving the phosphoester, anhydride, phosphofluoridate, or phosphocyanate bonds. Natural substrates of the paraoxonases appear to be lactones. In addition to catalyzing hydrolysis of organophosphates, the paraoxonase isoform PON1 associates with high-density lipoproteins and appears to play a role in removing oxidized lipids, thereby exerting a protective effect in atherosclerosis and inflammation (Costa et al., 2013; Harel et al., 2004; Mackness and Mackness, 2015). Wide variations in paraoxonase activity exist among animal species. Young animals are deficient in carboxylesterases and paraoxonases, which may account for age-related toxicities seen in newborn animals and suspected to be a basis for organophosphate toxicity in humans (Padilla et al., 2004).

Plasma and hepatic carboxylesterases (aliesterases) and plasma BChE are inhibited irreversibly by organophosphates (Costa et al., 2013; Lockridge, 2015); their scavenging capacity for organophosphates can afford partial protection against inhibition of AChE in the nervous system. The carboxylesterases also catalyze hydrolysis of malathion and other organophosphates that contain carboxyl-ester linkages, rendering them less active or inactive. Because carboxylesterases are inhibited by organophosphates, toxicity from simultaneous exposure to two organophosphorus insecticides can prove synergistic.

Toxicology

Scope of the Problem

The toxicological aspects of the anti-ChE agents are of practical importance to clinicians. In addition to cases of accidental intoxication from the use and manufacture of organophosphorus compounds as agricultural insecticides, because of their availability, these agents have been used frequently for homicidal and suicidal purposes. Organophosphates account for as many as 80% of pesticide-related hospital admissions. The World Health Organization documents pesticide toxicity as a widespread global problem associated with over 300,000 deaths a year; most poisonings occur in Southeast Asia (Eddleston and Chowdhury, 2015; Eddleston and Clark, 2011). Occupational exposure occurs most commonly by the dermal and pulmonary routes, while oral ingestion is most common in cases of nonoccupational poisoning.

Sources of Information

In the U.S., the Environmental Protection Agency (EPA), by virtue of revised risk assessments and the Food Quality Protection Act of 1996, has placed several organophosphate insecticides, including diazinon and chlorpyrifos, on restricted use and phased-out status in consumer products for home and garden use. A primary concern relates to exposure in pregnancy and to infants and children because the developing nervous system may be particularly susceptible to certain of these agents (Eaton et al., 2008). The National Pesticide Information Center (http://npic.orst.edu/) and the Office of Pesticide Programs of the EPA (https://www.epa.gov/pesticides) provide frequent updates of the status of organophosphate pesticides, their tolerance reassessments, and revisions of risk assessments through their websites.

Acute Intoxication

Acute intoxication by anti-ChE agents is manifested by muscarinic and nicotinic signs and symptoms and, except for quaternary cationic compounds of low lipid solubility, by signs referable to the CNS. Systemic effects appear within minutes after inhalation of vapors or aerosols, while onset of symptoms is delayed after GI and percutaneous absorption. The duration of toxic symptoms is determined largely by the properties of the compound: its lipid solubility, whether it must be activated to form the oxon, the stability of the organophosphate-AChE bond, and whether "aging" of the phosphorylated enzyme has occurred.

After local exposure to vapors or aerosols or after their inhalation, ocular and respiratory effects generally appear first. Ocular manifestations include marked miosis, ocular pain, conjunctival congestion, diminished vision, ciliary spasm, and brow ache. With acute systemic absorption, miosis may not be evident due to sympathetic discharges in response to hypotension. In addition to rhinorrhea and hyperemia of the upper respiratory tract, respiratory responses consist of tightness in the chest and wheezing caused by the combination of bronchoconstriction and increased bronchial secretion. GI symptoms occur earliest after ingestion and include anorexia, nausea and vomiting, abdominal cramps, and diarrhea. With percutaneous absorption of liquid, localized sweating and muscle fasciculations in the immediate vicinity are generally the earliest symptoms. Severe intoxication is manifested by extreme salivation, involuntary defecation and urination, sweating, lacrimation, penile erection, bradycardia, and hypotension.

Nicotinic actions at the neuromuscular junctions of skeletal muscle usually consist of fatigability and generalized weakness, involuntary twitchings, scattered fasciculations, and eventually severe weakness and paralysis. The most serious consequence is paralysis of the respiratory muscles.

A broad spectrum of effects of acute AChE inhibition on the CNS includes confusion, ataxia, slurred speech, loss of reflexes, Cheyne-Stokes respiration, generalized convulsions, coma, and central respiratory paralysis. Actions on the vasomotor and other cardiovascular centers in the medulla oblongata lead to hypotension.

The time of death after a single acute exposure may range from less than 5 min to nearly 24 h, depending on the dose, route, and agent. The cause of death primarily is respiratory failure, usually accompanied by a secondary cardiovascular component. Peripheral muscarinic and nicotinic as well as central actions all contribute to respiratory compromise; effects include laryngospasm, bronchoconstriction, increased tracheobronchial and salivary secretions, and compromised voluntary control of the diaphragm and intercostal muscles. Blood pressure may fall to alarmingly low levels, and cardiac arrhythmias may result from hypoxemia.

Delayed symptoms appearing after 1 to 4 days and marked by persistent low blood ChE and severe muscle weakness are termed the *intermediate syndrome* (Lotti, 2002). Delayed neurotoxicity and recurrent seizures also may be evident after severe intoxication (discussed below in Reactivation and Disposition).

Diagnosis and Treatment

The diagnosis of severe, acute anti-ChE intoxication is made readily from the history of exposure and the characteristic signs and symptoms. In suspected cases of milder acute or chronic intoxication, determination of AChE activities in erythrocytes and BChE in plasma generally will establish the diagnosis (Storm et al., 2000). Although these values vary considerably in the normal population, they usually are depressed well below the normal range before symptoms are evident.

Atropine in sufficient dosage (described further in the chapter) effectively antagonizes the actions at muscarinic receptor sites, including increased tracheobronchial and salivary secretion, bronchoconstriction, and bradycardia. Larger doses are required to get appreciable concentrations of *atropine* into the CNS. *Atropine* is virtually without effect against the peripheral neuromuscular compromise, which can be reversed by *pralidoxime* (2-PAM), a cholinesterase reactivator.

In moderate or severe intoxication with an organophosphorus anti-ChE agent, the recommended adult dose of 2-PAM is 1 to 2 g, slowly infused intravenously. If weakness is not relieved or if it recurs after 20 to 60 min, the dose should be repeated. Early treatment is important to ensure that the oxime reaches the phosphorylated AChE while the latter still can be reactivated. Many of the alkylphosphates are extremely lipid soluble, and if extensive partitioning into body fat has occurred and desulfuration is required for inhibition of AChE, toxicity will persist.

General supportive measures are important and include:

- Termination of exposure, by removal of the patient or application of a gas mask if the atmosphere remains contaminated, removal and destruction of contaminated clothing, copious washing of contaminated skin or mucous membranes with water, or gastric lavage
- Maintenance of a patent airway, including endobronchial aspiration
- Artificial respiration and administration of O_2, if required
- Alleviation of persistent convulsions with *diazepam* (5–10 mg IV)
- Treatment of shock

Atropine should be given in doses sufficient to cross the blood-brain barrier. Following an initial injection of 2 to 4 mg, given intravenously if possible, otherwise intramuscularly, 2 mg should be given every 5 to 10 min until muscarinic symptoms disappear, if they reappear, or until signs of atropine toxicity appear. More than 200 mg may be required on the first day. AChE reactivating agents and mild degree of *atropine* block then should be maintained for as long as symptoms are evident.

Although the phosphorylated esteratic site of AChE undergoes hydrolytic regeneration at a slow or negligible rate, nucleophilic agents, such as hydroxylamine (NH_2OH), hydroxamic acids (RCONH–OH), and oximes (RCH=NOH), reactivate the enzyme more rapidly than does spontaneous hydrolysis. Froede and Wilson (1971) reasoned that selective reactivation could be achieved by a site-directed nucleophile, wherein interaction of a quaternary nitrogen with the negative subsite of the active center would place the nucleophile in close apposition to the conjugated phosphorus. The oxime is oriented proximally to exert a nucleophilic attack on the phosphorus; a phosphoryloxime is formed, leaving the regenerated enzyme (Figure 12–2E).

Several *bis*-quaternary aldoximes are even more potent as reactivators for insecticide and nerve agent poisoning, for instance, *obidoxime* and HI-6, which are used in Europe as antidotes (Steinritz et al., 2016). However, these compounds do not cross the blood-brain barrier, limiting their effectiveness to peripheral nervous system sites. The oximate ionization state of the oximes is the acting nucleophile. Enhanced penetration of the blood-brain barrier is achieved with amphipathic oximes (Chambers, 2020) or zwitterionic oximes (Shyong et al., 2021; Taylor, 2021), where a neutral species and the cationic, anionic, and zwitterionic ionization species are in rapid equilibrium, allowing both access to the CNS and periphery, in addition to target selectivity (Shyong et al., 2021).

Certain phosphorylated AChE conjugates can undergo a fairly rapid process of "aging," so that within the course of minutes or hours they become completely resistant to the reactivators. Aging is due to the loss of one alkoxy group, leaving a much more stable monoalkyl- or monoalkoxyphosphoryl-AChE (Figure 12–2D and 12–2E). Organophosphorus compounds containing tertiary alkoxy groups, such as soman, are more prone to aging than are congeners containing the secondary or primary alkoxy groups. The oximes are not effective in antagonizing the toxicity of the more rapidly hydrolyzing carbamoyl ester inhibitors; since 2-PAM itself has weak anti-ChE activity, it is not recommended for the treatment of overdosage with *neostigmine* or *physostigmine* or poisoning with carbamoylating insecticides such as carbaryl.

Reactivation and Disposition

The reactivating action of oximes *in vivo* is most marked at the skeletal neuromuscular junction. Antidotal effects are less striking at autonomic effector sites, and the quaternary ammonium group restricts entry into the CNS (Eddleston and Clark, 2011; Shyong et al., 2021).

Since high doses or accumulation of oximes inhibit AChE and cause neuromuscular blockade, the offending organophosphate should be identified. Current antidotal therapy for organophosphate exposure resulting from warfare or terrorism includes parenteral *atropine*, an oxime (2-PAM, HI-6, or *obidoxime*), and *diazepam* or *midazolam* as anticonvulsants (King and Aaron, 2015). Oxime entry to the CNS is still limited since the capillary endothelial cell contains extrusion transporters that limit entry into functioning cholinergic synapses. The reactivating oximes are readily eliminated by the kidney, so repetitive dosing is recommended. Hence, a transport inhibitor limiting brain extrusion of the antidote and slowing its renal elimination may constitute improvements in antidote therapy (Shyong et al., 2021).

Parenterally administered human BChE and recombinant DNA-expressed paraoxonases and phosphotriesterases with selected mutations are also under development to scavenge the organophosphate at its portal of entry or in the plasma before it reaches peripheral and central tissue sites (Cerasoli et al., 2005; Mata et al., 2014; Worek et al., 2014). Catalytic enzyme scavengers are limited by their stabilities in the field of use, their slow distribution from intramuscular sites, and costs of production of catalytic proteins with high turnover.

Certain fluorine-containing organophosphorus anti-ChE agents (e.g., DFP, mipafox) may induce a delayed neurotoxicity shared with the triarylphosphates, of which triorthocresyl phosphate (TOCP) is the classic example. The clinical picture is a long-term polyneuropathy manifested initially by sensory disturbances, ataxia, muscle fatigue, and twitching. In severe cases, the weakness may progress to flaccid paralysis and muscle wasting. Toxicity from this organophosphate-induced delayed polyneuropathy is due to a distinct esterase, termed a *neurotoxic esterase* (Johnson, 1993). This enzyme has a specificity for hydrophobic esters, but its natural substrate and function remain unknown (Glynn, 2006; Read et al., 2009).

Therapeutic Uses of AChE Inhibitors

Current use of anti-AChE agents is limited to four conditions in the periphery:

- Atony of the smooth muscle of the intestinal tract and urinary bladder
- Glaucoma, via drop instillation into the eye
- Myasthenia gravis, via parenteral and oral therapy
- Reversal of the paralysis of competitive neuromuscular blocking drugs, via parenteral routes

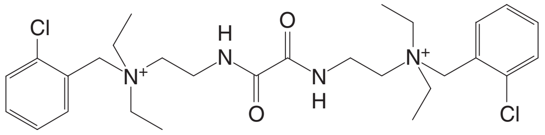
Long-acting and hydrophobic ChE inhibitors are the only inhibitors with documented efficacy, albeit limited, in the treatment of dementia symptoms of Alzheimer's disease. *Physostigmine*, with its shorter duration of action, is used to treat intoxication by *atropine* and several drugs with anticholinergic side effects (discussed further in the chapter); it also is indicated for the treatment of Friedreich or other inherited ataxias. *Edrophonium* has been used for terminating attacks of paroxysmal supraventricular tachycardia.

Available Therapeutic Agents

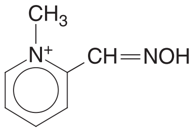
The compounds described here are those commonly used as anti-ChE drugs and ChE reactivators in the U.S. Preparations used solely for ophthalmic purposes are described in Chapter 74. Conventional dosages and routes of administration are given in the further discussion of therapeutic applications.

Physostigmine salicylate is available for injection. *Physostigmine sulfate* ophthalmic ointment and *physostigmine salicylate* ophthalmic solution also are available. *Pyridostigmine bromide* is available for oral or parenteral use. *Neostigmine bromide* is available for oral use. *Neostigmine methylsulfate* is marketed for parenteral injection. *Ambenonium chloride* is available for oral use. *Tacrine, donepezil*, *rivastigmine*, and *galantamine* have been approved for the treatment of Alzheimer's disease.

Pralidoxime chloride is the only AChE reactivator currently available in the U.S. and can be obtained in a parenteral formulation. HI-6 is available in several European and Middle Eastern countries.



AMBENONIUM



PRALIDOXIME (2-PAM)

Paralytic Ileus and Atony of the Urinary Bladder

In the treatment of both paralytic ileus and urinary bladder atony, *neostigmine* generally is preferred among the anti-ChE agents. Directly acting muscarinic agonists (see Chapter 11) are employed for the same purposes.

Neostigmine is used for the relief of abdominal distension and acute colonic pseudo-obstruction from a variety of medical and surgical causes (Ponec et al., 1999). The usual subcutaneous dose of *neostigmine methylsulfate* for postoperative paralytic ileus is 0.5 mg, given as needed. Peristaltic activity commences 10 to 30 min after parenteral administration, whereas 2 to 4 h are required after oral administration of *neostigmine bromide* (15–30 mg). It may be necessary to assist evacuation with a small low enema or gas with a rectal tube.

When *neostigmine* is used for the treatment of atony of the detrusor muscle of the urinary bladder, postoperative dysuria is relieved. The drug is used in a similar dose and manner as in the management of paralytic ileus. *Neostigmine* should not be used when the intestine or urinary bladder is obstructed, when peritonitis is present, when the viability of the bowel is doubtful, or when bowel dysfunction results from inflammatory bowel disease.

Glaucoma and Other Ophthalmologic Indications

Glaucoma is a complex disease characterized by an increase in intraocular pressure. If the pressure is sufficiently high and persistent, it will damage the optic disc at the juncture of the optic nerve and the retina; irreversible blindness can result. Of the three types of glaucoma—primary, secondary, and congenital—anti-AChE agents are of value in the management of the primary as well as of certain categories of the secondary type (e.g., aphakic glaucoma, following cataract extraction); congenital glaucoma rarely responds to any therapy other than surgery. Primary glaucoma is subdivided into narrow-angle (acute congestive) and wide-angle (chronic simple) types, based on the configuration of the angle of the anterior chamber where the aqueous humor is reabsorbed.

Narrow-angle glaucoma is nearly always a medical emergency in which drugs are essential in controlling the acute attack, but the long-range management is often surgical (e.g., peripheral or complete iridectomy). Wide-angle glaucoma, on the other hand, has a gradual, insidious onset and is not generally amenable to surgical improvement; in this type, control of intraocular pressure usually is dependent on continuous drug therapy.

Because the cholinergic agonists and ChE inhibitors also block accommodation and induce myopia, these agents produce transient blurring of far vision, limited visual acuity in low light, and loss of vision at the margin when instilled in the eye. With long-term administration of the cholinergic agonists and anti-ChE agents, these compromises of vision diminish. Nevertheless, other agents without these side effects, such as prostaglandin analogues, β adrenergic receptor antagonists, and carbonic anhydrase inhibitors, have become the primary topical therapies for open-angle glaucoma. AChE inhibitors are held in reserve for the chronic conditions when patients become refractory to the agents mentioned. Topical treatment with long-acting ChE inhibitors such as *echothiophate* gives rise to symptoms characteristic of systemic ChE inhibition. (For a complete account of the use of anti-ChE agents in ocular therapy, see Chapter 74.)

Myasthenia Gravis

Myasthenia gravis is a neuromuscular disease of complex genetic etiology characterized by exacerbations and remissions of weakness and marked fatigability of skeletal muscle (Drachman, 1994; Renton et al., 2015).

The relative importance of prejunctional and postjunctional defects in myasthenia gravis was unknown until Patrick and Lindstrom (1973) found that rabbits immunized with nicotinic receptor slowly developed muscular weakness and respiratory difficulties that resembled the symptoms of myasthenia gravis. This animal model prompted intense investigation into whether the natural disease represented an autoimmune response directed toward the ACh receptor. Antireceptor antibodies are detectable in sera of 90% of patients with the disease, although the clinical status of the patient does not correlate precisely with antibody titers (Drachman, 1994). Sequences in the α_1 ACh receptor subunit constituting the main immunogenic region are well defined (Lindstrom, 2008).

The picture that emerges is that myasthenia gravis is caused by an autoimmune response primarily to the ACh receptor at the postjunctional end plate. These antibodies reduce the number of receptors detectable either by snake α-neurotoxin–binding assays (Fambrough et al., 1973) or by electrophysiological measurements of ACh sensitivity (Drachman, 1994). Immune complexes along with marked ultrastructural abnormalities appear in the synaptic cleft and enhance receptor degradation through complement-mediated lysis in the end plate.

In a subset of about 10% of patients presenting with a myasthenic syndrome, muscle weakness has a congenital rather than an autoimmune basis. Characterization of biochemical and genetic bases of the congenital condition has demonstrated mutations in the ACh receptor that affect ligand-binding, channel-opening kinetics and durations; receptor biosynthesis; and synaptic location of receptors (Engel et al., 2012; Sine and Engel, 2006). Other mutations occur as a deficiency in the form of AChE that contains the collagen-like tail unit, in presynaptic transporters involved in the uptake of choline, and in vesicular storage of ACh. In myasthenic patients, identification of the mutation in the complement of synaptic proteins is essential for ascertaining whether a specific pharmacological treatment is warranted.

Diagnosis

Although the diagnosis of autoimmune myasthenia gravis usually can be made from the history, signs, and symptoms, its differentiation from certain neurasthenic, infectious, endocrine, congenital, neoplastic, and degenerative neuromuscular diseases can be challenging. However, in autoimmune myasthenia gravis, the aforementioned deficiencies and enhancement of muscle strength can be improved dramatically by anti-ChE medication. The *edrophonium* test for initial diagnosis relies on these responses. The *edrophonium* test is performed by rapid intravenous injection of 2 mg of *edrophonium chloride*, followed 45 sec later by an additional 8 mg if the first dose is without effect. A positive response consists of brief improvement in strength, unaccompanied by lingual fasciculation (which generally occurs in nonmyasthenic patients).

An excessive dose of an anti-ChE drug results in a *cholinergic crisis*. The condition is characterized by weakness resulting from generalized depolarization of the motor end plate; other symptoms result from overstimulation of muscarinic receptors. The weakness resulting from depolarization blockade may resemble myasthenic weakness, which is manifest when anti-ChE medication is insufficient. The distinction is of obvious practical importance because the former is treated by withholding, and the latter by administering, the anti-ChE agent. Detection of antireceptor antibodies in muscle biopsies or plasma is now widely employed to establish the diagnosis.

Treatment of Myasthenia Gravis

Pyridostigmine, *neostigmine*, and *ambenonium* are the standard anti-ChE drugs used in the symptomatic treatment of myasthenia gravis. All can increase the response of myasthenic muscle to repetitive nerve impulses, primarily by the preservation of released endogenous ACh. Following AChE inhibition, receptors over a greater cross-sectional area of the end plate presumably are exposed to concentrations of ACh sufficient for channel opening and production of a postsynaptic end-plate potential.

Unpredictable exacerbations and remissions of the myasthenic state may require adjustment of dosage. *Pyridostigmine* is available in sustained-release tablets containing a total of 180 mg, of which 60 mg are released immediately and 120 mg are released over several hours; this preparation is of value in maintaining patients for 6- to 8-h periods but should be limited to use at bedtime. Muscarinic cardiovascular and GI side effects of anti-ChE agents generally can be controlled by *atropine* or other anticholinergic drugs (see Chapter 11). However, these anticholinergic drugs mask many side effects of an excessive dose of an anti-ChE agent. In most patients, tolerance develops eventually to the muscarinic effects. Several drugs, including curariform agents and certain antibiotics and general anesthetics, interfere with neuromuscular transmission (see Chapter 13); their administration to patients with myasthenia gravis requires proper adjustment of anti-ChE dosage and other precautions.

Other therapeutic measures are essential elements in the management of this disease. Glucocorticoids promote clinical improvement in a high percentage of patients. However, when treatment with steroids is continued over prolonged periods, a high incidence of side effects may result (see Chapter 50). Initiation of steroid treatment augments muscle weakness; however, as the patient improves with continued administration of steroids, doses of anti-ChE drugs can be reduced (Drachman, 1994). Other immunosuppressive agents, such as *azathioprine* and *cyclosporine* and high-dose *cyclophosphamide* (Drachman et al., 2008), have also been beneficial in more refractory cases (see Chapter 39). Thymectomy should be considered in myasthenia associated with a thymoma or when the disease is not controlled adequately by anti-ChE agents and steroids. Because the thymus contains myoid cells with nicotinic receptors (Schluep et al., 1987) and a predominance of patients have thymic abnormalities, the thymus may be responsible for the initial pathogenesis. It also is the source of autoreactive T-helper cells.

Alzheimer's Disease

A deficiency of intact cholinergic neurons, particularly those extending from subcortical areas such as the nucleus basalis, has been observed in patients with progressive dementia of the Alzheimer's type (see Chapter 21). Using a rationale similar to that in other CNS degenerative diseases, therapy for enhancing concentrations of cholinergic and other neurotransmitters in the CNS has been investigated.

In 1993, the U.S. Food and Drug Administration (FDA) approved *tacrine* (tetrahydroaminoacridine) for use in mild-to-moderate Alzheimer's disease, but a high incidence of enhanced alanine aminotransferase and hepatotoxicity limited the utility of this drug.

Subsequently, *donepezil* was approved for clinical use and has emerged as the primary agent for treatment in multiple countries (Lee et al., 2015). Initially, 5-mg doses are administered daily, and if tolerated, doses are increased to 10 mg for mild-to-moderate conditions. Recent clinical trials in moderate-to-severe Alzheimer's disease have confirmed benefits for a 23-mg/day sustained-release form. Most studies are carried out for periods of 24 weeks, although treatment periods have been extended, usually extending the treatment baseline, but without further improvement or some decline after 6 months. Adverse side effects, attributed to excessive peripheral cholinergic stimulation, include nasopharyngitis, diarrhea, nausea, and vomiting. Rhabdomyolysis reportedly occurs, requiring discontinuation of the drug. Co-treatment with *memantine* did not result in significant improvement over the higher-dose *donepezil* treatment (Howard et al., 2012).

Rivastigmine, a more lipid-soluble, longer-acting carbamylating inhibitor, is approved for use in the U.S. and Europe in both oral and skin patch forms. While having similar side effects to other cholinesterase inhibitors, *rivastigmine* is reported to have shown a higher incidence of fatalities than other cholinesterase inhibitors used in Alzheimer's dementias (Ali et al., 2015). It has not been determined whether the increase relates to misuse of the transdermal form of administration. *Galantamine* is another FDA-approved agent for Alzheimer's dementias, acting as a reversible AChE inhibitor with a side effect profile similar to that of *donepezil*.

These three cholinesterase inhibitors, which have the requisite affinity and hydrophobicity to cross the blood-brain barrier and exhibit a prolonged duration of action, along with an excitatory amino acid transmitter mimic, *memantine*, constitute current modes of therapy. These agents are not disease modifying and lack well-documented actions on the pathology of disease. However, the bulk of the evidence indicates that they slow the decline in cognitive function and behavioral manifestation for limited intervals of time (see Chapter 21). Associated symptoms, such as depression, may be preferentially delayed (Lu et al., 2009). Current clinical research efforts are directed to synergistic actions of arresting inflammatory processes or neurodegeneration, combining cholinesterase inhibition with selective cholinergic receptor modulation and agents that affect amyloid precursor protein (APP) and tau (see Chapter 21).

Prophylaxis in Cholinesterase Inhibitor Poisoning

Studies in experimental animals have shown that pretreatment with *pyridostigmine* reduces the incapacitation and mortality associated with nerve agent poisoning, particularly for agents such as soman that show rapid aging. The first large-scale administration of *pyridostigmine* to humans occurred in 1990 in anticipation of nerve agent attack in the first Gulf War. At an oral dose of 30 mg every 8 h, the incidence of side effects was around 1%; fewer than 0.1% of the subjects had responses sufficient to warrant discontinuing the drug in the setting of military action (Keeler et al., 1991). Long-term follow-up indicates that veterans of the Gulf War who received *pyridostigmine* showed a low incidence of a neurological syndrome, now termed the *Persian Gulf War syndrome*. It is characterized by impaired cognition, ataxia, confusion, myoneuropathy, adenopathy, weakness, and incontinence (Haley et al., 1997).

Controversy still surrounds the basis of Gulf War syndrome or illness, despite multiple reports and reviews by the U.S. Department of Veterans Affairs in 2008 and the National Academy of Medicine (Committee on Gulf War and Health Reports, 2013; Institute of Medicine, 2013). Although several origins of the syndrome, such as *pyridostigmine* administration, have been ruled out as unlikely, the constellation of symptoms reflect an interplay of chemical toxicants and psychological factors, encompassing widespread pesticide use and exposure from postwar demolition bombing of munitions facilities likely containing chemical warfare agents (sarin and mustards). Psychological factors, emerging as posttraumatic stress disorders, have been documented in prolonged wars since the early 20th century.

Intoxication by Anticholinergic Drugs

In addition to *atropine* and other muscarinic agents, many other drugs, such as the phenothiazines, antihistamines, and tricyclic antidepressants, have central and peripheral anticholinergic activity. *Physostigmine* is potentially useful in reversing the central anticholinergic syndrome produced by overdosage or an unusual reaction to these drugs (Nilsson, 1982). While the effectiveness of *physostigmine* in reversing anticholinergic side effects has been documented, other toxic effects of the tricyclic antidepressants and phenothiazines (see Chapters 18 and 19), such as intraventricular conduction deficits and ventricular arrhythmias, are not reversed by *physostigmine*. In addition, *physostigmine* may precipitate seizures; hence, its usually small potential benefit must be weighed against these risks. The use of anti-ChE agents to reverse the effects of competitive neuromuscular blocking agents is discussed in Chapter 13.

Drug Facts for Your Personal Formulary: *Anticholinesterase Agents*

Drugs	Therapeutic Uses	Major Toxicity and Clinical Pearls
Noncovalent Reversible Inhibitors		
Edrophonium Tacrine Donepezil Propidium Fasciculin Galantamine	• Edrophonium can be used to diagnose myasthenia gravis • Tacrine, donepezil, and galantamine used for Alzheimer's disease	• Edrophonium, tacrine, and donepezil bind reversibly to the choline subsite in AChE, sterically blocking ACh entry for catalysis • Edrophonium has a short duration of action because of rapid renal elimination; effects are limited to the peripheral nervous system • Donepezil and tacrine: higher affinity for AChE, more hydrophobic, can cross blood-brain barrier • Tacrine: high incidence of hepatotoxicity • Donepezil binds with higher affinity to the active center gorge of AChE • Propidium and fasciculin: bind peripheral anionic site on AChE
Carbamate Inhibitors		
"Reversible" carbamate inhibitors Physostigmine Neostigmine Pyridostigmine Ambenonium Rivastigmine	• Pyridostigmine, neostigmine, and ambenonium are used for treatment of myasthenia gravis • Neostigmine is used for paralytic ileus and atony of the urinary bladder • Rivastigmine is a very lipid-soluble alternative for treating Alzheimer's disease • Pyridostigmine is used prophylactically for protection in nerve agent attacks	• Drugs with carbamoyl ester linkage: AChE substrates that block by carbamylation of AChE active center serine, are hydrolyzed slowly; regarded as hemi-substrate blockers • Neostigmine and pyridostigmine are poorly absorbed after oral administration • Pyridostigmine is available in sustained-release tablets; oral dose >> parenteral dose • Rivastigmine can cross the blood-brain barrier, has longer duration of action, and is available in oral and epidermal patch formulations

Drug Facts for Your Personal Formulary: *Anticholinesterase Agents* (*continued*)

Drugs	Therapeutic Uses	Major Toxicity and Clinical Pearls
Carbamate insecticides Carbaryl Propoxur Aldicarb	• Garden insecticides	• Symptoms of poisoning resemble those of organophosphates but are more readily reversed and less toxic
Organophosphates		
Echothiophate	• Treatment of glaucoma	• Instilled locally in the eye • Stable in aqueous solution
Nerve agents DFP Tabun Sarin Soman Cyclosarin VX	• Alkylphosphates are the most potent synthetic toxins: typically methylphosphonates • React covalently with the active site serine • Potent and irreversible inactivators of ChE • Recent documented use in terrorism	• Form a stable conjugate with the active site serine by phosphorylation/phosphonylation • Hydrolyzed by hepatic carboxyesterases and paraoxonases • Low molecular weight, hydrophobic, rapidly penetrate into CNS from pulmonary inhalation of the vapor or aerosol • Tabun, sarin, and cyclosarin are volatile and extremely toxic "nerve agents" • VX is absorbed through the skin, has slower onset, but high toxicity • 2-PAM and related aldoximes are used to reactivate organophosphate-ChE conjugates • Resistance to organophosphate-AChE reactivation is enhanced through "aging" that results from loss of one alkyl group of the conjugated organophosphate
Pesticides Parathion Methylparathion Malathion Diazinon Chlorpyrifos	• Insecticides, largely agricultural • Malathion is used topically in the treatment of pediculosis in cases of permethrin resistance • Lethal dose of malathion in mammals is 1 g/kg • Diazinon and chlorpyrifos are used widely in agriculture; indoor use is discouraged	• Metabolism of these *thion* pesticides to the corresponding *oxon* activates pesticide activity and toxicity; thought to occur more rapidly in insects • Malathion: detoxified by plasma carboxylesterases, a reaction that is more rapid in mammals and birds than insects, yielding a further margin of safety
Antidotal therapy for Organophosphate Exposure		
Cholinesterase reactivators 2-PAM HI-6 Obidoxime	• Quaternary pyridinium aldoxime reactivators indicated for insecticide and nerve agent poisoning • Improved reactivators acting centrally and peripherally are in development	• Reactivates organophosphate-AChE conjugate by attacking the conjugated phosphorus to form phospho-oxime and regenerate the active enzyme • Dose is injected IM with autoinjector; dosing should be repeated frequently • Early treatment helps ensure that the oxime reaches the phosphorylated enzyme prior to complete "aging" • Quaternary reactivators do not cross the blood-brain barrier rapidly to reactivate CNS AChE
Anticholinergic agents Atropine	• Blocks symptoms mediated through muscarinic receptors	• Given parenterally in 2- to 4-mg doses every few minutes until muscarinic symptoms subside (see Chapter 11)
Benzodiazepines Diazepam Midazolam (recent FDA approval)	• Minimize seizures and associated neuronal toxicity	• Administered parenterally after exposure

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Chapter 13

Neuromuscular Junction and Autonomic Ganglia; Nicotine, Muscle Relaxants, and Spasmolytics

Ryan E. Hibbs and Alexander C. Zambon

THE NICOTINIC ACETYLCHOLINE RECEPTOR
- Perspective
- Structure of Nicotinic Receptors

TRANSMISSION AT THE NEUROMUSCULAR JUNCTION
- Neuromuscular Blocking Agents
- Clinical Pharmacology

SPASMOLYTICS
- Central Acting Spasmolytics
- Peripherally Acting Antispasmodics

GANGLIONIC NEUROTRANSMISSION
- The Neural Nicotinic Receptor and Postsynaptic Potentials
- Ganglionic Stimulating Agents
- Ganglionic Blocking Agents

NICOTINE ADDICTION AND SMOKING CESSATION
- Nicotine Replacement Therapy
- Cytisine
- Varenicline

The Nicotinic Acetylcholine Receptor

The nicotinic ACh receptor mediates neurotransmission postsynaptically at the neuromuscular junction and peripheral autonomic ganglia; in the CNS, it plays a major role in modulating the release of neurotransmitters from presynaptic sites. The receptor is called the *nicotinic ACh receptor* because both the alkaloid nicotine and the neurotransmitter ACh can stimulate the receptor. Distinct subtypes of nicotinic receptors, defined by their subunit composition, exist at the neuromuscular junction (N_m), in autonomic ganglia, and in the CNS (the neuronal form, N_n). The binding of ACh to the nicotinic ACh receptor initiates an EPP in muscle or an EPSP in peripheral ganglia by directly mediating cation influx into the postsynaptic cell (see Chapter 10).

Perspective

Classical studies of the actions of curare and nicotine defined the concept of the nicotinic ACh receptor over a century ago and made this the prototypical pharmacological receptor. By taking advantage of specialized structures that have evolved to mediate cholinergic neurotransmission and natural toxins that block motor activity, nicotinic receptors were isolated and characterized (Changeux and Edelstein, 2005). These accomplishments represent historical landmarks in the development of molecular pharmacology.

Cholinergic neurotransmission mediates motor activity in marine vertebrates and mammals, and a large number of peptide, terpenoid, and alkaloid toxins that block the nicotinic receptors have evolved to enhance predation or protect plant and animal species from predation (Taylor et al., 2007; Tsetlin et al., 2021). Among these toxins are the α-toxins: peptides of about 7 kDa from venoms of the krait, *Bungarus multicinctus*, and varieties of the cobra, *Naja naja*. These toxins potently inhibit neuromuscular transmission, are readily radiolabeled, and provide excellent probes for the nicotinic receptor.

The electrical organs from the aquatic species of *Electrophorus* and *Torpedo* are rich sources of nicotinic receptors; up to 40% of the surface of the electric organ's membrane is excitable and contains cholinergic receptors, in contrast to vertebrate skeletal muscle, in which motor end plates occupy 0.1% or less of the cell surface. Using the α-toxin probes, researchers have purified the receptor from *Torpedo*, isolated the cDNAs of the subunits, and cloned the genes for the multiple receptor subunits from mammalian neurons and muscle (Numa et al., 1983). By simultaneously expressing various permutations of the genes that encode the individual subunits in cellular systems and then measuring binding and the electrophysiological events that result from activation by agonists, researchers have been able to correlate functional properties with details of primary structures of the receptor subtypes (Changeux and Edelstein, 2005; Karlin, 2002; Sine et al., 2008).

Structure of Nicotinic Receptors

The nicotinic receptor shares homology with other pentameric ligand-gated ion channels, which include the $5HT_3$ receptors (Chapter 15) and receptors for the inhibitory amino acids (GABA and glycine; Chapter 16). Each of the subunits in the pentameric receptor has a molecular mass of 40 to 60 kDa. In each subunit, around 210 residues of the amino-terminal constitute a large extracellular domain. This is followed by four domains that span the membrane; the region between TM3 and TM4 forms most of the cytoplasmic component (Figure 13–1, A and B).

In vertebrates, the nicotinic receptors of skeletal muscle N_m are pentamers composed of four distinct subunits (α, β, γ, and δ) in a stoichiometric ratio of 2:1:1:1 (Changeux and Edelstein, 2005; Gharpure et al., 2020; Karlin, 2002) (Figure 13–1C). In mature, innervated muscle end plates, the γ subunit is replaced by ε, a closely related subunit. The individual subunits are about 40% identical in their amino acid sequences. The five subunits of the nicotinic ACh receptor are arranged around a pseudo-axis of symmetry to circumscribe a channel. The resulting receptor is an asymmetrical molecule (16 × 8 nm) of 290 kDa, with the bulk of the non-membrane–spanning domain on the extracellular surface. The receptor is present at high densities ($10,000/\mu m^2$) in junctional areas (i.e., the motor end plate in skeletal muscle and the ventral surface of the *Torpedo* electrical organ). Agonist-binding sites occur at the subunit interfaces; in muscle, only two of the five subunit interfaces, αγ and αδ, bind agonists (Figures 13–1 and 13–2). Both of the subunits forming the subunit interface contribute to ligand specificity. Neuronal nicotinic N_n receptors found in ganglia and the CNS also exist as pentamers of one or more types of subunits. Subunit types α_2 through α_{10} and β_2 through β_4 are found in neuronal tissues. Although not all pentameric combinations of α and β subunits lead to functional receptors, the diversity in subunit composition is large and exceeds the capacity of ligands to distinguish subtypes on the basis of their selectivity.

Agonist-mediated changes in ion permeability occur through a cation channel intrinsic to the receptor structure. Measurements of membrane conductance demonstrate rates of ion translocation of 5×10^7 ions/sec. The channel is generally nonselective among cations; while highly permeable to Na^+, K^+, and in some cases Ca^{2+}, the majority of the current is carried by

Abbreviations

ACh: acetylcholine
AChE: acetylcholinesterase
anti-ChE: anticholinesterase
AUC: area under the curve
CNS: central nervous system
EPP: end-plate potential
EPSP: excitatory postsynaptic potential
FDA: Food and Drug Administration
GABA: γ-aminobutyric acid
GI: gastrointestinal
5HT: 5-hydroxytryptamine (serotonin)
IPSP: inhibitory postsynaptic potential
MAO: monoamine oxidase
M_x: muscarinic receptor subtype x (x = 1, 2, 3, 4, or 5)
N_m: nicotinic ACh receptor in skeletal muscle
N_n: nicotinic ACh receptor in neurons
NRT: nicotine replacement therapy
SIF: small, intensely fluorescent
TM: transmembrane
VMAT2: vesicular monoamine transporter 2

Na^+ ions (see Figure 13–2). The channel is lined by the five TM_2 α-helices of the channel subunits. The agonist-binding site is intimately coupled with the ion channel; in the N_m, simultaneous binding of two agonist molecules results in a rapid conformational change that opens the channel.

HISTORY Curare

In the mid-19th century, Claude Bernard demonstrated that the locus of action of curare was at or near the neuromuscular junction. Curare is a generic term for various South American arrow poisons. The drug has been used for centuries by Indians along the Amazon and Orinoco Rivers for immobilizing and paralyzing wild animals used for food; death results from paralysis of skeletal muscles. The preparation of curare was long shrouded in mystery and was entrusted only to tribal shamans. Soon after Europeans invaded South American, European explorers and botanists became interested in curare, and late in the 16th century, samples of the native preparations were brought to Europe. Following the pioneering work of scientist/explorer von Humboldt in 1805, the botanical sources of curare became the object of much field research. The curares from eastern Amazonia come from *Strychnos* species, which contain chiefly quaternary neuromuscular blocking alkaloids. The Asiatic, African, and Australian species nearly all contain tertiary strychnine-like alkaloids.

In the mid-19th century, Claude Bernard showed that the locus of curare's action was at or near the neuromuscular junction. Research on curare accelerated when Gill, after prolonged and intimate study of the native methods of preparing curare, brought to the U.S. a sufficient amount of the authentic drug to permit chemical and pharmacological investigations. The modern clinical use of curare apparently dates from 1932, when West employed highly purified fractions in patients with tetanus and spastic disorders. King established the essential structure of tubocurarine in 1935 (see Figure 13–3). Griffith and Johnson reported the first trial of curare for promoting muscular relaxation in general anesthesia in 1942.

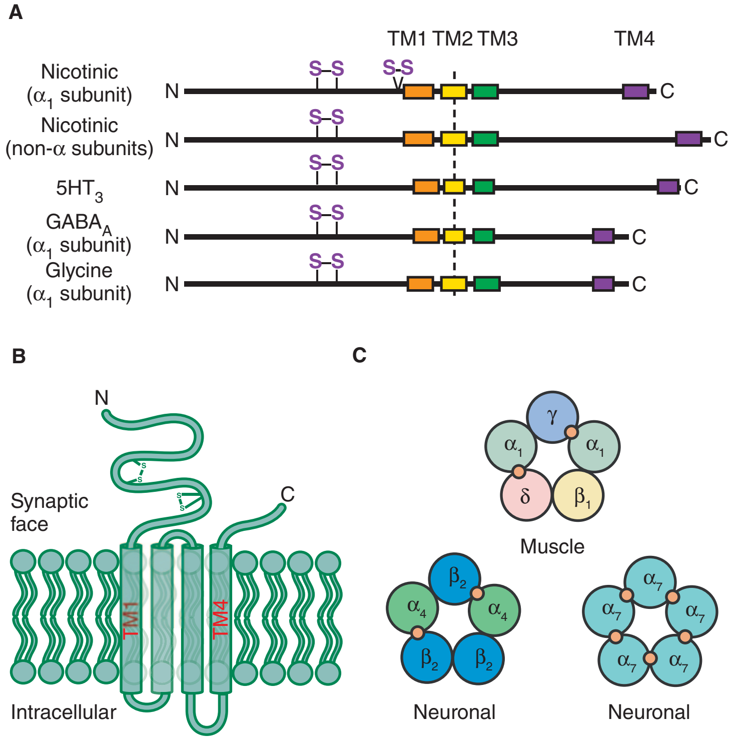


Figure 13–1 *Subunit organization of pentameric ligand-gated ion channels; nicotinic receptor subunit assembly.* **A.** Ligand-gated channels responding to ACh, 5HT, GABA and glycine share a common pentameric architecture. In each subunit, the amino (N)-terminal region of approximately 210 amino acids is found at the extracellular surface. This large extracellular region is followed by the four hydrophobic membrane-spanning regions (TM1 -TM4), leaving the small carboxyl (C) terminus on the extracellular surface. The TM2 region is α-helical, and TM2 regions from each subunit of the pentameric receptor line the internal pore of the receptor. Two disulfide loops at positions 128 to 142 and 192 to 193 are found in the α subunit of the nicotinic receptor. The 128 to 142 motif is conserved in the superfamily of pentameric receptors, whereas the vicinal cysteines at 192 and 193 distinguish α subunits from non-α subunits in the nicotinic receptor. **B.** This diagram illustrates the membrane topology for one of the five subunits of the nicotinic ACh receptor and depicts the regions shown in Panel A. **C.** These transverse sections show representative pentamers for muscle and neuronal nicotinic ACh receptors. Agonist binding sites (small red circles) occur at α subunit-containing interfaces. A total of 16 functional receptor isoforms have been observed in mammals, with different ligand specificities, relative Na^+/ Ca^{2+} permeabilities, and physiological functions as determined by their subunit composition. The only isoform found at the neuromuscular junction is the one shown here, with the caveat that an ε subunit replaces γ in mature muscle. The receptor isoforms found at autonomic ganglia and in the CNS are homo- and hetero-pentamers of α (α_{2-7} and α_{9-10}) and β (β_{2-4}) subunits.

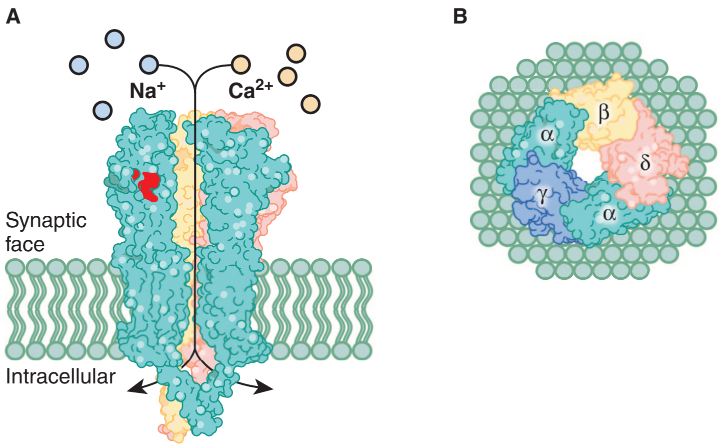


Figure 13–2 *Molecular structure of the nicotinic ACh receptor.* **A.** Longitudinal view of muscle receptor structure recently determined by electron microscopy, with the γ subunit removed (Rahman et al., 2020). The remaining subunits, two copies of α, one of β, and one of δ, are shown to surround an internal channel with an outer vestibule and its constriction located deep in the membrane bilayer region. Spans of α-helices form the perimeter of the channel and come from the TM2 region of the linear sequence (Figure 13–1). ACh-binding sites, indicated by red surface, occur at the αγ and αδ (not visible) interfaces. **B.** Muscle nicotinic receptor structure viewed from perspective of neuromuscular junction. Intrinsic channel is down the central axis defined by the pseudosymmetric arrangement of receptor subunits.

Transmission at the Neuromuscular Junction

Neuromuscular Blocking Agents

Modern-day neuromuscular blocking agents fall generally into two classes, depolarizing and competitive/nondepolarizing. At present, only a single depolarizing agent, *succinylcholine*, is in general clinical use; multiple competitive or nondepolarizing agents are available (Figure 13–3). Neuromuscular blocking agents are most commonly used for facilitating endotracheal intubation and to relax skeletal muscle during surgery.

Chemistry

Early structure-activity studies led to the development of the polymethylene bis-trimethyl-ammonium series (referred to as the methonium compounds, or depolarizing blockers). The most potent of these agents at the neuromuscular junction was the compound with 10 carbon atoms between the quaternary nitrogens: *decamethonium* (see Figure 13–3). The compound with 6 carbon atoms in the chain, *hexamethonium*, was found to be essentially devoid of neuromuscular blocking activity but particularly effective as a ganglionic blocking agent (see following discussion).

Several structural features distinguish competitive and depolarizing neuromuscular blocking agents. The competitive agents (e.g., *tubocurarine*, the benzylisoquinolines, the amino steroids, and the asymmetric mixed-onium chlorofumarates) are relatively bulky, rigid molecules, whereas the depolarizing agents (e.g., *decamethonium* [no longer marketed in the U.S.] and *succinylcholine*) generally have more flexible structures that enable free bond rotations.

Mechanism of Action

Competitive antagonists bind the N_m and thereby competitively block the binding of ACh. The depolarizing agents, such as *succinylcholine*, depolarize the membrane by opening channels in the same manner as ACh. However, they persist longer at the neuromuscular junction primarily because of their resistance to AChE. The depolarization is thus longer lasting, resulting in a brief period of repetitive excitation that may elicit transient and repetitive muscle excitation (fasciculations), followed by blocking of neuromuscular transmission and flaccid paralysis (called *phase I block*). The block arises because, after an initial opening, perijunctional Na$^+$ channels close and will not reopen until the end plate is repolarized. At this point, neural release of ACh results in the binding of ACh to receptors on an already-depolarized end plate. These closed perijunctional channels keep the depolarization signal from affecting downstream channels and effectively shield the rest of the muscle from activity at the motor end plate. This sequence is influenced by such factors as the anesthetic agent used concurrently, the type of muscle, and the rate of drug administration. The characteristics of depolarization and competitive blockade are contrasted in Table 13–1.

Under clinical conditions, with increasing concentrations of *succinylcholine* and over time, the block may convert slowly from a depolarizing phase I block to a nondepolarizing *phase II block* (Durant and Katz, 1982). While the response to peripheral stimulation during phase II block resembles that of the competitive agents, reversal of phase II block by administration of anti-AChE agents (e.g., with *neostigmine*) is difficult to predict and should be undertaken cautiously. Table 13–2 summarizes the characteristics of phase I and phase II blocks.

Many drugs and toxins block neuromuscular transmission by other mechanisms, such as interference with the synthesis or release of ACh (see Figure 10–6), but most of these agents are not employed clinically for neuromuscular blockade. One exception is the group of botulinum toxins, which are administered locally into muscles of the orbit in the management of ocular blepharospasm and strabismus and have been used to control other muscle spasms and to facilitate facial muscle relaxation (see Table 10–7 and Chapter 74). This toxin also has been injected into the lower esophageal sphincter to treat achalasia (Chapter 54). Botulinum toxins are discussed in detail in the later Spasmolytics section. The sites of action and interrelationship of several agents that serve as pharmacological tools are shown in Figure 13–4.

Sequence and Characteristics of Paralysis

Following intravenous injection of an appropriate dose of a *competitive blocking agent*, motor weakness progresses to total flaccid paralysis. Small, rapidly moving muscles such as those of the eyes, jaw, and larynx relax before those of the limbs and trunk. Ultimately, the intercostal muscles and finally the diaphragm are paralyzed, and respiration then ceases. Recovery of muscles usually occurs in the reverse order to that of their paralysis, and thus the diaphragm ordinarily is the first muscle to regain function (Brull and Meistelman, 2020).

After a single intravenous dose (10–30 mg) of the *depolarizing blocking agent succinylcholine*, muscle fasciculations, particularly over the chest and abdomen, occur briefly; then, relaxation occurs within 1 min, becomes maximal within 2 min, and generally disappears within 5 min. Transient apnea usually occurs at the time of maximal effect. Muscle relaxation of longer duration is achieved by continuous intravenous infusion. After infusion is discontinued, the effects of the drug usually disappear rapidly because of its efficient hydrolysis by plasma and hepatic butyrylcholinesterase. Muscle soreness may follow the administration of *succinylcholine*.

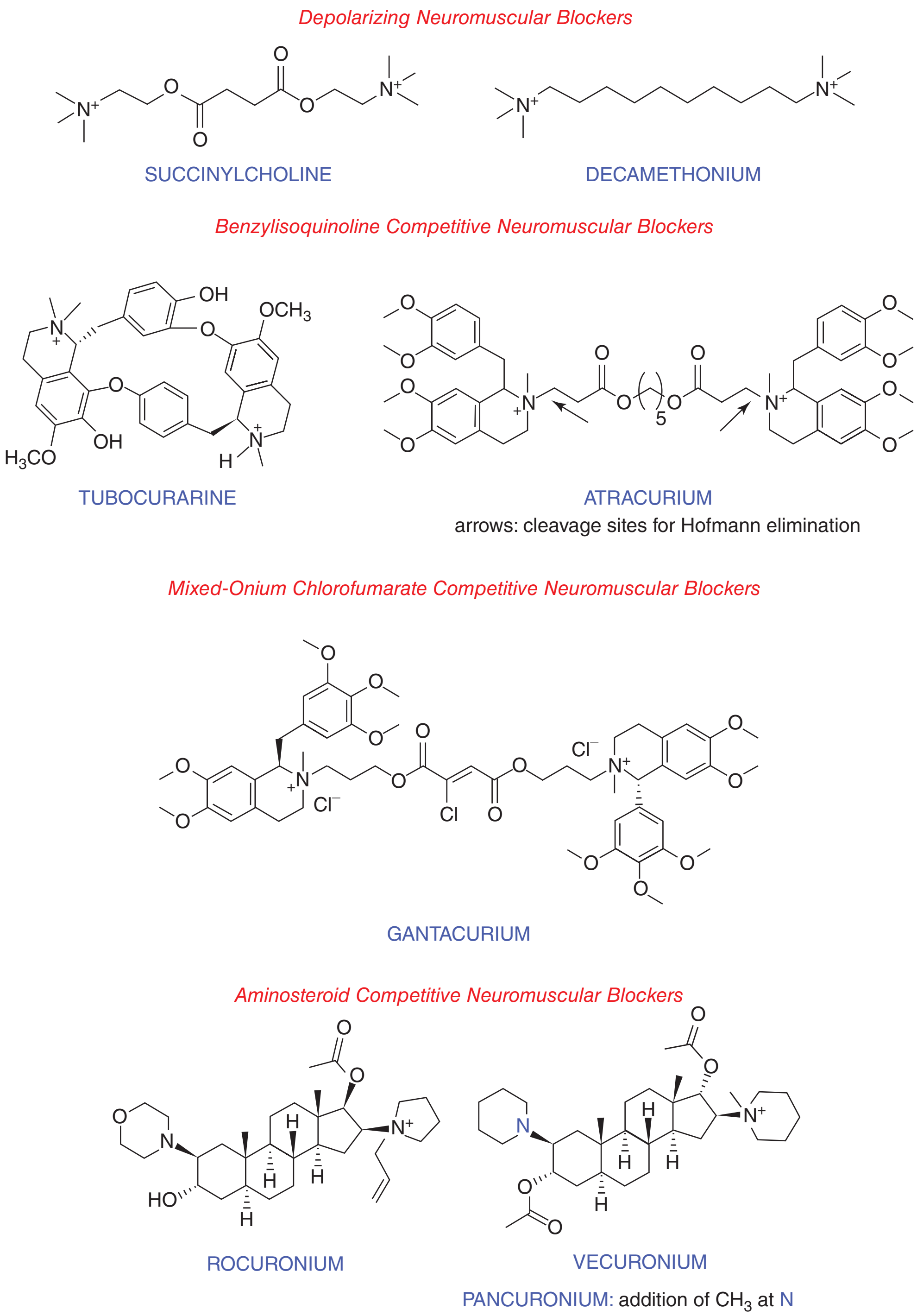

Depolarizing Neuromuscular Blockers
SUCCINYLCHOLINE
DECAMETHONIUM
Benzylisoquinoline Competitive Neuromuscular Blockers
TUBOCURARINE
ATRACURIUM
arrows: cleavage sites for Hofmann elimination
Mixed-Onium Chlorofumarate Competitive Neuromuscular Blockers
GANTACURIUM
Aminosteroid Competitive Neuromuscular Blockers
ROCURONIUM
VECURONIUM
PANCURONIUM: addition of CH3 at N

Figure 13–3 *Structural formulas of major neuromuscular blocking agents.*

During prolonged depolarization, muscle cells may lose significant quantities of K^+ and gain Na^+, Cl^-, and Ca^{2+}. In patients with extensive injury to soft tissues, the efflux of K^+ following continued administration of *succinylcholine* can be life-threatening. There are many conditions for which *succinylcholine* administration is contraindicated or should be undertaken with great caution. The change in the nature of the blockade produced by *succinylcholine* (from phase I to phase II) presents an additional complication with long-term infusions.

Effects in CNS and at Ganglia

Tubocurarine and other quaternary neuromuscular blocking agents are virtually devoid of central effects following ordinary clinical doses because of their inability to penetrate the blood-brain barrier.

Neuromuscular blocking agents show variable potencies in producing ganglionic blockade. Ganglionic blockade by *tubocurarine* and other stabilizing drugs is reversed or antagonized by anti-ChE agents (e.g., *edrophonium, neostigmine, pyridostigmine*).

Clinical doses of *tubocurarine* produce partial blockade both at autonomic ganglia and at the adrenal medulla, resulting in a fall in blood pressure and tachycardia. *Pancuronium* shows less ganglionic blockade at common clinical doses. *Atracurium, vecuronium, doxacurium, pipecuronium, mivacurium*, and *rocuronium* are even more selective, showing less ganglionic blockade (Brull and Meistelman, 2020). The maintenance of cardiovascular reflex responses usually is desired during anesthesia. *Pancuronium* has a vagolytic action, presumably from blockade of muscarinic receptors, which leads to tachycardia.

TABLE 13–1 ■ COMPARISON OF COMPETITIVE (D-TUBOCURARINE) AND DEPOLARIZING (DECAMETHONIUM) BLOCKING AGENTS

	D-TUBOCURARINE	DECAMETHONIUM
Effect of D-tubocurarine administered previously	Additive	Antagonistic
Effect of decamethonium administered previously	No effect or antagonistic	Some tachyphylaxis, but may be additive
Effect of anticholinesterase agents on block	Reversal of block	No reversal
Effect on motor end plate	Elevated threshold to acetylcholine; no depolarization	Partial, persisting depolarization
Initial excitatory effect on striated muscle	None	Transient fasciculations
Character of muscle response to indirect tetanic stimulation during *partial* block	Poorly sustained contraction	Well-sustained contraction

TABLE 13–2 ■ CLINICAL RESPONSES AND MONITORING OF PHASE I AND PHASE II NEUROMUSCULAR BLOCKADE BY SUCCINYLCHOLINE INFUSION

RESPONSE	PHASE I	PHASE II
End-plate membrane potential	Depolarized to −55 mV	Repolarization toward −80 mV
Onset	Immediate	Slow transition
Dose dependence	Lower	Usually higher or follows prolonged infusion
Recovery	Rapid	More prolonged
Train of four and tetanic stimulation	No fade	Fade[a]
Acetylcholinesterase inhibition	Augments	Reverses or antagonizes
Muscle response	Fasciculations → flaccid paralysis	Flaccid paralysis

[a] Posttetanic potentiation follows fade.

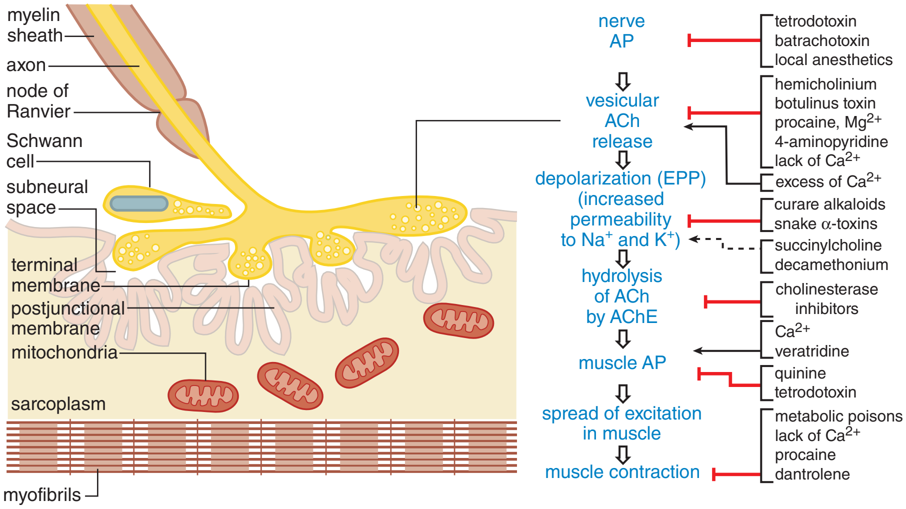

myelin sheath
axon
node of Ranvier
Schwann cell
subneural space
terminal membrane
postjunctional membrane
mitochondria
sarcoplasm
myofibrils
nerve AP
vesicular ACh release
depolarization (EPP) (increased permeability to Na$^+$ and K$^+$)
hydrolysis of ACh by AChE
muscle AP
spread of excitation in muscle
muscle contraction
tetrodotoxin
batrachotoxin
local anesthetics
hemicholinium
botulinus toxin
procaine, Mg^{2+}
4-aminopyridine
lack of Ca^{2+}
excess of Ca^{2+}
curare alkaloids
snake α-toxins
succinylcholine
decamethonium
cholinesterase inhibitors
Ca^{2+}
veratridine
quinine
tetrodotoxin
metabolic poisons
lack of Ca^{2+}
procaine
dantrolene

Figure 13–4 *A pharmacologist's view of the motor end plate.* The structures of the motor end plate (left side of figure) facilitate the series of physiological events leading from nerve action potential (AP) to skeletal muscle contraction (center column). Pharmacological agents can modify neurotransmission and excitation-contraction coupling at myriad sites (right-hand column). ←——, enhancement; ⊢——, blockade; ←- - - -, depolarization and phase II block.

Of the depolarizing agents, *succinylcholine* at doses producing neuromuscular relaxation rarely causes effects attributable to ganglionic blockade. However, cardiovascular effects are sometimes observed, probably owing to the successive stimulation of vagal ganglia (manifested by bradycardia) and sympathetic ganglia (resulting in hypertension and tachycardia).

ADME

Quaternary ammonium neuromuscular blocking agents are poorly absorbed from the GI tract. Absorption is adequate from intramuscular sites. Rapid onset is achieved with intravenous administration. The more potent agents must be given in lower concentrations, and diffusional requirements slow their rate of onset.

When long-acting competitive blocking agents such as *D-tubocurarine* and *pancuronium* are administered, blockade may diminish after 30 min owing to redistribution of the drug, yet residual blockade and plasma levels of the drug persist. Subsequent doses show diminished redistribution *as tissues become saturated*. Long-acting agents may accumulate with multiple doses.

The amino steroids contain ester groups that are hydrolyzed in the liver. Typically, the metabolites have about one-half the activity of the parent compound and contribute to the total relaxation profile. Amino steroids of intermediate duration of action, such as *vecuronium* and *rocuronium* (Table 13–3), are cleared more rapidly by the liver than is *pancuronium*. The more rapid decay of neuromuscular blockade with compounds of intermediate duration argues for sequential dosing of these agents rather than administering a single dose of a long-duration neuromuscular blocking agent.

Atracurium is converted to less-active metabolites by plasma esterases and by spontaneous degradation in plasma and tissue (Hofmann elimination). *Cisatracurium* is also subject to this spontaneous degradation. Because of these alternative routes of metabolism, *atracurium* and *cisatracurium* do not exhibit an increased $t_{1/2}$ of elimination in patients with impaired renal function and therefore are good choices in this setting (Brull and Meistelman, 2020; Fisher et al., 1986).

The extremely brief duration of action of *succinylcholine* is due largely to its rapid hydrolysis by the butyrylcholinesterase synthesized by the liver and found in the plasma. Among the occasional patients who exhibit prolonged apnea following the administration of *succinylcholine* or *mivacurium*, most have an atypical plasma cholinesterase owing to allelic variations such as the Asp70Gly polymorphism, which can prolong recovery 3 to 8 times longer for heterozygous individuals and up to 60 times longer for homozygous individuals. Additionally, hepatic or renal disease or a nutritional disturbance can also delay recovery (Brull and Meistelman, 2020).

Gantacurium is degraded by two chemical mechanisms, a rapid cysteine adduction and a slower hydrolysis of the ester bond adjacent to the chlorine. Both processes are purely chemical and hence not dependent on enzymatic activities. The adduction process has a $t_{1/2}$ of 1 to 2 min and is likely the basis for the ultrashort duration of action of gantacurium. Administration of exogenous cysteine, which may have excitotoxic side effects, can accelerate the antagonism of gantacurium-induced neuromuscular blockade (Naguib and Brull, 2009).

Clinical Pharmacology

Choice of Agent

Therapeutic selection of a neuromuscular blocking agent should be based on achieving a pharmacokinetic profile consistent with the duration of the interventional procedure and minimizing cardiovascular compromise or other side effects, with attention to drug-specific modes of elimination in patients with renal or hepatic failure (see Drug Facts Table).

Two characteristics are useful in distinguishing side effects and pharmacokinetic behavior of neuromuscular blocking agents:

- ***The chemical nature of the agents (Figure 13–3 and Table 13–3).*** Apart from a shorter duration of action, newer agents exhibit greatly diminished frequency of side effects, chiefly ganglionic blockade, block of vagal responses, and histamine release.

TABLE 13–3 ■ NEUROMUSCULAR BLOCKING AGENTS

AGENT **Chemical Class** ***Type of action***	**ONSET (min)[a]**	**DURATION (min)[a]**	**MODE OF ELIMINATION**
Ultrashort and short duration			
Succinylcholine DCE, *depolarizing*	0.8–1.4	6–11	Hydrolysis by plasma cholinesterases
Gantacurium[c] MOCF, *competitive*	1–2	5–10	Cysteine adduction, ester hydrolysis
Mivacurium BIQ, *competitive*	2–3	15–21	Hydrolysis by plasma cholinesterases
Intermediate duration			
Vecuronium AS, *competitive*	2–3	25–40	Hepatic and renal elimination
Atracurium BIQ, *competitive*	3	45	Hofmann elimination; ester hydrolysis
Rocuronium AS, *competitive*	0.5–2	36–73	Hepatic elimination
Cisatracurium BIQ, *competitive*	2–8	45–90	Hofmann elimination; renal elimination
Long duration			
Pipecuronium[b] AS, *competitive*	3–6	30–90	Renal elimination; hepatic metabolism/clearance
D-Tubocurarine[b] CBI, *competitive*	6	80	Renal and hepatic elimination
Pancuronium AS, *competitive*	3–4	85–100	Renal and hepatic elimination
Metocurine[b] BIQ, *competitive*	4	110	Renal elimination
Doxacurium[b] BIQ, *competitive*	4–8	120	Renal elimination

[a] As achieved from dose ranges in Table 11–4.
[b] Not commercially available in the U.S.
[c] Gantacurium is in investigational status.
Abbreviations: AS, aminosteroid; BIQ, benzylisoquinoline; CBI (natural alkaloid), cyclic benzylisoquinoline; DCE, dicholine ester; MOCF, assymetric mixed-onium chlorofumarate.

- ***Duration of drug action.*** These agents are categorized as long-, intermediate-, short-, or ultrashort-acting agents. Often, the long-acting agents are the more potent, requiring the use of low doses (Table 13–4). The necessity of administering potent agents at low concentrations delays their onset.

The prototypical amino steroid *pancuronium* induces virtually no histamine release; however, it blocks muscarinic receptors, an antagonism manifested primarily by vagal blockade and tachycardia. Tachycardia is eliminated in the newer amino steroids *vecuronium* and *rocuronium*. The benzylisoquinolines appear to be devoid of vagolytic and ganglionic blocking actions but show a slight propensity to cause histamine release. The unusual metabolism of the prototype compound *atracurium* and its congener *mivacurium* confers special indications for use of these compounds. For example, *atracurium*'s disappearance from the body depends on hydrolysis of the ester moiety by plasma esterases and by a spontaneous or Hofmann degradation (cleavage of the *N*-alkyl portion in the benzylisoquinoline). Hence, two routes for termination of effect are available, both of which remain functional in renal failure. *Mivacurium* is extremely sensitive to catalysis by cholinesterase or other plasma

TABLE 13–4 ■ DOSING RANGES FOR NEUROMUSCULAR BLOCKING AGENTS

AGENT	INITIATION DOSE (mg/kg)	INTERMITTENT INJECTION (mg/kg)	CONTINUOUS INFUSION (µg/kg/min)
Succinylcholine	0.3–1	0.04–0.07	N/A
D-Tubocurarine[a]	0.6	0.25–0.5	2–3
Metocurine[a]	0.4	0.5–1	N/A
Atracurium	0.3–0.5	0.08–0.2	2–15
Cisatracurium	0.15–0.2	0.03	1–3
Mivacurium	0.15–0.25	0.1	9–10
Doxacurium[a]	0.03–0.06	0.005–0.01	N/A
Pancuronium	0.04–0.1	0.01	1[b]
Rocuronium	0.45–1.2	0.1–0.2	10–12
Vecuronium	0.04–0.28	0.01–0.015	0.8–1.2
Gantacurium[a]	0.2–0.5	N/A	N/A

[a] Not commercially available in the U.S.

[b] Off-label use.

hydrolases, accounting for its short duration of action. Side effects are not yet fully characterized for *gantacurium*, but transient adverse cardiovascular effects suggestive of histamine release have been observed at doses over three times the ED_{95} (Belmont et al., 2004).

Muscle Relaxation

The main clinical use of the neuromuscular blocking agents is as an adjuvant in surgical anesthesia to obtain relaxation of skeletal muscle, particularly of the abdominal wall, to facilitate operative manipulations. With muscle relaxation no longer dependent on the depth of general anesthesia, a much lighter level of anesthesia suffices. Thus, the risk of respiratory and cardiovascular depression is minimized, and postanesthetic recovery is shortened. Neuromuscular blocking agents of short duration often are used to facilitate endotracheal intubation and have been used to facilitate laryngoscopy, bronchoscopy, and esophagoscopy in combination with a general anesthetic agent. Neuromuscular blocking agents are administered parenterally, nearly always intravenously. These agents may be administered by continuous infusion in the intensive care setting for improving chest wall compliance and eliminating ventilator dyssynchrony.

Measurement of Neuromuscular Blockade in Humans

Assessment of neuromuscular block is usually performed by stimulation of the ulnar nerve. Responses are monitored from compound action potentials or muscle tension developed in the adductor pollicis (thumb) muscle. Responses to repetitive or tetanic stimuli are most useful for evaluation of blockade of transmission. Rates of onset of blockade and recovery are more rapid in the airway musculature (jaw, larynx, and diaphragm) than in the thumb. Hence, tracheal intubation can be performed before onset of complete block at the adductor pollicis, whereas partial recovery of function of this muscle allows sufficient recovery of respiration for extubation.

Preventing Trauma During Electroshock Therapy

Electroconvulsive therapy of psychiatric disorders occasionally is complicated by trauma to the patient; the seizures induced may cause dislocations or fractures. Inasmuch as the muscular component of the convulsion is not essential for benefit from the procedure, neuromuscular blocking agents, usually *succinylcholine*, and a short-acting barbiturate, usually *methohexital*, are employed.

Synergisms and Antagonisms

The comparison of interactions between competitive and depolarizing neuromuscular blocking agents is instructive (Table 13–1) and a good test of one's understanding of the drugs' actions. In addition, many other drugs affect transmission at the neuromuscular junction and thus can affect the choice and dosage of neuromuscular blocking agent used.

Because the anti-ChE agents *neostigmine, pyridostigmine*, and *edrophonium* preserve endogenous ACh and also act at the neuromuscular junction, they have been used in the treatment of overdosage with competitive blocking agents. Similarly, on completion of the surgical procedure, many anesthesiologists employ *neostigmine* or *edrophonium* to reverse and decrease the duration of competitive neuromuscular blockade. A muscarinic antagonist (*atropine* or *glycopyrrolate*) is used concomitantly to prevent stimulation of muscarinic receptors and thereby to avoid bradycardia. Anti-ChE agents will not reverse depolarizing neuromuscular blockade; instead, they enhance it.

Many inhalational anesthetics exert a stabilizing effect on the postjunctional membrane and therefore potentiate the activity of competitive blocking agents. Consequently, when such blocking drugs are used for muscle relaxation as adjuncts to these anesthetics, their doses should be reduced. The rank order of potentiation is *desflurane* > *sevoflurane* > *isoflurane* > *halothane* > nitrous oxide-barbiturate-opioid or *propofol* anesthesia (Brull and Meistelman, 2020).

Aminoglycoside antibiotics produce neuromuscular blockade by inhibiting ACh release from the preganglionic terminal (through competition with Ca^{2+}) and to a lesser extent by noncompetitively blocking the receptor. The blockade is antagonized by Ca^{2+} salts but only inconsistently by anti-ChE agents (see Chapter 59). The tetracyclines also can produce neuromuscular blockade, possibly by chelation of Ca^{2+}. Additional antibiotics that have neuromuscular blocking action, through both presynaptic and postsynaptic actions, include *polymyxin B, colistin, clindamycin*, and *lincomycin*. Ca^{2+} channel blockers enhance neuromuscular blockade produced by both competitive and depolarizing antagonists. When neuromuscular blocking agents are administered to patients receiving these agents, dose adjustments should be considered.

Miscellaneous drugs that may have significant interactions with either competitive or depolarizing neuromuscular blocking agents include *trimethaphan*, *lithium*, opioid analgesics, *procaine*, *lidocaine*, *quinidine*, *phenelzine*, *carbamazepine*, *phenytoin*, *propranolol*, *dantrolene*, *azathioprine*, *tamoxifen*, magnesium salts, corticosteroids, digitalis glycosides, *chloroquine*, catecholamines, and diuretics.

Adverse Effects

The important untoward responses of the neuromuscular blocking agents include prolonged apnea, cardiovascular collapse, those resulting from histamine release, and, rarely, anaphylaxis. Related factors may include alterations in body temperature; electrolyte imbalance, particularly of K^+; low plasma butyrylcholinesterase levels, resulting in a reduction in the rate of destruction of *succinylcholine*; the presence of latent myasthenia gravis or of malignant disease such as small cell carcinoma of the lung with Eaton-Lambert myasthenic syndrome; reduced blood flow to skeletal muscles, causing delayed removal of the blocking drugs; and decreased elimination of the muscle relaxants secondary to hepatic dysfunction (*cisatracurium, rocuronium, vecuronium*) or reduced renal function (*pancuronium*). Great care should be taken when administering neuromuscular blockers to dehydrated or severely ill patients. Depolarizing agents can cause rapid release of K^+ from intracellular sites; this may be a factor in production of the prolonged apnea in patients who receive these drugs while in electrolyte imbalance. *Succinylcholine*-induced hyperkalemia is a life-threatening complication of that drug.

Malignant Hyperthermia. Malignant hyperthermia is a potentially life-threatening event triggered by the administration of certain anesthetics and neuromuscular blocking agents. The clinical features include contracture, rigidity, and heat production from skeletal muscle, resulting in severe hyperthermia (increases of up to 1°C/5 min), accelerated muscle metabolism, metabolic acidosis, and tachycardia. Uncontrolled release of Ca^{2+} from the sarcoplasmic reticulum of skeletal muscle is the initiating event. Although the halogenated hydrocarbon anesthetics (e.g., *halothane, isoflurane*, and *sevoflurane*) and *succinylcholine* alone have been reported to precipitate the response, most of the incidents arise from the combination of depolarizing blocking agent and anesthetic. Susceptibility to malignant hyperthermia, an autosomal dominant trait, is associated with certain congenital myopathies, such as *central core disease*. In the majority of cases, however, no clinical signs are visible in the absence of anesthetic intervention.

Treatment entails intravenous administration of *dantrolene*, which blocks Ca^{2+} release from the sarcoplasmic reticulum of skeletal muscle (see section on antispasmodics below). Rapid cooling, inhalation of 100% O_2, and control of acidosis should be considered adjunct therapy in malignant hyperthermia.

Respiratory Paralysis. Treatment of respiratory paralysis arising from an adverse reaction or overdose of a neuromuscular blocking agent should be by positive-pressure artificial respiration with O_2 and maintenance of a patent airway until recovery of normal respiration is ensured. With the competitive blocking agents, this may be hastened by the administration of *neostigmine methylsulfate* (0.5–2 mg IV) or *edrophonium* (10 mg IV, repeated as required up to a total of 40 mg) (Watkins, 1994). In the case of overdose, a muscarinic cholinergic antagonist (*atropine* or *glycopyrrolate*) may be added to prevent undue slowing of the heart (see Synergisms and Antagonisms).

Histamine Release From Mast Cells. Some clinical responses to neuromuscular blocking agents (e.g., bronchospasm, hypotension, excessive bronchial and salivary secretion) appear to be caused by the release of histamine. *Succinylcholine*, *mivacurium*, and *atracurium* cause histamine release, but to a lesser extent than *tubocurarine* unless administered rapidly. The amino steroids *pancuronium*, *vecuronium*, *pipecuronium*, and *rocuronium* have even less tendency to release histamine after intradermal or systemic injection (Basta, 1992; Watkins, 1994). Histamine release typically is a direct action of the muscle relaxant on the mast cell rather than anaphylaxis mediated by immunoglobulin E.

Interventional Strategies for Toxic Effects

Neostigmine effectively antagonizes only the skeletal muscular blocking action of the competitive blocking agents and may aggravate such side effects as hypotension or induce bronchospasm. In such circumstances, sympathomimetic amines may be given to support the blood pressure. *Atropine* or *glycopyrrolate* is administered to counteract muscarinic stimulation. Antihistamines are beneficial to counteract the responses that follow the release of histamine, particularly when administered before the neuromuscular blocking agent.

Reversal of Effects by Chelation Therapy. *Sugammadex*, a modified γ-cyclodextrin, is a chelating agent specific for *rocuronium* and *vecuronium*. *Sugammadex* at doses greater than 2 mg/kg is able to reverse neuromuscular blockade from *rocuronium* within 3 min. *Sugammadex* clearance is markedly reduced in patients with impaired renal function, and use of this agent should be avoided. *Sugammadex* was approved for use in the U.S. in 2015. Side effects include dysgeusia and rare hypersensitivity.

Pediatric and Geriatric Indications and Problems

Because the neuromuscular junction is not fully developed at birth, additional care must be taken in administration of neuromuscular blocking agents to infants and children. *Succinylcholine* is not safe for routine use in pediatric patients, and its use must be reserved for extreme emergency situations where immediate securing of the airway is necessary and other options for neuromuscular blockade are not available. Competitive blocking agents, however, are commonly used in pediatric patients; generally, dosage is similar to adults, but both rate of block onset and clearance are faster. *Atracurium* is an exception: The dosage and duration of action are not significantly different between children older than 2 years and adults, and the same dose (0.25–0.5 mg/kg) can be used among these populations for tracheal intubation. *Vecuronium, cisatracurium, rocuronium*, and *mivacurium* are also commonly administered to children for short procedures where only a single intubating dose is required.

There are normal changes at the neuromuscular junction in elderly patients that may affect pharmacodynamics of neuromuscular blocking agents. With aging, the distance between the terminus of the motor neuron and the end plate increases, the end-plate invaginations become flatter, the amount of transmitter per synaptic vesicle decreases, the vesicle release probability is lower, and the density of receptors at the end plate decreases. The result of these changes is decreased efficiency of neuromuscular transmission. General physiological changes in aging patients, including decreases in body water and muscle, increases in total body fat, and decreases in renal and hepatic function, also contribute to the action of neuromuscular blockers. The dosing of *succinylcholine* is not significantly altered in the geriatric population. Among the competitive blocking agents, initial dose requirements are unchanged; however, the onset of blockade is delayed in an age-related manner, and block is prolonged. For compounds dependent on the kidney, liver, or both for clearance, such as *pancuronium, vecuronium*, and *rocuronium*, plasma clearance times are prolonged by 30% to 50% (Brull and Meistelman, 2020). For compounds such as *atracurium* that are not dependent on hepatic or renal blood flow for their elimination, pharmacodynamics and kinetics are largely unaltered.

Spasmolytics

Centrally Acting Spasmolytics

A variety of clinical manifestations, such as spinal cord injury, stroke, and multiple sclerosis, can cause lesions that disrupt the normal somatic control of skeletal muscle, resulting in muscle spasms, repetitive and uncontrolled contractions of skeletal muscle that are involuntary. The underlying mechanism is the hyperexcitability of the alpha motor neurons in the spinal cord. Spasmolytics are agents that act in the brain and higher centers of the spinal cord to reduce spasms, with the objective of increasing functional capacity and relieving discomfort. Unfortunately, due to significant adverse effects, many of these therapeutic agents are recommended for short-term use only. These orally administered agents include *baclofen*, several anticonvulsants/hypnotics/sedatives (benzodiazepines and *gabapentin*), α_2 adrenergic agonists (*clonidine* and *tizanidine*), and *cyclobenzaprine.*

Baclofen

Baclofen is an orally effective $GABA_B$ receptor agonist that suppresses neurotransmission by multiple mechanisms in both the brain and spinal cord. *Baclofen* acts at pre- and postsynaptic sites to cause membrane hyperpolarization, thereby reducing the probability of membrane depolarization and thus the Ca^{2+} influx necessary to induce release of excitatory transmitter release and inhibiting spinal reflexes.

Benzodiazepines and Gabapentin

Diazepam and other benzodiazepines are effective in treating muscle rigidity but are limited in utility due to high abuse potential and substantial CNS depressant effects. A prominent effect of benzodiazepines is enhancing GABAergic neurotransmission, resulting in enhanced inhibitory signaling in GABA-sensitive synapses.

Gabapentin, a cyclic analogue of GABA (GABA liganded to cyclohexane), interacts not with the GABA receptor but reportedly with two membrane proteins involved in excitation-response coupling: the α_2-δ subunit of Ca_V, the voltage-gated Ca^{2+} channel (Gee et al., 1996), and the voltage-gated K^+ channels KCNQ2/3 (the molecular correlate of neuronal M-currents) and homomeric KCNQ3 and KCNQ5 (Manville and Abbott, 2018). The proposed responses to *gabapentin*'s binding at these sites are the reduction of neuronal Ca^{2+} currents (via interaction with Ca_V) and activation of KCNQ2/3 and homomeric KCNQ3 and KCNQ5 channels, leading to hyperpolarization of susceptible neurons, with concomitant reduction in excitability.

Chapter 22 describes the pharmacology of *diazepam* and other benzodiazepines in detail. Chapter 20 describes the use of both benzodiazepines and *gabapentin* as antiseizure drugs.

α_2 Adrenergic Agonists

Tizanidine is an α_2 adrenergic receptor agonist. It provides effective relief from spasticity due to multiple sclerosis and spinal cord injury. Although a precise mechanism of action in relieving spasms is not understood, *tizanidine* is thought to have actions similar to those of *clonidine*. The advantage of *tizanidine* over *clonidine* for this indication is *tizanidine*'s lesser effect on lowering blood pressure at an effective concentration to relieve spasm. *Tizanidine* has a short $t_{1/2}$ and is taken at a dose of 2 mg every 6 h for up to three doses daily; the dosage can be gradually ramped up to a daily maximum of 36 mg. In reverse fashion, the drug must be withdrawn slowly, tapering by 2 to 4 mg daily, to avoid a rebound of spasms, tachycardia, and hypertension. Common side effects include dizziness, sedation, and dry mouth. Use of *tizanidine* with a potent CYP1A2 inhibitor (e.g., *fluvoxamine*, *ciprofloxacin*, *cimetidine*) can substantially elevate *tizanidine*'s AUC. *Tizanidine* can interact additively with CNS depressants; its combination with ethanol should be avoided; antibiotics, antiarrhythmics, and hypotensive agents should be used with caution.

Cyclobenzaprine

Cyclobenzaprine is pharmacologically related to tricyclic antidepressants. It can be used for short-term reduction of painful muscle spasms such as those resulting from an acute peripheral injury. It is not useful for spasms resulting from neurological conditions of central origin. *Cyclobenzaprine* acts centrally by a mechanism of action likely related to its activity as a 5HT$_2$ receptor antagonist. The drug is administered by mouth, 5 mg three times daily, scheduled or as needed, with one of the doses administered at bedtime, for a duration of only 2 weeks. This agent should not be given concomitantly with MAO inhibitors and should be used with caution with inhibitors of 5HT uptake. *Cyclobenzaprine* can interact with most CNS depressants.

Peripherally Acting Antispasmodics

Botulinum Toxin

Botulinum toxins act peripherally to reduce muscle contraction. There are numerous nonequivalent preparations: *abobotulinumtoxinA, incobotulinumtoxinA, onabotulinumtoxinA, prabotulinumtoxinA-xvfs*, and *rimabotulinumtoxinB*; all work by blocking ACh release. The botulinum toxins bind to cholinergic neurons, enter the cell, and cleave SNARE proteins, thereby inhibiting vesicular release of ACh. The result is flaccid paralysis of skeletal muscle and diminished activity of parasympathetic and sympathetic cholinergic synapses. Inhibition lasts from several weeks to 3 to 4 months, and restoration of function requires nerve sprouting.

Originally approved for the treatment of the ocular conditions of strabismus and blepharospasm and for hemifacial spasms, botulinum toxins have been used to treat spasms and dystonias and spasms associated with the lower esophageal sphincter and anal fissures. Botulinum toxin treatments also have become a popular cosmetic procedure for those seeking a wrinkle-free face. Like the bloom of youth, the reduction of wrinkles is temporary; unlike the bloom of youth, the effect of botulinum toxin can be renewed by readministration.

Botulinum toxins are extremely poisonous and must be administered with great caution. Lethal doses of pure toxin are, roughly, approximately 1 ng/kg when administered IM or IV, approximately 10 ng/kg when inhaled, and approximately 1000 ng/kg when ingested (Arnon et al., 2001). The FDA requires a boxed warning on preparations of botulinum toxin, alerting practitioners and patients to the risk of respiratory paralysis from unexpected spread of the toxin from the site of injection (uses are described in Chapter 75). The FDA requires the highly specific names of the various products to emphasize that different preparations of botulinum toxin are not interchangeable; that is, different preparations' units of biological activity cannot safely be compared. Patients showing signs of botulism should be treated promptly with antitoxin and given long-term supportive therapy.

Dantrolene

Dantrolene inhibits Ca^{2+} release from the sarcoplasmic reticulum of skeletal muscle by limiting the capacity of Ca^{2+} and calmodulin to activate the ryanodine receptor, RYR1. *Dantrolene* is indicated for chronic muscle spasticity associated with upper motor neuron disorders (spinal cord injury, stroke, cerebral palsy, or multiple sclerosis). *Dantrolene* is initiated at 25 mg daily for 7 days and then titrated up every 7 days up to a maximum dose of 400 mg/day. It is also indicated for malignant hyperthermia and used off-label for neuroleptic malignant syndrome. With its peripheral action, it causes generalized weakness. Thus, its use should be reserved to nonambulatory patients with severe spasticity. Hepatotoxicity has been reported with chronic use, requiring frequent liver function tests and use of the lowest possible oral dose.

Miscellaneous Agents

A number of other agents used as muscle relaxants seem to rely on sedative properties and blockade of nociceptive pathways; this group includes *carisoprodol*, which is metabolized to meprobamate (see Chapter 22); *metaxalone*; *methocarbamol*; and *orphenadrine*. *Tetrabenazine* is available for treatment of the chorea associated with Huntington's disease; the drug inhibits VMAT2, resulting in depletion of vesicular stores of dopamine in dopaminergic neurons in the CNS (see Chapters 10, 15, and 16).

Ganglionic Neurotransmission

The Neural Nicotinic Receptor and Postsynaptic Potentials

Neurotransmission in autonomic ganglia involves release of ACh by preganglionic fibers and the rapid depolarization of postsynaptic membranes via the activation of neuronal nicotinic (N_n) receptors by ACh. Unlike the neuromuscular junction, ganglia do not have discrete end plates with focal localization of receptors; rather, the dendrites and nerve cell bodies contain the receptors. The characteristics of nicotinic receptor channels of the ganglia and the neuromuscular junction are similar. There are multiple nicotinic receptor subunits (e.g., α_3, α_5, α_7, β_2, and β_4) in ganglia, with α3 and β4 most abundant and important. The ganglionic nicotinic ACh receptors are sensitive to classical blocking agents such as *hexamethonium* and *trimethaphan* (see discussion that follows). Measurements of single-channel conductance indicate that the characteristics of nicotinic receptor channels of the ganglia and the neuromuscular junction are similar.

Intracellular recordings from postganglionic neurons indicate that at least four different changes in postsynaptic membrane potential can be elicited by stimulation of the preganglionic nerve (Figure 13–5):

- An initial EPSP (via nicotinic receptors) that may result in an action potential
- An IPSP mediated by M$_2$ (G_i/G_o-coupled) muscarinic receptors
- A secondary slow EPSP mediated by M$_1$ (G_q/G_{11}-coupled) muscarinic receptors
- A late, slow EPSP mediated by myriad peptides

An action potential is generated in the postganglionic neuron when the initial EPSP achieves a threshold potential. The events that follow the initial depolarization (IPSP; slow EPSP; late, slow EPSP) are insensitive to *hexamethonium* or other N_n antagonists. Electrophysiological and neurochemical evidence suggests that catecholamines participate in the generation of the IPSP. Dopamine and norepinephrine cause hyperpolarization of ganglia; however, in some ganglia, IPSPs are mediated by M$_2$ muscarinic receptors.

The slow EPSP is generated by ACh activation of M$_1$ muscarinic receptors and is blocked by *atropine* and M$_1$-selective antagonists (see Chapter 11). The slow EPSP has a longer latency and greater duration (10–30 sec) than the initial EPSP. Slow EPSPs result from decreased K$^+$ conductance, the *M current* that regulates the sensitivity of the cell to repetitive fast-depolarizing events. By contrast, the late, slow EPSP lasts for several minutes and is mediated by peptides released from presynaptic nerve endings or interneurons in specific ganglia (see next section). The peptides and ACh may be coreleased at the presynaptic nerve terminals; the relative stability of the peptides in the ganglion extends its sphere of influence to postsynaptic sites beyond those in the immediate proximity of the nerve ending.

Secondary synaptic events modulate the initial EPSP. A variety of peptides, including gonadotropin-releasing hormone, substance P,

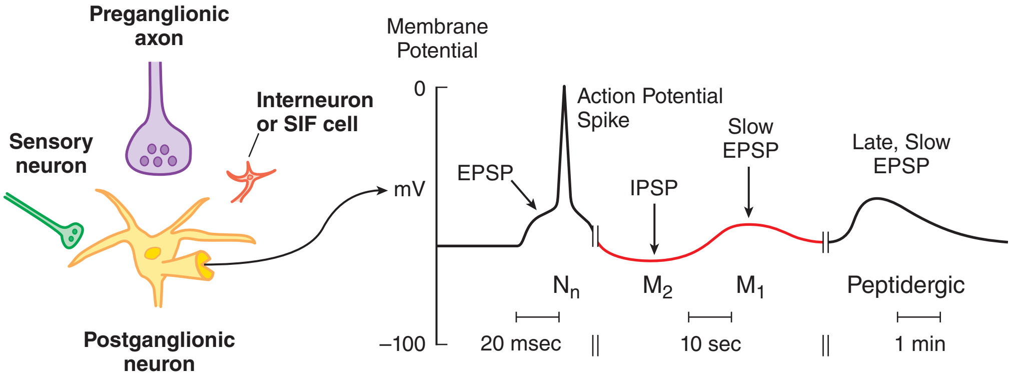


Figure 13–5 *Postsynaptic potentials recorded from an autonomic postganglionic nerve cell body after stimulation of the preganglionic nerve fiber.* The preganglionic nerve releases ACh onto postganglionic cells. The initial EPSP results from the inward Na$^+$ current (and perhaps Ca^{2+} current) through the nicotinic receptor channel. If the EPSP is of sufficient magnitude, it triggers an action potential spike, which is followed by a slow IPSP, a slow EPSP, and a late, slow EPSP. The slow IPSP and slow EPSP are not seen in all ganglia. The electrical events subsequent to the initial EPSP are thought to modulate the probability that a subsequent EPSP will reach the threshold for triggering a spike. Other interneurons, such as catecholamine-containing SIF cells, and axon terminals from sensory, afferent neurons also release transmitters, which may influence the slow potentials of the postganglionic neuron. A number of cholinergic, peptidergic, adrenergic, and amino acid receptors are found on the dendrites and soma of the postganglionic neuron and the interneurons. The preganglionic fiber releases ACh and peptides; the interneurons store and release catecholamines, amino acids, and peptides; the sensory afferent nerve terminals release peptides. The initial EPSP is mediated through nicotinic (N_n) receptors, the slow IPSP and EPSP through M_2 and M_1 muscarinic receptors, and the late, slow EPSP through several types of peptidergic receptors.

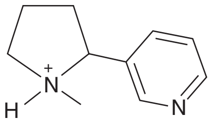
angiotensin, calcitonin gene–related peptide, vasoactive intestinal polypeptide, neuropeptide Y, and enkephalins, have been identified in ganglia. They appear localized to particular cell bodies, nerve fibers, or small, intensely fluorescent (SIF) cells; are released on nerve stimulation; and are presumed to mediate the late, slow EPSP. Other neurotransmitter substances (e.g., 5HT and GABA) can modulate ganglionic transmission.

Ganglionic Stimulating Agents

Drugs that stimulate N_n cholinergic receptors on autonomic ganglia have been essential for analyzing the mechanism of ganglionic function; however, these ganglionic agonists have limited therapeutic use. They can be grouped into two categories. The first group consists of drugs with specificities similar to nicotine: *lobeline*, *tetramethylammonium*, and *dimethylphenylpiperazinium*. Nicotine's excitatory effects on ganglia are rapid in onset, are blocked by ganglionic nicotinic receptor antagonists, and mimic the initial EPSP. The second group consists of muscarinic receptor agonists such as *muscarine*, *McN-A-343*, and *methacholine* (see Chapter 11); their excitatory effects on ganglia are delayed in onset, blocked by *atropine*-like drugs, and mimic the slow EPSP.

Nicotine

Nicotine is of considerable medical significance because of its toxicity, presence in tobacco, and propensity for conferring dependence on its users. The chronic effects of nicotine and the untoward effects of the chronic use of tobacco are considered in Chapter 28. Nicotine is one of the few natural liquid alkaloids. It is a colorless, volatile base ($pK_a = 8.5$) that turns brown and acquires the odor of tobacco on exposure to air.



NICOTINE

Mechanism of Action. In addition to the actions of nicotine on a variety of neuroeffector and chemosensitive sites, the alkaloid can both stimulate and desensitize receptors, making nicotine's effects complex and unpredictable. The ultimate response of any one system represents the summation of stimulatory and inhibitory effects of nicotine. Nicotine can increase heart rate by excitation of sympathetic ganglia or by paralysis of parasympathetic cardiac ganglia, and it can slow heart rate by paralysis of sympathetic or stimulation of parasympathetic cardiac ganglia. The effects of the drug on the chemoreceptors of the carotid and aortic bodies and on regions of the CNS also can influence heart rate, as can the compensatory baroreceptor reflexes resulting from changes in blood pressure caused by nicotine. Finally, nicotine can stimulate secretion of epinephrine from the adrenal medulla, which accelerates heart rate and raises blood pressure.

Effects on Physiological Systems

Peripheral Nervous System. The major action of nicotine consists initially of transient stimulation and then a more persistent depression of all autonomic ganglia. Small doses of nicotine stimulate the ganglion cells directly and may facilitate impulse transmission. Following larger doses, the initial stimulation is followed by a blockade of transmission. Whereas stimulation of the ganglion cells coincides with their depolarization, depression of transmission by adequate doses of nicotine occurs both during the depolarization and after it has subsided. Nicotine also possesses a biphasic action on the adrenal medulla: Small doses evoke the discharge of catecholamines; larger doses prevent their release in response to splanchnic nerve stimulation.

The effects of high doses of nicotine on the neuromuscular junction are similar to those on ganglia. However, the stimulant phase is obscured largely by the rapidly developing paralysis. In the latter stage, nicotine also produces neuromuscular blockade by receptor desensitization. At lower concentrations, such as those typically achieved by recreational tobacco use (~200 nM), nicotine's effects reflect its higher affinity for a neuronal nicotinic receptor ($\alpha_4\beta_2$) than for the neuromuscular junction receptor ($\alpha_1\beta_1\gamma\delta$) (Xiu et al., 2009).

Nicotine, like ACh, stimulates a number of sensory receptors. These include mechanoreceptors that respond to stretch or pressure of the skin, mesentery, tongue, lung, and stomach; chemoreceptors of the carotid body; thermal receptors of the skin and tongue; and pain receptors. Prior administration of *hexamethonium* prevents stimulation of the sensory receptors by nicotine but has little, if any, effect on the activation of sensory receptors by physiological stimuli.

Central Nervous System. Nicotine markedly stimulates the CNS. Low doses produce weak analgesia; higher doses cause tremors, leading to convulsions at toxic doses. The excitation of respiration is a prominent action of nicotine: Large doses act directly on the medulla oblongata, whereas smaller doses augment respiration reflexly by excitation of the chemoreceptors of the carotid and aortic bodies. Stimulation of the CNS with large doses is followed by depression, and death results from failure of respiration owing to both central paralysis and peripheral blockade of the diaphragm and intercostal muscles that facilitate respiration.

Nicotine induces vomiting by both central and peripheral actions. The central component of the vomiting response is due to stimulation of the emetic chemoreceptor trigger zone in the area postrema of the medulla oblongata. In addition, nicotine activates vagal and spinal afferent nerves that form the sensory input of the reflex pathways involved in the act of vomiting. The primary sites of action of nicotine in the CNS are prejunctional, causing the release of other transmitters. The stimulatory and pleasure-reward actions of nicotine appear to result from release of excitatory amino acids, dopamine, and other biogenic amines from various CNS centers (Dorostkar and Boehm, 2008).

Chronic exposure to nicotine in several systems causes a marked increase in the density or number of nicotinic receptors, possibly contributing to tolerance and dependence. Nicotine is thought to act as an intracellular pharmacological chaperone; it is uncharged at physiological pH and readily permeates the plasma membrane. Inside the cell, it upregulates receptor expression by stabilizing nascent subunits in pentamers in the endoplasmic reticulum. Chronic low-dose exposure to nicotine also significantly increases the $t_{1/2}$ of nicotinic receptors on the cell surface (Kuryatov et al., 2005; Srinivasan et al., 2014).

Cardiovascular System. In general, the cardiovascular responses to nicotine are due to stimulation of sympathetic ganglia and the adrenal medulla, together with the discharge of catecholamines from sympathetic nerve endings. Contributing to the sympathomimetic response to nicotine is the activation of chemoreceptors of the aortic and carotid bodies, which reflexly results in vasoconstriction, tachycardia, and elevated blood pressure.

GI Tract. The combined activation of parasympathetic ganglia and cholinergic nerve endings by nicotine results in increased tone and motor activity of the bowel. Nausea, vomiting, and occasionally diarrhea are observed following systemic absorption of nicotine in an individual who has not been exposed to nicotine previously.

Exocrine Glands. Nicotine causes an initial stimulation of salivary and bronchial secretions that is followed by inhibition.

ADME. Nicotine is readily absorbed from the respiratory tract, buccal membranes, and skin. Severe poisoning has resulted from percutaneous absorption. As a relatively strong base, nicotine has limited absorption from the stomach. Intestinal absorption is far more efficient. Nicotine in chewing tobacco, because it is absorbed more slowly than inhaled nicotine, has a longer duration of effect. The average cigarette contains 6 to 11 mg nicotine and delivers about 1 to 3 mg nicotine systemically to the smoker; bioavailability can increase as much as 3-fold with the intensity of puffing and technique of the smoker (Benowitz, 1998).

Approximately 80% to 90% of nicotine is altered in the body, mainly in the liver but also in the kidney and lung. Cotinine is the major metabolite. The $t_{1/2}$ of nicotine following inhalation is about 2 h. Nicotine and its metabolites are eliminated rapidly by the kidney. The rate of urinary excretion of nicotine diminishes when the urine is alkaline. Nicotine also is excreted in the milk of lactating women who smoke; the milk of heavy smokers may contain 0.5 mg/L.

Acute Adverse Effects. Poisoning from nicotine may occur from accidental ingestion of nicotine-containing insecticide sprays or in children from ingestion of tobacco products. The acutely fatal dose of nicotine for an adult is probably about 60 mg. Smoking tobacco usually contains 1% to 2% nicotine. The gastric absorption of nicotine from tobacco taken by mouth is delayed because of slowed gastric emptying, so vomiting caused by the central effect of the initially absorbed fraction may remove much of the tobacco remaining in the GI tract.

The onset of symptoms of acute, severe nicotine poisoning is rapid; they include nausea, salivation, abdominal pain, vomiting, diarrhea, cold sweat, headache, dizziness, disturbed hearing and vision, mental confusion, and marked weakness. Faintness and prostration ensue; the blood pressure falls; breathing is difficult; the pulse is weak, rapid, and irregular; and collapse may be followed by terminal convulsions. Death may result within a few minutes from respiratory failure.

For treating nicotine poisoning, vomiting may be induced, or gastric lavage should be performed. Alkaline solutions should be avoided. A slurry of activated charcoal is then passed through the tube and left in the stomach. Respiratory assistance and treatment of shock may be necessary.

Ganglionic Blocking Agents

There are two categories of agents that block ganglionic nicotinic receptors. The prototype of the first group, nicotine, initially stimulates the ganglia by an ACh-like action and then blocks them by causing persistent depolarization (Volle, 1980). Compounds in the second category (e.g., *trimethaphan* and *hexamethonium*) impair transmission. *Trimethaphan* acts by competition with ACh, analogous to the mechanism of action of curare at the neuromuscular junction. *Hexamethonium* appears to block the channel after it opens; this action shortens the duration of current flow because the open channel either becomes occluded or closes. Thus, the initial EPSP is blocked, and ganglionic transmission is inhibited. Representative diverse chemicals that block autonomic ganglia without first causing stimulation are shown in Figure 13–6.

Ganglionic blocking agents were the first effective therapy for the treatment of hypertension. However, due to the role of ganglionic transmission in both the sympathetic and parasympathetic branches of the autonomic nervous system, the antihypertensive action of ganglionic blocking agents is accompanied by numerous undesirable side effects (Table 13–5). *Mecamylamine*, a secondary amine with a channel block mechanism similar to *hexamethonium*, is available as an antihypertensive agent with good oral bioavailability for moderate to severe hypertension. Dosing of *mecamylamine* is 2.5 mg twice daily and can be increased in increments of 2.5 mg/day at intervals of 2 or more days until desired blood pressure response is achieved.

Mechanism of Action

Nearly all the physiological alterations observed after the administration of ganglionic blocking agents can be anticipated with reasonable accuracy by a careful inspection of Figure 10–1 and Table 10–1, and by knowing which division of the autonomic nervous system exercises dominant control of various organs (see Table 13–5). For example, blockade of sympathetic ganglia interrupts adrenergic control of arterioles and results in vasodilation, improved peripheral blood flow in some vascular beds, and a fall in blood pressure.

Generalized ganglionic blockade also may result in atony of the bladder and GI tract, cycloplegia, xerostomia, diminished perspiration, and, by abolishing circulatory reflex pathways, postural hypotension. These changes represent the generally undesirable features of ganglionic blockade that severely limit the therapeutic efficacy of ganglionic blocking agents.

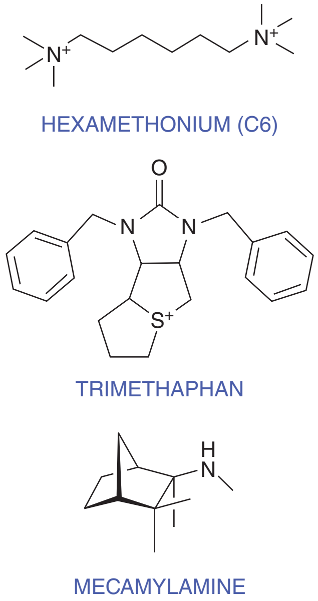


Figure 13–6 *Ganglionic blocking agents.*

Cardiovascular Effects

Existing sympathetic tone is a critical determinant of the degree ganglionic blockade will lower blood pressure. Thus, blood pressure may decrease only minimally in recumbent normotensive subjects but may fall markedly in sitting or standing subjects. Postural hypotension limits the use of ganglionic blockers in ambulatory patients. Changes in heart rate following ganglionic blockade depend largely on existing vagal tone. In humans, only mild tachycardia usually accompanies the hypotension, a sign that indicates fairly complete ganglionic blockade. However, a decrease may occur if the heart rate is high initially. Cardiac output often is reduced by ganglionic blocking drugs in patients with normal cardiac function, because of venodilation, peripheral pooling of blood, and the resulting decrease in venous return. In patients with cardiac failure, ganglionic blockade frequently results in increased cardiac output owing to a reduction in peripheral resistance. In hypertensive subjects, cardiac output, stroke volume, and left ventricular work are diminished. Although total systemic vascular resistance is decreased in patients who receive ganglionic blocking agents, changes in blood flow and vascular resistance of individual vascular beds are variable. Reduction of cerebral blood flow is small unless mean systemic blood pressure falls below 50 to 60 mmHg. Skeletal muscle blood flow is unaltered, but splanchnic and renal blood flow decrease.

ADME

The absorption of quaternary ammonium and sulfonium compounds from the enteric tract is incomplete and unpredictable. This is due both to the limited ability of these ionized substances to penetrate cell membranes and to the depression of propulsive movements of the small intestine and gastric emptying by parasympathetic blockade. Although the absorption of *mecamylamine* is less erratic, reduced bowel activity and paralytic ileus are a danger. After absorption, the quaternary ammonium- and sulfonium-blocking agents are confined primarily to the extracellular space and are excreted mostly unchanged by the kidney. *Mecamylamine* concentrates in the liver and kidney and is excreted slowly in an unchanged form.

Therapeutic Uses; Adverse Effects

Trimethaphan was once used for the induction of controlled hypotension during surgery to reduce bleeding and for the rapid reduction of blood pressure in the treatment of hypertensive emergencies; however, the agent is no longer marketed in the U.S. Adverse reactions are consistent with ganglionic blockade, as shown in Table 13–5.

Nicotine Addiction and Smoking Cessation

As a therapeutic, nicotine is primarily used to aid in smoking cessation. Two goals of the pharmacotherapy of smoking cessation are the reduction of the craving for nicotine and inhibition of the reinforcing effects of smoking. Myriad approaches and drug regimens are used, including NRT, *bupropion* (a CNS-active nicotinic antagonist; see Chapter 18), and partial agonists of the nicotinic ACh receptor (e.g., *varenicline*). More recently, electronic cigarettes (vaping) have been marketed as a safer alternative to combustible cigarettes, a questionable claim in the face of their reported popularity with teenagers smoking flavored tobaccos via vaping.

Current consensus is that NRT, *bupropion*, and *varenicline* all help smokers to quit their smoking habit. *Cytisine* (not approved for use in Europe or the U.S.) also appears effective. The safety and efficacy of NRT are clear. The highest rates of smoking cessation (~30% success at maintaining abstinence from smoking for 6 months) result from the combination of NRT (e.g., patch plus inhaler) and *varenicline* (Cahill et al., 2013). Meta-analysis of clinical studies indicates that behavioral support (e.g., counseling, phone support) in addition to pharmacological treatment increases the likelihood of smoking cessation by about 10% to 25% (Hartman-Boyce et al., 2019).

Nicotine Replacement Therapy

NRT is available in several dosage forms to help achieve abstinence from tobacco use. Nicotine is marketed for over-the-counter use as a gum or lozenge or transdermal patch and by prescription as a nasal spray or vapor inhaler. Different nicotine delivery systems produce different patterns of exposure (see Figure 28–10; St. Helen et al., 2016). The efficacy of these dosage forms in producing abstinence from smoking is enhanced when linked to counseling and motivational therapy (Prochaska and Benowitz, 2016).

Cytisine

Cytisine is a plant alkaloid and a partial agonist at nicotinic ACh receptors, with an affinity for the $\alpha_4\beta_2$ subtype. *Cytisine* is taken orally, has a half-life of about 5 h, and can produce mild GI side effects. In a recent small trial, *cytisine* was effective in producing effects similar to those of NRT and *varenicline* (Walker et al., 2014).

Varenicline

Varenicline is a *cytisine*-based therapeutic for smoking cessation. It is administered initially at 0.5 mg once per day for 3 days then twice daily at the same dose. After 8 days, it is maintained at 1 mg twice daily. The drug interacts with nicotinic ACh receptors. In model systems, *varenicline* is a selective partial agonist at $\alpha_4\beta_2$ receptors, which is thought to be the principal nicotinic receptor subtype involved in nicotine addiction. Binding of *varenicline* to the $\alpha_4\beta_2$ receptors blocks the ability of nicotine to generate the reward experience and, due to its partial agonist activity, does not promote the withdrawal effects as would an antagonist. *Varenicline*

TABLE 13–5 ■ USUAL PREDOMINANCE OF SYMPATHETIC OR PARASYMPATHETIC TONE AT VARIOUS EFFECTOR SITES AND CONSEQUENCES OF AUTONOMIC GANGLIONIC BLOCKADE

SITE	PREDOMINANT TONE	EFFECT OF GANGLIONIC BLOCKADE
Arterioles	Sympathetic (adrenergic)	Vasodilation; increased peripheral blood flow; hypotension
Veins	Sympathetic (adrenergic)	Dilation: peripheral pooling of blood; decreased venous return; decreased cardiac output
Heart	Parasympathetic (cholinergic)	Tachycardia
Iris	Parasympathetic (cholinergic)	Mydriasis
Ciliary muscle	Parasympathetic (cholinergic)	Cycloplegia—focus to far vision
Gastrointestinal tract	Parasympathetic (cholinergic)	Reduced tone and motility; constipation; decreased gastric and pancreatic secretions
Urinary bladder	Parasympathetic (cholinergic)	Urinary retention
Salivary glands	Parasympathetic (cholinergic)	Xerostomia
Sweat glands	Sympathetic (cholinergic)	Anhidrosis
Genital tract	Sympathetic and parasympathetic	Decreased stimulation

is a lower-affinity full agonist at the α_7 subtype and exhibits weak activity toward $\alpha_3\beta_2$- and α_6-containing receptors. *Varenicline* has been shown to be more effective than placebo, nicotine patch, or *bupropion* in helping smokers achieve abstinence (Anthenelli et al., 2016).

ADME

Varenicline has a high oral bioavailability (~90%) and is excreted unchanged in the urine. Reduction in dosage is recommended for those with renal function impairment.

Adverse Effects

Varenicline had previously carried an FDA black-box warning of serious psychiatric adverse effects (e.g., suicide) in some patients with underlying psychiatric disorders; however, recent studies show no significant increase in such events attributable to *varenicline* relative to nicotine patch or placebo (Anthenelli et al., 2016). Nonetheless, patients should be monitored for behavioral or psychiatric changes that typically resolve following discontinuation.

Drug Facts for Your Personal Formulary: *Agents Acting at the Neuromuscular Junction and Autonomic Ganglia; Antispasmodics; Nicotine*

Drug	Therapeutic Uses	Clinical Pharmacology and Tips
Nicotinic ACh Receptor Agonists		
Succinylcholine^{US} (N_m agonist)	Induction of neuromuscular blockade in surgery and during intubation	• Induces rapid depolarization of motor end plate, inducing phase I block • Resistant to and augments AChE inhibition; induces fasciculations, then flaccid paralysis • Influenced by anesthetic agent, type of muscle, and rate of administration • Leads to phase II block after prolonged use • Metabolized by butyrylcholinesterase; not safe for infants and children • Contraindications: history of malignant hyperthermia, muscular dystrophy
Dexamethonium (depolarizer)	• Not used clinically in the U.S.	
Nicotine (N_n agonist)	• Smoking cessation	• Low dose induces postganglionic depolarization • High doses induce ganglionic transmission blockade
Varenicline (N_n [$\alpha_4\beta_2$ subtype])	• Smoking cessation • FDA warning about mood and behavioral changes revised down in 2016	• Partial nicotinic receptor agonist preventing nicotine stimulation and decreasing craving • May cause seizures with alcohol use; excreted largely unchanged in urine
Competitive Nicotinic ACh Receptor Antagonists (Nondepolarizing Neuromuscular Blocking Agents)		
D-Tubocurarine^{a,L}	• Induction of neuromuscular blockade in surgery and during intubation • All neuromuscular blocking agents are administered parenterally	• No longer used clinically in the U.S. or Canada • Produces partial blockade of ganglionic ACh transmission that can produce hypertension and reflex tachycardia • Can induce histamine release
Mivacurium^{S}		• Short acting due to rapid hydrolysis by plasma cholinesterase • Use with caution in patients with renal or hepatic insufficiency
Pancuronium^{L}		• Shows antimuscarinic receptor activity • Renal and hepatic elimination • Vagolytic activity may cause tachycardia, hypertension, and increased cardiac output
Rocuronium^{I}		• Amino steroid • Stable in solution • More rapid onset than vecuronium and cisatracurium • Hepatic elimination
Vecuronium^{I}		• Amino steroid • Not stable in solution • Hepatic and renal elimination
Metocurine^{a,L}		• Three times more potent than tubocurarine • Less histamine release
Atracurium^{I}	• Preferred agent for patients with renal failure	• Susceptible to Hofmann elimination and ester hydrolysis • Same dosage for infants >1 month, children, and adults
Cisatracurium^{I}		• More potent than atracurium, Hofmann elimination, no histamine release (unlike atracurium)
Doxacurium^{a,L}		• Renal elimination
Pipecuronium^{a,L}		• Hepatic metabolism, renal elimination

Drug Facts for Your Personal Formulary: *Agents Acting at the Neuromuscular Junction and Autonomic Ganglia; Antispasmodics; Nicotine* (continued)

Drug	Therapeutic Uses	Clinical Pharmacology and Tips
Competitive Nicotinic ACh Receptor Antagonists (Nondepolarizing Neuromuscular Blocking Agents) (cont.)		
Gantacurium[b,US]		• New compound class; in clinical trial stage • Fastest onset and shortest acting • Metabolism: rapid cysteine adduction, slow ester hydrolysis
Hexamethonium	• Not used therapeutically	• N_n receptor antagonist; blocks ganglionic transmission
Trimethaphan	• Hypertensive crisis • No longer used	• N_n receptor antagonist; blocks ganglionic transmission
CNS-Active Agents		
Baclofen Benzodiazepines Tizanidine Cyclobenzaprine	• Control of muscle spasms	• See Chapter 21
Carisoprodol Metaxalone Methocarbamol Orphenadrine Tetrabenazine	• Muscle relaxants acting in CNS, having, in general, a depressant effect	• CYP2C19 metabolizes carisoprodol to largely to meprobamate • Tetrabenazine is a VCAT2 inhibitor and depletes neuronal monoamine stores
Agents That Block ACh Release		
AbobotulinumtoxinA	• Cervical dystonia • Glabellar lines (moderate to severe)	• Spread of toxin effect may induce paralysis of nontargeted muscle, rarely if administered carefully • Paralysis of swallowing and respiration can be life-threatening
IncobotulinumtoxinA	• Blepharospasm, cervical dystonia • Glabellar lines (moderate to severe)	
OnabotulinumtoxinA	• Botox: axillary hyperhidrosis (severe) • Blepharospasm associated with dystonia; cervical dystonia; migraine (chronic) prophylaxis • Overactive bladder; strabismus; upper limb spasticity (severe); urinary incontinence (due to detrusor overactivity associated with a neurologic condition)	
PrabotulinumtoxinA-xvfs	• Moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity in adult patients	
RimabotulinumtoxinB	• Cervical dystonia	
Inhibitor of Release of Ca^{2+} From the Sarcoplasmic Reticulum		
Dantrolene	• Management and prevention of malignant hyperthermia • Treatment of spasticity associated with upper motor neuron disorders (e.g., spinal cord injury, stroke, cerebral palsy, or multiple sclerosis)	• Hepatic metabolism • Can cause significant hepatotoxicity

Duration of action: [L]long (> ~80 min); [I]intermediate (~20–80 min); [S]short (~15–20 min); [US]ultrashort (< ~15 min).

[a] Not available in the U.S.

[b] Gantacurium is in investigational status.

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Chapter 14

Adrenergic Agonists and Antagonists

Douglas G. Tilley, Steven R. Houser, and Walter J. Koch

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- Inverse and Biased Agonism at Adrenergic Receptors
- False-Transmitter Concept

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ADRENERGIC RECEPTOR ANTAGONISTS

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- Nonselective α Adrenergic Antagonists
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Introduction

Agents or drugs that act on the adrenergic system may mimic the actions of endogenous catecholamines, block their synthesis or release, or antagonize their effects at the level of adrenergic receptors on cell membranes. Catecholamines are released from neurons within the sympathetic nervous system and adrenal medulla. Norepinephrine (NE) is the primary catecholamine neurotransmitter in the peripheral sympathetic nervous system while epinephrine (EPI) is the primary catecholamine hormone released from the adrenal medulla. NE therefore acts in innervated tissues locally and EPI, secreted into blood circulation, acts as a hormone, with effects dependent upon its circulating concentration. NE and EPI are released and activated by several stimuli including physical and psychological stress. The diversity of their actions accounts for the fact that drugs acting to alter sympathetic and adrenergic responses are used for a myriad of clinical disorders including hypertension, asthma, heart failure, and anaphylactic reactions. Many of these uses are discussed elsewhere (see Chapters 32, 33, and 44). Dopamine is a third endogenous catecholamine; it is predominantly located in the central nervous system (CNS), and its central effects are discussed elsewhere (see Chapter 15), although there are some dopamine receptors in the periphery.

Actions of agents that activate or antagonize adrenergic receptors primarily follow known physiological effects of endogenous catecholamines. Most available adrenergic agonists are structural analogues of EPI and NE. Although EPI and NE are sometimes used, modifications of these parent structures offer therapeutic benefits including improved bioavailability, duration of action, and receptor subtype specificity. The actions of NE and EPI are similar at some sites but quite different at others due to subtypes of adrenergic receptors that are expressed by different organs and tissues. There are nine adrenergic receptor subtypes, and these can dictate specific effects of adrenergic agonists and antagonists throughout the body. Subtype selectivity of newer agonists and antagonists adds to the therapeutic benefits.

Abbreviations

ADHD: attention-deficit/hyperactivity disorder
AV: atrioventricular
BPH: benign prostatic hyperplasia
COMT: catechol-*O*-methyltransferase
COPD: chronic obstructive pulmonary disease
CYP: cytochrome P450
DA: dopamine
ECG: electrocardiogram
EPI: epinephrine
GI: gastrointestinal
HDL: high-density lipoprotein
5HT: 5-hydroxytryptamine (serotonin)
INE: isopropylnorepinephrine, isoproterenol
LDL: low-density lipoprotein
MAO: monoamine oxidase
NE: norepinephrine
SA: sinoatrial
VLABA: very long-acting or ultra β_2 adrenergic receptor agonist

HISTORICAL PERSPECTIVE

The pressor effects of adrenal extracts were first demonstrated by Oliver and Schafer in 1895. The active component was named epinephrine by J.J. Abel in 1899, who isolated the monobenzoyl derivative. Takamine isolated the "pure, stable, crystalline form" and termed it adrenalin (see Arthur, 2015). Henry Dale worked on a series of synthetic amines related to EPI that were termed sympathomimetics (Barger and Dale, 1910). Cannon and Rosenblueth described the actions of the body's "fight and flight" mechanisms, observations that later led to the discovery that NE is the sympathetic neurotransmitter (see Bacq, 1983). In the mid-20th century, Ahlquist hypothesized that the myriad organ-level effects of catecholamines were mediated by activation of two distinct populations of receptors, which he termed distinct α and β receptors. This theory, although not universally embraced at the time (see Lefkowitz, 2018), provided the initial impetus for the synthesis and pharmacological evaluation of β receptor antagonists. The first β-selective agent was dichloroisoproterenol, a partial agonist. James Black and his colleagues initiated a program in the late 1950s to develop additional β blockers, with the resulting synthesis and characterization of *propranolol*. Only in the 1980s were α and β receptors purified, giving physical proof that the receptors were distinct entities. Researchers in the general area of adrenergic signaling have garnered a number of Nobel Prizes: Sutherland (1971) for the discovery of cyclic AMP and a mechanism of transmembrane hormone action; Black, Hitchings, and Elion (1988) for development of β blockers and H_2 antagonists; Krebs and Fischer (1992) for the discovery of cyclic AMP–dependent protein kinase; Rodbell and Gilman (1994) for the discovery of G proteins and their roles in transmembrane signaling; and Lefkowitz and Kobilka (2012) for studies of G protein-coupled receptors, principally the β_2 adrenergic receptor.

Overview: Actions of Catecholamines and Sympathomimetic Drugs

Most of the actions of catecholamines and sympathomimetic agents can be classified into seven broad types:

1. *A peripheral excitatory action* on certain types of smooth muscle, such as those in blood vessels supplying skin, kidney, and mucous membranes; and on gland cells, such as those in salivary and sweat glands
2. *A peripheral inhibitory action* on certain other types of smooth muscle, such as those in the wall of the gut, in the bronchial tree, and in blood vessels supplying skeletal muscle
3. *A cardiac excitatory action* that increases heart rate, force and rate, and extent of contraction, and rate of relaxation
4. *Metabolic actions*, such as an increase in the rate of glycogenolysis in liver and muscle and liberation of free fatty acids from adipose tissue
5. *Endocrine actions*, such as modulation (increasing or decreasing) of the secretion of insulin, renin, and pituitary hormones
6. *Actions in the CNS*, such as respiratory stimulation, an increase in wakefulness and psychomotor activity, and a reduction in appetite
7. *Prejunctional actions* that either inhibit or facilitate the release of neurotransmitters, the inhibitory action being physiologically more important

Not all sympathomimetic drugs show each of the types of action to the same degree; however, many of the differences in their effects are only quantitative. The pharmacological properties of these drugs as a class are described in detail for the prototypical agent, EPI. Appreciation of the pharmacological properties of the drugs described in this chapter depends on an understanding of the classification, distribution, and mechanism of action of α and β adrenergic receptors.

Classification of Sympathomimetic Drugs

Catecholamines and sympathomimetic drugs are classified as *direct-acting, indirect-acting, or mixed-acting sympathomimetics* (Figure 14–1). Direct-acting sympathomimetic drugs act directly on one or more of the adrenergic receptors. These agents may exhibit considerable selectivity for a specific receptor subtype (e.g., *phenylephrine* for α_1, *terbutaline* for β_2) or may have no or minimal selectivity and act on several receptor types (e.g., EPI for α_1, α_2, β_1, β_2, and β_3 receptors; NE for α_1, α_2, and β_1 receptors).

Indirect-acting drugs increase the availability of NE or EPI to stimulate adrenergic receptors by several mechanisms:

- By releasing or displacing NE from sympathetic nerve varicosities
- By inhibiting the transport of NE into sympathetic neurons (e.g., *cocaine*), thereby increasing the dwell time of the transmitter at the receptor
- By blocking the metabolizing enzymes, monoamine oxidase (MAO) (e.g., *pargyline*) or catechol-*O*-methyltransferase (COMT) (e.g., *entacapone*), effectively increasing transmitter supply

Drugs that indirectly release NE and directly activate receptors are referred to as *mixed-acting sympathomimetic drugs* (e.g., *ephedrine*). A feature of *direct-acting sympathomimetic drugs* is that their responses are not reduced by prior treatment with *reserpine* or *guanethidine*, which deplete NE from sympathetic neurons. After transmitter depletion, the actions of direct-acting sympathomimetic drugs may actually increase because the loss of the neurotransmitter induces compensatory changes that upregulate receptors or enhance the signaling pathway. By contrast, the responses of indirect-acting sympathomimetic drugs (e.g., *amphetamine, tyramine*) are abolished by prior treatment with *reserpine* or *guanethidine*. The cardinal feature of mixed-acting sympathomimetic drugs is that their effects are blunted, but not abolished, by prior treatment with *reserpine* or *guanethidine*.

Because *the actions of NE are more pronounced on α and* β_1 *receptors than on* β_2 *receptors*, many noncatecholamines that induce the release of NE have predominantly α receptor–mediated and cardiac effects. However, certain noncatecholamines with both direct and indirect effects on adrenergic receptors show significant β_2 activity and are used clinically for these effects. Thus, *ephedrine*, although dependent on release of NE for some of its effects, relieves bronchospasm by its action on β_2 receptors in bronchial smooth muscle, an effect not seen with NE. Moreover, some noncatecholamines (e.g., *phenylephrine*) act primarily and directly on target cells. Thus, it is not possible to predict precisely the effects of noncatecholamines solely on their ability to provoke NE release.

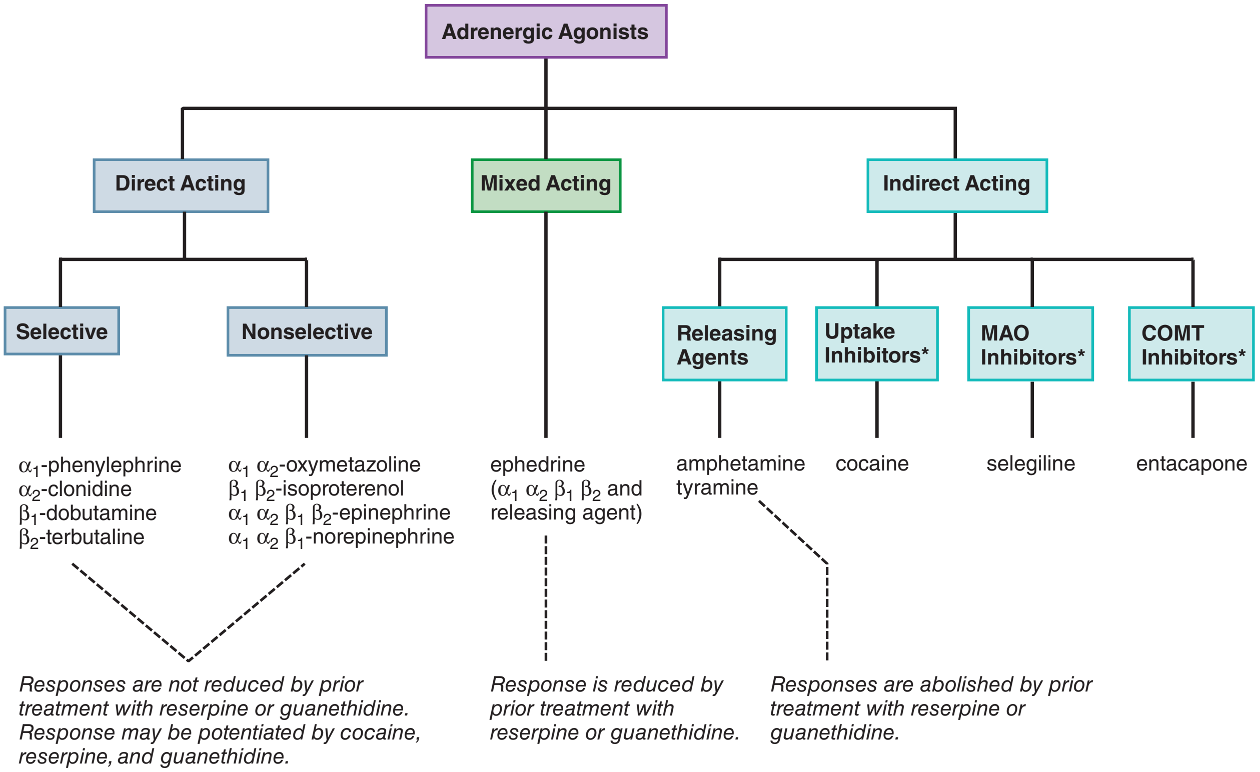

Adrenergic Agonists
Direct Acting
Mixed Acting
Indirect Acting
Selective
Nonselective
Releasing Agents
Uptake Inhibitors*
MAO Inhibitors*
COMT Inhibitors*
α1-phenylephrine
α2-clonidine
β1-dobutamine
β2-terbutaline
α1 α2-oxymetazoline
β1 β2-isoproterenol
α1 α2 β1 β2-epinephrine
α1 α2 β1-norepinephrine
ephedrine
(α1 α2 β1 β2 and releasing agent)
amphetamine
tyramine
cocaine
selegiline
entacapone
Responses are not reduced by prior treatment with reserpine or guanethidine. Response may be potentiated by cocaine, reserpine, and guanethidine.
Response is reduced by prior treatment with reserpine or guanethidine.
Responses are abolished by prior treatment with reserpine or guanethidine.

Figure 14–1 *Classification of adrenergic receptor agonists (sympathomimetic amines) or drugs that produce sympathomimetic-like effects.* For each category, a prototypical drug is shown. (*Not actually sympathomimetic drugs but produce sympathomimetic-like effects.)

Structure-Activity Relationship of Sympathomimetic Amines

β-Phenylethylamine, *consisting of a benzene ring and an ethylamine side chain* (parent structure in Table 14–1), can be viewed as the parent compound of the sympathomimetic amines. The structure permits substitutions to be made on the aromatic ring, the α- and β-carbon atoms, and the terminal amino group to yield a variety of compounds with sympathomimetic activity. *NE (norepinephrine), EPI (epinephrine), DA (dopamine), INE (isopropylnorepinephrine, isoproterenol),* and a few other agents have hydroxyl groups substituted at positions 3 and 4 of the benzene ring. Because *o-dihydroxybenzene* is also known as *catechol*, sympathomimetic amines with these hydroxyl substitutions in the aromatic ring are termed *catecholamines*. By far the greatest sympathomimetic activity occurs when two carbon atoms separate the ring from the amino group. This rule applies with few exceptions to all types of action.

Many directly acting sympathomimetic drugs influence both α and β receptors, but the ratio of activities varies among drugs in a continuous spectrum from predominantly α activity (*phenylephrine*) to predominantly β activity (INE). Despite the multiplicity of the sites of action of sympathomimetic amines, several generalizations can be made (Table 14–1).

Substitution on the Amino Group

The effects of amino substitution are most readily seen in the actions of catecholamines on α and β receptors. *Increase in the size of the alkyl substituent increases β receptor activity (e.g., INE).* NE has, in general, rather feeble β_2 activity; this activity is greatly increased in EPI by the addition of a methyl group. A notable exception is *phenylephrine*, which has an *N*-methyl substituent but is an α-selective agonist. β_2-Selective compounds require a large amino substituent but also depend on other substitutions to define selectivity for β_2 over β_1 receptors. *In general, the smaller the substitution on the amino group, the greater is the selectivity for α activity, although N-methylation increases the potency of primary amines. Thus, α activity is maximal in EPI, less in NE, and almost absent n INE.*

Substitution on the Aromatic Nucleus

Maximal α and β activity depends on the presence of hydroxyl groups on positions 3 and 4. When one or both of these groups are absent, with no other aromatic substitution, the overall potency is reduced. *Phenylephrine* is thus less potent than EPI at both α and β receptors, with β_2 activity almost completely absent. Studies of the β adrenergic receptor suggest that the catechol hydroxyl groups at positions 3 and 4 can interact with specific hydroxyl groups on serine residues within the ligand binding pocket.

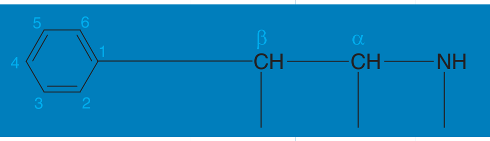
Hydroxyl groups in positions 3 and 5 confer β_2 receptor selectivity on compounds with large amino substituents. Thus, *terbutaline* and similar compounds relax the bronchial musculature in patients with asthma but cause less-direct cardiac stimulation than do the nonselective drugs. The response to noncatecholamines is partly determined by their capacity to release NE from storage sites. These agents thus cause effects that are mostly mediated by α and β_1 receptors because NE is a weak β_2 agonist. Phenylethylamines that lack hydroxyl groups on the ring and the β-hydroxyl group on the side chain act almost exclusively by causing the release of NE from sympathetic nerve terminals.

Because substitution of polar groups on the phenylethylamine structure makes the resultant compounds less lipophilic, unsubstituted or alkyl-substituted compounds cross the blood-brain barrier more readily and have more central activity. Thus, *ephedrine*, *amphetamine*, and *methamphetamine* exhibit considerable CNS activity. As noted, the absence of polar hydroxyl groups results in a loss of direct sympathomimetic activity.

Catecholamines have only a brief duration of action and are ineffective when administered orally because they are rapidly inactivated in the intestinal mucosa and in the liver before reaching the systemic circulation. Compounds without one or both hydroxyl substituents are not acted on by COMT, and their oral effectiveness and duration of action are enhanced.

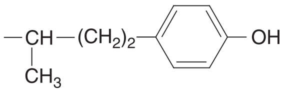
Groups other than hydroxyls have been substituted on the aromatic ring. In general, potency at α receptors is reduced, and β receptor activity is minimal; the compounds may even block β receptors. For example, *methoxamine*, with methoxy substituents at positions 2 and 5, has highly

TABLE 14–1 ■ STRUCTURES AND MAIN CLINICAL USES OF IMPORTANT SYMPATHOMIMETIC DRUGS



					MAIN CLINICAL USES							
					α RECEPTOR				β RECEPTOR			
					A	N	P	V	B	C	U	CNS
Phenylethylamine		H	H	H								
Epinephrine	3-OH, 4-OH	OH	H	CH_3	A		P	V	B	C		
Norepinephrine	3-OH, 4-OH	OH	H	H			P			C^a		
Dopamine	3-OH, 4-OH	H	H	H			P					
Droxidopa	3-OH, 4-OH	OH	COOH	H			P			C^a		
Dobutamine	3-OH, 4-OH	H	H	X						C		
Isoproterenol	3-OH, 4-OH	OH	H	$CH(CH_3)_2$					B	C		
Terbutaline	3-OH, 5-OH	OH	H	$C(CH_3)_3$					B		U	
Metaraminol	3-OH	OH	CH_3	H			P					
Phenylephrine	3-OH	OH	H	CH_3		N	P					
Methoxamine	$2\text{-}OCH_3$, $5\text{-}OCH_3$	OH	CH_3	H			P					
Albuterol	$3\text{-}CH_2OH$, 4-OH	OH	H	$C(CH_3)_3$					B		U	
Amphetamine		H	CH_3	H								++
Methamphetamine		H	CH_3	CH_3								++
Ephedrine		OH	CH_3	CH_3		N	P		B	C		

X:



α Activity: A, allergic reactions (includes β action); N, nasal decongestion; P, pressor (may include β action); V, other local vasoconstriction.
β Activity: B, bronchodilator; C, cardiac; U, uterus.
[a]Direct effects reduced by compensatory baroreceptor reflex.

selective α stimulating activity and in large doses blocks β receptors. *Albuterol*, a β_2-selective agonist, has a substituent at position 3 and is an important exception to the general rule of low β receptor activity.

Substitution on the α-Carbon Atom

The substitution on the α-carbon atom blocks oxidation by MAO, greatly prolonging the duration of action of noncatecholamines because their degradation depends largely on the action of this enzyme. The duration of action of drugs such as *ephedrine* or *amphetamine* is thus measured in hours rather than in minutes. Similarly, compounds with an α-methyl substituent persist in the nerve terminals and are more likely to release NE from storage sites. Agents such as *metaraminol* exhibit a greater degree of indirect sympathomimetic activity.

Substitution on the β-Carbon Atom

Substitution of a hydroxyl group on the β-carbon generally decreases actions within the CNS, largely because it lowers lipid solubility. However, such substitution greatly enhances agonist activity at both α and β adrenergic receptors. Although *ephedrine* is less potent than *methamphetamine* as a central stimulant, it is more powerful in dilating bronchioles and increasing blood pressure and heart rate.

Optical Isomerism

Substitution on either α- or β-carbon yields optical isomers. Levorotatory substitution on the β-carbon confers the greater peripheral activity, so that the naturally occurring *l*-EPI and *l*-NE are at least 10 times more potent than their unnatural *d*-isomers. Dextrorotatory substitution on the α-carbon generally results in a more potent compound. *d*-Amphetamine is more potent than *l*-amphetamine in central but not peripheral activity.

Physiological Basis of Adrenergic Responsiveness

Important factors in the response of any cell or organ to sympathomimetic amines are the density and relative proportion of α and β adrenergic receptors. For example, NE has relatively little capacity to increase bronchial airflow because the receptors in bronchial smooth muscle are largely of the β_2 subtype. In contrast, INE and EPI are potent bronchodilators. Cutaneous blood vessels physiologically express almost exclusively α receptors; thus, NE and EPI cause constriction of such vessels, whereas INE has little effect. The smooth muscle of blood vessels that supply skeletal muscles has both β_2 and α receptors; activation of β_2 receptors causes vasodilation, and stimulation of α receptors constricts these vessels. In such vessels, the threshold concentration for activation of β_2 receptors by EPI is lower than that for α receptors, but when both types of receptors are activated at high concentrations of EPI, the response to α receptors predominates. Physiological concentrations of EPI primarily cause vasodilation.

The ultimate response of a target organ to sympathomimetic amines is dictated not only by the direct effects of the agents but also by the reflex homeostatic adjustments of the organism. One of the most striking effects of many sympathomimetic amines is a rise in arterial blood pressure caused by stimulation of vascular α adrenergic receptors. This stimulation elicits compensatory reflexes that are mediated by the carotid-aortic baroreceptor system. As a result, sympathetic tone is diminished and vagal tone is enhanced; each of these responses leads to slowing of the heart rate. Conversely, when a drug (e.g., a β_2 agonist) lowers mean blood pressure at the mechanoreceptors of the carotid sinus and aortic arch, the baroreceptor reflex works to restore pressure by reducing parasympathetic (vagal) outflow from the CNS to the heart and increasing sympathetic outflow to the heart and vessels. The baroreceptor reflex effect is of special importance for drugs that have little capacity to activate β receptors directly. With diseases such as atherosclerosis, which may impair baroreceptor mechanisms, the effects of sympathomimetic drugs may be magnified.

Inverse and Biased Agonism at Adrenergic Receptors

The observed physiologic effects of adrenergic agonists are broadly attributable to their activation of G proteins, which rapidly initiate downstream cellular responses including generation of the second messenger cyclic AMP or mobilization of intracellular Ca^{2+}. For instance, EPI stimulation of β_2 adrenergic receptors induces G_s-dependent airway smooth muscle relaxation through the generation of cAMP, while its stimulation of α_1 adrenergic receptors induces G_q-dependent vascular smooth muscle constriction via Ca^{2+} mobilization. The ability of adrenergic receptor ligands (agonists or antagonists) to regulate G protein activation is related to how their chemical structures interact with and alter receptor conformation (Wingler and Lefkowitz, 2020). GPCRs exist within a spectrum of conformations, the stabilization of which by their ligands directs G protein accessibility. While agonists generally promote receptor conformations that increase G protein binding, antagonists stabilize receptor structures that prevent G protein binding. Some receptors have structural arrangements that allow G protein binding and some level of activity in the absence of an agonist. Thus, antagonists for these receptors have the property of *inverse agonism* (see Chapter 3); these drugs can decrease the basal activity of their receptor by shifting the equilibrium of spontaneously active receptors toward an inactive state.

In addition to engagement of G proteins, stimulation of GPCRs for prolonged periods or at high concentrations of agonist results in G protein-coupled receptor kinase–dependent phosphorylation of the receptor's C-terminal tail and subsequent recruitment of β-arrestins, which were originally identified as mediators of receptor desensitization (see Chapter 3). β-Arrestins also orchestrate prolonged GPCR trafficking and signaling events due to their capacity as scaffold proteins that can regulate additional cellular responses (Wingler and Lefkowitz, 2020). The ability of a ligand to elicit a stronger G protein- or β-arrestin–dependent signaling response in comparison to a reference agonist, such as the natural endogenous agonist, is termed *biased agonism* (Kenakin, 2021). The identification of biased adrenergic receptor ligands, including both classically described agonists and antagonists, has demonstrated their ability to differentially engage particular G proteins or β-arrestin scaffolds to induce quantitatively distinct signaling responses. Thus far, the most well-characterized biased adrenergic receptor ligands include β_2 receptor agonists, such as *salmeterol* with bias toward G_s protein activation, and β receptor antagonists, such as *carvedilol* with bias toward β-arrestin–dependent signaling (Ippolito and Benovic, 2021). Whether adrenergic receptor ligands with biased agonist properties exert measurable clinical effects that are attributable specifically to their biased engagement of downstream signaling pathways remains to be determined.

False-Transmitter Concept

Indirectly acting amines are taken up into sympathetic nerve terminals and storage vesicles, where they replace NE in the storage complex. Phenylethylamines that lack a β-hydroxyl group are retained there poorly, but β-hydroxylated phenylethylamines and compounds that subsequently become hydroxylated in the synaptic vesicle by DA β-hydroxylase are retained in the synaptic vesicle for relatively long periods of time. Such substances can produce a persistent diminution in the content of NE at functionally critical sites. When the nerve is stimulated, the contents of a relatively constant number of synaptic vesicles are released by exocytosis. If these vesicles contain phenylethylamines that are much less potent than NE, activation of postsynaptic α and β receptors will be diminished.

This hypothesis, known as the *false-transmitter concept*, is a possible explanation for some of the effects of MAO inhibitors. Phenylethylamines normally are synthesized in the GI (gastrointestinal) tract as a result of the action of bacterial tyrosine decarboxylase. The *tyramine* formed in this fashion usually is oxidatively deaminated in the GI tract and the liver, and the amine does not reach the systemic circulation in significant concentrations. However, when an MAO inhibitor is administered, tyramine may be absorbed systemically and transported into sympathetic nerve terminals, where its catabolism again is prevented because of the inhibition of MAO at this site; the tyramine then is β-hydroxylated to octopamine and stored in the vesicles in this form. As a consequence, NE gradually is displaced, and stimulation of the nerve terminal results in the release of a relatively small amount of NE along with a fraction of octopamine, which has relatively little ability to activate either α or β receptors. Thus, a functional impairment of sympathetic transmission parallels long-term administration of MAO inhibitors.

Despite such functional impairment, patients who have received MAO inhibitors may experience severe hypertensive crises if they ingest cheese, beer, or red wine. These and related foods, which are produced by fermentation, are enriched in tyramine and, to a lesser degree, other phenylethylamines. When GI and hepatic MAO are inhibited, the large quantity of tyramine that is ingested is absorbed rapidly and reaches the systemic circulation in high concentration. A massive and precipitous release of NE can result, causing hypertension severe enough to precipitate a myocardial infarction or a stroke. The properties of various MAO inhibitors are discussed in Chapters 10 and 18.

Endogenous Catecholamines

Epinephrine

Epinephrine (adrenaline) is a potent stimulant of both α and β adrenergic receptors; thus, its effects on target organs are complex (see Table 10–1). Some responses of EPI such as sweating, piloerection, and mydriasis depend on the physiological state of the subject. However, the most prominent actions of EPI are on the heart and on vascular and other smooth muscle.

Actions on Organ Systems

Effects on Blood Pressure. Epinephrine is one of the most potent vasopressor substances known. If a pharmacological dose is given rapidly by an intravenous route, it evokes a characteristic effect on blood pressure, which rises rapidly to a peak that is proportional to the dose. The increase in systolic pressure is greater than the increase in diastolic pressure, so that the pulse pressure increases. As the response wanes, the mean pressure may fall below normal before returning to control levels.

The mechanism of the rise in blood pressure due to EPI is a triad of effects:

- a direct myocardial stimulation that increases the strength of ventricular contraction due to stimulation of β adrenergic receptors (*positive inotropic action*);
- an increased heart rate due to β receptors (*positive chronotropic action*); and
- vasoconstriction in many vascular beds—especially in the *precapillary resistance vessels* of skin, mucosa, and kidney—along with marked constriction of the veins due to net stimulation of α adrenergic receptors.

The pulse rate, at first accelerated, may be slowed markedly at the height of the rise of blood pressure by compensatory vagal discharge (baroreceptor reflex). Small doses of EPI (0.1 μg/kg) may cause the blood pressure to fall. The depressor effect of small doses and the biphasic response to larger doses are due to greater sensitivity to EPI of vasodilator β_2 receptors than of constrictor α receptors.

Absorption of EPI after subcutaneous injection is slow due to local vasoconstrictor action; the effects of doses as large as 0.5 to 1.5 mg can be duplicated by intravenous infusion at a rate of 10 to 30 μg/min. There is a moderate increase in systolic pressure due to increased cardiac contractile force and a rise in cardiac output (Figure 14–2). Peripheral resistance decreases, owing to a dominant action on β_2 receptors within vessels in skeletal muscle, where blood flow is enhanced; as a consequence, diastolic pressure usually falls. Because the mean blood pressure is not, as a rule, greatly elevated, compensatory baroreceptor reflexes do not appreciably antagonize the direct cardiac actions. Heart rate, cardiac output, stroke volume, and left ventricular work per beat are increased as a result of direct cardiac stimulation and increased venous return to the heart, which is reflected by an increase in right atrial pressure. At slightly higher rates of infusion, there may be no change or a slight rise in peripheral resistance and diastolic pressure, depending on the dose and the resultant ratio of α to β responses in the various vascular beds; compensatory reflexes also may come into play. The details of the effects of intravenous infusion of EPI, NE, and INE in humans are compared in Figure 14–2 and of EPI and NE in Table 14–2.

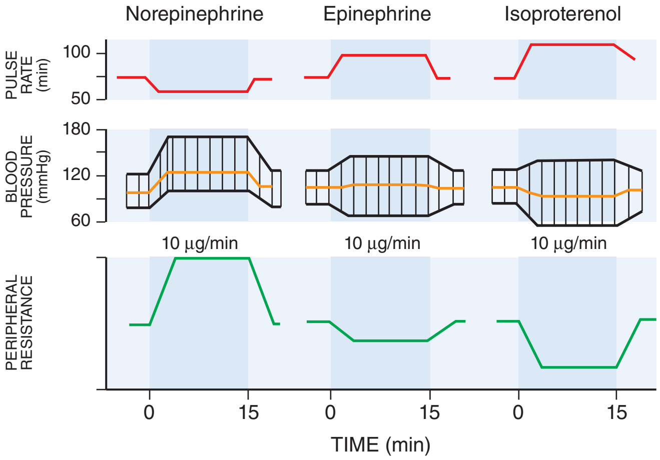


Figure 14–2 *Comparative effects of intravenous infusion of NE, EPI, and INE.* (Reproduced with permission from Allwood MJ, Cobbold AF, Ginsberg J. Peripheral vascular effects of noradrenaline, isopropylnoradrenaline, and dopamine. *Br Med Bull*, **1963**, *19*:132–136. With permission from Oxford University Press.)

TABLE 14–2 ■ COMPARATIVE EFFECTS OF INFUSIONS OF EPINEPHRINE AND NOREPINEPHRINE IN HUMAN BEINGS[a]

EFFECT	EPI	NE
Cardiac		
Heart rate	+	–[b]
Stroke volume	++	++
Cardiac output	+++	0, –
Arrhythmias	++++	++++
Coronary blood flow	++	++
Blood pressure		
Systolic arterial	+++	+++
Mean arterial	+	++
Diastolic arterial	+, 0, –	++
Mean pulmonary	++	++
Peripheral circulation		
Total peripheral resistance	–	++
Cerebral blood flow	+	0, –
Muscle blood flow	+++	0, –
Cutaneous blood flow	–	–
Renal blood flow	–	–
Splanchnic blood flow	+++	0,+
Metabolic effects		
Oxygen consumption	++	0, +
Blood glucose	+++	0, +
Blood lactic acid	+++	0, +
Eosinopenic response	+	0
CNS		
Respiration	+	+
Subjective sensations	+	+

+, increase; 0, no change; –, decrease. Data from Goldenberg M, et al. *Arch Intern Med.* **1950**;*86*:823.

[a] 0.1–0.4 μg/kg per minute.

[b] After atropine.

Vascular Effects. In the vasculature, *EPI acts chiefly on the smaller arterioles and precapillary sphincters,* although veins and large arteries also respond. Various vascular beds react differently, which results in a substantial redistribution of blood flow. Injected EPI markedly decreases cutaneous blood flow, constricting precapillary vessels and small venules. Cutaneous vasoconstriction accounts for a marked decrease in blood flow in the hands and feet. Blood flow to skeletal muscles is increased by therapeutic doses in humans. This is due in part to a powerful β_2-mediated vasodilator action that is only partially counterbalanced by a vasoconstrictor action on the α receptors that also are present in the vascular bed. The effect of EPI on cerebral circulation is related to systemic blood pressure. In usual therapeutic doses, EPI has relatively little constrictor action on cerebral arterioles. Indeed, autoregulatory mechanisms tend to limit the increase in cerebral blood flow caused by increased blood pressure.

Doses of EPI that have little effect on mean arterial pressure consistently increase renal vascular resistance and reduce renal blood flow by as much as 40%. All segments of the renal vascular bed contribute to the increased resistance. Because the glomerular filtration rate is only slightly and variably altered, the filtration fraction is consistently increased. Excretion of Na^+, K^+, and Cl^- is decreased; urine volume may be increased, decreased, or unchanged. Maximal tubular reabsorptive and excretory capacities are unchanged. *The secretion of renin is increased due to the direct action of EPI on β_1 adrenergic receptors in the renal juxtaglomerular cells.*

Arterial and venous pulmonary pressures are raised. Although direct pulmonary vasoconstriction occurs, redistribution of blood from the systemic to the pulmonary circulation, due to constriction of the more powerful musculature in the systemic great veins, doubtless plays an important part in the increase in pulmonary pressure. Very high concentrations of EPI may cause pulmonary edema precipitated by elevated pulmonary capillary filtration pressure and possibly by "leaky" capillaries.

Coronary blood flow is enhanced by EPI or by cardiac sympathetic stimulation under physiological conditions. The increased flow, which occurs even with doses that do not increase the aortic blood pressure, is the result of two factors. The first is the increased relative duration of diastole at higher heart rates (described later in this chapter); this is partially offset by decreased blood flow during systole because of more forceful contraction of the surrounding myocardium and an increase in mechanical compression of the coronary vessels. The increased flow during diastole is further enhanced if aortic blood pressure is elevated by EPI; as a consequence, total coronary flow may be increased. The second factor is a metabolic dilator effect that results from the increased strength of contraction and myocardial O_2 consumption due to the direct effects of EPI on cardiac myocytes via β_1. This vasodilation is mediated in part by adenosine released from cardiac myocytes, which tends to override a direct vasoconstrictor effect of EPI that results from activation of α receptors in coronary vessels.

Cardiac Effects. *Epinephrine is a powerful cardiac stimulant.* It acts directly on the predominant β_1 adrenergic receptors on cardiomyocytes and on cells within pacemaker and conducting tissues; β_2, β_3, and α receptors also are present in the heart, although there are considerable species differences. The heart rate increases, and the rhythm often is altered by EPI. Cardiac systole is shorter and more powerful, cardiac output is enhanced, and the work of the heart and its oxygen consumption are markedly increased. Cardiac efficiency (work done relative to oxygen consumption) is lessened. *Direct responses to EPI include increases in contractile force (inotropy), accelerated rate of rise of isometric tension, enhanced rate of relaxation (lusitropy), decreased time to peak tension, increased excitability, acceleration of the rate of spontaneous beating (chronotropy), and induction of automaticity in specialized regions of the heart.*

In accelerating the heart, EPI preferentially shortens systole so that the duration of diastole usually is not reduced. Indeed, activation of β receptors increases the rate of relaxation of ventricular muscle. EPI speeds the heart by accelerating the slow depolarization of SA (sinoatrial) nodal cells that takes place during diastole, that is, during phase 4 of the action potential (see Chapter 34). Consequently, the transmembrane potential of the pacemaker cells rises more rapidly to the threshold level of action potential initiation. The amplitude of the action potential and the maximal rate of depolarization (phase 0) also are increased. A shift in the location of the pacemaker within the SA node often occurs, owing to activation of latent pacemaker cells. In Purkinje fibers, EPI also accelerates diastolic depolarization and may activate latent pacemaker cells. These changes do not occur in atrial and ventricular muscle fibers, where EPI has little effect on the stable, phase 4 membrane potential after repolarization. If large doses of EPI are given, premature ventricular contractions occur and may herald more serious ventricular arrhythmias. This rarely is seen with conventional doses in humans, but ventricular extrasystoles, tachycardia, or even fibrillation may be precipitated by release of endogenous EPI when the heart has been sensitized to this action of EPI by certain anesthetics or by myocardial ischemia. The mechanism of induction of these cardiac arrhythmias is not clear (see Chapter 34 for further details).

Some effects of EPI on cardiac tissues are largely secondary to the increase in heart rate and are small or inconsistent when the heart rate is kept constant. For example, the effect of EPI on repolarization of atrial muscle, Purkinje fibers, or ventricular muscle is small if the heart rate is unchanged. When the heart rate is increased, the duration of the action potential is consistently shortened, and the refractory period is correspondingly decreased. Conduction through the Purkinje system depends on the level of membrane potential at the time of excitation. Excessive reduction of this potential results in conduction disturbances, ranging from slowed conduction to complete block. EPI often increases the membrane potential and improves conduction in Purkinje fibers that have been excessively depolarized.

Epinephrine normally shortens the refractory period of the human atrioventricular (AV) node by direct effects on the heart, although doses of EPI that slow the heart through reflex vagal discharge may indirectly tend to prolong it. EPI also decreases the grade of AV block that occurs as a result of disease, drugs, or vagal stimulation. Supraventricular arrhythmias are apt to occur from the combination of EPI and cholinergic stimulation. Depression of sinus rate and AV conduction by vagal discharge probably plays a part in EPI-induced ventricular arrhythmias because various drugs that block the vagal effect confer some protection. The actions of EPI in enhancing cardiac automaticity and in causing arrhythmias are effectively antagonized by β receptor antagonists such as *propranolol*. However, α_1 receptors exist in most regions of the heart, and their activation prolongs the refractory period and strengthens myocardial contractions. Cardiac arrhythmias have been seen in patients after inadvertent intravenous administration of conventional subcutaneous doses of EPI. Premature ventricular contractions can appear, which may be followed by multifocal ventricular tachycardia or ventricular fibrillation. Pulmonary edema also may occur.

Epinephrine decreases the amplitude of the T wave of the electrocardiogram (ECG) in normal persons. In animals given relatively larger doses, additional effects are seen on the T wave and the ST segment. After decreasing in amplitude, the T wave may become biphasic, and the ST segment can deviate either above or below the isoelectric line. Such ST segment changes are similar to those seen in patients with angina pectoris during spontaneous or EPI-induced attacks of pain. These electrical changes therefore have been attributed to myocardial ischemia. Also, EPI as well as other catecholamines may cause myocardial cell death, particularly after intravenous infusions. Acute toxicity is associated with contraction band necrosis and other pathological changes. Recent interest has focused on the possibility that prolonged sympathetic stimulation of the heart, such as in congestive heart failure, may promote apoptosis of cardiomyocytes and replacement by fibrotic tissue that would dampen proper electrical propagation (see Frangogiannis, 2021).

Effects on Nonvascular Smooth Muscles. The effects of EPI on the smooth muscles of different organs and systems depend on the type of adrenergic receptor in the muscle. In general, *EPI relaxes GI smooth muscle* due to activation of both α and β receptors. Intestinal tone and the frequency and amplitude of spontaneous contractions are reduced. The stomach usually is relaxed and the pyloric and ileocecal sphincters are contracted, but these effects depend on the preexisting tone of the muscle. If tone already is high, EPI causes relaxation; if low, contraction.

The responses of *uterine muscle* to EPI vary with species, phase of the sexual cycle, state of gestation, and dose given. During the last month of pregnancy and at parturition, EPI inhibits uterine tone and contractions. Effects of adrenergic agents and other drugs on the uterus are discussed further in this chapter and in Chapter 48. *EPI relaxes the detrusor muscle of the bladder as a result of activation of β receptors and contracts the trigone and sphincter muscles owing to its α agonist activity.* This can result in hesitancy in urination and may contribute to retention of urine in the bladder. Activation of smooth muscle contraction in the prostate promotes urinary retention.

Respiratory Effects. *Epinephrine has a powerful bronchodilator action*, most evident when bronchial muscle is contracted because of disease, as in bronchial asthma, or in response to drugs or various autacoids. The beneficial effects of EPI in asthma also may arise from inhibition of antigen-induced release of inflammatory mediators from mast cells and to a lesser extent from diminution of bronchial secretions and congestion within the mucosa. *Inhibition of mast cell secretion is mediated by β_2 receptors, while the effects on the mucosa are mediated by α receptors*; however, other drugs, such as glucocorticoids and leukotriene receptor antagonists, have much more profound anti-inflammatory effects in asthma (see Chapter 44).

Effects on the CNS. Because EPI is a polar compound, it penetrates poorly into the CNS and thus is not a powerful CNS stimulant. While the drug may cause restlessness, apprehension, headache, and tremor in many persons, these effects in part may be secondary to the effects of EPI on the cardiovascular system, skeletal muscles, and intermediary metabolism; that is, they may be the result of somatic manifestations of anxiety.

Metabolic Effects. Epinephrine elevates the concentrations of glucose and lactate in blood. EPI inhibits secretion of insulin through an interaction with α_2 receptors, whereas activation of β_2 receptors enhances insulin secretions; the predominant effect of EPI is inhibition. Glucagon secretion is enhanced via activation of β receptors of the α cells of pancreatic islets. EPI also decreases the uptake of glucose by peripheral tissues, at least in part not only because of its effects on the secretion of insulin, but also possibly due to direct effects on skeletal muscle. Glycosuria rarely occurs. The effect of EPI to stimulate glycogenolysis in most tissues and in most species involves β receptors. EPI raises the concentration of free fatty acids in blood by stimulating β receptors in adipocytes, primarily β_3 receptors. The result is activation of triglyceride lipase, which accelerates the triglyceride breakdown to free fatty acids and glycerol. The calorigenic action of EPI (increase in metabolism) is reflected in humans by an increase of 20% to 30% in O_2 consumption after conventional doses, an effect due mainly to enhanced breakdown of triglycerides in brown adipose tissue (via β_3 receptors), providing an increase in oxidizable substrate.

Miscellaneous Effects. Epinephrine reduces circulating plasma volume by loss of protein-free fluid to the extracellular space, thereby increasing hematocrit and plasma protein concentration. However, conventional doses of EPI do not significantly alter plasma volume or packed red cell volume under normal conditions, although such doses may have variable effects in the presence of shock, hemorrhage, hypotension, or anesthesia. EPI rapidly increases the number of circulating polymorphonuclear leukocytes, likely due to β receptor–mediated demargination of these cells. EPI accelerates blood coagulation and promotes fibrinolysis.

The effects of EPI on secretory glands are not marked; in most glands, secretion usually is inhibited, partly owing to the reduced blood flow caused by vasoconstriction. EPI stimulates lacrimation and scanty mucus secretion from salivary glands. Sweating and pilomotor activity are minimal after systemic administration of EPI but occur after intradermal injection of very dilute solutions of either EPI or NE. Such effects are inhibited by α receptor antagonists.

Mydriasis occurs with physiological sympathetic stimulation but not when EPI is instilled into the conjunctival sac of normal eyes. However, EPI usually lowers intraocular pressure, possibly the result of reduced production of aqueous humor due to vasoconstriction and enhanced outflow (see Chapter 74).

Although EPI does not directly excite *skeletal muscle, it facilitates neuromuscular transmission*, particularly that following prolonged rapid stimulation of motor nerves. In apparent contrast to the effects of α receptor activation at presynaptic nerve terminals in the autonomic nervous system (α_2 receptors), stimulation of α receptors causes a more rapid increase in transmitter release from the somatic motor neuron, perhaps as a result of enhanced influx of Ca^{2+}. These responses likely are mediated by α_1 receptors. These actions may explain in part the ability of EPI (given intra-arterially) to briefly increase strength of the injected limb of patients with myasthenia gravis. EPI also acts directly on white, fast-twitch muscle fibers to prolong the active state, thereby increasing peak tension. Of greater physiological and clinical importance is the capacity of EPI and selective β_2 agonists to increase physiological tremor, at least in part due to β receptor–mediated enhancement of discharge of muscle spindles.

Epinephrine promotes a fall in plasma K^+, largely due to stimulation of K^+ uptake into cells, particularly skeletal muscle, due to activation of β_2 receptors. This is associated with decreased renal K^+ excretion. These receptors have been exploited in the management of hyperkalemic familial periodic paralysis, which is characterized by episodic flaccid paralysis, hyperkalemia, and depolarization of skeletal muscle. The β_2-selective agonist *albuterol* apparently ameliorates the impairment in the ability of the muscle to accumulate and retain K^+.

The administration of large or repeated doses of EPI or other sympathomimetic amines to experimental animals damages arterial walls and myocardium, even inducing necrosis in the heart that is indistinguishable from myocardial infarction. The mechanism of this injury is not yet clear, but α and β receptor antagonists and Ca^{2+} channel blockers may afford substantial protection against the damage. Similar lesions occur in many patients with pheochromocytoma or after prolonged infusions of NE.

ADME

Epinephrine is not effective after oral administration because it is rapidly conjugated and oxidized in the GI mucosa and liver. Absorption from subcutaneous tissues occurs relatively slowly because of local vasoconstriction. Absorption is more rapid after intramuscular injection. In emergencies, it may be necessary to administer EPI intravenously. When relatively concentrated solutions are nebulized and inhaled, the actions of the drug largely are restricted to the respiratory tract; however, systemic reactions such as arrhythmias may occur, particularly if larger amounts are used.

Epinephrine is rapidly inactivated in the liver by COMT and MAO (see Figure 10–9). Although only small amounts appear in the urine of normal persons, the urine of patients with pheochromocytoma may contain relatively large amounts of EPI, NE, and their metabolites.

Epinephrine is available in a variety of formulations geared for different clinical indications and routes of administration, including self-administration for anaphylactic reactions. EPI is unstable in alkaline solution; when exposed to air or light, it turns pink from oxidation to adrenochrome and then brown from formation of polymers. Injectable EPI is available in solutions of 1, 0.5, and 0.1 mg/mL. A subcutaneous dose ranges from 0.3 to 0.5 mg. The intravenous route is used cautiously if an immediate and reliable effect is mandatory. If the solution is given by vein, it must be adequately diluted and injected very slowly. The dose is seldom as much as 0.25 mg, except for cardiac arrest, when larger doses may be required.

Toxicity, Adverse Effects, and Contraindications

Epinephrine may cause restlessness, throbbing headache, tremor, and palpitations. The effects rapidly subside with rest, quiet, recumbency, and reassurance. More serious reactions include cerebral hemorrhage and cardiac arrhythmias. The use of large doses or the accidental, rapid intravenous injection of EPI may result in cerebral hemorrhage from the sharp rise in blood pressure. Ventricular arrhythmias may follow the administration of EPI. Angina may be induced by EPI in patients with coronary artery disease. *The use of EPI generally is contraindicated in patients who are receiving nonselective β receptor antagonists because its unopposed actions on vascular α_1 receptors may lead to severe hypertension and cerebral hemorrhage.*

Therapeutic Uses

A major use of EPI is to provide rapid, emergency relief of hypersensitivity reactions, including anaphylaxis, to drugs and other allergens. EPI also is used to prolong the action of local anesthetics, presumably by decreasing local blood flow and reducing systemic absorption (see Chapter 25). Its cardiac effects may be of use in restoring cardiac rhythm in patients with cardiac arrest due to various causes. It also is used as a topical hemostatic agent on bleeding surfaces, such as in the mouth or in bleeding peptic ulcers during endoscopy of the stomach and duodenum. Systemic absorption of the drug can occur with dental application. Inhalation of EPI may be useful in the treatment of postintubation and infectious croup.

Norepinephrine

Norepinephrine (*levarterenol*, *l*-noradrenaline, *l*-β-[3,4-dihydroxyphenyl]-α-aminoethanol) is a major chemical mediator liberated by mammalian *postganglionic sympathetic nerves*. It differs from EPI only by lacking the methyl substitution in the amino group (see Table 14–1). NE constitutes 10% to 20% of the catecholamine content of human adrenal medulla and as much as 97% in some pheochromocytomas, which may not express the enzyme phenylethanolamine-*N*-methyltransferase.

Pharmacological Properties

The physiological consequences of pharmacological doses of NE and EPI are compared in Table 14–2. Both drugs are direct agonists on effector cells, and their actions differ mainly in the ratio of their effectiveness in stimulating α and β_2 receptors. *They are approximately equipotent in stimulating β_1 receptors. NE is a potent α agonist and has relatively little action on β_2 receptors*; however, it is somewhat less potent than EPI on the α receptors of most organs.

Cardiovascular Effects

In response to intravenous infusion of NE in humans (see Figure 14–2), *systolic and diastolic pressures, and usually pulse pressure, are increased. Cardiac output is unchanged or decreased, and total peripheral resistance is raised.* Compensatory vagal reflex activity slows the heart, overcoming a direct cardioaccelerator action, and stroke volume is increased. The peripheral vascular resistance increases in most vascular beds, and renal blood flow is reduced. NE constricts mesenteric vessels and reduces splanchnic and hepatic blood flow. Coronary flow usually is increased, probably owing both to indirectly induced coronary dilation, as with EPI, and to elevated blood pressure. Although generally a poor β_2 receptor agonist, NE may increase coronary blood flow directly by stimulating β_2 receptors on coronary vessels. Patients with Prinzmetal variant angina may be supersensitive to the α adrenergic vasoconstrictor effects of NE.

Unlike EPI, NE in small doses does not cause vasodilation or lower blood pressure because the blood vessels of skeletal muscle constrict rather than dilate; α adrenergic receptor antagonists therefore abolish the pressor effects but do not cause significant reversal (i.e., hypotension).

Other Effects

Other responses to NE are not prominent in humans. The drug causes hyperglycemia and other metabolic effects similar to those produced by EPI, but these are observed only when large doses are given because NE is not as effective a "hormone" as EPI. Intradermal injection of suitable doses causes sweating that is not blocked by *atropine*.

ADME

Norepinephrine, like EPI, is ineffective when given orally and is absorbed poorly from sites of subcutaneous injection. It is rapidly inactivated in the body by COMT and MAO. Small amounts are normally present in the urine; the excretion rate may be greatly increased in patients with pheochromocytoma.

Toxicity, Adverse Effects, and Precautions

The untoward effects of NE are similar to those of EPI, although there typically is greater elevation of blood pressure with NE. Excessive doses can cause severe hypertension. Care must be taken that necrosis and sloughing do not occur at the site of intravenous injection owing to extravasation of the drug. The infusion should be made high in the limb, preferably through a long plastic cannula extending centrally. Impaired circulation at injection sites, with or without extravasation of NE, may be relieved by infiltrating the area with *phentolamine*, an α receptor antagonist. Blood pressure must be determined frequently during the infusion, particularly during adjustment of the rate of the infusion. Reduced blood flow to organs such as kidney and intestines is a constant danger with the use of NE.

Therapeutic Uses

Norepinephrine is used as a vasoconstrictor to raise or support blood pressure under certain intensive care conditions (discussed further in this chapter).

Droxidopa, a Synthetic Prodrug of Norepinephrine

Droxidopa (L-threo-3,4,-dihydroxyphenylserine) is a synthetic prodrug that is converted by L-aromatic amino acid decarboxylase into NE. It is approved by the FDA (Food and Drug Administration) for the treatment of orthostatic dizziness and light-headiness in adults with symptomatic neurogenic orthostatic hypotension associated with primary autonomic failure and impaired compensatory autonomic reflexes (Keating, 2015). The pharmacological effects of *droxidopa* are thought to be mediated through NE rather than through the parent drug or other metabolites. *Droxidopa* can cross the blood-brain barrier, presumably as the substrate of an amino acid transporter.

Dopamine

Dopamine (3,4-dihydroxyphenylethylamine) (see Table 14–1) is the immediate metabolic precursor of NE and EPI; it is a central neurotransmitter particularly important in the regulation of movement (see Chapter 15) and possesses important intrinsic pharmacological properties. In the periphery, it is synthesized in epithelial cells of the proximal tubule and is thought to exert local diuretic and natriuretic effects. DA is a substrate for both MAO and COMT and, thus, is ineffective when administered orally. Classification of DA receptors, GPCRs structurally similar to adrenergic receptors, is described in Chapter 15.

Pharmacological Properties and Cardiovascular Effects

The cardiovascular effects of DA are mediated by several distinct types of receptors that vary in their affinity for DA (see Chapter 15). At low concentrations, the primary interaction of DA is with vascular D_1 receptors, especially in the renal, mesenteric, and coronary beds. By activating adenylyl cyclase and raising intracellular concentrations of cyclic AMP, D_1 receptor stimulation leads to vasodilation. Infusion of low doses of DA causes an increase in glomerular filtration rate, renal blood flow, and Na^+ excretion. Activation of D_1 receptors on renal tubular cells decreases Na^+ transport by cyclic AMP–dependent and cyclic AMP–independent mechanisms. Increasing cyclic AMP production in the proximal tubular cells and the medullary part of the thick ascending limb of the loop of Henle inhibits the Na^+-H^+ exchanger and the Na^+,K^+-ATPase pump. The renal tubular actions of DA that cause natriuresis may be augmented by the increase in renal blood flow and the small increase in the glomerular filtration rate that follows its administration. The resulting increase in hydrostatic pressure in the peritubular capillaries and reduction in oncotic pressure may contribute to diminished reabsorption of Na^+ by the proximal tubular cells. Accordingly, DA has pharmacologically appropriate effects in the management of states of low cardiac output associated with compromised renal function, such as severe congestive heart failure.

At higher concentrations and because of its catecholamine structure, DA exerts a positive inotropic effect on the myocardium, acting on β_1 adrenergic receptors. DA also causes the release of NE from nerve terminals, thereby affecting the heart indirectly via NE. Tachycardia is less prominent during infusion of DA than of INE (discussed further in the chapter). DA usually increases systolic blood pressure and pulse pressure and either has no effect on diastolic blood pressure or increases it slightly. Total peripheral resistance usually is unchanged when low or intermediate doses of DA are given, probably because of the ability of DA to reduce regional arterial resistance in some vascular beds, such as mesenteric and renal, while causing only minor increases in others. At high concentrations, DA activates vascular α_1 receptors, leading to more general vasoconstriction.

CNS Effects

Dopamine is a major neurotransmitter in the CNS; however, injected DA usually has no central effects because it does not readily cross the blood-brain barrier.

Precautions, Adverse Reactions, and Contraindications

Before DA is administered to patients in shock, hypovolemia should be corrected by transfusion of whole blood, plasma, or other appropriate fluid. Untoward effects due to overdosage generally are attributable to excessive sympathomimetic activity (although this also may be the response to worsening shock). Nausea, vomiting, tachycardia, anginal pain, arrhythmias, headache, hypertension, and peripheral vasoconstriction may be encountered during DA infusion. Extravasation of large amounts of DA during infusion may cause ischemic necrosis and sloughing. Rarely, gangrene of the fingers or toes has followed prolonged infusion of the drug. DA should be avoided or used at much lower doses if the patient has received a MAO inhibitor. Careful adjustment of dosage also is necessary in patients who are taking tricyclic antidepressants.

Therapeutic Uses

Dopamine is used in the treatment of severe acute decompensated heart failure, particularly in patients with oliguria and low or normal peripheral vascular resistance. The drug also may improve physiological parameters in the treatment of cardiogenic and septic shock. While DA may acutely improve cardiac and renal function in severely ill patients with chronic heart disease or renal failure, there is relatively little evidence supporting long-term benefit in clinical outcome (Doggrell, 2002).

Dopamine hydrochloride is used only intravenously, preferably into a large vein to prevent perivascular infiltration; extravasation may cause necrosis and sloughing of the surrounding tissue. The use of a calibrated infusion pump to control the rate of flow is necessary. The drug is administered at a rate of 2 to 5 μg/kg per min; this rate may be increased gradually up to 20 to 50 μg/kg per min or more as the clinical situation dictates. During the infusion, patients require clinical assessment of myocardial function, perfusion of vital organs such as the brain, and the production of urine. Reduction in urine flow, tachycardia, or the development of arrhythmias may be indications to slow or terminate the infusion. The duration of action of DA is brief, and hence, the rate of administration can be used to control the intensity of effect.

Fenoldopam and Dopexamine. *Fenoldopam*, a benzazepine derivative, is a rapidly acting vasodilator used for not more than 48 h for control of severe hypertension (e.g., malignant hypertension with end-organ damage) in hospitalized patients. *Fenoldopam* is an agonist for peripheral D_1 receptors and binds with moderate affinity to α_2 adrenergic receptors; it has no significant affinity for D_2 receptors or α_1 or β adrenergic receptors. *Fenoldopam* is a racemic mixture; the R-isomer is the active component. It dilates a variety of blood vessels, including coronary arteries, afferent and efferent arterioles in the kidney, and mesenteric arteries (Murphy et al., 2001). *Fenoldopam* must be administered using a calibrated infusion pump; the usual dose rate ranges from 0.01 to 1.6 μg/kg per min. Less than 6% of an orally administered dose is absorbed because of extensive first-pass formation of sulfate, methyl, and glucuronide conjugates. The elimination $t_{1/2}$ of intravenously infused *fenoldopam* is about 10 min. Adverse effects are related to the vasodilation and include headache, flushing, dizziness, and tachycardia or bradycardia.

Dopexamine is a synthetic analogue related to DA with intrinsic activity at DA D_1 and D_2 receptors as well as at β_2 receptors; it may have other effects, such as inhibition of catecholamine uptake (Frishman and Hotchkiss, 1996). It has favorable hemodynamic actions in patients with severe congestive heart failure, sepsis, and shock. In patients with low cardiac output, *dopexamine* infusion significantly increases stroke volume with a decrease in systemic vascular resistance. Tachycardia and hypotension can occur, but usually only at high infusion rates. *Dopexamine* is not approved for use in the U.S.

α Adrenergic Receptor Agonists

α_1-Selective Adrenergic Receptor Agonists

The major effects of a number of sympathomimetic drugs are due to activation of α_1 adrenergic receptors in vascular smooth muscle. As a result, peripheral vascular resistance is increased, and blood pressure is maintained or elevated. The clinical utility of these drugs is limited to the treatment of some patients with hypotension, including orthostatic hypotension, or shock. *Phenylephrine* and *methoxamine* (discontinued in the U.S.) are direct-acting vasoconstrictors and are selective activators of α_1 adrenergic receptors. *Mephentermine* and *metaraminol* act both directly and indirectly. *Midodrine* is a prodrug that is converted, after oral administration, to *desglymidodrine*, a direct-acting α_1 adrenergic receptor agonist.

Phenylephrine

Phenylephrine is an α_1-selective agonist; it activates β receptors only at much higher concentrations. The pharmacological effects of *phenylephrine* are similar to those of *methoxamine*. The drug causes marked arterial vasoconstriction during intravenous infusion. *Phenylephrine* also is used as a nasal decongestant and as a mydriatic in various nasal and ophthalmic formulations (see Chapter 74).

Metaraminol

Metaraminol exerts *direct effects* on vascular α_1 adrenergic receptors and acts *indirectly* by stimulating the release of NE. The drug has been used in the treatment of hypotensive states or off-label to relieve attacks of paroxysmal atrial tachycardia, particularly those associated with hypotension (see Chapter 34).

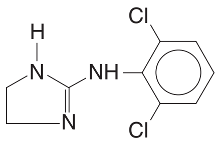
Midodrine

Midodrine is an orally effective α_1 receptor agonist. It is a prodrug, converted to an active metabolite, desglymidodrine, which achieves peak concentrations about 1 h after a dose of *midodrine*. The $t_{1/2}$ of desglymidodrine is about 3 h; its duration of action is about 4 to 6 h. *Midodrine*-induced rises in blood pressure are associated with contraction of both arterial and venous smooth muscle. This is advantageous in the treatment of patients with autonomic insufficiency and postural hypotension (McClellan et al., 1998). A frequent complication in these patients is supine hypertension. This can be minimized by administering the drug during periods when the patient will remain upright, avoiding dosing within 4 h of bedtime, and elevating the head of the bed. Very cautious use of a short-acting antihypertensive drug at bedtime may be useful in some patients. Typical dosing, achieved by careful titration of blood pressure responses, varies between 2.5 and 10 mg three times daily.

α_2-Selective Adrenergic Receptor Agonists

α_2-Selective adrenergic receptor agonists are used primarily for the treatment of systemic hypertension. Their efficacy as antihypertensive agents is somewhat surprising, because many blood vessels contain postsynaptic α_2 adrenergic receptors that promote vasoconstriction. *Clonidine*, an α_2-selective agonist, is an imidazoline derivative that was developed as a vasoconstricting nasal decongestant; it lowers blood pressure by activating α_2 receptors in the CNS, thereby suppressing sympathetic outflow from the brain. The α_2 receptor agonists also reduce intraocular pressure by decreasing the production of aqueous humor. Two derivatives of *clonidine*, *apraclonidine* and *brimonidine*, applied topically to the eye, decrease intraocular pressure with little or no effect on systemic blood pressure.

Clonidine



CLONIDINE

Mechanisms of Action and Pharmacological Effects. Intravenous infusion of *clonidine* causes an acute rise in blood pressure because of activation of postsynaptic α_2 adrenergic receptors in vascular smooth muscle. This transient vasoconstriction (not usually seen with oral administration) is followed by a more prolonged hypotensive response that results from decreased sympathetic outflow from the CNS. The effect appears to result, at least in part, from activation of α_2 receptors in the lower brainstem region. *Clonidine* also stimulates parasympathetic outflow, which may contribute to the slowing of heart rate. In addition, some of the antihypertensive effects of *clonidine* may be mediated by activation of presynaptic α_2 receptors that suppress the release of NE, ATP, and NPY (neuropeptide Y) from postganglionic sympathetic nerves. *Clonidine* decreases the plasma concentration of NE and reduces its excretion in the urine.

Since *clonidine* is an imidazoline derivative, there are questions about it also acting on imidazoline receptors. Importantly, studies in knockout animals have demonstrated the requirement for a functional α_2 adrenergic receptor for the hypotensive effect of *clonidine*. Imidazoline receptors, of which there are three subtypes (I_1, I_2, and I_3), are widely distributed in the body, including the CNS. Activation of the I_1 receptor appears to reduce sympathetic outflow from the CNS. Whether activation of the CNS I_1 imidazoline receptor also plays a role in the hypotensive effects of *clonidine* and its congeners is a topic of ongoing research. The current hypothesis is that I_1 receptors are upstream from the hypotensive α_2 adrenergic receptors in the CNS and work in tandem with them, such that activation of the I_1 receptors results in catecholamine release onto α_2 receptors (Lowry and Brown, 2014; Nikolic and Agbaba, 2012), thereby reducing sympathetic outflow and reducing blood pressure.

Clonidine does decrease discharges in sympathetic preganglionic fibers in the splanchnic nerve and in postganglionic fibers of cardiac nerves. These effects are blocked by α_2-selective antagonists such as *yohimbine*. *Clonidine* also stimulates parasympathetic outflow, which may contribute to the slowing of heart rate as a consequence of increased vagal tone and diminished sympathetic drive. In addition, some of the antihypertensive effects of *clonidine* may be mediated by activation of presynaptic α_2 receptors that suppress the release of NE, ATP, and NPY from postganglionic sympathetic nerves. *Clonidine* decreases the plasma concentration of NE and reduces its excretion in the urine.

ADME. *Clonidine* is well absorbed after oral administration, with bioavailability about 100%. Peak concentration in plasma and the maximal hypotensive effect are observed 1 to 3 h after an oral dose. The elimination $t_{1/2}$ is 6 to 24 h (mean about 12 h). About half of an administered dose can be recovered unchanged in the urine; the $t_{1/2}$ of the drug may increase with renal failure. A transdermal delivery patch permits continuous administration of *clonidine* as an alternative to oral therapy. The drug is released at an approximately constant rate for a week; 3 to 4 days are required to reach steady-state concentrations in plasma. When the patch is removed, plasma concentrations remain stable for about 8 h and then decline gradually over a period of several days; this decrease is associated with a rise in blood pressure.

Therapeutic Uses. *Clonidine* is used mainly in the treatment of hypertension (see Chapter 32). *Clonidine* also has apparent efficacy in the off-label treatment of a range of other disorders: in reducing diarrhea in some diabetic patients with autonomic neuropathy; in treating and preparing addicted subjects for withdrawal from narcotics, alcohol, and tobacco (see Chapter 28) by ameliorating some of the adverse sympathetic nervous activity associated with withdrawal and decreasing craving for the drug; and in reducing the incidence of menopausal hot flashes (transdermal application). Acute administration of *clonidine* has been used in the differential diagnosis of patients with hypertension and suspected pheochromocytoma. Among the other off-label uses of *clonidine* are atrial fibrillation, attention-deficit/hyperactivity disorder (ADHD), constitutional growth delay in children, *cyclosporine*-associated nephrotoxicity, Tourette syndrome, hyperhidrosis, mania, posthepatic neuralgia, psychosis, restless leg syndrome, ulcerative colitis, and allergy-induced inflammatory reactions in patients with extrinsic asthma.

Adverse Effects. The major adverse effects of *clonidine* are dry mouth and sedation, which may diminish in intensity after several weeks of therapy.

Sexual dysfunction also may occur. Marked bradycardia is observed in some patients. These effects of *clonidine* frequently are related to dose, and their incidence may be lower with transdermal administration of *clonidine*. About 15% to 20% of patients develop contact dermatitis when using the transdermal system. Withdrawal reactions follow abrupt discontinuation of long-term therapy with *clonidine* in some hypertensive patients (see Chapter 32).

Apraclonidine

Apraclonidine is a relatively selective α_2 receptor agonist that is used topically to reduce intraocular pressure with minimal systemic effects. This agent does not cross the blood-brain barrier and is more useful than *clonidine* for ophthalmic therapy. *Apraclonidine* is useful as short-term adjunctive therapy in patients with glaucoma whose intraocular pressure is not well controlled by other pharmacological agents. The drug also is used to control or prevent elevations in intraocular pressure that occur in patients after laser trabeculoplasty or iridotomy (see Chapter 74).

Brimonidine

Brimonidine is a clonidine derivative and α_2-selective agonist that is administered ocularly to lower intraocular pressure in patients with ocular hypertension or open-angle glaucoma. Unlike *apraclonidine*, *brimonidine* can cross the blood-brain barrier and can produce hypotension and sedation, although these CNS effects are slight compared to those of *clonidine*.

Guanfacine

Guanfacine is an α_2 receptor agonist that is more selective than *clonidine* for α_2 receptors. Like *clonidine*, *guanfacine* lowers blood pressure by activation of brainstem receptors with resultant suppression of sympathetic activity. A sustained-release form is FDA approved for treatment of ADHD in children aged 6 to 17 years.

Clinical Use. *Guanfacine* is well absorbed after oral administration. About 50% of the drug appears unchanged in the urine; the rest is metabolized, predominantly by CYP3A4; thus, inhibitors and inducers of CYP3A4 can alter the area under the curve. The $t_{1/2}$ for elimination ranges from 12 to 24 h. *Guanfacine* and *clonidine* appear to have similar efficacy for the treatment of hypertension and a similar pattern of adverse effects. A withdrawal syndrome may occur after the abrupt discontinuation, but it is less frequent and milder than the syndrome that follows *clonidine* withdrawal; this difference may relate to the longer $t_{1/2}$ of *guanfacine*.

Guanabenz

Guanabenz is a centrally acting α_2 agonist that decreases blood pressure by a mechanism similar to those of *clonidine* and *guanfacine*. *Guanabenz* has a $t_{1/2}$ of 4 to 6 h and is extensively metabolized by the liver. Dosage adjustment may be necessary in patients with hepatic cirrhosis. The adverse effects caused by *guanabenz* (e.g., dry mouth and sedation) are similar to those seen with *clonidine*.

Methyldopa

Methyldopa (α-methyl-3,4-dihydroxyphenylalanine) is a centrally acting antihypertensive agent. It is metabolized to α-methylnorepinephrine in the brain, and this compound is thought to activate central α_2 receptors and lower blood pressure in a manner similar to that of *clonidine* (see Chapter 32).

Tizanidine

Tizanidine is a muscle relaxant used for the treatment of spasticity associated with cerebral and spinal disorders (see Chapter 13). It is also an α_2 agonist with some properties similar to those of *clonidine*.

Moxonidine

Moxonidine is a mixed α_2 receptor and imidazole I_1 receptor agonist. It acts to reduce sympathetic outflow from the CNS and thereby reduces blood pressure. *Moxonidine* also has analgesic activity, interacts synergistically with opioid agonists, and is used in treating neuropathic pain.

β Adrenergic Receptor Agonists

β Adrenergic receptor agonists play a major role only in the treatment of bronchoconstriction in patients with asthma (reversible airway obstruction) or COPD. Minor uses include management of preterm labor, treatment of complete heart block in shock, and short-term treatment of cardiac decompensation after surgery or in patients with congestive heart failure or myocardial infarction. The development of β_2-selective agonists has resulted in drugs with more valuable characteristics, including adequate oral bioavailability, lack of α adrenergic activity, and relative lack of β_1 adrenergic activity, and thus diminished likelihood of adverse cardiovascular effects. These agents are useful in the treatment of asthma and are more widely used than the nonselective β agonist *isoproterenol* or the primarily β_1-selective agonist *dobutamine*. Emerging β_3-selective adrenergic receptor agonists are being used for overactive bladder syndrome and are in preclinical testing for a host of disorders (Schena and Caplan, 2019).

β Receptor agonists, especially the β_1-selective agonist *dobutamine*, may be used to stimulate the rate and force of cardiac contraction and relaxation. The chronotropic effect is useful in the emergency treatment of arrhythmias such as *torsades de pointes*, bradycardia, or heart block (see Chapter 34), whereas the inotropic effect is useful when it is desirable to augment myocardial contractility.

Nonselective Adrenergic Receptor Agonists

Isoproterenol

Isoproterenol (INE, isopropyl norepinephrine, isoprenaline, isopropylarterenol, isopropyl noradrenaline, *d,l*-β-[3,4-dihydroxyphenyl]-α-isopropylaminoethanol) (see Table 14–1) is a potent, nonselective β adrenergic receptor agonist with very low affinity for α receptors. Consequently, INE has powerful effects on all β receptors and almost no action at α receptors; it is often used experimentally to activate all cellular β receptors.

Pharmacological Actions. The major cardiovascular effects of INE (compared with EPI and NE) are illustrated in Figure 14–2. Intravenous infusion of INE lowers peripheral vascular resistance, primarily in skeletal muscle but also in renal and mesenteric vascular beds. Diastolic pressure falls. Systolic blood pressure may remain unchanged or rise, although mean arterial pressure typically falls. Cardiac output is increased because of the positive inotropic and chronotropic effects of the drug in the face of diminished peripheral vascular resistance. The cardiac effects of INE may lead to palpitations, sinus tachycardia, and more serious arrhythmias; large doses of INE cause myocardial cell death in experimental animals.

Isoproterenol relaxes almost all varieties of smooth muscle when the tone is high, an action that is most pronounced on bronchial and GI smooth muscle. It also prevents or relieves bronchoconstriction. The effects of INE in asthma may be due in part to an additional action to inhibit antigen-induced release of histamine and other mediators of inflammation, an action shared by β_2-selective stimulants.

ADME. *Isoproterenol* is readily absorbed when given parenterally or as an aerosol. It is metabolized by COMT, primarily in the liver but also by other tissues. Of note, INE is a relatively poor substrate for MAO and NET (NE transporter) and is not taken up by sympathetic neurons to the same extent as are EPI and NE. The duration of action of INE therefore may be longer than that of EPI but is still relatively brief.

Therapeutic Uses. *Isoproterenol* may be used in emergencies to stimulate heart rate in patients with bradycardia or heart block, particularly in anticipation of inserting an artificial cardiac pacemaker or in patients with the ventricular arrhythmia torsades de pointes. In disorders such as asthma and shock, INE largely has been replaced by other sympathomimetic drugs (see further in this chapter and in Chapter 44).

Adverse Effects. Palpitations, tachycardia, headache, and flushing are common. Cardiac ischemia and arrhythmias may occur, particularly in patients with underlying coronary artery disease.

β_1-Selective Adrenergic Receptor Agonists

Dobutamine

Dobutamine resembles DA structurally but possesses a bulky aromatic substituent on the amino group (Table 14–1). The pharmacological effects of *dobutamine* are due to direct interactions with α and β receptors; its actions do not appear to result from release of NE from sympathetic nerve endings, and they are not exerted by dopaminergic receptors. *Dobutamine* possesses a center of asymmetry; both enantiomeric forms are present in the racemate used clinically. The (–) isomer of *dobutamine* is a potent α_1 agonist and can cause marked pressor responses. In contrast, (+)-*dobutamine* is a potent α_1 receptor antagonist, which can block the effects of (–)-*dobutamine* on a receptor and the primary effect is potent β_1 receptor stimulation as both isomers are full agonists at β_1 receptors. The (+) isomer of *dobutamine* is a more potent β agonist than the (–) isomer by approximately 10-fold.

Cardiovascular Effects. The cardiovascular effects of racemic *dobutamine* represent a composite of the distinct pharmacological properties of the (–) and (+) stereoisomers. Compared to INE, *dobutamine* has relatively more prominent inotropic than chronotropic effects on the heart due to its selectivity to the β_1 receptor. Although not completely understood, this useful selectivity may arise because peripheral resistance is relatively unchanged. At equivalent inotropic doses, *dobutamine* enhances automaticity of the sinus node to a lesser extent than does INE; however, enhancement of AV and intraventricular conduction is similar for both drugs. Heart rate increases only modestly when *dobutamine* is administered at less than 20 μg/kg per min. After administration of nonselective or β_1-selective β receptor antagonists, infusion of *dobutamine* fails to increase cardiac output, but total peripheral resistance increases, confirming that *dobutamine* has some modest effects on α adrenergic receptors in the vasculature.

ADME. *Dobutamine* has a $t_{1/2}$ of about 2 min; the major metabolites are conjugates of *dobutamine* and 3-*O*-methyldobutamine. The onset of effect is rapid. Steady-state concentrations generally are achieved within 10 min of initiation of the infusion by calibrated infusion pump. The rate of infusion required to increase cardiac output typically is between 2.5 and 10 μg/kg per min, although higher infusion rates occasionally are required. The rate and duration of the infusion are determined by the clinical and hemodynamic responses of the patient.

Therapeutic Uses. *Dobutamine* is indicated for the short-term treatment of cardiac decompensation that may occur after cardiac surgery or in patients with acute decompensated congestive heart failure requiring hospitalization or acute myocardial infarction. *Dobutamine* increases cardiac output and stroke volume in such patients, usually without a marked increase in heart rate. Alterations in blood pressure or peripheral resistance usually are minor, although some patients may have marked increases in blood pressure or heart rate. An infusion of *dobutamine* in combination with echocardiography is useful in the noninvasive assessment of patients with coronary artery disease.

Adverse Effects. Blood pressure and heart rate may increase significantly during *dobutamine* administration, requiring reduction of infusion rate. Patients with a history of hypertension may exhibit an exaggerated pressor response more frequently. Because *dobutamine* facilitates AV conduction, patients with atrial fibrillation are at risk of marked increases in ventricular response rates; *digoxin* or other measures may be required to prevent this from occurring. Some patients may develop ventricular ectopic activity. *Dobutamine* may increase the size of a myocardial infarct by increasing myocardial O_2 demand, a property common to inotropic agents. The efficacy of *dobutamine* over a period of more than a few days is uncertain; there is evidence for the development of tolerance, which is due to receptor desensitization mediated by GPCR kinases (Sato et al., 2015).

β_2-Selective Adrenergic Receptor Agonists

β_2-Selective agents have been developed to avoid adverse effects on the heart by INE and *dobutamine* acting on β_1 receptors when used for asthma or COPD (Billington et al., 2017). This selectivity for β_2 receptors, however, is not absolute and is lost at high concentrations of these drugs. Moreover, up to 40% of the β receptors in the human heart are β_2 receptors, activation of which can also cause cardiac stimulation (Rockman et al., 2002).

A second strategy that has increased the usefulness of several β_2-selective agonists in the treatment of asthma and COPD has been structural modification that results in lower rates of metabolism and enhanced oral bioavailability. Modifications have included placing the hydroxyl groups at positions 3 and 5 of the phenyl ring or substituting another moiety for the hydroxyl group at position 3. This has yielded drugs such as *metaproterenol*, *terbutaline*, and *albuterol*, which are not substrates for COMT. Bulky substituents on the amino group of catecholamines contribute to potency at β receptors with decreased activity at α receptors and decreased metabolism by MAO.

A final strategy to enhance preferential activation of pulmonary β_2 receptors is the administration by inhalation of small doses of the drug in aerosol form. This approach typically leads to effective activation of β_2 receptors in the bronchi but very low systemic drug concentrations. Consequently, there is less potential to activate cardiac β_1 or β_2 receptors or to stimulate β_2 receptors in skeletal muscle, which can cause tremor and thereby limit oral therapy.

Subcutaneous injection also causes prompt bronchodilation; for an orally administered agent, the peak effect may be delayed for several hours. Administration of β receptor agonists by aerosol (see Chapter 44) typically leads to a very rapid therapeutic response, generally within minutes, although some agonists such as *salmeterol* have a delayed onset of action. Aerosol therapy depends on the delivery of drug to the distal airways. This, in turn, depends on the size of the particles in the aerosol and respiratory parameters such as inspiratory flow rate, tidal volume, breath-holding time, and airway diameter. Only about 10% of an inhaled dose actually enters the lungs; much of the remainder is swallowed and ultimately may be absorbed. Successful aerosol therapy requires that each patient master the technique of drug administration. In some patients, particularly children and the elderly, spacer devices may enhance the efficacy of inhalation therapy.

In the treatment of asthma and COPD, β_2 receptor agonists are used to activate pulmonary receptors that relax bronchial smooth muscle and decrease airway resistance. These drugs also may suppress the release of leukotrienes and histamine from mast cells in lung tissue, enhance mucociliary function, decrease microvascular permeability, and possibly inhibit phospholipase A_2. Airway inflammation also contributes airway hyperresponsiveness; consequently, the use of anti-inflammatory drugs such as inhaled steroids has primary importance. Most authorities recommend that long-acting β_2 agonists should not be used without concomitant anti-inflammatory therapy in the treatment of asthma (see Chapter 44).

Short-Acting β_2 Adrenergic Agonists

Metaproterenol. *Metaproterenol* (called *orciprenaline* in Europe), along with *terbutaline* and *fenoterol,* belongs to the structural class of resorcinol bronchodilators that have hydroxyl groups at positions 3 and 5 of the phenyl ring (rather than at positions 3 and 4 as in catechols) (see Table 14–1). Consequently, *metaproterenol* is resistant to methylation by COMT, and a substantial fraction (40%) is absorbed in active form after oral administration. It is excreted primarily as glucuronic acid conjugates. *Metaproterenol* is considered β_2 selective, although it probably is less selective than *albuterol* or *terbutaline* and hence is more prone to cause cardiac stimulation. Effects occur within minutes of inhalation and persist for several hours. After oral administration, onset of action is slower, but effects last 3 to 4 h. *Metaproterenol* is used for the long-term treatment of obstructive airway diseases and asthma and for treatment of acute bronchospasm (see Chapter 44). Side effects are similar to the short- and intermediate-acting sympathomimetic bronchodilators.

Albuterol. *Albuterol* is a selective β_2 adrenergic receptor agonist with pharmacological properties and therapeutic indications similar to those of *terbutaline*. It can be administered by inhalation or orally for the symptomatic relief of bronchospasm. When administered by inhalation, it produces significant bronchodilation within 15 min, and effects persist for 3 to 4 h. The cardiovascular effects of *albuterol* are much weaker than those of INE when doses that produce comparable bronchodilation are administered by inhalation. Oral *albuterol* has the potential to delay preterm labor. Although rare, CNS and respiratory side effects are sometimes observed.

Albuterol has been made available in a metered-dose inhaler free of CFCs (chlorofluorocarbons). The alternate propellant, HFA (hydrofluoroalkane), is inert in the human airway, but unlike CFCs, it does not deplete stratospheric ozone.

Levalbuterol. *Levalbuterol* is the *R*-enantiomer of *albuterol*, a racemate used to treat asthma and COPD. Although originally available only as a solution for a nebulizer, it is now available as a CFC-free metered-dose inhaler. *Levalbuterol* is β_2 selective and acts like other β_2 adrenergic agonists. In general, *levalbuterol* has similar pharmacokinetic and pharmacodynamic properties as *albuterol*.

Pirbuterol. *Pirbuterol* is a relatively selective β_2 adrenergic receptor agonist. Its structure differs from that of *albuterol* by the substitution of a pyridine ring for the benzene ring. *Pirbuterol acetate* is available for inhalation therapy; dosing is typically every 4 to 6 h. *Pirbuterol* is the only preparation available in a breath-activated metered-dose inhaler, a device meant to optimize medication delivery by releasing a spray of medication only on the patient's initiation of inspiration.

Terbutaline. *Terbutaline* is a β_2-selective bronchodilator. It contains a resorcinol ring and thus is not a substrate for COMT methylation. It is effective when taken orally or subcutaneously or by inhalation (not marketed for inhalation in the U.S.). Effects are observed rapidly after inhalation or parenteral administration; after inhalation, its action may persist 3 to 6 h. With oral administration, the onset of effect may be delayed 1 to 2 h. *Terbutaline* is used for the long-term treatment of obstructive airway diseases and for treatment of acute bronchospasm; it also is available for parenteral use for the emergency treatment of status asthmaticus (see Chapter 44). It should not be used as a tocolytic (FDA boxed warning).

Fenoterol. *Fenoterol* is a β_2-selective receptor agonist. After inhalation, it has a prompt onset of action, and its effect typically is sustained for 4 to 6 h. A possible association of *fenoterol* use with increased deaths from asthma, although controversial (Billington et al., 2017), has led to its withdrawal from the market. The dysrhythmias and cardiac effects associated with *fenoterol* are likely due to effects on β_1 adrenergic receptors.

Procaterol. *Procaterol* is a β_2-selective receptor agonist. After inhalation, it has a prompt onset of action that is sustained for about 5 h. *Procaterol* is not available in the U.S.

Long-Acting β_2 Adrenergic Agonists (LABAs)

Salmeterol

Mechanism of Action. *Salmeterol* is a lipophilic β_2-selective agonist with a prolonged duration of action (>12 h) and a selectivity for β_2 receptors about 50-fold greater than that of *albuterol*. *Salmeterol* provides symptomatic relief and improves lung function and quality of life in patients with COPD. It is as effective as the cholinergic antagonist *ipratropium*, more effective than *theophylline*, and has additive effects when used in combination with inhaled *ipratropium* or oral *theophylline*. *Salmeterol* also may have anti-inflammatory activity and has been reported to be a G_s-biased agonist at β_2 receptors, although the impact of these characteristics on its clinical effects are not known.

ADME. The onset of action of inhaled *salmeterol* is relatively slow, so it is not suitable monotherapy for acute attacks of bronchospasm. *Salmeterol* is metabolized by cytochrome P450 (CYP) 3A4 to α-hydroxy-salmeterol, which is eliminated primarily in the feces.

Clinical Use, Precautions, and Adverse Effects. *Salmeterol* and *formoterol* (below) are the agents of choice for nocturnal asthma in patients who remain symptomatic despite anti-inflammatory agents and other standard management. *Salmeterol* is generally well tolerated but has the potential to increase heart rate and plasma glucose concentration, to produce tremors, and to decrease plasma K^+ concentration through effects on extrapulmonary β_2 receptors. *Salmeterol* should not be used more than twice daily (morning and evening) and should not be used to treat acute asthma symptoms, which should be treated with a short-acting β_2 agonist (e.g., *albuterol*) when breakthrough symptoms occur.

Patients with moderate or severe persistent asthma or COPD benefit from the use of LABAs like *salmeterol* in combination with an inhaled corticosteroid. For that reason, *salmeterol* is available in a single formulate combination with the corticosteroid *fluticasone*. These benefits must be counterbalanced against data, oft-criticized, showing that the addition of a LABA to "usual therapy" was associated with an increased risk of fatal or near-fatal asthmatic attacks, as compared with usual therapy alone. On the other hand, there is a lack of reports of increased asthma mortality among patients taking both a LABA and an inhaled corticosteroid (Fanta, 2009). Nevertheless, the FDA has placed a **black-box warning** in the labeling information for *salmeterol, formoterol*, and *arformoterol*. Expert panels (Fanta, 2009) recommend the use of LABAs only for patients in whom inhaled corticosteroids alone either failed to achieve good asthma control or for initial therapy.

Formoterol. *Formoterol* is a long-acting β_2-selective receptor agonist that induces significant bronchodilation that may persist for up to 12 h, with effects starting within minutes of inhalation of a therapeutic dose. It is highly lipophilic and has high affinity for β_2 receptors. Its major advantage over many other β_2-selective agonists is this prolonged duration of action, which may be particularly advantageous in settings such as nocturnal asthma. *Formoterol*'s sustained action is due to its insertion into the lipid bilayer of the plasma membrane, from which it gradually diffuses to provide prolonged stimulation of β_2 receptors. It is FDA approved for treatment of asthma and bronchospasm, prophylaxis of exercise-induced bronchospasm, and COPD. It can be used concomitantly with short-acting β_2 agonists, glucocorticoids (inhaled or systemic), and *theophylline* (Billington et al., 2017). *Formoterol* is also available as a single formulaic combination with the glucocorticoids *mometasone* or *budesonide* for treatment of COPD.

Arformoterol. *Arformoterol*, an enantiomer of *formoterol*, is a selective LABA that has twice the potency of racemic *formoterol*. It is FDA approved for the long-term treatment of bronchoconstriction in patients with COPD, including chronic bronchitis and emphysema (Matera and Cazzola, 2007). Systemic exposure to *arformoterol* is due to pulmonary absorption, with plasma levels reaching a peak in 0.25 to 1 h. It is primarily metabolized by direct conjugation to glucuronide or sulfate conjugates and secondarily by O-demethylation by CYP2D6 and CYP2C19. It does not inhibit any of the common CYPs (Fanta, 2009).

Very Long-Acting β_2 Adrenergic Agonists (VLABAs)

Very long-acting or ultra β_2 adrenergic receptor agonists have been developed primarily for treating COPD. These drugs are not recommended for treating asthma.

Indacaterol, the first once-daily VLABA approved for COPD, is a potent β_2 agonist with high intrinsic efficacy. It has a fast onset of action, appears well tolerated, and is effective in COPD with little tachyphylaxis on continued use. In contrast to *salmeterol*, *indacaterol* does not antagonize the bronchorelaxant effect of short-acting β_2 adrenergic agonists.

Olodaterol is also a once-daily VLABA approved for use in COPD. It is also offered in combination with *tiotropium bromide*, an antagonist at M_3 muscarinic receptors.

Vilanterol is a VLABA approved for use in combination with *fluticasone*. *Vilanterol* is available in Europe in combination with the long-acting muscarinic antagonist *umeclidinium*.

Other β_2-Selective Agonists

Ritodrine. *Ritodrine* is a β_2-selective agonist that was developed specifically for use as a uterine relaxant. Its pharmacological properties closely resemble those of the other agents in this group. The pharmacokinetic properties of *ritodrine* are complex and incompletely defined, especially in pregnant women. *Ritodrine* is rapidly but incompletely (30%) absorbed following oral administration: The drug may be administered intravenously to selected patients to arrest premature labor. β_2-Selective agonists may not have clinically significant benefits on perinatal mortality and may actually increase maternal morbidity. *Ritodrine* is not available in the U.S.

Adverse Effects of β_2-Selective Agonists

The major adverse effects of β_2 receptor agonists occur as a result of excessive activation of β adrenergic receptors. Patients with underlying cardiovascular disease are particularly at risk for significant reactions. However, the likelihood of adverse effects can be greatly decreased in patients with lung disease by administering the drug by inhalation rather than orally or parenterally. Tremor is a relatively common adverse effect of the β_2-selective adrenergic receptor agonists. Tolerance generally develops to this effect; it is not clear whether tolerance reflects desensitization of the β_2 receptors of skeletal muscle or adaptation within the CNS. This adverse effect can be minimized by starting oral therapy with a low dose of drug and progressively increasing the dose as tolerance to the tremor develops. Feelings of restlessness, apprehension, and anxiety may limit therapy with these drugs, particularly oral or parenteral administration.

Tachycardia is a common adverse effect of systemically administered β receptor agonists. Stimulation of heart rate occurs primarily with higher doses via activation of β_1 adrenergic receptors. It is uncertain to what extent the increase in heart rate also is due to activation of cardiac β_2 receptors or to reflex effects that stem from β_2 receptor–mediated peripheral vasodilation. During a severe asthma attack, heart rate actually may decrease during therapy with a β_2 agonist, presumably because of improvement in pulmonary function with consequent reduction in endogenous cardiac sympathetic stimulation. In patients without cardiac disease, β_2 agonists rarely cause significant arrhythmias or myocardial ischemia; however, patients with underlying coronary artery disease or preexisting arrhythmias are at greater risk. The risk of adverse cardiovascular effects also is increased in patients who are receiving MAO inhibitors. In general, at least 2 weeks should elapse between the use of MAO inhibitors and administration of β_2 agonists or other sympathomimetics.

When given parenterally, these drugs also may increase the concentrations of glucose, lactate, and free fatty acids in plasma and decrease the concentration of K^+. The decrease in K^+ concentration may be especially important in patients with cardiac disease, particularly those taking *digoxin* and diuretics. In some diabetic patients, hyperglycemia may be worsened by these drugs, and higher doses of insulin may be required. Side effects of LABAs and VLABAs include nasopharyngitis and increase in incidence of pneumonia. As a result of these side effects, postmarketing safety studies are under way.

β_3 Adrenergic Receptor Agonists

The existence of the β_3 adrenergic receptor subtype was first proposed in the 1970s but was not confirmed until the receptor was cloned in 1989 (Emorine et al., 1989). The β_3 adrenergic receptor couples to the G_s-cyclic AMP pathway and has a much stronger affinity for NE than EPI. There is also significant coupling to the G_i pathway that leads to activation of nitric oxide (NO) synthases, accumulation of NO, and cyclic GMP (Cannavo and Koch, 2017; Schena and Caplan, 2019). The β_3 receptor displays much lower affinities for classic β antagonists (such as *propranolol* or *atenolol*) that are better at blocking β_1 and β_2 receptors. In humans, the β_3 adrenergic receptor is expressed in brown adipose tissue, gallbladder, and ileum and to a lesser extent in white adipose tissue and the detrusor muscle of the bladder; however, there is appreciable expression in myocardium (Cannavo and Koch, 2017). To date, the major therapeutic target that has emerged from this field has been the development of β_3 receptor agonists for use in urinary incontinence (Schena and Caplan, 2019). β_3 Receptors are prominent in brown and white adipose tissues where, in rodents, their activation can increase brown fat and stimulate lipolysis. Such observations have made these receptors an attractive target for weight loss, with clinical trials proceeding in this area. The absence of cardioprotective NO-dependent signaling in β_3 receptor knockout mice (Cannavo and Koch, 2017) has led to preclinical studies and clinical trials of β_3 agonists for cardiovascular indications. In addition to the current indication for β_3 agonists for overactive bladder disease, there are ongoing clinical trials testing their efficacy in irritable bowel syndrome.

Mirabegron is a β_3-selective adrenergic receptor agonist approved for use against incontinence due to overactive bladder. Activation of this receptor in the bladder leads to detrusor muscle relaxation and increased bladder capacity. This action prevents voiding and provides relief for those with an overactive bladder and urinary incontinence. Side effects include increased blood pressure, increased incidence of urinary tract infection, and headache. *Mirabegron* is also a moderate CYP2D6 inhibitor, so care must be taken when prescribing with other drugs metabolized by CYP2D6, such as *digoxin*, *metoprolol*, and *desipramine*. *Mirabegron* is being used in clinical trials for induction of brown fat in humans and also heart failure.

Vibegron is another β_3-selective adrenergic receptor agonist also approved for overactive bladder syndrome and being tested preclinically and clinically for other indications.

Miscellaneous Sympathomimetic Agonists

Amphetamine

Amphetamine, racemic β phenylisopropylamine (see Table 14–1), has powerful CNS stimulant actions in addition to the peripheral α and β receptor actions common to indirect-acting sympathomimetic drugs. Unlike EPI, it is effective after oral administration, and its effects last for several hours.

Cardiovascular System

Amphetamine given orally raises both systolic and diastolic blood pressure. Heart rate often is slowed via reflex mechanisms; with large doses, cardiac arrhythmias may occur. Cardiac output is not enhanced by therapeutic doses, and cerebral blood flow does not change much. The *l*-isomer is slightly more potent than the *d*-isomer in its cardiovascular actions.

Other Smooth Muscles

In general, smooth muscles respond to *amphetamine* as they do to other sympathomimetic amines. The contractile effect on the sphincter of the urinary bladder is particularly marked, and for this reason, *amphetamine* has been used in treating enuresis and incontinence. Pain and difficulty in micturition occasionally occur. The GI effects of *amphetamine* are unpredictable. If enteric activity is pronounced, *amphetamine* may cause relaxation and delay the movement of intestinal contents; if the gut already is relaxed, the opposite effect may occur. The response of the human uterus varies, but there usually is an increase in tone.

CNS

Amphetamine is one of the most potent sympathomimetic amines in stimulating the CNS. It stimulates the medullary respiratory center, lessens the degree of central depression caused by various drugs, and produces other signs of CNS stimulation. In eliciting CNS excitatory effects, the *d*-isomer (dextroamphetamine) is three to four times more potent than the *l*-isomer. The psychic effects depend on the dose and the mental state and personality of the individual. The main results of an oral dose of 10 to 30 mg include wakefulness, alertness, and a decreased sense of fatigue; elevation of mood, with increased initiative, self-confidence, and ability to concentrate; often, elation and euphoria; and increase in motor and speech activities. Performance of simple mental tasks is improved, but, although more work may be accomplished, the number of errors may increase. Physical performance (e.g., in athletes) is improved, and the drug often is abused for this purpose. These effects are variable and may be reversed by overdosage or repeated usage. Prolonged use or large doses are nearly always followed by depression and fatigue. Many individuals given *amphetamine* experience headache, palpitation, dizziness, vasomotor disturbances, agitation, confusion, dysphoria, apprehension, delirium, or fatigue.

Fatigue and Sleep. In general, *amphetamine* prolongs the duration of adequate performance before fatigue appears, and the effects of fatigue are at least partly reversed, most strikingly when performance has been reduced by fatigue and lack of sleep. Such improvement may be partly due to alteration of unfavorable attitudes toward the task. However, *amphetamine* reduces the frequency of attention lapses that impair performance after prolonged sleep deprivation and thus improves execution of tasks requiring sustained attention. The need for sleep may be postponed, but it cannot be avoided indefinitely. When the drug is discontinued after long use, the pattern of sleep may take as long as 2 months to return to normal.

Analgesia. *Amphetamine* and some other sympathomimetic amines have a small analgesic effect that is not sufficiently pronounced to be therapeutically useful. However, *amphetamine* can enhance the analgesia produced by opiates.

Respiration. *Amphetamine* stimulates the respiratory center, increasing the rate and depth of respiration. In normal individuals, usual doses of the drug do not appreciably increase respiratory rate or minute volume. Nevertheless, when respiration is depressed by centrally acting drugs, *amphetamine* may stimulate respiration.

Appetite. *Amphetamine* and similar drugs have been used for the treatment of obesity, although the wisdom of this use is at best questionable. Weight loss in obese humans treated with *amphetamine* is almost entirely due to reduced food intake and only in small measure to increased metabolism. The site of action probably is in the lateral hypothalamic feeding center; injection of *amphetamine* into this area, but not into the ventromedial region, suppresses food intake. Neurochemical mechanisms of action are unclear but may involve increased release of NE or DA. In humans, tolerance to the appetite suppression develops rapidly. Hence, continuous weight reduction usually is not observed in obese individuals without dietary restriction.

Mechanisms of Action in the CNS

Amphetamine exerts most or all of its effects in the CNS by releasing biogenic amines from their storage sites in nerve terminals. The neuronal dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT2) appear to be two of the principal targets of *amphetamine*'s action (Fleckenstein, 2007; Sitte and Freissmuth, 2015). These mechanisms include *amphetamine*-induced exchange diffusion, reverse transport, channel-like transport phenomena, and effects resulting from the weakly basic properties of *amphetamine*. *Amphetamine* analogues affect monoamine transporters through phosphorylation, transporter trafficking, and the production of reactive oxygen and nitrogen species. These mechanisms may have potential implications for neurotoxicity as well as dopaminergic neurodegenerative diseases (discussed further in the chapter).

The alerting effect of *amphetamine*, its anorectic effect, and at least a component of its locomotor-stimulating action presumably are mediated by release of NE from central noradrenergic neurons. These effects can be prevented in experimental animals by inhibiting tyrosine hydroxylase and thus catecholamine synthesis. Some aspects of locomotor activity and the stereotyped behavior induced by *amphetamine* probably are a consequence of the release of DA from dopaminergic nerve terminals, particularly in the neostriatum. Higher doses are required to produce these behavioral effects, and this correlates with the higher concentrations of *amphetamine* required to release DA from brain slices or synaptosomes *in vitro*. With still higher doses of *amphetamine*, disturbances of perception and overt psychotic behavior occur. These effects may be due to release of 5HT [(5-hydroxytryptamine (serotonin)] from serotonergic neurons and of DA in the mesolimbic system. In addition, *amphetamine* may exert direct effects on CNS receptors for 5HT (see Chapter 15).

Toxicity and Adverse Effects

The acute toxic effects of *amphetamine* usually are extensions of its therapeutic actions and as a rule result from overdosage. CNS effects commonly include restlessness, dizziness, tremor, hyperactive reflexes, talkativeness, tenseness, irritability, weakness, insomnia, fever, and sometimes euphoria. Confusion, aggressiveness, changes in libido, anxiety, delirium, paranoid hallucinations, panic states, and suicidal or homicidal tendencies occur, especially in mentally ill patients. However, these psychotic effects can be elicited in any individual if sufficient quantities of *amphetamine* are ingested for a prolonged period. Fatigue and depression usually follow central stimulation. Cardiovascular effects are common and include headache, chilliness, pallor or flushing, palpitation, cardiac arrhythmias, anginal pain, hypertension or hypotension, and circulatory collapse. Excessive sweating occurs. GI symptoms include dry mouth, metallic taste, anorexia, nausea, vomiting, diarrhea, and abdominal cramps. Fatal poisoning usually terminates in convulsions and coma, and cerebral hemorrhages are the main pathological findings.

The toxic dose of *amphetamine* varies widely. Toxic manifestations occasionally occur as an idiosyncratic reaction after as little as 2 mg but are rare with doses less than 15 mg. Severe reactions have occurred with 30 mg, yet doses of 400 to 500 mg are not uniformly fatal. Larger doses can be tolerated after chronic use of the drug. Treatment of acute *amphetamine* intoxication may include acidification of the urine by administration of ammonium chloride; this enhances the rate of elimination. Sedatives may be required for the CNS symptoms. Severe hypertension may require administration of sodium nitroprusside or an α adrenergic receptor antagonist.

Chronic intoxication with *amphetamine* causes symptoms similar to those of acute overdosage, but abnormal mental conditions are more common. Weight loss may be marked. A psychotic reaction with vivid hallucinations and paranoid delusions, often mistaken for schizophrenia, is the most common serious effect. Recovery usually is rapid after withdrawal of the drug, but occasionally the condition becomes chronic. In these persons, *amphetamine* may act as a precipitating factor hastening the onset of incipient schizophrenia.

The abuse of *amphetamine* as a means of overcoming sleepiness and of increasing energy and alertness should be discouraged. The drug should be used only under medical supervision. All *amphetamines* are schedule II drugs under federal regulations. The additional contraindications and precautions for the use of *amphetamine* generally are similar to those described for EPI. *Amphetamine* use is inadvisable in patients with anorexia, insomnia, asthenia, psychopathic personality, or a history of homicidal or suicidal tendencies.

Dependence and Tolerance

Psychological dependence often occurs when *amphetamine* or *dextroamphetamine* is used chronically, as discussed in Chapter 28. Tolerance almost invariably develops to the anorexigenic effect of *amphetamines* and often is seen also in the need for increasing doses to maintain improvement of mood in psychiatric patients. Tolerance is striking in individuals who are dependent on the drug; a daily intake of 1.7 g without apparent ill effects has been reported. Development of tolerance is not invariable, and cases of narcolepsy have been treated for years without requiring an increase in the initially effective dose.

Therapeutic Uses

Amphetamine is used chiefly for its CNS effects. *Dextroamphetamine*, with greater CNS action and less peripheral action, is FDA approved for the treatment of narcolepsy and ADHD (see discussion later in this chapter).

Methamphetamine

Methamphetamine is closely related chemically to *amphetamine* and *ephedrine* (see Table 14–1). The drug acts centrally to release DA and other biogenic amines and to inhibit neuronal and VMATs as well as MAO. Small doses have prominent central stimulant effects without significant peripheral actions; somewhat larger doses produce a sustained rise in systolic and diastolic blood pressures, due mainly to cardiac stimulation. Cardiac output is increased, although the heart rate may be slowed via reflex action. Venous constriction causes peripheral venous pressure to increase. These factors tend to increase the venous return and thus cardiac output; pulmonary arterial pressure is raised.

Methamphetamine is widely abused as a cheap, accessible recreational drug. Illegal production of *methamphetamine* in clandestine laboratories throughout the U.S. is common. It is used principally for its central effects, which are more pronounced than those of *amphetamine* and are accompanied by less-prominent peripheral actions.

Methylphenidate

Methylphenidate is a piperidine derivative that is structurally related to *amphetamine*. *Methylphenidate* is a mild CNS stimulant with more prominent effects on mental than on motor activities. However, large doses produce signs of generalized CNS stimulation that may lead to convulsions. The effects of *methylphenidate* resemble those of the *amphetamines*.

Methylphenidate also shares the abuse potential of the *amphetamines* and is listed as a schedule II controlled substance in the U.S. *Methylphenidate* is effective in the treatment of narcolepsy and ADHD (described below). *Methylphenidate* is readily absorbed after oral administration, reaching a peak C_p in about 2 h. The drug is a racemate; its more potent (+) enantiomer has a $t_{1/2}$ of about 6 h; the less-potent (−) enantiomer has a $t_{1/2}$ of approximately 4 h. Concentrations in the brain exceed those in plasma. The main urinary metabolite is a de-esterified product, ritalinic acid, which accounts for 80% of the dose. The use of *methylphenidate* is contraindicated in patients with glaucoma.

Dexmethylphenidate

Dexmethylphenidate is the *d*-threo enantiomer of racemic *methylphenidate*. It is FDA approved for the treatment of ADHD and is listed as a schedule II controlled substance in the U.S.

Pemoline

Pemoline is structurally dissimilar to *methylphenidate* but elicits similar changes in CNS function with minimal effects on the cardiovascular system. It is employed in treating ADHD. It can be given once daily because of its long $t_{1/2}$. Clinical improvement may require treatment for 3 to 4 weeks. Use of *pemoline* has been associated with severe hepatic failure. The drug was discontinued in the U.S. in 2006.

Lisdexamphetamine

Lisdexamphetamine is a therapeutically inactive prodrug that is converted primarily in the blood to lysine and D-amphetamine, the active component (Childress and Berry, 2012). It is approved for the treatment of ADHD in children, adolescents, and adults. The drug produces mild-to-moderate side effects, including decreased appetite, dizziness, dry mouth, fatigue, headache, insomnia, irritability, nasal congestion, nasal pharyngitis, upper respiratory infection, vomiting, and decreased weight.

Ephedrine

Ephedrine is an agonist at both α and β receptors; in addition, it enhances release of NE from sympathetic neurons and thus is a mixed-acting sympathomimetic (see Table 14–1 and Figure 14–1). Only *l*-ephedrine and racemic ephedrine are used clinically.

ADME and Pharmacological Actions

Ephedrine is effective after oral administration; effects may persist for several hours. *Ephedrine* is eliminated in the urine largely as unchanged drug, with a $t_{1/2}$ of 3 to 6 h. The drug stimulates heart rate and cardiac output and variably increases peripheral resistance; as a result, *ephedrine* usually increases blood pressure. Stimulation of the α adrenergic receptors of smooth muscle cells in the bladder base may increase the resistance to the outflow of urine. Activation of β adrenergic receptors in the lungs promotes bronchodilation. *Ephedrine* is a potent CNS stimulant.

Therapeutic Uses and Untoward Effects

The use of *ephedrine* as a bronchodilator in asthmatic patients is less common with the availability of β_2-selective receptor agonists. *Ephedrine* has been used to promote urinary continence. Indeed, the drug may cause urinary retention, particularly in men with benign prostatic hyperplasia (BPH). *Ephedrine* also has been used to treat the hypotension that may occur with spinal anesthesia.

Untoward effects of *ephedrine* include hypertension and insomnia. Tachyphylaxis may occur with repetitive dosing. Usual or higher-than-recommended doses may cause important adverse effects in susceptible individuals, especially in patients with underlying cardiovascular disease that might be unrecognized. Large amounts of herbal preparations containing *ephedrine* (ma huang, ephedra) are utilized around the world. There can be considerable variability in the content of *ephedrine* in these preparations, which may result in inadvertent consumption of higher-than-usual doses of *ephedrine* and its isomers, leading to significant toxicity and death. Thus, the FDA has banned the sale of dietary supplements containing *ephedra*. In addition, the Combat Methamphetamine Epidemic Act of 2005 regulates the sale of *ephedrine*, *phenylpropanolamine*, and *pseudoephedrine*, which can be used as precursors in the illicit manufacture of *amphetamine* and *methamphetamine*.

Other Sympathomimetic Agents

Several sympathomimetic drugs (e.g., *propylhexedrine, naphazoline, oxymetazoline*, and *xylometazoline*) are used primarily as vasoconstrictors for local application to the nasal mucous membrane or the eye.

Phenylephrine, *pseudoephedrine* (a stereoisomer of *ephedrine*), and *phenylpropanolamine* are the sympathomimetic drugs that have been used most commonly in oral preparations for the relief of nasal congestion. *Pseudoephedrine* is available without a prescription in a variety of solid and liquid dosage forms. *Phenylpropanolamine* shares the pharmacological properties of *ephedrine* and is approximately equal in potency except that it causes less CNS stimulation. Due to concern about the possibility that *phenylpropanolamine* increases the risk of hemorrhagic stroke, the drug is no longer licensed for marketing in the U.S.

Therapeutic Uses of Sympathomimetic Drugs

Shock

Shock is a clinical syndrome characterized by inadequate perfusion of tissues; it usually is associated with hypotension and ultimately with the failure of organ systems. Shock is an immediately life-threatening impairment of delivery of O_2 and nutrients to the organs of the body. Causes of shock include hypovolemia; cardiac failure; obstruction to cardiac output (due to pulmonary embolism, pericardial tamponade, or aortic dissection); and peripheral circulatory dysfunction (sepsis or anaphylaxis). The treatment of shock consists of specific efforts to reverse the underlying pathogenesis as well as nonspecific measures aimed at correcting hemodynamic abnormalities. The accompanying fall in blood pressure generally leads to marked activation of the sympathetic nervous system. This, in turn, causes peripheral vasoconstriction and an increase in the rate and force of cardiac contraction. In the initial stages of shock, these mechanisms may maintain blood pressure and cerebral blood flow, although blood flow to the kidneys, skin, and other organs may be decreased, leading to impaired production of urine and metabolic acidosis.

The initial therapy of shock involves basic life support measures. It is essential to maintain blood volume, which often requires monitoring of hemodynamic parameters. Specific therapy (e.g., antibiotics for patients in septic shock) should be initiated immediately. If these measures do not lead to an adequate therapeutic response, it may be necessary to use vasoactive drugs in an effort to improve abnormalities in blood pressure and flow. Many of these pharmacological approaches, while apparently clinically reasonable, are of uncertain efficacy. Adrenergic receptor agonists may be used in an attempt to increase myocardial contractility or to modify peripheral vascular resistance. In general terms, β adrenergic receptor agonists increase heart rate and force of contraction, α adrenergic receptor agonists increase peripheral vascular resistance, and DA promotes dilation of renal and splanchnic vascular beds, in addition to activating β and α receptors.

Cardiogenic shock due to myocardial infarction has a poor prognosis; therapy is aimed at improving peripheral blood flow. Medical intervention is designed to optimize cardiac filling pressure (preload), myocardial contractility, and peripheral resistance (afterload). Preload may be increased by administration of intravenous fluids or reduced with drugs such as diuretics and nitrates. A number of sympathomimetic amines have been used to increase the force of contraction of the heart. Some of these drugs have disadvantages: INE is a powerful chronotropic agent and can greatly increase myocardial O_2 demand; NE intensifies peripheral vasoconstriction; and EPI increases heart rate and may predispose the heart to dangerous arrhythmias. DA is an effective inotropic agent that causes less increase in heart rate than does INE. DA also promotes renal arterial dilation; this may be useful in preserving renal function. When given in high doses (>10–20 μg/kg per min), DA activates α receptors, causing peripheral and renal vasoconstriction. *Dobutamine* has complex pharmacological actions that are mediated by its stereoisomers; the clinical effects of the drug are to increase myocardial contractility with little increase in heart rate or peripheral resistance.

In some patients in shock, hypotension is so severe that vasoconstricting drugs are required to maintain a blood pressure that is adequate for CNS perfusion. The α agonists such as NE, *phenylephrine*, *metaraminol*, *mephentermine*, *midodrine*, *ephedrine*, EPI, DA, and *methoxamine* all have been used for this purpose. This approach may be advantageous in patients with hypotension due to failure of the sympathetic nervous system (e.g., after spinal anesthesia or injury). However, in patients with other forms of shock, such as cardiogenic shock, reflex vasoconstriction generally is intense, and α receptor agonists may further compromise blood flow to organs such as the kidneys and gut and adversely increase the work of the heart. Indeed, vasodilating drugs such as *nitroprusside* are more likely to improve blood flow and decrease cardiac work in such patients by decreasing afterload if a minimally adequate blood pressure can be maintained.

The hemodynamic abnormalities in septic shock are complex and poorly understood. Most patients with septic shock initially have low or barely normal peripheral vascular resistance, possibly owing to excessive effects of endogenously produced NO as well as normal or increased cardiac output. If the syndrome progresses, myocardial depression, increased peripheral resistance, and impaired tissue oxygenation occur. The primary treatment of septic shock is antibiotics. Therapy with drugs such as DA or *dobutamine* is guided by hemodynamic monitoring.

Hypotension

Drugs with predominantly α adrenergic receptor agonist activity can be used to raise blood pressure in patients with decreased peripheral resistance in conditions such as spinal anesthesia or intoxication with antihypertensive medications. However, hypotension per se is not an indication for treatment with these agents unless there is inadequate perfusion of organs such as the brain, heart, or kidneys. Furthermore, adequate replacement of fluid or blood may be more appropriate than drug therapy for many patients with hypotension.

Patients with orthostatic hypotension (excessive fall in blood pressure with standing) often represent a pharmacological challenge. There are diverse causes for this disorder, including the Shy-Drager syndrome and idiopathic autonomic failure. Therapeutic approaches include physical maneuvers and a variety of drugs (*fludrocortisone*, prostaglandin synthesis inhibitors, somatostatin analogues, caffeine, vasopressin analogues, and DA antagonists). A number of sympathomimetic drugs also have been used in treating this disorder. The ideal agent would enhance venous constriction prominently and produce relatively little arterial constriction to avoid supine hypertension. No such agent currently is available. Drugs used in this disorder to activate α_1 receptors include both direct- and indirect-acting agents. *Midodrine* shows promise in treating this challenging disorder.

Hypertension

Centrally acting α_2 adrenergic receptor agonists such as *clonidine* are useful in the treatment of hypertension. Drug therapy of hypertension is discussed in Chapter 32.

Cardiac Arrhythmias

Cardiopulmonary resuscitation in patients with cardiac arrest due to ventricular fibrillation, electromechanical dissociation, or asystole may be facilitated by drug treatment. EPI is an important therapeutic agent in patients with cardiac arrest; EPI and other α agonists increase diastolic pressure and improve coronary blood flow. The α receptor agonists also help to preserve cerebral blood flow during resuscitation. Cerebral blood vessels are relatively insensitive to the vasoconstricting effects of catecholamines, and perfusion pressure is increased. Consequently, during external cardiac massage, EPI facilitates distribution of the limited cardiac output to the cerebral and coronary circulations. The optimal dose of EPI in patients with cardiac arrest is unclear. Once a cardiac rhythm has been restored, it may be necessary to treat arrhythmias, hypotension, or shock.

In patients with paroxysmal supraventricular tachycardias, particularly those associated with mild hypotension, careful infusion of an α agonist (e.g., *phenylephrine*) to raise blood pressure to about 160 mmHg may end the arrhythmia by increasing vagal tone. However, this method of treatment has been replaced largely by Ca^{2+} channel blockers with clinically significant effects on the AV node, β antagonists, adenosine, and electrical cardioversion (see Chapter 34). A β adrenergic receptor agonist such as INE may be used as adjunctive or temporizing therapy with *atropine* in patients with marked bradycardia who are compromised hemodynamically; if long-term therapy is required, a cardiac pacemaker usually is the treatment of choice.

Congestive Heart Failure

At first glance, sympathetic stimulation of β receptors in the heart would appear to be an important compensatory mechanism for maintenance of cardiac function in patients with congestive heart failure. However, the failing heart does not respond well to excess sympathetic stimulation. *Dobutamine* and other β_1-selective agonists are used acutely in hospitalized decompensated heart failure patients and do improve short-term cardiac contractility; their long-term effects are deleterious in congestive heart failure (Butta et al., 2020). In chronic heart failure, *dobutamine* can add to the already toxic nature of increased catecholamines; moreover, β adrenergic receptors are also desensitized and downregulated in part to increased GPCR kinase activity (Sato et al., 2015). Further, β agonists may increase cardiac output in acute emergency settings such as shock but, again, are indicated only for short-term use. Chronically, β adrenergic receptor antagonists are now part of the standard of care in the treatment of patients with congestive heart failure, a topic covered in detail in Chapter 33.

Local Vascular Effects

Epinephrine is used in surgical procedures in the nose, throat, and larynx to shrink the mucosa and improve visualization by limiting hemorrhage. Simultaneous injection of EPI with local anesthetics retards their absorption and increases the duration of anesthesia (see Chapter 25). Injection of α adrenergic receptor agonists into the penis may be useful in reversing priapism, a complication of the use of α receptor antagonists or phosphodiesterase 5 (PDE5) inhibitors (e.g., *sildenafil*) in the treatment of erectile dysfunction. Both *phenylephrine* and *oxymetazoline* are efficacious vasoconstrictors when applied locally during sinus surgery.

Nasal Decongestion

α Adrenergic receptor agonists are used as nasal decongestants in patients with allergic or vasomotor rhinitis and in acute rhinitis in patients with upper respiratory infections. These drugs probably decrease resistance to airflow by decreasing the volume of the nasal mucosa; this may occur by activation of α receptors in venous capacitance vessels in nasal tissues that have erectile characteristics. The receptors that mediate this effect appear to be α_1 adrenergic receptors. The use of α_2 adrenergic receptor agonists may mediate contraction of arterioles that supply nutrition to the nasal mucosa. Intense constriction of these vessels may cause structural damage to the mucosa. A major limitation of therapy with nasal decongestants is loss of efficacy, “rebound” hyperemia, and worsening of symptoms with chronic use or when the drug is stopped. Although mechanisms are uncertain, possibilities include receptor desensitization and damage to the mucosa. Agonists that are selective for α_1 receptors may be less likely to induce mucosal damage.

In general, α adrenergic receptor agonists for nasal decongestion may be administered either orally or topically. Sympathomimetic decongestants should be used with great caution in patients with hypertension and in men with prostatic enlargement; these agents are contraindicated in patients who are taking MAO inhibitors. Topical decongestants are particularly useful in acute rhinitis because of their more selective site of action, but they are apt to be used excessively by patients, leading to rebound congestion. Oral decongestants are much less likely to cause rebound congestion but carry a greater risk of inducing adverse systemic effects. Patients with uncontrolled hypertension or ischemic heart disease generally should avoid the oral consumption of over-the-counter products or herbal preparations containing sympathomimetic drugs.

Asthma

Use of β adrenergic agonists in the treatment of asthma and COPD is discussed in Chapter 44.

Allergic Reactions

Epinephrine is the drug of choice to reverse the manifestations of serious acute hypersensitivity reactions (e.g., from food, bee sting, or drug allergy). A subcutaneous injection of EPI rapidly relieves itching, hives, and swelling of lips, eyelids, and tongue. In some patients, careful intravenous infusion of EPI may be required to ensure prompt pharmacological effects. This treatment may be life-saving when edema of the glottis threatens airway patency or when there is hypotension or shock in patients with anaphylaxis. In addition to its cardiovascular effects, EPI is thought to activate β receptors that suppress the release from mast cells of mediators such as histamine and leukotrienes. Although glucocorticoids and antihistamines frequently are administered to patients with severe hypersensitivity reactions, EPI remains the mainstay. EPI autoinjectors are employed widely for the emergency self-treatment of anaphylaxis.

Ophthalmic Uses

Ophthalmic uses are discussed in Chapter 74.

Narcolepsy and Sleep/Wake Imbalance

Hypocretin neurons activate wake-promoting pathways in the CNS. A deficiency of hypocretin, likely due to autoimmune destruction of hypocretin neurons, produces narcolepsy, a condition of hypersomnia, including excessive daytime sleepiness and attacks of sleep that may occur suddenly under conditions that are not normally conducive to sleep. Hypocretin agonists will likely be available in the future. At present, treatment relies on the fact that monoamine pathways promote wakefulness; thus, current treatments utilize CNS stimulants, including those that enhance transmission in monoamine pathways (Black et al., 2017).

The CNS stimulants *modafinil* (a mixture of *R*- and *S*-enantiomers) and *armodafinil* (the *R*-enantiomer of modafinil) are first-line agents for narcolepsy. In the U.S., *modafinil* is a schedule IV controlled substance. Its mechanism of action in narcolepsy is unclear. *Methylphenidate* and *amphetamines* are also used. Therapy with *amphetamines* is complicated by the risk of abuse and the likelihood of the development of tolerance. Depression, irritability, and paranoia also may occur. *Amphetamines* may disturb nocturnal sleep, which increases the difficulty of avoiding daytime attacks of sleep in these patients. *Armodafinil* is also indicated to improve wakefulness in shift workers and to combat excessive sleepiness in patients with obstructive sleep apnea-hypopnea syndrome. See previous sections for more details on these agents.

Sodium γ-hydroxybutyrate (Na^+-oxybate) is FDA approved for treating the sleep/wake imbalance and cataplexy of narcolepsy. The mechanism of action of *oxybate* is unknown but likely relates to its structural similarity to glutamate and GABA and to actions on NE and DA neurons mediated by $GABA_B$ receptors. *Oxybate* is a schedule III controlled substance, available through a special program with the manufacturer. *Oxybate* carries an FDA boxed warning about severe CNS depressants and must be used with great caution (see FDA, 2012).

Weight Reduction

Amphetamine promotes weight loss by suppressing appetite rather than by increasing energy expenditure. Other anorexic drugs include *methamphetamine*, *dextroamphetamine* (and a prodrug form, *lisdexamfetamine*), *phentermine*, *benzphetamine*, *phendimetrazine*, *phenmetrazine*, *diethylpropion*, *mazindol*, *phenylpropanolamine*, and *sibutramine* (a mixed adrenergic/serotonergic drug). *Phenmetrazine*, *mazindol*, and *phenylpropanolamine* have been discontinued in the U.S. Available evidence does not support the isolated use of these drugs in the absence of a more comprehensive program that stresses exercise and modification of diet under medical supervision.

Preclinical data show that β_3-selective adrenergic receptor agonists have remarkable antiobesity and antidiabetic effects in rodents, and the drug *mirabegron* (above) has some promising effects in humans (Cypess et al., 2015). β_3 Agonists are being studied in humans, but their use for the treatment of obesity in humans remains a possibility for the future (Dehvari et al., 2018).

Attention-Deficit/Hyperactivity Disorder

The ADHD syndrome, usually first evident in childhood, is characterized by excessive motor activity, difficulty in sustaining attention, and impulsiveness. Children with this disorder frequently are troubled by difficulties in school, impaired interpersonal relationships, and excitability. Academic underachievement is an important characteristic. A substantial number of children with this syndrome have characteristics that persist into adulthood. Behavioral therapy may be helpful in some patients.

Catecholamines may be involved in the control of attention at the level of the cerebral cortex. A variety of stimulant drugs have been utilized in the treatment of ADHD, and they are particularly indicated in moderate-to-severe cases (Cortese, 2020). *Dextroamphetamine* has been demonstrated to be more effective than placebo. *Methylphenidate* is effective in children with ADHD and is the most common intervention. Treatment may start with a dose of 5 mg of *methylphenidate* in the morning and at lunch; the dose is increased gradually over a period of weeks depending on the response as judged by parents, teachers, and the clinician. The total daily dose generally should not exceed 60 mg; because of its short duration of action, most children require two or three doses of *methylphenidate* each day. The timing of doses is adjusted individually in accordance with rapidity of onset of effect and duration of action.

Methylphenidate, *dextroamphetamine*, and *amphetamine* probably have similar efficacy in ADHD and are the preferred drugs in this disorder (Cortese, 2020). Sustained-release preparations of *dextroamphetamine*, *methylphenidate*, *dexmethylphenidate*, and *amphetamine* may be used once daily in children and adults. *Lisdexamfetamine* can be administered once daily, and a transdermal formulation of *methylphenidate* is marketed for daytime use. Potential adverse effects of these medications include insomnia, abdominal pain, anorexia, and weight loss, which may be associated with suppression of growth in children. Minor symptoms may be transient or may respond to adjustment of dosage or administration of the drug with meals. Other drugs that have been utilized include tricyclic antidepressants, antipsychotic agents, and *clonidine*. A sustained-release formulation of guanfacine, an α_{2A} adrenergic receptor agonist, is also being used for ADHD (Cortese, 2020).

Adrenergic Receptor Antagonists

Many types of drugs interfere with the function of the sympathetic nervous system and thus have profound effects on the physiology of sympathetically innervated organs. Several of these drugs are important in clinical medicine, particularly for the treatment of cardiovascular diseases.

The remainder of this chapter focuses on the pharmacology of adrenergic receptor antagonists, drugs that inhibit the interaction of NE, EPI, and other sympathomimetic drugs with α and β adrenergic receptors throughout peripheral tissues (Figure 14–3). Most of these agents are competitive antagonists; an important exception is *phenoxybenzamine*, an irreversible antagonist that binds covalently to α adrenergic receptors.

There are important structural differences among adrenergic receptors, differences that have permitted development of compounds with substantially different affinities for the various receptors. Thus, it is possible to interfere selectively with responses that result from stimulation of the sympathetic nervous system. The selectivity is relative, not absolute.

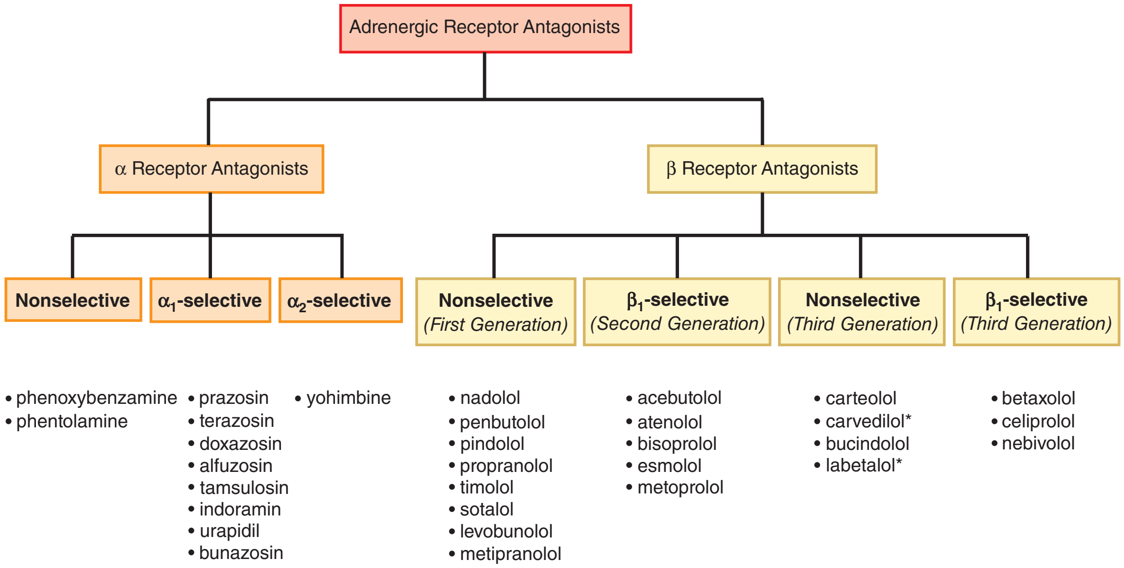


Figure 14–3 *Classification of adrenergic receptor antagonists.* Drugs marked by an asterisk (*) also block α_1 receptors.

Nonetheless, selective antagonists of β_1 adrenergic receptors block most actions of epinephrine and NE on the heart, while having less effect on β_2 receptors in bronchial smooth muscle and no effect on responses mediated by α_1 or α_2 adrenergic receptors. Accordingly, detailed knowledge of the autonomic nervous system and the sites of action of drugs that act on adrenergic receptors is essential for understanding the pharmacological properties and therapeutic uses of this important class of drugs (see Chapter 10). Agents that block dopaminergic receptors are considered in Chapter 15.

α Adrenergic Receptor Antagonists

Overview

The α adrenergic receptors mediate many of the important actions of endogenous catecholamines as detailed above. α_1 Adrenergic receptors mediate contraction of arterial, venous, and visceral smooth muscle, while the α_2 receptors are involved in suppressing sympathetic output, increasing vagal tone, facilitating platelet aggregation, inhibiting the release of NE and acetylcholine from nerve endings, and regulating metabolic effects (e.g., suppression of insulin secretion and inhibition of lipolysis). α_2 Adrenergic receptors also mediate contraction of some arteries and veins.

Some of the most important effects of α receptor antagonists observed clinically are on the cardiovascular system. Actions in both the CNS and the periphery are involved; the outcome depends on the cardiovascular status of the patient at the time of drug administration and the relative selectivity of the agent for α_1 and α_2 receptors.

Antagonists against α adrenergic receptors have a wide spectrum of pharmacological specificities and are chemically heterogeneous. Some of these drugs have markedly different affinities for α_1 and α_2 adrenergic receptors. For example, *prazosin* is much more potent in blocking α_1 than α_2 receptors (i.e., α_1 selective), whereas *yohimbine* is α_2 selective; *phentolamine* has similar affinities for both of these receptor subtypes and is considered a nonselective α-adrenergic receptor antagonist. More recently, agents that discriminate among the various subtypes of a particular receptor have become available; for example, *tamsulosin* has higher potency at α_{1A} than at α_{1B} receptors (Docherty, 2019).

Catecholamines increase the output of glucose from the liver; in humans, this effect is mediated predominantly by β adrenergic receptors, although α adrenergic receptors may contribute. Thus, α receptor antagonists may reduce glucose release. Receptors of the α_{2A} subtype facilitate platelet aggregation; the effect of blockade of platelet α_2 receptors *in vivo* is not clear. Activation of α_2 receptors in the pancreatic islets suppresses insulin secretion; conversely, blockade of pancreatic α_2 adrenergic receptors may facilitate insulin release (see Chapter 51).

Nonselective α Adrenergic Antagonists

Phenoxybenzamine and Phentolamine

Phenoxybenzamine and *phentolamine* are nonselective α adrenergic receptor antagonists. *Phenoxybenzamine*, a haloalkylamine, produces an irreversible antagonism, while *phentolamine*, an imidazaline, produces a competitive antagonism. Both of these drugs cause a progressive decrease in peripheral resistance due to antagonism of α adrenergic receptors in the vasculature and an increase in cardiac output that is due in part to reflex sympathetic nerve stimulation. The cardiac stimulation is accentuated by enhanced release of NE from cardiac sympathetic nerve due to antagonism of presynaptic α_2 adrenergic receptors by these nonselective α blockers. Postural hypotension is a prominent feature with these drugs, and this, accompanied by reflex tachycardia that can precipitate cardiac arrhythmias, severely limits the use of these drugs to treat essential hypertension. The α_1-selective receptor antagonists, such as *prazosin*, have replaced the "classical" α-blockers in the management of essential hypertension. However, *phenoxybenzamine* and *phentolamine* are still marketed for several specialized uses.

Therapeutic Uses. *Phenoxybenzamine* is used in the treatment of pheochromocytomas, tumors of the adrenal medulla and sympathetic neurons that secrete enormous quantities of catecholamines into the circulation. The usual result is hypertension, which may be episodic and severe. The vast majority of pheochromocytomas are treated surgically; *phenoxybenzamine* is often used in preparing the patient for surgery. The drug controls episodes of severe hypertension and minimizes other adverse effects of catecholamines, such as contraction of plasma volume and injury of the myocardium. A conservative approach is to initiate treatment with *phenoxybenzamine* (at a dosage of 10 mg twice daily) 1 to 3 weeks before the operation. The dose is increased every other day until the desired effect on blood pressure is achieved. The usual daily dose of *phenoxybenzamine* in patients with pheochromocytoma is 40 to 120 mg given in two or three divided portions. Prolonged treatment with *phenoxybenzamine* may be necessary in patients with inoperable or malignant pheochromocytoma. In some patients, particularly those with malignant disease, administration of *metyrosine*, a competitive inhibitor of tyrosine hydroxylase (the rate-limiting enzyme in the synthesis of catecholamines), may be a useful adjunct. β Adrenergic receptor antagonists also are used to treat pheochromocytoma, but only after the administration of an α receptor antagonist (described later in the chapter). *Phenoxybenzamine* has been used off-label to control the manifestations of autonomic hyperreflexia in patients with spinal cord transection.

Phentolamine can also be used in short-term control of hypertension in patients with pheochromocytoma. Rapid infusions of *phentolamine* may cause severe hypotension, so the drug should be administered cautiously. *Phentolamine* may be useful in relieving pseudo-obstruction of the bowel in patients with pheochromocytoma.

Phentolamine has been used locally to prevent dermal necrosis after the inadvertent extravasation of an α receptor agonist. The drug also may be useful for the treatment of hypertensive crises that follow the abrupt withdrawal of *clonidine* or that may result from the ingestion of tyramine-containing foods during the use of nonselective MAO inhibitors. Although excessive activation of α receptors is important in the development of severe hypertension in these settings, there is little information about the safety and efficacy of *phentolamine* compared with those of other antihypertensive agents in the treatment of such patients. Buccally or orally administered *phentolamine* may have efficacy in some men with sexual dysfunction.

Phentolamine is FDA approved for reversing or limiting the duration of soft tissue local anesthesia. Sympathomimetics are frequently administered with local anesthetics to slow the removal of the anesthetic by causing vasoconstriction. When the need for anesthesia is over, *phentolamine* can help reverse it by antagonizing the α receptor–induced vasoconstriction.

Toxicity and Adverse Effects. Hypotension is the major adverse effect of *phenoxybenzamine* and *phentolamine*. In addition, reflex cardiac stimulation may cause alarming tachycardia, cardiac arrhythmias, and ischemic cardiac events, including myocardial infarction. Reversible inhibition of ejaculation may occur due to impaired smooth muscle contraction in the vas deferens and ejaculatory ducts. *Phentolamine* stimulates GI smooth muscle, an effect antagonized by *atropine*, and enhances gastric acid secretion due in part to histamine release. Thus, *phentolamine* should be used with caution in patients with a history of peptic ulcer. *Phenoxybenzamine* is mutagenic in the Ames test, and repeated administration of this drug to experimental animals causes peritoneal sarcomas and lung tumors.

α_1-Selective Adrenergic Receptor Antagonists

General Pharmacological Properties

Blockade of α_1 adrenergic receptors inhibits vasoconstriction induced by endogenous catecholamines; vasodilation may occur in both arteriolar resistance vessels and veins. The result is a fall in blood pressure due to decreased peripheral resistance. The magnitude of such effects depends on the activity of the sympathetic nervous system at the time the antagonist is administered and thus is less in supine than in upright subjects and is particularly marked if there is hypovolemia. For most α_1 adrenergic receptor antagonists, the fall in blood pressure is opposed by baroreceptor reflexes that cause increases in heart rate and cardiac output, as well as fluid retention. These reflexes are exaggerated if the antagonist also blocks α_2 adrenergic receptors on peripheral sympathetic nerve endings, leading to enhanced release of NE and increased stimulation of postsynaptic β_1 adrenergic receptors in the heart and on juxtaglomerular cells (Gilsbach and Hein, 2008). Although stimulation of α_1 receptors in the heart may cause an increased force of contraction, the importance of blockade at this site in humans is uncertain.

Blockade of α_1 adrenergic receptors also inhibits vasoconstriction and the increase in blood pressure produced by the administration of a sympathomimetic amine. The pattern of effects depends on the adrenergic agonist that is administered: Pressor responses to *phenylephrine* can be completely suppressed; those to NE are only incompletely blocked because of residual stimulation of cardiac β_1 receptors; and pressor responses to EPI may be transformed to vasodepressor effects because of residual stimulation of β_2 adrenergic receptors in the vasculature with resultant vasodilation.

Blockade of α_1 adrenergic receptors can alleviate some of the symptoms of BPH. The symptoms of BPH include a resistance to urine outflow. This results from mechanical pressure on the urethra due to an increase in smooth muscle mass and an α adrenergic receptor–mediated increase in smooth muscle tone in the prostate and neck of the bladder. Antagonism of α_1 receptors permits relaxation of the smooth muscle and decreases the resistance to the outflow of urine.

Prazosin. *Prazosin* is the prototypical α_1-selective adrenergic receptor antagonist; accordingly, it and several similar agents exhibit greater clinical utility and have largely replaced the nonselective haloalkylamine (e.g., *phenoxybenzamine*) and imidazoline (e.g., *phentolamine*) α receptor antagonists. The affinity of *prazosin* for α_1 adrenergic receptors is about 1000-fold greater than that for α_2 adrenergic receptors. *Prazosin* has similar potencies at α_{1A}, α_{1B}, and α_{1D} subtype. *Prazosin* and the related α receptor antagonists *doxazosin* and *tamsulosin* frequently are used for the treatment of hypertension (see Chapter 32).

Pharmacological Effects. The major effects of *prazosin* result from its antagonism of α_1 adrenergic receptors in arterioles and veins. This leads to a fall in peripheral vascular resistance and in venous return to the heart. Unlike other vasodilating drugs, administration of *prazosin* usually does not increase heart rate. Because *prazosin* has little or no α_2 receptor–blocking effect, it probably does not promote the release of NE from sympathetic nerve endings in the heart. *Prazosin* decreases cardiac preload and has little effect on cardiac output and rate, in contrast to vasodilators such as *hydralazine* that have minimal dilating effects on veins. Although the combination of reduced preload and selective α_1 adrenergic receptor blockade might be sufficient to account for the relative absence of reflex tachycardia, *prazosin* also may act in the CNS to suppress sympathetic outflow. *Prazosin* appears to depress baroreflex function in hypertensive patients. *Prazosin* and related drugs in this class decrease LDLs and triglycerides and increase concentrations of HDLs.

ADME. *Prazosin* is well absorbed after oral administration, and bioavailability is about 50% to 70%. Peak concentrations of *prazosin* in plasma generally are reached 1 to 3 h after an oral dose. The drug is tightly bound to plasma proteins (primarily α_1-acid glycoprotein), and only 5% of the drug is free in the circulation; diseases that modify the concentration of this protein (e.g., inflammatory processes) may change the free fraction. *Prazosin* is extensively metabolized in the liver, and little unchanged drug is excreted by the kidneys. The plasma $t_{1/2}$ is about 3 h (may be prolonged to 6–8 h in congestive heart failure). The duration of action is approximately 7 to 10 h in the treatment of hypertension.

The initial dose should be 1 mg, usually given at bedtime so that the patient will remain recumbent for at least several hours to reduce the risk of syncopal reactions that may follow the first dose of *prazosin*. The dose is titrated upward depending on the blood pressure. A maximal effect generally is observed with a total daily dose of 20 mg in patients with hypertension. In the off-label treatment of BPH, doses from 1 to 5 mg twice daily typically are used.

Terazosin. *Terazosin,* a close structural analogue of *prazosin*, is less potent than *prazosin* but retains high specificity for α_1 adrenergic receptors; *terazosin* does not discriminate among α_{1A}, α_{1B}, and α_{1D} receptors. The major distinction between the two drugs is in their pharmacokinetic properties. *Terazosin* is more soluble in water than is *prazosin*, and its bioavailability is high (>90%). The $t_{1/2}$ of elimination of *terazosin* is about 12 h, and its duration of action usually extends beyond 18 h. Consequently, the drug may be taken once daily to treat hypertension and BPH in most patients. *Terazosin* and *doxazosin* induce apoptosis in prostate smooth muscle cells. This apoptosis may lessen the symptoms associated with chronic BPH by limiting cell proliferation. The apoptotic effect of *terazosin* and *doxazosin* appears to be related to the quinazoline moiety rather than α_1 receptor antagonism; *tamsulosin*, a nonquinazoline α_1 receptor antagonist, does not produce apoptosis (Kyprianou, 2003). Only about 10% of *terazosin* is excreted unchanged in the urine. An initial first dose of 1 mg is recommended. Doses are slowly titrated upward depending on the therapeutic response. Doses of 10 mg/day may be required for maximal effect in BPH.

Doxazosin. *Doxazosin* is another congener of *prazosin* and a highly selective antagonist at α_1 adrenergic receptors. It is nonselective among α_1 receptor subtypes and differs from *prazosin* in its pharmacokinetic profile. The $t_{1/2}$ of *doxazosin* is about 20 h, and its duration of action may extend to 36 h. The bioavailability and extent of metabolism of *doxazosin* and *prazosin* are similar. Most *doxazosin* metabolites are eliminated in the feces. The hemodynamic effects of *doxazosin* appear to be similar to those of *prazosin*. *Doxazosin* should be given initially as a 1-mg dose in the treatment of hypertension or BPH. *Doxazosin* also may have beneficial actions in the long-term management of BPH related to apoptosis that are independent of α_1 receptor antagonism. *Doxazosin* is typically administered once daily. An extended-release formulation marketed for BPH is not recommended for the treatment of hypertension.

Alfuzosin. *Alfuzosin* is a quinazoline-based α_1 receptor antagonist with similar affinity at all of the α_1 receptor subtypes. It has been used extensively in treating BPH; it is not approved for treatment of hypertension. Alfuzosin has a $t_{1/2}$ of 3 to 5 h. *Alfuzosin* is a substrate of CYP3A4, and the concomitant administration of CPY3A4 inhibitors (e.g., *ketoconazole*, *clarithromycin*, *itraconazole*, *ritonavir*) is contraindicated. *Alfuzosin* should be avoided in patients at risk for prolonged QT syndrome. The recommended dosage is one 10-mg extended-release tablet daily to be taken after the same meal each day.

Tamsulosin. *Tamsulosin*, a benzenesulfonamide, is an α_1 receptor antagonist with some selectivity for α_{1A} (and α_{1D}) subtypes over the α_{1B} subtype (Kenny et al., 1996). This selectivity may favor blockade of α_{1A} receptors in the prostate. *Tamsulosin* is efficacious in the treatment of BPH with little effect on blood pressure (Beduschi et al., 1998) and is not approved for the treatment of hypertension. *Tamsulosin* is well absorbed and has a $t_{1/2}$ of 5 to 10 h. It is extensively metabolized by CYPs. *Tamsulosin* may be administered at a 0.4-mg starting dose; a dose of 0.8 mg ultimately will be more efficacious in some patients. Abnormal ejaculation is an adverse effect of *tamsulosin*, experienced by about 18% of patients receiving the higher dose.

Silodosin. *Silodosin* exhibits selectivity for the α_{1A}, over the α_{1B}, adrenergic receptor. The drug is metabolized by several pathways; the main metabolite is a glucuronide formed by UGT2B7; coadministration with inhibitors of this enzyme (e.g., *probenecid*, *valproic acid*, *fluconazole*) increases systemic exposure to *silodosin*. The drug is approved for the treatment of BPH and has lesser effects on blood pressure than the non–α_1-subtype selective antagonists. Nevertheless, dizziness and orthostatic hypotension can occur. The chief side effect of *silodosin* is retrograde ejaculation (in 28% of those treated). *Silodosin* is available as 4- and 8-mg capsules.

Adverse Effects

A major potential adverse effect of *prazosin* and its congeners is the first-dose effect: Marked postural hypotension and syncope sometimes are seen 30 to 90 min after an initial dose of *prazosin* and 2 to 6 h after an initial dose of *doxazosin*. Syncopal episodes also have occurred with a rapid increase in dosage or with the addition of a second antihypertensive drug to the regimen of a patient already taking a large dose of *prazosin*. The risk of the first-dose phenomenon is minimized by limiting the initial dose (e.g., 1 mg at bedtime), by increasing the dosage slowly, and by introducing additional antihypertensive drugs cautiously.

Because orthostatic hypotension may be a problem during long-term treatment with *prazosin* or its congeners, it is essential to check standing as well as recumbent blood pressure. Nonspecific adverse effects such as headache, dizziness, and asthenia rarely limit treatment with *prazosin*.

Therapeutic Uses

Hypertension. *Prazosin* and its congeners have been used successfully in the treatment of essential hypertension (see Chapter 32). Pleotropic effects of these drugs include improved lipid profiles and glucose-insulin metabolism in patients with hypertension who are at risk for atherosclerotic disease (Deano and Sorrentino, 2012). Catecholamines are also powerful stimulators of vascular smooth muscle hypertrophy, acting by α_1 adrenergic receptors. To what extent these effects of α_1 antagonists have clinical significance in diminishing the risk of atherosclerosis is not known.

Congestive Heart Failure. Although α adrenergic receptor antagonists have been used in the treatment of congestive heart failure, they are not the drugs of choice. Short-term effects of α adrenergic receptor blockade in these patients are due to dilation of both arteries and veins, resulting in a reduction of preload and afterload, which increases cardiac output and reduces pulmonary congestion. In contrast to results obtained with inhibitors of angiotensin-converting enzyme or a combination of *hydralazine* and an organic nitrate, *prazosin* has not been found to prolong life in patients with congestive heart failure.

Benign Prostatic Hyperplasia (BPH). In a significant percentage of older men, BPH produces symptomatic urethral obstruction that leads to weak stream, increased urinary frequency, and nocturia. These symptoms are due to a combination of mechanical pressure on the urethra due to the increase in smooth muscle mass and the α_1 adrenergic receptor–mediated increase in smooth muscle tone in the prostate and neck of the bladder (Kyprianou, 2003). α_1 Receptors in the trigone muscle of the bladder and urethra contribute to the resistance to outflow of urine. *Prazosin* reduces this resistance in some patients with impaired bladder emptying caused by prostatic obstruction or parasympathetic decentralization from spinal injury.

Finasteride and *dutasteride*, two drugs that inhibit conversion of testosterone to dihydrotestosterone (see Chapter 49) and can reduce prostate volume in some patients, are approved as monotherapy and in combination with α receptor antagonists. Drugs that are α_1-selective antagonists have efficacy in BPH owing to relaxation of smooth muscle in the bladder neck, prostate capsule, and prostatic urethra. These agents rapidly improve urinary flow, whereas the actions of *finasteride* are typically delayed for months. Combination therapy with *doxazosin* and *finasteride* reduces the risk of overall clinical progression of BPH significantly more than treatment with either drug alone (McConnell et al., 2003). *Tamsulosin* at the recommended dose of 0.4 mg daily and *silodosin* at 0.8 mg are less likely to cause orthostatic hypotension than are the other drugs. The predominant α_1 subtype expressed in the human prostate is the α_{1A} receptor (Kenny et al., 1996). Developments in this area will provide the basis for the selection of α receptor antagonists with specificity for the relevant subtype of α_1 receptor. However, the possibility remains that some of the symptoms of BPH are due to α_1 receptors in other sites, such as bladder, spinal cord, or brain.

Other Disorders. Some studies indicated that *prazosin* can decrease the incidence of digital vasospasm in patients with Raynaud syndrome; however, its relative efficacy as compared with Ca^{2+} channel blockers is not known. *Prazosin* may have some benefit in patients with other vasospastic disorders. *Prazosin* may be useful for the treatment of patients with mitral or aortic valvular insufficiency, presumably by reducing afterload.

α_2-Selective Adrenergic Receptor Antagonists

Activation of presynaptic α_2 adrenergic receptors inhibits the release of NE and other co-transmitters from peripheral sympathetic nerve endings. Activation of α_2 receptors in the pontomedullary region of the CNS inhibits sympathetic nervous system activity and leads to a fall in blood pressure; these receptors are a site of action for drugs such as *clonidine*. Blockade of α_2 adrenergic receptors with selective antagonists such as *yohimbine* thus can increase sympathetic outflow and potentiate the release of NE from nerve endings, leading to activation of α_1 and β_1 adrenergic receptors in the heart and peripheral vasculature with a consequent rise in blood pressure. Antagonists that also block α_1 receptors give rise to similar effects on sympathetic outflow and release of NE, but the net increase in blood pressure is prevented by inhibition of vasoconstriction.

Although certain vascular beds contain α_2 adrenergic receptors that promote contraction of smooth muscle, it is thought that these receptors are preferentially stimulated by circulating catecholamines, whereas α_1 receptors are activated by NE released from sympathetic nerve fibers. In other vascular beds, α_2 adrenergic receptors reportedly promote vasodilation by stimulating the release of NO from endothelial cells. The physiological role of vascular α_2 receptors in the regulation of blood flow within various vascular beds is uncertain. α_2 Receptors contribute to smooth muscle contraction in the human saphenous vein, whereas α_1 receptors are more prominent in dorsal hand veins. The effects of α_2 adrenergic receptor antagonists on the cardiovascular system are dominated by actions in the CNS and on sympathetic nerve endings.

Yohimbine

Yohimbine is a competitive antagonist that is selective for α_2 adrenergic receptors. The compound is an indolealkylamine alkaloid and is found in the bark of the tree *Pausinystalia yohimbe* and in *Rauwolfia* root; its structure resembles that of *reserpine*, an alkaloid also found in *Rauwolfia*. *Yohimbine* readily enters the CNS, where it acts to increase blood pressure and heart rate; it also enhances motor activity and produces tremors. These actions are opposite to those of *clonidine*, a selective α_2 adrenergic receptor agonist. *Yohimbine* also antagonizes effects of 5HT. In the past, it was used extensively to treat male sexual dysfunction; however, the efficacies of PDE5 inhibitors (e.g., *sildenafil*, *vardenafil*, and *tadalafil*) and *apomorphine* (off-label) have been much more conclusively demonstrated in oral treatment of erectile dysfunction. Some studies suggested that *yohimbine* may be useful for diabetic neuropathy and in the treatment of postural hypotension. In the U.S., *yohimbine* can be legally sold as a dietary supplement; however, labeling claims that it will arouse or increase sexual desire or improve sexual performance are prohibited. *Yohimbine* is approved in veterinary medicine for the reversal of xylazine anesthesia.

Additional α Adrenergic Receptor Antagonists

Ergot Alkaloids

The ergot alkaloids were the first adrenergic receptor antagonists to be discovered. Ergot alkaloids exhibit a complex variety of pharmacological properties. To varying degrees, these agents act as partial agonists or antagonists at α adrenergic receptors, DA receptors, and 5HT receptors. Chapter 15 presents additional information about the ergot alkaloids.

Indoramin

Indoramin is a selective, competitive, α_1-selective receptor antagonist that also antagonizes H_1 and 5HT receptors. *Indoramin* lowers blood pressure with minimal tachycardia. The drug is not available in the U.S.; outside the U.S., *indoramin* is used for the treatment of hypertension and BPH and in the prophylaxis of migraine. The drug also decreases the incidence of attacks of Raynaud syndrome. Some of the adverse effects of *indoramin* include sedation, dry mouth, and failure of ejaculation.

Ketanserin

Although developed as a 5HT receptor antagonist, *ketanserin* also blocks α_1 receptors. *Ketanserin* (not available in the U.S.) is discussed in Chapter 15.

Urapidil

Urapidil is a selective α_1 receptor antagonist that has a chemical structure distinct from those of *prazosin* and related compounds; the drug is not commercially available in the U.S. Blockade of peripheral α_1 adrenergic receptors appears to be primarily responsible for the hypotension produced by *urapidil*, although it has actions in the CNS as well.

Bunazosin

Bunazosin is an α_1-selective antagonist of the quinazoline class that can lower blood pressure in patients with hypertension. *Bunazosin* is not available in the U.S.

Neuroleptic Agents

Chlorpromazine, *haloperidol*, and other neuroleptic drugs of the phenothiazine and butyrophenone types produce significant blockade of both α adrenergic and dopaminergic D_2 receptors in humans.

β Adrenergic Receptor Antagonists

Overview

Competitive antagonists of β adrenergic receptors, or β blockers, have received enormous clinical attention because of their efficacy in the treatment of hypertension, ischemic heart disease, congestive heart failure, and certain arrhythmias.

The myriad β antagonists can be distinguished by the following properties:

- Relative affinity for β_1 and β_2 receptors (and to some extent β_3 receptors)
- Intrinsic sympathomimetic activity
- Blockade of α receptors
- Differences in lipid solubility (CNS penetration)
- Capacity to induce vasodilation
- Pharmacokinetic parameters

Propranolol is a competitive nonselective β adrenergic receptor antagonist and remains the prototype to which other β blockers are compared. *Propranolol* has equal affinity for β_1 and β_2 adrenergic receptors and a lower affinity for β_3 receptors. Agents such as *metoprolol*, *atenolol*, *acebutolol*, *bisoprolol*, and *esmolol* have somewhat greater affinity for β_1 than for β_2 receptors; and are examples of β_1-selective antagonists, even though the selectivity is not absolute and higher concentrations will block β_2 receptors. There currently are no selective β_2 or β_3 adrenergic receptor antagonists used clinically, but there are some for experimental use (Schena and Caplan, 2019).

Several β blockers (e.g., *pindolol* and *acebutolol*) can actually activate β adrenergic receptors partially in the absence of catecholamines; however, the intrinsic activities of these drugs are less than that of a full agonist such as INE. These partial agonists have *intrinsic sympathomimetic activity*; this slight residual activity may prevent profound bradycardia or negative inotropy in a resting heart. The potential clinical advantage of this property, however, is unclear and may be disadvantageous in the context of secondary prevention of myocardial infarction. *Propranolol* is a pure antagonist, and it has no capacity to activate β adrenergic receptors.

Several β adrenergic receptor antagonists also have local anesthetic or membrane-stabilizing activity, independent of β blockade. Such drugs include *propranolol*, *acebutolol*, and *carvedilol*. *Pindolol*, *metoprolol*, *betaxolol*, and *labetalol* have slight membrane-stabilizing effects. Although most β adrenergic receptor antagonists do not block α adrenergic receptors, *labetalol*, *carvedilol*, and *bucindolol* block both α_1 and β adrenergic receptors. In addition to *carvedilol*, *labetalol*, and *bucindolol*, other β receptor antagonists have vasodilating properties due to mechanisms discussed in the following material. These include *celiprolol*, *nebivolol*, *nipradilol*, *carteolol*, *betaxolol*, *bopindolol*, and *bevantolol* (Toda, 2003).

Pharmacological Properties

The pharmacological properties of β adrenergic receptor antagonists can be deduced and explained largely from knowledge of the responses elicited by the receptors in the various tissues and the activity of the sympathetic nerves that innervate these tissues (see Chapter 10, especially Table 10–1). For example, β adrenergic receptor blockade has relatively little effect on the normal heart of an individual at rest but has profound effects when sympathetic control of the heart is dominant, as during exercise or stress.

The β adrenergic receptor antagonists are generally classified as non–subtype-selective ("first generation"), β_1 selective ("second generation"), and non–subtype- or subtype-selective *with additional cardiovascular actions* ("third generation"). These last drugs have additional cardiovascular properties (especially vasodilation) that seem unrelated to β receptor blockade. Table 14–3 summarizes the pharmacological and pharmacokinetic properties of β receptor antagonists.

Cardiovascular System. The major therapeutic effects of β adrenergic receptor antagonists are on the cardiovascular system. It is important to distinguish these effects in normal subjects from those in subjects with cardiovascular disease such as hypertension or myocardial ischemia. As discussed above, catecholamines have positive chronotropic and inotropic actions; therefore, β adrenergic receptor antagonists have the converse actions including slowing heart rate and decreasing myocardial contractility, but only if there is sympathetic tone, which normally is present. When tonic stimulation of β adrenergic receptors is low, the effect of β blockade is correspondingly modest. However, when the sympathetic nervous system is activated, as during exercise, during stress, or in disease states, β receptor antagonists produce more robust cardiac depressant effects.

TABLE 14–3 ■ PHARMACOLOGICAL/PHARMACOKINETIC PROPERTIES OF β ADRENERGIC RECEPTOR BLOCKING AGENTS

DRUG	MEMBRANE STABILIZING ACTIVITY	INTRINSIC AGONIST ACTIVITY	LIPID SOLUBILITY	EXTENT OF ABSORPTION (%)	ORAL AVAILABILITY (%)	PLASMA $t_{1/2}$ (HOURS)	PROTEIN BINDING (%)
Classical nonselective β blockers: First generation							
Nadolol	0	0	Low	30	30–50	20–24	30
Penbutolol	0	+	High	~100	~100	~5	80–98
Pindolol	+	+++	Low	>95	~100	3–4	40
Propranolol	++	0	High	<90	30	3–5	90
Timolol	0	0	Low to moderate	90	75	4	<10
β_1 Selective blockers: Second generation							
Acebutolol	+	+	Low	90	20–60	3–4	26
Atenolol	0	0	Low	90	50–60	6–7	6–16
Bisoprolol	0	0	Low	≤90	80	9–12	~30
Esmolol	0	0	Low	NA	NA	0.15	55
Metoprolol	+[a]	0	Moderate	~100	40–50	3–7	12
Nonselective β blockers with additional actions: Third generation							
Carteolol	0	++	Low	85	85	6	23–30
Carvedilol	++	0	Moderate	>90	~30	7–10	98
Labetalol	+	+	Low	>90	~33	3–4	~50
β_1-selective blockers with additional actions: Third generation							
Betaxolol	+	0	Moderate	>90	~80	15	50
Celiprolol	0	+	Low	~74	30–70	5	4–5
Nebivolol	0	0	Low	NA	NA	11–30	98

NA, not applicable.

[a] Detectable only at doses much greater than required for β blockade.

Short-term administration of β adrenergic receptor antagonists decreases cardiac output; peripheral resistance increases in proportion to maintain blood pressure as a result of blockade of vascular β_2 adrenergic receptors and compensatory reflexes, such as increased sympathetic nervous system activity, leading to activation of vascular α adrenergic receptors. However, with long-term use of β antagonists, total peripheral resistance returns to initial values or decreases in patients with hypertension (Man in't Veld et al., 1988). With β adrenergic receptor antagonists that also are α_1 receptor antagonists, such as *labetalol*, *carvedilol*, and *bucindolol*, cardiac output is maintained with a greater fall in peripheral resistance. This also is seen with β receptor antagonists that are direct vasodilators.

The β adrenergic receptor antagonists have significant effects on cardiac rhythm and automaticity. Although it had been thought that these effects were due exclusively to blockade of β_1 receptors, β_2 receptors likely also regulate heart rate in humans (Altschuld and Billman, 2000). As discussed above, β_3 adrenergic receptors also have been identified in normal myocardial tissue (Schena and Caplan, 2019). Signal transduction for β_3 adrenergic receptors is complex and includes not only G_s but also G_i/G_o stimulation and signaling through NO and cyclic GMP (Cannavo and Koch, 2017). While β_3 receptor agonists are being explored as possible treatments for heart failure, the potential utility of β_3-adrenergic receptor antagonists in human heart disease is unknown.

Antagonizing β adrenergic receptors reduces the sinus rate, decreases the rate of spontaneous depolarization of ectopic pacemakers, slows conduction in the atria and in the AV node, and increases the functional refractory period of the AV node. Although high concentrations of many β blockers exert a membrane-stabilizing activity, it is doubtful that this is significant at usual therapeutic doses. However, this effect may be important when there is overdosage. *d*-Propranolol may suppress ventricular arrhythmias independently of β receptor blockade.

The cardiovascular effects of β adrenergic receptor antagonists are most evident during dynamic exercise when there is more sympathetic tone and higher levels of catecholamines. In the presence of β blockade, exercise-induced increases in heart rate and myocardial contractility are attenuated. However, the exercise-induced increase in cardiac output is less affected because of an increase in stroke volume. The effects of β adrenergic receptor antagonists on exercise are somewhat analogous to the changes that occur with normal aging. In healthy elderly persons, catecholamine-induced increases in heart rate are smaller than in younger individuals; however, the increase in cardiac output in older people may be preserved because of an increase in stroke volume during exercise. β Blockers tend to decrease work capacity, as assessed by their effects on intense short-term or more prolonged steady-state exertion. Exercise performance may be impaired to a lesser extent by β_1-selective agents than by nonselective antagonists. Blockade of β_2 receptors blunts the increase in blood flow to active skeletal muscle during submaximal exercise and may attenuate catecholamine-induced activation of glucose metabolism and lipolysis.

Coronary artery blood flow increases during exercise or stress to meet the metabolic demands of the heart. By increasing heart rate, contractility, and systolic pressure, catecholamines increase myocardial O_2 demand. However, in patients with coronary artery disease, fixed narrowing of these vessels attenuates the expected increase in flow, leading to myocardial ischemia. β Adrenergic receptor antagonists decrease the effects of catecholamines on the determinants of myocardial O_2 consumption.

TABLE 14–4 ■ THIRD-GENERATION β RECEPTOR ANTAGONISTS WITH PUTATIVE ADDITIONAL MECHANISMS OF VASODILATION

NITRIC OXIDE PRODUCTION	β_2 RECEPTOR AGONISM	α_1 RECEPTOR ANTAGONISM	Ca^{2+} ENTRY BLOCKADE	K^+ CHANNEL OPENING	ANTIOXIDANT ACTIVITY
Celiprolol[a]	Celiprolol[a]	Carvedilol	Carvedilol	Tilisolol[a]	Carvedilol
Nebivolol	Carteolol	Bucindolol[a]	Betaxolol		
Carteolol	Bopindolol[a]	Bevantolol[a]	Bevantolol[a]		
Bopindolol[a]		Nipradilol[a]			
Nipradilol[a]		Labetalol			

[a]Not currently available in the U.S.

However, these agents may tend to increase the requirement for O_2 by increasing end-diastolic pressure and systolic ejection period. Usually, the net effect is to improve the relationship between cardiac O_2 supply and demand; exercise tolerance generally is improved in patients with angina, whose capacity to exercise is limited by the development of chest pain (see Chapter 31).

Antihypertensive Activity. β Adrenergic receptor antagonists generally do not reduce blood pressure in patients with normal blood pressure. However, these drugs lower blood pressure in patients with hypertension, but the mechanisms responsible for this important clinical effect are not fully understood. The release of renin from the juxtaglomerular cells is stimulated by the sympathetic nervous system by means of β_1 adrenergic receptors, and this effect is blocked by β receptor antagonists (see Chapter 30). Some investigators have found that the antihypertensive effect of β blockade is most marked in patients with elevated concentrations of plasma renin, compared to patients with low or normal concentrations of renin. However, β adrenergic receptor antagonists are effective even in patients with low plasma renin.

Presynaptic β adrenergic receptors (primarily β_2 receptors) enhance the release of NE from sympathetic neurons, and diminished release of NE from β blockade is a possible response. Although β blockers would not be expected to decrease the contractility of vascular smooth muscle, long-term administration of these drugs to hypertensive patients ultimately leads to a fall in peripheral vascular resistance (Man in't Veld et al., 1988). The mechanism for this effect is not known, but this delayed fall in peripheral vascular resistance in the face of a persistent reduction of cardiac output appears to account for much of the antihypertensive effect of these drugs.

Some β adrenergic receptor antagonists have additional effects that may contribute to their capacity to lower blood pressure. These drugs all produce peripheral vasodilation; at least six properties have been proposed to contribute to this effect, including production of NO, partial activation of β_2 adrenergic receptors, blockade of α_1 adrenergic receptors, blockade of Ca^{2+} entry, opening of K^+ channels, and antioxidant activity (see Table 14–4). These mechanisms appear to contribute to the antihypertensive effects by enhancing hypotension, increasing peripheral blood flow, and decreasing afterload. *Celiprolol* and *nebivolol* also have been observed to produce vasodilation and thereby reduce preload (see below).

Nonselective β adrenergic receptor antagonists inhibit the vasodilation caused by INE and augment the pressor response to EPI. This is particularly significant in patients with pheochromocytoma, in whom β receptor antagonists should be used only after adequate α receptor blockade has been established. This avoids uncompensated α adrenergic receptor–mediated vasoconstriction caused by EPI secreted from the tumor.

Pulmonary System. Nonselective β receptor antagonists such as *propranolol* block β_2 receptors in bronchial smooth muscle. This usually has little effect on pulmonary function in normal individuals. However, in patients with COPD, such blockade can lead to life-threatening bronchoconstriction. Although β_1-selective antagonists or antagonists with intrinsic sympathomimetic activity are less likely than *propranolol* to increase airway resistance in patients with asthma, these drugs should be used only with great caution, if at all, in patients with bronchospastic diseases. Drugs such as *celiprolol*, with β_1 receptor selectivity and β_2 receptor partial agonism, are of potential promise, although clinical experience is limited.

Metabolic Effects. The β adrenergic receptor antagonists modify the metabolism of carbohydrates and lipids. Catecholamines promote glycogenolysis and mobilize glucose in response to hypoglycemia. Nonselective β blockers may delay recovery from hypoglycemia in type 1 (insulin-dependent) diabetes mellitus, but infrequently in type 2 diabetes mellitus. In addition to blocking glycogenolysis, β receptor antagonists can interfere with the counterregulatory effects of catecholamines secreted during hypoglycemia by blunting the perception of symptoms such as tremor, tachycardia, and nervousness. Thus, β adrenergic receptor antagonists should be used with great caution in patients with labile diabetes and frequent hypoglycemic reactions. If such a drug is indicated, a β_1-selective antagonist is preferred because these drugs are less likely to delay recovery from hypoglycemia (Dunne et al., 2001).

The β receptors mediate activation of hormone-sensitive lipase in fat cells, leading to release of free fatty acids into the circulation. This increased flux of fatty acids is an important source of energy for exercising muscle. β Receptor antagonists can attenuate the release of free fatty acids from adipose tissue. Nonselective β receptor antagonists consistently reduce HDL (high-density lipoprotein) cholesterol, increase LDL (low-density lipoprotein) cholesterol, and increase triglycerides. In contrast, β_1-selective antagonists, including *celiprolol*, *carteolol*, *nebivolol*, and *bevantolol*, reportedly improve the serum lipid profile of dyslipidemic patients. While drugs such as *propranolol* and *atenolol* increase triglycerides, plasma triglycerides are reduced with chronic use of *celiprolol*, *carvedilol*, and *carteolol* (Toda, 2003).

In contrast to classical β blockers, which decrease insulin sensitivity, the vasodilating β receptor antagonists (e.g., *celiprolol*, *nipradilol*, *carteolol*, *carvedilol*, and *dilevalol*) increase insulin sensitivity in patients with insulin resistance. Together with their cardioprotective effects, improvement in insulin sensitivity from vasodilating β adrenergic receptor antagonists may partially counterbalance the hazard from worsened lipid abnormalities associated with diabetes.

When β blockers are required, β_1-selective or vasodilating β receptor antagonists are preferred. In addition, it may be necessary to use β receptor antagonists in conjunction with other drugs (e.g., 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) to ameliorate adverse metabolic effects (Dunne et al., 2001).

The β receptor agonists decrease the plasma concentration of K^+ by promoting its uptake, predominantly into skeletal muscle. At rest, an infusion of EPI causes a decrease in the plasma concentration of K^+. The marked increase in the concentration of EPI that occurs with stress (such as myocardial infarction) may cause hypokalemia, which could predispose to cardiac arrhythmias. In preclinical studies, the hypokalemic effect of EPI is blocked by an experimental antagonist, ICI 118551, which has a high affinity for β_2 and, to a lesser degree, β_3 receptors. Exercise causes an increase in the efflux of K^+ from skeletal muscle. Catecholamines tend to buffer the rise in K^+ by increasing its influx into muscle. β Blockers negate this buffering effect.

Other Effects. β Adrenergic receptor antagonists block catecholamine-induced tremor. They also block inhibition of mast cell degranulation by catecholamines.

Adverse Effects and Precautions

Cardiovascular System. β Adrenergic receptor blockade, although initially contraindicated in chronic heart failure, is now standard of care, and the β_1-selective antagonist *metoprolol* and nonselective β blocker *carvedilol* have been shown to improve the mortality and morbidity of heart failure patients (Rockman et al., 2002). The use of β blockers in congestive heart failure is discussed in more detail in Chapter 33.

Bradycardia is a normal response to β adrenergic receptor blockade; however, in patients with partial or complete AV conduction defects, β antagonists may cause life-threatening bradyarrhythmias. Particular caution is indicated in patients who are taking other drugs, such as *verapamil* or various antiarrhythmic agents, which may impair sinus node function or AV conduction.

Some patients complain of cold extremities while taking β receptor antagonists. Symptoms of peripheral vascular disease may occasionally worsen, or Raynaud syndrome may develop.

Abrupt discontinuation of β receptor antagonists after long-term treatment can exacerbate angina and may increase the risk of sudden death. There is enhanced sensitivity to β adrenergic receptor agonists in patients who have undergone long-term treatment with certain β receptor antagonists after the blocker is withdrawn abruptly. This increased sensitivity is evident several days after stopping a β receptor antagonist and may persist for at least 1 week. Such enhanced sensitivity can be attenuated by tapering the dose of the β blocker for several weeks before discontinuation. Super-sensitivity to INE also has been observed after abrupt discontinuation of *metoprolol*, but not of *pindolol*. This enhanced β responsiveness may result from upregulation of β receptors and downregulation of GPCR kinases (Sato et al., 2015). The number of β adrenergic receptors on circulating lymphocytes is increased in subjects who have received *propranolol* for long periods; *pindolol*, with its slight agonist activity, has the opposite effect. For discontinuation of β blockers, it is prudent to decrease the dose gradually and to restrict exercise during this period.

Pulmonary Function. A major adverse effect of β adrenergic receptor antagonists is caused by blockade of β_2 receptors in bronchial smooth muscle. These receptors are particularly important for promoting bronchodilation in patients with bronchospastic disease, and β_2 receptor blockade may cause a life-threatening increase in airway resistance in such patients. Drugs with selectivity for β_1 adrenergic receptors or those with intrinsic sympathomimetic activity at β_2 adrenergic receptors seem less likely to induce bronchospasm. β Blockers should be avoided if at all possible in patients with asthma. However, in selected patients with COPD and cardiovascular disease, the advantages of using β_1 receptor antagonists may outweigh the risk of worsening pulmonary function (Salpeter et al., 2005).

CNS. The adverse effects of β adrenergic receptor antagonists that enter the CNS may include fatigue, sleep disturbances (including insomnia and nightmares), and depression. Interest has focused on the relationship between the incidence of the adverse effects of β adrenergic receptor antagonists and their lipophilicity; however, no clear correlation has emerged.

Metabolism. β Adrenergic receptor blockade may blunt recognition of hypoglycemia by patients; it also may delay recovery from insulin-induced hypoglycemia. β Receptor antagonists should be used with great caution in patients with diabetes who are prone to hypoglycemic reactions; β_1-selective agents may be preferable for these patients.

Sexual Function and Reproduction. The incidence of sexual dysfunction in men with hypertension who are treated with β adrenergic receptor antagonists is not clearly defined. Although experience with the use of β adrenergic receptor antagonists in pregnancy is increasing, information about the safety of these drugs during pregnancy still is limited.

Overdosage. The manifestations of poisoning with β adrenergic receptor antagonists depend on the pharmacological properties of the ingested drug, particularly its β_1 selectivity, intrinsic sympathomimetic activity, and membrane-stabilizing properties. Hypotension, bradycardia, prolonged AV conduction times, and widened QRS complexes are common manifestations of overdosage. Seizures and depression may occur. Hypoglycemia and bronchospasm can occur. Significant bradycardia should be treated initially with *atropine*, but a cardiac pacemaker often is required. Large doses of INE or an α adrenergic receptor agonist may be necessary to treat hypotension. *Glucagon*, acting through its own GPCR and independently of the β adrenergic receptor, has positive chronotropic and inotropic effects on the heart, and the drug has been useful in some patients who have an overdose of a β receptor antagonist.

Drug Interactions. Aluminum salts, *cholestyramine*, and *colestipol* may decrease the absorption of β blockers. Drugs such as *phenytoin*, *rifampin*, and *phenobarbital*, as well as smoking, induce hepatic biotransformation enzymes and may decrease plasma concentrations of β adrenergic receptor antagonists that are metabolized extensively (e.g., *propranolol*). *Cimetidine* and *hydralazine* may increase the bioavailability of agents such as *propranolol* and *metoprolol* by affecting hepatic blood flow. β Receptor antagonists can impair the clearance of *lidocaine*.

Additive effects on blood pressure by β blockers and other antihypertensive agents often are employed to clinical advantage. However, the antihypertensive effects of β adrenergic receptor antagonists can be opposed by *indomethacin* and other nonsteroidal anti-inflammatory drugs (see Chapter 42).

Therapeutic Uses

Cardiovascular Diseases. β Adrenergic receptor antagonists are used extensively in the treatment of hypertension, angina and acute coronary syndromes, and congestive heart failure (see Chapters 31–33). These drugs also are used frequently in the treatment of supraventricular and ventricular arrhythmias (see Chapter 34). β Receptor antagonists are used in the treatment of hypertrophic obstructive cardiomyopathy, relieving angina, palpitations, and syncope in patients with this disorder. Efficacy probably is related to partial relief of the pressure gradient along the outflow tract. β Blockers also may attenuate catecholamine-induced cardiomyopathy in pheochromocytoma.

β Blockers are used frequently in the medical management of acute dissecting aortic aneurysm; their usefulness comes from reduction in the force of myocardial contraction and the rate of development of such force. *Nitroprusside* is an alternative, but when given in the absence of β adrenergic receptor blockade, it causes an undesirable reflex tachycardia. Chronic treatment with β receptor antagonists may be efficacious in slowing the progression of aortic dilation and its complications in patients with Marfan syndrome, although surgical aortic repair is still warranted as aortic diameter expands; *losartan*, an angiotensin-converting enzyme inhibitor, is showing promise as a more effective treatment (Hiratzka et al., 2010).

Glaucoma. The β adrenergic receptor antagonists are used in the treatment of chronic open-angle glaucoma (see Chapter 74). These agents decrease the production of aqueous humor, which appears to be the mechanism for their clinical effectiveness.

Other Uses. Many of the signs and symptoms of hyperthyroidism are reminiscent of the manifestations of increased sympathetic nervous system activity. β Adrenergic receptor antagonists control many of the cardiovascular signs and symptoms of hyperthyroidism and are useful adjuncts to more definitive therapy. In addition, *propranolol* inhibits the peripheral conversion of thyroxine to triiodothyronine, an effect that may be independent of β receptor blockade (see Chapter 47).

Propranolol, *timolol*, and *metoprolol* are effective for the prophylaxis of migraine; these drugs are not useful for treatment of acute attacks of migraine.

Propranolol and other β blockers are effective in controlling acute panic symptoms in individuals who are required to perform in public or in other anxiety-provoking situations. Tachycardia, muscle tremors, and other evidence of increased sympathetic activity are reduced.

β Adrenergic receptor antagonists may be of some value in the treatment of patients undergoing withdrawal from alcohol or those with akathisia. *Propranolol* and *nadolol* are efficacious in the primary prevention of variceal bleeding in patients with portal hypertension caused by cirrhosis of the liver (Bosch, 1998).

Clinical Selection of a β Adrenergic Receptor Antagonist

The various β receptor antagonists that are used for the treatment of hypertension and angina appear to have similar efficacies. Selection of the most appropriate drug for an individual patient should be based on pharmacokinetic and pharmacodynamic differences among the drugs, cost, and whether there are concurrent medical problems. β_1-Selective antagonists are preferable in patients with bronchospasm, diabetes, peripheral vascular disease, or Raynaud syndrome. Although no clinical advantage of β adrenergic receptor antagonists with intrinsic sympathomimetic activity has been clearly established, such drugs may be preferable in patients with bradycardia. In addition, third-generation β antagonists that block α_1 adrenergic receptors, stimulate β_2 adrenergic receptors, enhance NO production, block Ca^{2+} entry, open K^+ channels, or possess antioxidant properties may offer therapeutic advantages.

Nonselective β Adrenergic Receptor Antagonists

Propranolol

Propranolol (Table 14–5) interacts with β_1 and β_2 receptors with equal affinity, lacks intrinsic sympathomimetic activity, does not block α receptors, and has lower affinity for β_3 adrenergic receptors.

ADME. *Propranolol* is highly lipophilic and almost completely absorbed after oral administration. Much of the drug is metabolized by the liver during its first passage through the portal circulation; only about 25% reaches the systemic circulation. In addition, there is great interindividual variation in the presystemic clearance of *propranolol* by the liver; this contributes to enormous variability in plasma concentrations (~20-fold) after oral administration of the drug and to the wide dosage range for clinical efficacy. The degree of hepatic extraction of *propranolol* declines as the dose is increased. The bioavailability of *propranolol* may be increased by the concomitant ingestion of food and during long-term administration of the drug.

Propranolol readily enters the CNS. Approximately 90% of the drug in the circulation is bound to plasma proteins. It is extensively metabolized, with most metabolites appearing in the urine. One product of hepatic metabolism is 4-hydroxypropranolol, which has some β adrenergic antagonist activity. Analysis of the distribution of *propranolol*, its clearance by the liver, and its activity is complicated by the stereospecificity of these processes (Walle et al., 1988). The (–) enantiomers of *propranolol* and other β blockers are the active forms. The (–) enantiomer of *propranolol* appears to be cleared more slowly from the body than is the inactive enantiomer. The clearance of *propranolol* may vary with hepatic blood flow and liver disease and also may change during the administration of other drugs that affect hepatic metabolism.

Despite its short $t_{1/2}$ in plasma (~4 h), twice-daily administration suffices to produce the antihypertensive effect in some patients. Sustained-release formulations of *propranolol* maintain therapeutic concentrations of *propranolol* in plasma throughout a 24-h period. For the treatment of hypertension and angina, the initial oral dose of *propranolol* generally is 40 to 80 mg/day. The dose may then be titrated upward until the optimal response is obtained. For the treatment of angina, the dose may be increased at intervals of less than 1 week, as indicated clinically. In hypertension, the full blood pressure response may not develop for several weeks. Typically, doses are less than 320 mg/day. If *propranolol* is taken twice daily for hypertension, blood pressure should be measured just prior to a dose to ensure that the duration of effect is sufficiently prolonged. Adequacy of β adrenergic receptor blockade can be assessed by measuring suppression of exercise-induced tachycardia (see Table 14–5).

Propranolol may be administered intravenously for the management of life-threatening arrhythmias or to patients under anesthesia. Under these circumstances, the usual dose is 1 to 3 mg, administered slowly (<1 mg/min) with careful and frequent monitoring of blood pressure, ECG, and cardiac function. If an adequate response is not obtained, a second dose may be given after several minutes. If bradycardia is excessive, *atropine* should be administered to increase heart rate. A change to oral therapy should be initiated as soon as possible.

Nadolol

Nadolol is a long-acting antagonist with equal affinity for β_1 and β_2 adrenergic receptors. It is devoid of both membrane-stabilizing and intrinsic sympathomimetic activity. A distinguishing characteristic of *nadolol* is its relatively long $t_{1/2}$. It can be used to treat hypertension and angina pectoris. Unlabeled uses have included migraine prophylaxis, parkinsonian tremors, and variceal bleeding in portal hypertension.

ADME. *Nadolol* is very soluble in water and is incompletely absorbed from the gut; its bioavailability is about 35%. Interindividual variability is less than with *propranolol*. The low lipid solubility of *nadolol* may result in lower concentrations of the drug in the brain. *Nadolol* is not extensively metabolized and is largely excreted intact in the urine. The $t_{1/2}$ of the drug in plasma is about 20 h; consequently, it generally is administered once daily. *Nadolol* may accumulate in patients with renal failure, and dosage should be reduced in such individuals.

Timolol

Timolol is a potent, nonselective β adrenergic receptor antagonist with no intrinsic sympathomimetic or membrane-stabilizing activity. It is used for hypertension, congestive heart failure, acute myocardial infarction, and migraine prophylaxis. In ophthalmology, *timolol* has been used in the treatment of open-angle glaucoma and intraocular hypertension. The drug appears to reduce aqueous humor production through blockade of β receptors on the ciliary epithelium.

ADME. *Timolol* is well absorbed from the GI tract. It is metabolized extensively by CYP2D6 in the liver. Only a small amount of unchanged drug appears in the urine. The $t_{1/2}$ in plasma is about 4 h. The ocular formulation of *timolol* may be absorbed systemically (see Chapter 74) and produce adverse effects in susceptible patients, such as those with asthma or congestive heart failure. The systemic administration of *cimetidine* with topical ocular *timolol* increases the degree of β adrenergic receptor blockade, resulting in a reduction of resting heart rate, intraocular pressure, and exercise tolerance (Ishii et al., 2000). For ophthalmic use, *timolol* is available combined with other medications (e.g., with *dorzolamide* or *travoprost*). *Timolol* also provides benefits to patients with coronary heart disease: In the acute period after myocardial infarction, *timolol* produced a 39% reduction in mortality in the Norwegian Multicenter Study.

Pindolol

Pindolol is a nonselective β receptor antagonist *with intrinsic sympathomimetic activity*. It has low membrane-stabilizing activity and low lipid solubility. It is used to treat angina pectoris and hypertension. β Blockers with slight partial agonist activity may be preferred as antihypertensive agents in individuals with diminished cardiac reserve or a propensity for bradycardia. Nonetheless, the clinical significance of partial agonism has not been substantially demonstrated in controlled trials but may be of importance in individual patients.

ADME. *Pindolol* is almost completely absorbed after oral administration; the drug has a moderately high bioavailability and plasma $t_{1/2}$ of about 4 h. Approximately 50% of *pindolol* ultimately is metabolized in the liver; the remainder is excreted unchanged in the urine. Clearance is reduced in patients with renal failure.

β_1-Selective Adrenergic Receptor Antagonists

Metoprolol

Metoprolol is a β_1-selective adrenergic receptor antagonist that is devoid of intrinsic sympathomimetic activity and membrane-stabilizing activity.

ADME. *Metoprolol* is almost completely absorbed after oral administration, but bioavailability is relatively low (~40%) due to first-pass metabolism. Plasma concentrations of the drug vary widely (up to 17-fold), possibly due to genetically determined differences in the rate of metabolism in the liver by CYP2D6. Only 10% of the administered drug is recovered unchanged in the urine. The $t_{1/2}$ of *metoprolol* is 3 to 4 h, but can increase to 7 to 8 h in CYP2D6 poor metabolizers who have a

TABLE 14–5 ■ SUMMARY OF ADRENERGIC AGONISTS AND ANTAGONISTS

SUBCLASS	DRUGS	PROMINENT PRINCIPAL PHARMACOLOGICAL ACTIONS	THERAPEUTIC APPLICATIONS	UNTOWARD EFFECTS	COMMENTS
Direct-acting nonselective agonists					
	Epinephrine (α_1, α_2, β_1, β_2, β_3)	↑ Heart rate; ↑ blood pressure; ↑ contractility; slight ↓ in PVR; ↑ cardiac output; vasoconstriction (viscera); vasodilation (skeletal muscle); ↑ blood glucose and lactate	Open-angle glaucoma With local anesthetics to prolong action Anaphylactic shock Complete heart block or cardiac arrest Bronchodilator in asthma	Palpitation Cardiac arrhythmias Cerebral hemorrhage Headache Tremor Restlessness	Not given orally Life saving in anaphylaxis or cardiac arrest
	Norepinephrine (α_1, α_2, $\beta_1 >> \beta_2$)	↑ Systolic and diastolic blood pressure; vasoconstriction; ↑ PVR; direct ↑ in heart rate and contraction; reflex ↓ in heart rate	Hypotension	Similar to EPI Hypertension	Not absorbed orally
β Receptor agonists					
Nonselective ($\beta_1 + \beta_2$)	Isoproterenol	↓ PVR; ↑ cardiac output; bronchodilation	Bronchodilator in asthma Complete heart block or cardiac arrest Shock	Palpitations Tachycardia Tachyarrhythmias Headache Flushed skin Cardiac ischemia in patients with coronary artery disease	Intravenous administration Administered by inhalation in asthma
β_1 Selective	Dobutamine	↑ Contractility; some ↑ heart rate; ↑ AV conduction	Short-term treatment of cardiac decompensation after surgery or patients with congestive heart failure or myocardial infarction	↑ Blood pressure and heart rate	Intravenous only Use with caution in patients with hypertension or cardiac arrhythmias
β_2 Selective (intermediate acting)	Albuterol Bitolterol Fenoterol Isoetharine Levalbuterol Metaproterenol Pirbuterol Procaterol Terbutaline	Relaxation of bronchial smooth muscle Relaxation of uterine smooth muscle Activation of other β_2 receptors after systemic administration	Bronchodilators for treatment of asthma and COPD Short-/intermediate-acting drugs for acute bronchospasm	Skeletal muscle tremor Tachycardia and other cardiac effects seen after systemic administration (much less with inhalational use)	Use with caution in patients with cardiovascular disease (reduced by inhalational administration) Minimal side effects

(*Continued*)

TABLE 14–5 ■ SUMMARY OF ADRENERGIC AGONISTS AND ANTAGONISTS (*CONTINUED*)

SUBCLASS	DRUGS	PROMINENT PRINCIPAL PHARMACOLOGICAL ACTIONS	THERAPEUTIC APPLICATIONS	UNTOWARD EFFECTS	COMMENTS
(Long acting)	Formoterol Salmeterol Arformoterol Carniterol Indacaterol Ritodrine	Relaxation of bronchial smooth muscle Relaxation of uterine smooth muscle	Bronchodilators for treatment of COPD Best choice for prophylaxis due to long action Ritodrine, to stop premature labor	Contraindicated in asthma	Long action, favored for prophylaxis
β_3 Selective	Mirabegron Vibegron	Increases nitric oxide Relaxes nonvascular smooth muscle	Urinary incontinence Overactive bladder syndrome	May increase levels of drugs that are metabolized by CYP2D6	Clinical trials being conducted for heart failure and irritable bowel syndrome Potential use for obesity
α Receptor agonists					
α_1 Selective	Methoxamine Phenylephrine Mephentermine Metaraminol Midodrine	Vasoconstriction	Nasal congestion (used topically) Postural hypotension	Hypertension Reflex bradycardia Dry mouth, sedation, rebound hypertension on abrupt withdrawal	Mephentermine and metaraminol also act indirectly to release NE Midodrine, a prodrug activated *in vivo*
α_2 Selective	Clonidine Apraclonidine Guanfacine Guanabenz Brimonidine α-Methyldopa	↓ Sympathetic outflow from brain to periphery resulting in ↓ PVR and blood pressure ↓ Nerve-evoked release of sympathetic transmitters ↓ Production of aqueous humor	Adjunctive therapy in shock Hypertension To reduce sympathetic response to withdrawal from narcotics, alcohol, and tobacco Glaucoma		Apraclonidine and brimonidine used topically for glaucoma and ocular hypertension Methyldopa is converted in CNS to α-methyl NE, an effective α_2 agonist
Indirect acting	Amphetamine Methamphetamine Methylphenidate (releases NE peripherally; NE, DA, 5HT centrally)	CNS stimulation ↑ Blood pressure Myocardial stimulation	Treatment of ADHD Narcolepsy Obesity (rarely)	Restlessness Tremor Insomnia Anxiety Tachycardia Hypertension Cardiac arrhythmias	Schedule II drugs Marked tolerance occurs Chronic use leads to dependence Can result in hemorrhagic stroke in patients with underlying disease Long-term use can cause paranoid schizophrenia

Mixed acting	Dopamine (α_1, α_2, β_1, D_1; releases NE)	Vasodilation (coronary, renal mesenteric beds) ↑ Glomerular filtration rate and natriuresis ↑ Heart rate and contractility ↑ Systolic blood pressure	Cardiogenic shock Congestive heart failure Treatment of acute renal failure	High doses lead to vasoconstriction Restlessness	Important for its ability to maintain renal blood flow Administered intravenously
	Ephedrine (α_1, α_2, β_1, β_2; releases NE)	Similar to epinephrine but longer lasting CNS stimulation	Bronchodilator for treatment of asthma Nasal congestion Treatment of hypotension and shock	Tremor Insomnia Anxiety Tachycardia Hypertension	Administered by all routes Not commonly used
α Blockers					
Nonselective (classical α blockers)	PBZ Phentolamine Tolazoline	↓ PVR and blood pressure Venodilation	Treatment of catecholamine excess (e.g., pheochromocytoma)	Postural hypotension Failure of ejaculation	Cardiac stimulation due to initiation of reflexes and to enhanced release of NE via α_2 receptor blockade PBZ produces long-lasting α receptor blockade, can block neuronal and extraneuronal uptake of amines
α_1 Selective	Prazosin Terazosin Doxazosin Trimazosin Alfuzosin Tamsulosin Silodosin	↓ PVR and blood pressure Relax smooth muscles in neck of urinary bladder and in prostate	Primary hypertension Increase urine flow in BPH	Postural hypotension when therapy instituted	Prazosin and related quinazolines are selective for α_1 receptors Tamsulosin exhibits some selectivity for α_{1A} receptors
β Blockers					
Nonselective (first generation)	Nadolol Penbutolol Pindolol Propranolol Timolol	↓ Heart rate ↓ Contractility ↓ Cardiac output Slow conduction in atria and AV node ↑ Refractory period, AV node Bronchoconstriction Prolonged hypoglycemia ↓ Plasma free fatty acids ↓ HDL cholesterol ↑ LDL cholesterol and triglycerides Hypokalemia	Angina pectoris Hypertension Cardiac arrhythmias Congestive heart failure Pheochromocytoma Glaucoma Hypertrophic obstructive cardiomyopathy Hyperthyroidism Migraine prophylaxis Acute panic symptoms Substance abuse withdrawal Variceal bleeding in portal hypertension	Bradycardia Negative inotropy ↓ Cardiac output Bradyarrhythmias ↓ AV conduction Bronchoconstriction Fatigue Sleep disturbances (insomnia, nightmares) Prolongation of hypoglycemia Sexual dysfunction in men Drug interactions	Effects depend on sympathoadrenal tone Bronchoconstriction (do not use in asthma and COPD) Hypoglycemia (of concern in hypoglycemics and diabetics) Membrane-stabilizing effect (propranolol, and betaxolol) ISA (strong for pindolol; weak for penbutolol, carteolol, and betaxolol)

(*Continued*)

TABLE 14–5 ■ SUMMARY OF ADRENERGIC AGONISTS AND ANTAGONISTS (*CONTINUED*)

SUBCLASS	DRUGS	PROMINENT PRINCIPAL PHARMACOLOGICAL ACTIONS	THERAPEUTIC APPLICATIONS	UNTOWARD EFFECTS	COMMENTS
β_1 Selective (second generation)	Acebutolol Atenolol Bisoprolol Betaxolol Esmolol Metoprolol	Similar to above but with less adverse effect on bronchial constriction	Similar to above	Similar to above	Effects depend on sympathoadrenal tone Bronchoconstriction effect is less than for nonspecific agents but use only with great caution in asthma and COPD
Nonselective (third-generation) vasodilators	Carteolol Carvedilol Bucindolol Labetalol	See text. These agents affect multiple receptor types and signaling pathways. They are used to treat hypertension; carvedilol is also used to treat heart failure. Effects and applications generally resemble those of other β blockers with some α blocking properties: • α_1 adrenergic receptor blockade (labetalol, carvedilol, bucindolol) • increased production of NO (celiprolol, nebivolol, carteolol) • β_2 agonist properties (celiprolol, carteolol) • Ca^{2+} entry blockade (carvedilol) • antioxidant action (carvedilol)			Vasodilation seen in third-generation drugs; multiple mechanisms (see Figure 14–4) Weak ISA for labetalol Receptor polymorphisms affect response to bucindolol's anti-arrhythmic properties
β_1 Selective (third-generation) vasodilators	Celiprolol Nebivolol				

ISA, intrinsic sympathomimetic activity; NO, nitric oxide; PBZ, phenoxybenzamine; PVR, pulmonary vascular resistance.

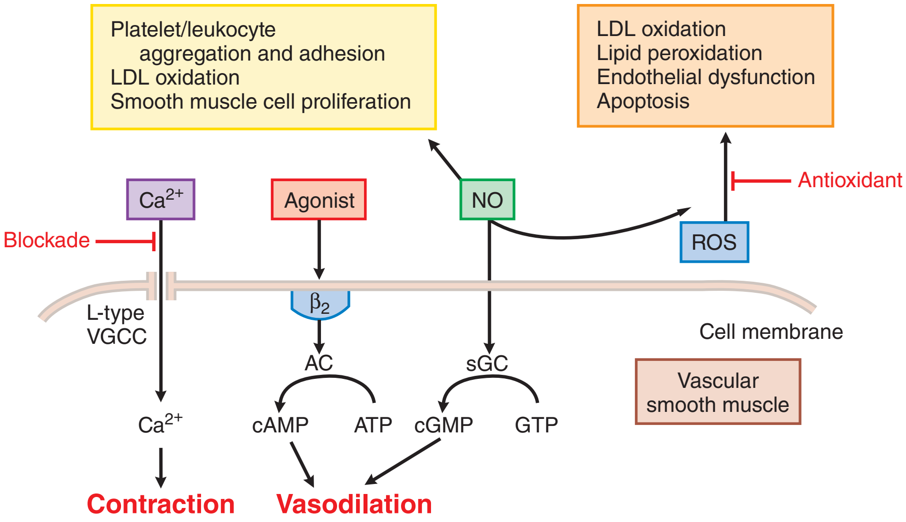


Figure 14–4 *Mechanisms underlying actions of vasodilating β blockers in blood vessels.* Multiple mechanisms contribute to the vasodilating effects of the newer β antagonists. See Table 14–4 for details. AC: adenylyl cyclase; sGC: soluble guanylyl cyclase; NO: nitric oxide; ROS: reactive oxygen species; VGCC: voltage-gated Ca^{2+} channel. (Modified with permission from Toda, 2003. Copyright © Elsevier.)

5-fold higher risk for developing adverse effects (Wuttke et al., 2002). An extended-release formulation is available for once-daily administration.

Therapeutic Uses. *Metoprolol* has been used to treat essential hypertension, angina pectoris, tachycardia, heart failure, and vasovagal syncope and as secondary prevention after myocardial infarction, an adjunct in treatment of hyperthyroidism, and for migraine prophylaxis. For the treatment of hypertension, the usual initial dose is 100 mg/day. The drug sometimes is effective when given once daily, although it frequently is used in two divided doses. Dosage may be increased at weekly intervals until optimal reduction of blood pressure is achieved. *Metoprolol* generally is used in two divided doses for the treatment of stable angina. For the initial treatment of patients with acute myocardial infarction, an intravenous formulation of *metoprolol tartrate* is available; oral dosing is initiated as soon as the clinical situation permits. *Metoprolol* generally is contraindicated for the treatment of acute myocardial infarction in patients with heart rates of less than 45 beats/min, heart block greater than first-degree (PR interval ≥0.24 sec), systolic blood pressure less than 100 mmHg, or moderate-to-severe heart failure.

Atenolol

Atenolol is a β_1-selective antagonist that is devoid of intrinsic sympathomimetic and membrane-stabilizing activity. *Atenolol* is very hydrophilic and appears to penetrate the CNS only to a limited extent.

ADME. *Atenolol* is available in 25-, 50-, and 100-mg oral tablets (initial dose is 50 mg/day). It is incompletely absorbed (~50%) and is excreted largely unchanged in the urine, with elimination $t_{1/2}$ of 5 to 8 h. The drug accumulates in patients with renal failure, and dosage should be adjusted for patients whose creatinine clearance is less than 35 mL/min.

Therapeutic Uses. *Atenolol* can be used to treat hypertension, coronary heart disease, arrhythmias, and angina pectoris and to treat or reduce the risk of heart complications following myocardial infarction. Recent meta-analysis and clinical trials demonstrated a lack of benefit compared with placebo or other antihypertensive agents for reduction of stroke, cardiovascular and all-cause, despite similar blood pressure reduction compared to other antihypertensive agents (Ripley and Saseen, 2014). Compared with other active treatments, *atenolol* was associated with increased risk of all-cause mortality, cardiovascular mortality, and stroke and had a neutral effect on myocardial infarction. *Atenolol* is also used to treat the hypertension that can accompany Graves disease until antithyroid medication can take effect. The initial dose of *atenolol* for the treatment of hypertension usually is 50 mg/day, given once daily. If an adequate therapeutic response is not evident within several weeks, the daily dose may be increased to 100 mg. *Atenolol* has been shown to be efficacious in combination with a diuretic in elderly patients with isolated systolic hypertension. *Atenolol* causes fewer CNS side effects (depression, nightmares) than most β blockers and few bronchospastic reactions due to its pharmacological and pharmacokinetic profile (Varon, 2008).

Esmolol

Esmolol is a β_1-selective antagonist with a rapid onset and a very short duration of action. It has little if any intrinsic sympathomimetic activity and lacks membrane-stabilizing actions. *Esmolol* is administered intravenously and is used when β blockade of short duration is desired or in critically ill patients in whom adverse effects of bradycardia, heart failure, or hypotension may necessitate rapid withdrawal of the drug. It is a class II antiarrhythmic agent (see Chapter 34).

ADME. *Esmolol* is given by slow intravenous injection. Because *esmolol* is used in urgent settings where immediate onset of β receptor blockade is warranted, a partial loading dose (500 μg/kg over 1 min) typically is administered, followed by a continuous infusion of the drug (maintenance dose of 50 μg/kg/min for 4 min). If an adequate therapeutic effect is not observed within 5 min, the same loading dose is repeated, followed by a maintenance infusion at a higher rate. This may need to be repeated until the desired end point (e.g., lowered heart rate or blood pressure) is approached. The drug is hydrolyzed rapidly by esterases in erythrocytes and has a $t_{1/2}$ of about 8 min. The $t_{1/2}$ of the carboxylic acid metabolite of *esmolol* is far longer (~4 h) and will accumulate during prolonged infusion of *esmolol*. However, this metabolite has very low potency as a β receptor antagonist (1/500 of the potency of *esmolol*); it is excreted in the urine.

Therapeutic Uses. *Esmolol* is commonly used in patients during surgery to prevent or treat tachycardia and in the treatment of supraventricular tachycardia. The onset and cessation of β receptor blockade with *esmolol* are rapid: Peak hemodynamic effects occur within 6 to 10 min of administration of a loading dose, and there is substantial diminution of β blockade within 20 min of stopping an infusion. *Esmolol* is particularly useful in severe postoperative hypertension and is a suitable agent in situations where cardiac output, heart rate, and blood pressure are increased. The American Heart Association/American College of Cardiology guidelines recommend against using *esmolol* in patients already on β blocker therapy, bradycardic patients, and patients with decompensated heart failure, as the drug may compromise their myocardial function (Varon, 2008). *Esmolol* is generally tolerated well, but it is associated with an increased risk of hypotension that is rapidly reversible (Garnock-Jones, 2012).

Acebutolol

Acebutolol is a β_1-selective adrenergic antagonist with some intrinsic sympathomimetic and membrane-stabilizing activity.

ADME. *Acebutolol* is administered orally (starting dose 200 mg twice daily titrated up to 1200 mg/day). It is well absorbed and undergoes significant first-pass metabolism to an active metabolite, diacetolol, which accounts for most of the drug's activity. Overall bioavailability is 35% to 50%. The elimination $t_{1/2}$ of *acebutolol* typically is approximately 3 h, but the $t_{1/2}$ of diacetolol is 8 to 12 h; it is excreted largely in the urine. *Acebutolol* is lipophilic and crosses the blood-brain barrier. It has no negative impact on serum lipids (cholesterol, triglycerides, or HDL).

Therapeutic Uses. *Acebutolol* has been used to treat hypertension, ventricular and atrial cardiac arrhythmias, acute myocardial infarction in high-risk patients, and Smith-Magenis syndrome. The initial dose of *acebutolol* in hypertension usually is 400 mg/day; it may be given as a single dose, but two divided doses may be required for adequate control of blood pressure. Optimal responses usually occur with doses of 400 to 800 mg/day (range 200–1200 mg).

Bisoprolol

Bisoprolol is a highly selective β_1 adrenergic receptor antagonist that lacks intrinsic sympathomimetic or membrane-stabilizing activity (McGavin and Keating, 2002). It has a higher degree of β_1 selectivity than *atenolol*, *metoprolol*, or *betaxolol* but less than *nebivolol*. It is approved for the treatment of hypertension.

Bisoprolol generally is well tolerated; side effects include dizziness, bradycardia, hypotension, and fatigue. *Bisoprolol* is well absorbed following oral administration, with bioavailability of about 90%. It is eliminated by renal excretion (50%) and liver metabolism to pharmacologically inactive metabolites (50%). *Bisoprolol* has a plasma $t_{1/2}$ of approximately 11 to 17 h. *Bisoprolol* can be considered a standard treatment option when selecting a β blocker for use in combination with angiotensin-converting enzyme inhibitors and diuretics in patients with stable, moderate-to-severe chronic heart failure and in treating hypertension (McGavin and Keating, 2002; Simon et al., 2003). It has also been used to treat arrhythmias and ischemic heart disease. *Bisoprolol* was associated with a 34% mortality benefit in the CIBIS-II (Cardiac Insufficiency Bisoprolol Study-II).

Betaxolol

Betaxolol is a selective β_1 adrenergic receptor antagonist with no partial agonist activity and slight membrane-stabilizing properties. *Betaxolol* is used to treat hypertension, angina pectoris, and glaucoma. The drug is well absorbed with high bioavailability; its elimination $t_{1/2}$ varies from 14 to 22 h. It is usually well tolerated; side effects are mild and transient.

β Adrenergic Receptor Antagonists With Additional Cardiovascular Effects ("Third-Generation" β Blockers)

In addition to the classical nonselective and β_1-selective adrenergic receptor antagonists, there are drugs that possess vasodilating actions (Toda, 2003). These effects are produced through a variety of mechanisms, including the following:

- *α_1 Adrenergic receptor blockade* (*labetalol, carvedilol, bucindolol, bevantolol, nipradilol*)
- *Increased production of NO* (*celiprolol, nebivolol, carteolol, bopindolol, nipradilol*)
- *β_2 Agonist properties* (*celiprolol, carteolol, bopindolol*)
- *Ca^{2+} entry blockade* (*carvedilol, betaxolol, bevantolol*)
- *Opening of K^+ channels* (*tilisolol*)
- *Antioxidant action* (*carvedilol*)

These actions are summarized in Table 14–4. Some third-generation β adrenergic receptor antagonists are not available in the U.S. but have undergone clinical trials and are available elsewhere.

Labetalol

Labetalol acts as a competitive antagonist at both α_1 and β receptors. *Labetalol* has two optical centers, and the formulation used clinically contains equal amounts of the four diastereomers. The pharmacological properties of the drug are complex because each isomer displays different relative activities. The properties of the mixture include selective blockade of α_1 adrenergic receptors (as compared with the α_2 subtype), blockade of β_1 and β_2 adrenergic receptors, partial agonist activity at β_2 receptors, and inhibition of neuronal uptake of NE (cocaine-like effect). The potency of the mixture for β adrenergic receptor blockade is 5- to 10-fold that for α_1 receptor blockade.

The pharmacological effects of *labetalol* have become clearer since the four isomers were separated and tested individually.

- *The R,R isomer* is about four times more potent as a β receptor antagonist than is racemic *labetalol* and accounts for much of the β blockade produced by the mixture of isomers. As an α_1 antagonist, this isomer is less than 20% as potent as the racemic mixture. The *R,R* isomer has some intrinsic sympathomimetic activity at β_2 adrenergic receptors; this may contribute to vasodilation.
- *The R,S isomer* is almost devoid of both α- and β-blocking effects.
- *The S,R isomer* has almost no β-blocking activity, yet is about five times more potent as an α_1 blocker than is racemic *labetalol*.
- *The S,S isomer* is devoid of β-blocking activity and has a potency similar to that of racemic *labetalol* as an α_1 receptor antagonist.

The actions of *labetalol* on both α_1 and β adrenergic receptors contribute to the fall in blood pressure observed in patients with hypertension. α_1 Receptor blockade leads to relaxation of arterial smooth muscle and vasodilation, particularly when the patient is upright. The β_1 blockade also contributes to a fall in blood pressure, in part by blocking reflex sympathetic stimulation of the heart. In addition, the intrinsic sympathomimetic activity of *labetalol* at β_2 adrenergic receptors may contribute to vasodilation, and the drug may have some direct vasodilating capacity.

Labetalol is available in oral form for therapy of chronic hypertension and as an intravenous formulation for use in hypertensive emergencies. *Labetalol* has been associated with hepatic injury in a limited number of patients. *Labetalol* has been recommended as treatment of acute severe hypertension (hypertensive emergency). Its hypotensive action begins within 2 to 5 min after intravenous administration, reaching its peak at 5 to 15 min and lasting about 2 to 4 h. Heart rate is either maintained or slightly reduced, and cardiac output is maintained. *Labetalol* reduces systemic vascular resistance without reducing total peripheral blood flow. Cerebral, renal, and coronary blood flow is maintained. It can be used in the setting of pregnancy-induced hypertensive crisis because little placental transfer occurs due to the poor lipid solubility of *labetalol*.

ADME. Although *labetalol* is completely absorbed from the gut, there is extensive first-pass clearance; bioavailability is about 20% to 40% but may be increased by food intake. The drug is rapidly metabolized in the liver; very little unchanged drug is found in the urine. The rate of metabolism of *labetalol* is sensitive to changes in hepatic blood flow. The elimination $t_{1/2}$ of the drug is about 8 h. The $t_{1/2}$ of the *R,R* isomer of *labetalol* is approximately 15 h.

Carvedilol

Carvedilol is a third-generation β receptor antagonist that has a unique pharmacological profile. It blocks β_1, β_2, and α_1 receptors similarly to *labetalol* but also has antioxidant and anti-inflammatory properties (DiNicolantonio et al., 2015), features that may be beneficial in treating congestive heart failure (see below). The drug has membrane-stabilizing activity but lacks intrinsic sympathomimetic activity. *Carvedilol* has also been demonstrated to be a β-arrestin–biased ligand that promotes β-arrestin recruitment to β_1 and β_2 receptors in the absence of G_s activation, the clinical relevance of which has yet to be determined.

Carvedilol reduces arterial blood pressure by decreasing vascular resistance and maintaining cardiac output while decreasing sympathetic vascular tone (DiNicolantonio et al., 2015; Zepeda et al., 2012). The hemodynamic effect exerted by *carvedilol* is similar to that of angiotensin-converting enzyme inhibitors and superior to that of traditional β blockers such as *propranolol*. *Carvedilol* is renoprotective and has favorable effects in patients with diabetes or metabolic syndrome. The drug is FDA approved for use in hypertension, congestive heart failure, and left ventricular dysfunction following myocardial infarction.

Carvedilol possesses two distinct antioxidant properties: It is a chemical antioxidant that can scavenge reactive oxygen species (ROS), and it can suppress the biosynthesis of ROS and oxygen radicals. *Carvedilol* is extremely lipophilic and protects cell membranes from lipid peroxidation. It prevents LDL oxidation, which in turn induces the uptake of LDL into the coronary vasculature. *Carvedilol* also inhibits ROS-mediated loss of myocardial contractility, stress-induced hypertrophy, apoptosis, and the accumulation and activation of neutrophils. At high doses, *carvedilol* exerts Ca^{2+} channel–blocking activity.

Numerous controlled trials have shown that *carvedilol* improves ventricular function and reduces mortality and morbidity in patients with mild-to-severe congestive heart failure (Chatterjee et al., 2013; Poole-Wilson et al., 2003). Several experts recommend it as the standard treatment option in this setting. In addition, *carvedilol*, in combination with conventional therapy, reduces mortality and attenuates myocardial infarction. In patients with chronic heart failure, *carvedilol* reduces cardiac sympathetic drive, but it is not clear whether blockade of α_1 adrenergic receptor–mediated vasodilation is maintained over long periods of time.

ADME. *Carvedilol* is rapidly absorbed following oral administration, with peak plasma concentrations occurring in 1 to 2 h. It is highly lipophilic and more than 95% protein bound. Hepatic CYPs 2D6 and 2C9 metabolize *carvedilol*, yielding a $t_{1/2}$ of 7 to 10 h. Stereoselective first-pass metabolism results in more rapid clearance of S(−)-carvedilol than R(+)-carvedilol. No significant changes in the pharmacokinetics of *carvedilol* are seen in elderly patients with hypertension, and no change in dosage is needed in patients with moderate-to-severe renal insufficiency (Keating and Jarvis, 2003). Because of extensive hepatic metabolism, *carvedilol*'s pharmacokinetics can be profoundly affected by drugs that induce or inhibit CYPs 2D6 and 2C9. These include the inducer *rifampin* and inhibitors such as *cimetidine, quinidine, fluoxetine*, and *paroxetine*.

Bucindolol

Bucindolol is a third-generation nonselective β adrenergic receptor antagonist with weak α_1 receptor–blocking properties. *Bucindolol* increases left ventricular systolic ejection fraction and decreases peripheral resistance, thereby reducing afterload. It increases plasma HDL cholesterol but does not affect plasma triglycerides. A large comprehensive clinical trial, BEST (β Blocker Evaluation of Survival Trial), was terminated early because of a lack of a demonstrable survival benefit with *bucindolol* versus placebo. Further analysis has demonstrated that polymorphisms in β_1 and α_{2c} adrenergic receptors predict the effect of *bucindolol* to prevent new-onset atrial fibrillation and ventricular arrhythmias (Cooper-DeHoff and Johnson, 2016; O'Connor et al., 2012).

Celiprolol

Celiprolol is a third-generation, cardioselective β adrenergic receptor antagonist used for treatment of hypertension and angina. It has low lipid solubility, is devoid of membrane-stabilizing activity, and possesses weak vasodilating and bronchodilating effects attributed to partial selective β_2 agonist activity and possibly *papaverine*-like relaxant effects on smooth muscle (including bronchial smooth muscle). It also has been reported to antagonize peripheral α_2 adrenergic receptor activity, to promote NO production, and to inhibit oxidative stress. Weak α_1 adrenergic receptor antagonistic properties are present but are not considered clinically significant at therapeutic doses (Toda, 2003). *Celiprolol* reduces heart rate and blood pressure and can increase the functional refractory period of the AV node. Oral bioavailability ranges broadly (30%–70%); peak plasma levels occur 2 to 4 h after oral administration. It is predominantly excreted in the urine, largely unchanged, and to a lesser extent in the feces.

Nebivolol

Nebivolol is a third-generation, long-acting, and highly selective β_1 adrenergic receptor antagonist that stimulates NO-mediated vasodilation via β_3 receptor agonism (Fongemie and Felix-Getzik, 2015). *Nebivolol* is devoid of intrinsic sympathomimetic effects as well as membrane-stabilizing activity and α_1 receptor–blocking properties.

ADME. *Nebivolol* is administered as the racemate containing equal amounts of the *d*- and *l*-enantiomers. The *d*-isomer is the active β-blocking component; the *l*-isomer is responsible for enhancing production of NO. *Nebivolol* is lipophilic, and concomitant administration of *chlorthalidone*, *hydrochlorothiazide*, *theophylline*, or *digoxin* with *nebivolol* may reduce its extent of absorption.

Nebivolol undergoes extensive first-pass metabolism, primarily by CYP2D6, with a mean terminal $t_{1/2}$ of about 10 h. Active metabolites (e.g., 4-OH nebivolol) contribute to the β-blocking effect of *nebivolol*. Polymorphisms in the CYP2D6 gene affect *nebivolol*'s metabolism but not its efficacy due to the production of active hydroxylated metabolites (Lefebvre et al., 2007).

Therapeutic Uses. *Nebivolol* is approved for treatment of hypertension and may be associated with better outcomes than first- and second-generation β receptor antagonists with reduced risk of hospitalization due to cardiovascular events (Olawi et al., 2019). *Nebivolol* lowers blood pressure by reducing peripheral vascular resistance and significantly increases stroke volume with preservation of cardiac output to maintain systemic blood flow to target organs. *Nebivolol* also reduces endothelial dysfunction and oxidative stress and may have favorable effects on both carbohydrate and lipid metabolism. These benefits are also observed in the presence of metabolic syndrome, which often presents along with hypertension (Ignarro, 2008). The NO-dependent vasodilating action of *nebivolol* and its high β_1 adrenergic receptor selectivity likely contribute to the drug's efficacy and comparative tolerability as an antihypertensive agent (e.g., less fatigue and sexual dysfunction in men) (Olawi et al., 2019). While *nebivolol* has potential utility in the treatment of heart failure with reduced ejection fraction, it may not reduce mortality to the same degree as *carvedilol*, *metoprolol*, or *bisoprolol* (Chatterjee et al., 2013).

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Chapter 15

5-Hydroxytryptamine (Serotonin) and Dopamine

Charles D. Nichols, Susan G. Amara, and David R. Sibley

INTRODUCTION

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Introduction

5-Hydroxytryptamine (5HT, serotonin) and dopamine (DA) are neurotransmitters in the central nervous system (CNS) that also have prominent peripheral actions. Although high concentrations of 5HT are present in the CNS, about 95% of all 5HT in the human body is located in the periphery, with high levels in enterochromaffin cells throughout the gastrointestinal (GI) tract and in storage granules in platelets. The highest concentrations of DA are found in the brain, but DA stores are also present peripherally in the adrenal medulla, in the plexuses of the GI tract, and in the enteric nervous system. Fourteen mammalian 5HT receptor subtypes, categorized into seven subfamilies, and five DA receptor subtypes, categorized into two subfamilies, have been delineated by structural and pharmacological analyses and are encoded by separate genes. For some receptors, alternative RNA splicing or editing creates additional heterogeneity; for example, over 30 isoforms of the $5HT_{2C}$ receptor subtype arise from RNA editing. The identification of individual receptor subtypes has facilitated development of subtype-selective drugs and elucidation of actions of 5HT and DA at a molecular level. Increasingly, therapeutic goals are being achieved by using drugs that selectively target one or more of the subtypes of 5HT or DA receptors or that act on a combination of both 5HT and DA receptors.

5-Hydroxytryptamine

HISTORICAL PERSPECTIVE

In the 1930s, Vittorio Erspamer began to study the distribution of enterochromaffin cells, which were stained with a reagent for indoles. The highest concentrations of these cells were found in GI mucosa, followed by platelets and the CNS. Subsequently, Irving Page and colleagues at the Cleveland Clinic isolated and characterized a vasoconstrictor substance released from platelets in clotting blood. This substance, named serotonin by Page, was identical to the indole, enteramine, isolated by Erspamer. Both enteramine and serotonin proved to be 5-hydroxytryptamine. Subsequent discovery of the biosynthetic and degradative pathways for 5HT and clinical presentation of patients with carcinoid tumors of intestinal enterochromaffin cells spurred interest in 5HT. In the mid-1950s, Betty Tvorog discovered that 5HT was also present in the brain. Subsequent discoveries that the pronounced behavioral effects of *reserpine* are accompanied by a profound decrease in brain 5HT led to the proposal that 5HT might be a neurotransmitter in the mammalian CNS. Numerous congeners of 5HT have pharmacological activity (see Figure 15–1). Many of the *N*- and *O*-methylated indoleamines have psychedelic or hallucinogenic properties. A close relative of 5HT, melatonin, is formed by sequential *N*-acetylation and *O*-methylation (Figures 15–1 and 15–2). Melatonin, not to be confused with the pigment melanin, is the principal indoleamine in the pineal gland, where it modulates circadian rhythms , especially those relating to sleep preparation, hence its use in the treatmnt of jet lag and insomnia.

Synthesis and Metabolism of 5-Hydroxytryptamine (Serotonin)

Synthesis of 5HT (5-hydroxytryptamine, serotonin) occurs by a two-step pathway from the essential amino acid tryptophan (Figure 15–2). Tryptophan is actively transported into the brain by L-type amino acid transporter 1 (LAT1), a heteromeric carrier protein that also transports other large neutral and branched-chain amino acids as well as some drugs.

Abbreviations

AADC: aromatic L-amino acid decarboxylase
ADHD: attention-deficit/hyperactivity disorder
BBB: blood-brain barrier
COMT: catechol-*O*-methyl transferase
CGRP: calcitonin gene-related peptide
CSF: cerebrospinal fluid
DA: dopamine
DAG: diacylglycerol
DAT: dopamine transporter
L-DOPA: 3,4-dihydroxyphenylalanine
DOPAC: 3,4-dihydroxyphenylacetic acid
EPI: epinephrine
EPS: extrapyramidal symptoms
GABA: γ-aminobutyric acid
GI: gastrointestinal
GPCR: G protein-coupled receptor
GSK-3: glycogen synthase kinase 3
5-HIAA: 5-hydroxyindole acetic acid
HSDD: hypoactive sexual desire disorder
5HT: 5-hydroxytryptamine, serotonin
HVA: homovanillic acid
LSD: lysergic acid diethylamide
MAO: monoamine oxidase
MPP$^+$: 1-methyl-4-phenylpyridinium
MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSAA: multifunctional serotonin agonists and antagonists
NE: norepinephrine
NET: norepinephrine transporter
NO: nitric oxide
NSAID: nonsteroidal anti-inflammatory drug
NSS: neurotransmitter–sodium symporter
6-OHDA: 6-hydroxydopamine
PD: Parkinson's disease
PFC: prefrontal cortex
PKC: protein kinase C
PLC: phospholipase C
RLS: restless leg syndrome
SERT: serotonin transporter
SNRI: serotonin-norepinephrine reuptake inhibitor
SSRI: selective serotonin reuptake inhibitor
TAAR1: trace amine-associated receptor 1
TCA: tricyclic antidepressants
VMAT2: vesicular monoamine transporter 2
VNTR: variable number of tandem repeat

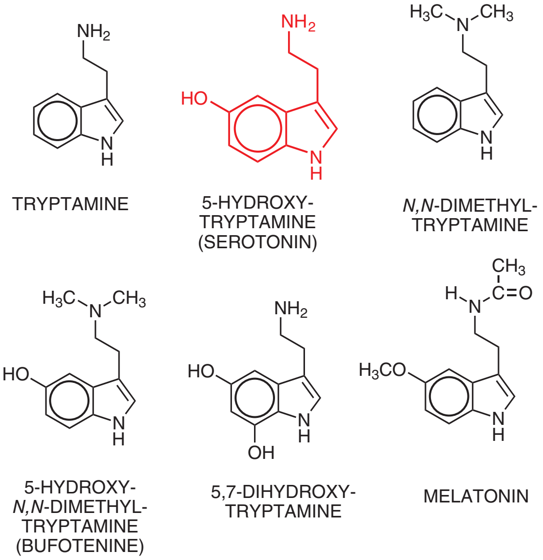

TRYPTAMINE
5-HYDROXY-TRYPTAMINE (SEROTONIN)
N,N-DIMETHYL-TRYPTAMINE
5-HYDROXY-*N,N*-DIMETHYL-TRYPTAMINE (BUFOTENINE)
5,7-DIHYDROXY-TRYPTAMINE
MELATONIN
NH2
HO
H3C
CH3
N
H
OH
H3CO
C=O

Figure 15–1 *Structures of representative indolealkylamines.*

Levels of tryptophan in the brain are influenced not only by its plasma concentration but also by the plasma concentrations of other amino acids that compete for the transporter. Tryptophan hydroxylase 1 (TPH1) is a mixed-function oxidase that requires molecular O_2 and a reduced pteridine cofactor for activity and is the rate-limiting enzyme in the synthetic pathway. TPH2, a brain-specific isoform of TPH1, is entirely responsible for the synthesis of brain 5HT. 5HT itself does not cross the blood-brain barrier (BBB) and thus must be synthesized locally within the CNS. Brain TPH2 is not generally saturated with substrate; consequently, the concentration of tryptophan in the brain influences the synthesis of 5HT.

L-5-hydroxytryptophan is converted to 5HT by aromatic L-amino acid decarboxylase (AADC). AADC is widely distributed and has broad substrate specificity. The synthesized product, 5HT, is transported into secretory granules by the vesicular monoamine transporter, VMAT2. *Reserpine* depletes vesicular stores of 5HT and other monoamines by selectively inhibiting VMAT2. Based on its ability to deplete norepinephrine (NE) or DA, *reserpine* was once used as an antihypertensive or antipsychotic agent, respectively; however, it is no longer used for these purposes. In the CNS, stored vesicular 5HT is released by exocytosis from serotonergic neurons in response to an action potential. 5HT signaling is either terminated through neuronal reuptake by the serotonin transporter (SERT), localized in the membrane of serotonergic axon terminals, or degraded by monoamine oxidase A (MAO-A). SERT is also found on the cellular membranes of platelets and is crucial for regulating and maintaining serotonin concentrations within platelets, which lack the ability to synthesize 5HT. Localized on the plasma membrane, SERT serves a function distinct from VMAT2, a vesicular monoamine transporter that concentrates monoamines into intracellular storage vesicles. SERT can be inhibited by selective serotonin reuptake inhibitors (SSRIs) that are used to treat depression and other mood disorders.

The principal route of metabolism of 5HT involves oxidative deamination by monoamine oxidase (MAO). The aldehyde intermediate formed is converted to 5-hydroxyindole acetic acid (5-HIAA) by aldehyde dehydrogenase (see Figure 15–2). An alternative route, reduction of the acetaldehyde to an alcohol to generate 5-hydroxytryptophol, is normally insignificant. 5-HIAA is actively transported out of the brain by a process that is sensitive to the nonspecific transport inhibitor *probenecid.* 5-HIAA produced from metabolism of 5HT is excreted in the urine along with small amounts of 5-hydroxytryptophol sulfate or glucuronide conjugates.

Of the two isoforms of MAO (see Chapter 10), MAO-A preferentially metabolizes 5HT and NE. Selective MAO-A inhibitors increase stores of 5HT and NE and are considered first-generation antidepressant agents (see Chapter 15). MAO-B prefers β-phenylethylamine and benzylamine as substrates. DA and tryptamine are metabolized equally well by both isoforms. Neurons contain both isoforms of MAO, which are found primarily in the outer membrane of mitochondria. MAO-B is the principal isoform in platelets, which contain large amounts of 5HT.

Serotonergic Projection Pathways in the Brain

In the CNS, 5HT is almost entirely synthesized by cells located in the raphe nuclei in the brainstem. These neurons exhibit extensive projections throughout the brain and spinal cord. These projections are so extensive that it has been hypothesized that every neuron in the brain may be in synaptic contact with a serotonergic projection fiber (Figure 15–3). Individual cell types both in the brain and body, however, produce small amounts of 5HT. The role of this intracellular 5HT is unclear, but it may be necessary for posttranslational modifications of intracellular proteins by "serotonylation" where 5HT is covalently attached to glutamine residues of proteins via the enzyme transglutaminase.

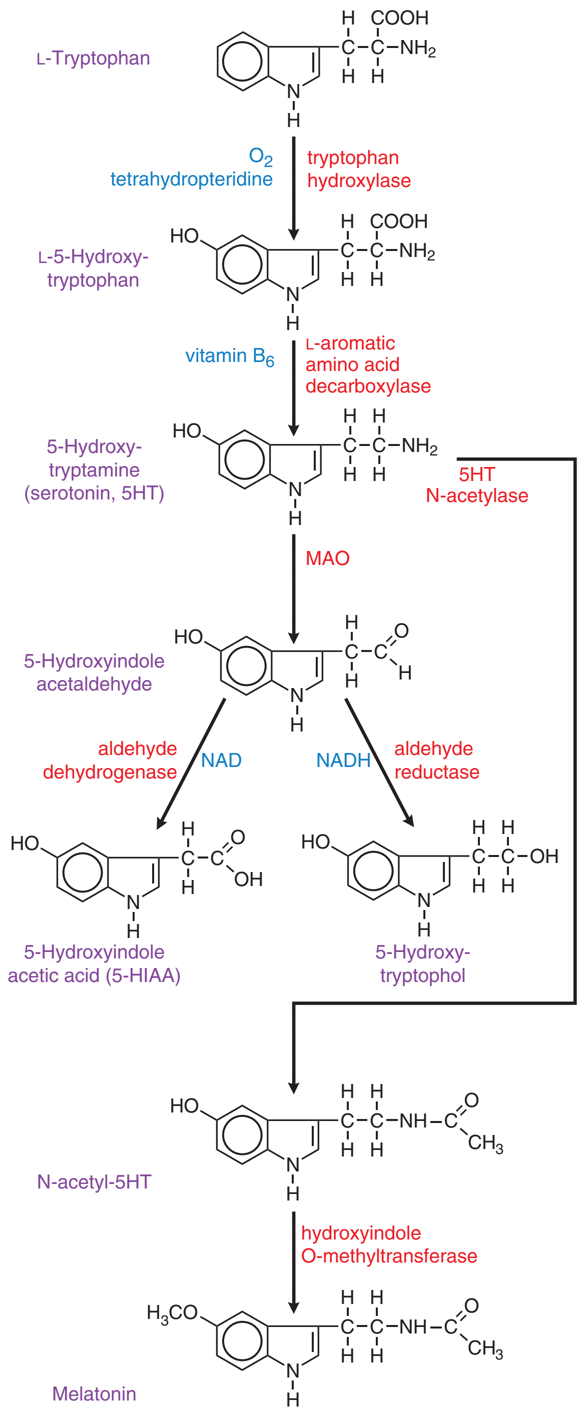


Figure 15–2 *Synthesis and inactivation of serotonin.* Enzymes are identified in red lettering, and cofactors are shown in *blue*.

A model of a serotonergic synapse is depicted in Figure 15–4. 5HT released from the nerve terminal activates cell-specific postsynaptic receptors, leading to activation of effectors and signal transduction. Presynaptic 5HT receptors (autoreceptors) also exist on the nerve terminal where they can act to modulate 5HT release via negative feedback. Reuptake of 5HT by the SERT transporter is the primary mechanism for termination of 5HT action and allows for either vesicular repackaging of transmitter or metabolism.

The 5HT Receptors

Multiple 5HT receptor subtypes and their splice and edited isoforms mediate serotonin's diverse array of physiological effects and comprise the largest known neurotransmitter/hormone receptor family (Barnes et al., 2020). The 5HT receptor subtypes are expressed in distinct but often overlapping patterns and couple with differing efficiencies to different transmembrane signaling mechanisms (Table 15–1). With the exception of the $5HT_3$ receptor, which is a ligand-gated ion channel, all of the 5HT receptor subtypes are G protein-coupled receptors (GPCRs) (see Figures 16–7 and 16–8).

The $5HT_1$ Receptor Subfamily

- The $5HT_1$ receptor family comprises five members, all of which preferentially couple to $G_{i/o}$ and inhibit adenylyl cyclase and cyclic AMP accumulation. $5HT_1$ receptors are also known to modulate K^+ and Ca^{2+} channels.
- The $5HT_{1A}$ and $5HT_{1B/1D}$ receptors can act as autoreceptors, either on the cell bodies ($5HT_{1A}$) or on the axon terminals ($5HT_{1B/1D}$) (Figure 15–5). The density of $5HT_{1A}$ receptors is high in the brain, where they have been implicated in depression and anxiety. $5HT_{1B}$ and $5HT_{1D}$ receptors are predominantly expressed in the brain, and these two receptors share significant co-localization in the CNS. Antimigraine triptan drugs are $5HT_{1B/1D}$ agonists (see further discussion).
- The $5HT_{1E}$ and $5HT_{1F}$ receptors are predominantly found in the brain; however, their precise functions remain unclear. A $5HT_{1F}$ receptor–selective agonist, *lasmiditan*, has been recently approved as a clinical therapeutic for migraines.

The $5HT_2$ Receptor Subfamily

- The three subtypes of $5HT_2$ receptors preferentially couple to G_q/G_{11} proteins and activate PLC-DAG/IP_3-Ca^{2+}-PKC pathways (see Table 15–1). $5HT_2$ receptor stimulation can also lead to activation of phospholipase A_2, promoting the release of arachidonic acid.
- $5HT_{2A}$ receptors are the most widespread of the 5HT receptors and expressed in nearly every type of tissue and cell type throughout the body. In the CNS, they are found at high densities in several brain structures including the prefrontal, parietal, and somatosensory cortices. In the periphery, they are found at high levels in blood platelets and smooth muscle cells, among other tissues. Many antipsychotic drugs inhibit $5HT_{2A}$ receptors, and psychedelics activate $5HT_{2A}$ receptors.
- The $5HT_{2B}$ receptor is not as widely expressed but is found at low levels in the brain and peripheral tissues. It is found at higher levels in vascular smooth muscle and certain immune cells.
- The $5HT_{2C}$ receptor is the only GPCR whose activity is regulated by RNA editing. Multiple $5HT_{2C}$ receptor isoforms generated by RNA editing exhibit modified G protein-coupling efficiencies (Barnes et al., 2020). The $5HT_{2C}$ receptor has been implicated in the control of cerebrospinal fluid (CSF) production, feeding behavior, and mood and is expressed most highly in the brain and choroid plexus.

$5HT_3$ Receptors

- The $5HT_3$ receptor is the only monoamine neurotransmitter receptor that functions as a ligand-gated ion channel.
- The functional $5HT_3$ receptor forms pentameric complexes consisting of three distinct subunits, and activation of these ligand-gated channels elicits a rapidly desensitizing depolarization, mediated by the gating of cations.
- $5HT_3$ receptors are located on parasympathetic terminals in the GI tract, including vagal and splanchnic afferents. In the CNS, a high density of $5HT_3$ receptors occurs in the solitary tract nucleus, the area postrema, and the vomiting center of the brainstem. $5HT_3$ receptors in both the GI tract and the CNS participate in the emetic response, providing a basis for the antiemetic property of FDA-approved $5HT_3$ receptor antagonists like *ondansetron*. The functional $5HT_3$ receptor is not the product of a single gene but can be composed of subunits expressed from one of five separate gene products ($5HT_{3A-E}$).

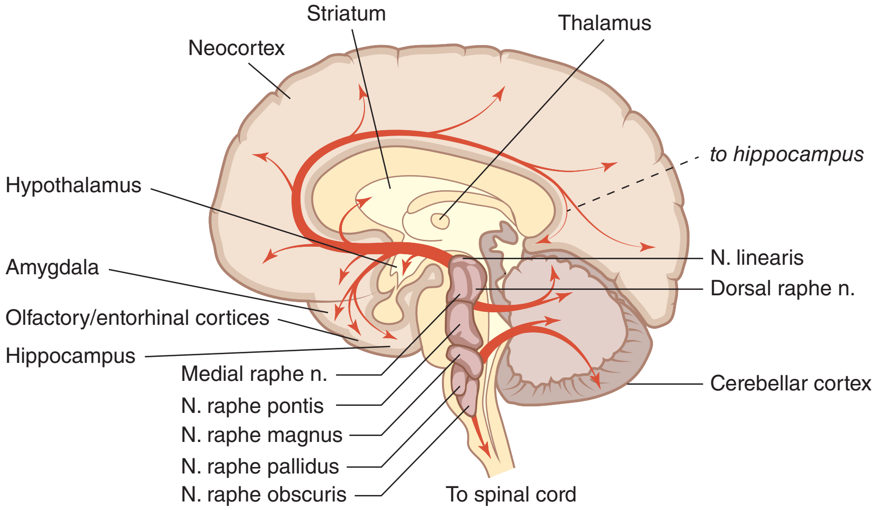


Figure 15–3 *Serotonergic pathways in the brain.* Serotonin is produced by several discrete brainstem nuclei, shown here in rostral and caudal clusters. The rostral nuclei, which include the nucleus, dorsal raphe, medial raphe, and raphe pontis, innervate most of the brain, including the cerebellum. The caudal nuclei, which comprise the raphe magnus, raphe pallidus, and raphe obscuris, have more limited projections that terminate in the cerebellum, brainstem, and spinal cord. Together, the rostral and caudal nuclei innervate most of the CNS. (Modified with permission from Nestler EJ et al., eds. *Molecular Neuropharmacology: A Foundation for Clinical Neuroscience*, 3rd ed. McGraw Hill, New York, **2015**.)

5HT$_4$ Receptors

- The 5HT$_4$ receptor subtype couples to G$_s$ to activate adenylyl cyclase and increase cyclic AMP production.
- In the CNS, high densities of 5HT$_4$ receptors are found on neurons of the superior and inferior colliculi and in the hippocampus. In the GI tract, 5HT$_4$ receptors are located on neurons of the myenteric plexus and on smooth muscle and secretory cells. Stimulation of the 5HT$_4$ receptor is thought to evoke secretion and to facilitate the peristaltic reflex. The latter effect may explain the utility of prokinetic benzamide drugs in GI disorders (see Chapter 54).
- Effects of pharmacological manipulation of 5HT$_4$ receptors on memory and feeding behaviors in animal models suggest possible clinical applications in the future.

5HT$_5$ Receptors

- The 5HT$_5$ subfamily preferentially couples to G$_{i/o}$ to inhibit adenylyl cyclase.
- Humans express a single functional 5HT$_{5A}$ receptor, whereas rodents express both 5HT$_{5A}$ and 5HT$_{5B}$ receptors. The human 5HT$_{5B}$ gene is interrupted by a stop codon and several other polymorphisms, leading to a nonfunctional gene.
- The 5HT$_{5A}$ receptor has high constitutive activity and is expressed widely in the CNS where it is linked to anxiety, circadian rhythms, and cognition. There is little to no expression of 5HT$_5$ receptors in the periphery.

5HT$_6$ Receptors

- The 5HT$_6$ receptor preferentially couples to G$_s$ to activate adenylyl cyclase and increase intracellular cyclic AMP levels.
- The 5HT$_6$ receptor is almost exclusively found in the CNS; its abundance in cortical, limbic, and extrapyramidal regions suggests that it is important for motor control and cognition. It is one of the few GPCRs found in primary neuronal cilia.
- 5HT$_6$ receptor antagonists are currently in clinical trials as a therapeutic modality for cognitive decline in patients with Alzheimer's disease and dementia.

5HT$_7$ Receptors

- The 5HT$_7$ receptor preferentially couples to G$_s$ to activate adenylyl cyclase and increase intracellular cyclic AMP. It is widely distributed throughout the CNS, including the trigeminal nerve. It is also found in the periphery at high levels in several tissues including the spleen, GI tract, and arteries.
- 5HT$_7$ receptors play a role in thermoregulation, sleep, nociception, cognitive function, and the relaxation of smooth muscle in the GI tract and the vasculature. Blockade of 5HT$_7$ receptor activity may play a role in the therapeutic action of some antipsychotic and antidepressant medications.
- There is a 5HT$_7$ receptor pseudogene in humans whose mRNA is widely expressed throughout the body but is not translated into a functional receptor.

The 5HT Transporter (SERT)

- The actions of 5HT are primarily terminated by SERT (SLC6A4), the transport protein responsible for the reuptake of 5HT into serotonergic neurons.
- Encoded by a single gene, SERT possesses 12 membrane-spanning domains and is a member of the neurotransmitter–sodium symporter (NSS) family that includes the transporters for DA, NE, γ-aminobutyric acid (GABA), and glycine. SERT is expressed prominently in central serotonergic neurons that originate in the raphe nucleus, but is also found in platelets, placenta, lung, gut, enteric nervous system, and the adrenal gland.
- SERT is a secondary active transporter that couples 5HT transport to the movement of sodium into the cell.
- SSRIs such as *fluoxetine*, *paroxetine*, *citalopram*, and *sertraline* bind to the SERT and inhibit serotonin transport. Tricyclic antidepressants (TCAs) and the newer class of serotonin-norepinephrine reuptake inhibitors (SNRIs) that includes *venlafaxine* and *duloxetine* block SERT, the NE transporter (NET), or both with varying degrees of selectivity.
- SSRIs and SNRIs are prescribed for major depressive disorder, obsessive-compulsive disorder, panic disorder, generalized anxiety disorder, fibromyalgia, and neuropathic pain, among others.

Actions of 5HT in Physiological Systems

Platelets

Platelets differ from other formed elements of blood in expressing mechanisms for uptake, storage, and exocytotic release of 5HT. 5HT is not synthesized in platelets but is taken up from the circulation via the SERT and

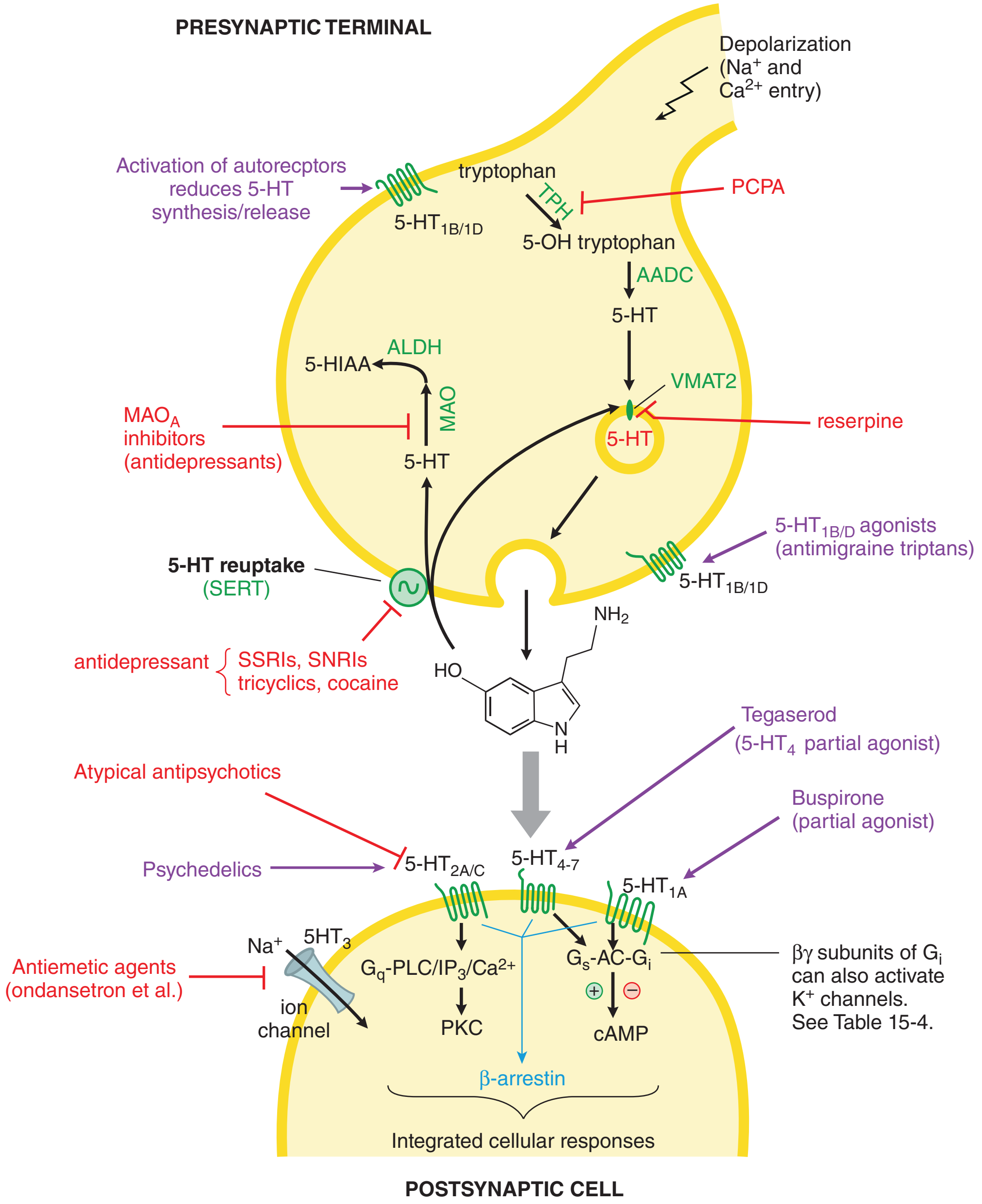

PRESYNAPTIC TERMINAL
Depolarization (Na+ and Ca2+ entry)
Activation of autorecptors reduces 5-HT synthesis/release
5-HT1B/1D
tryptophan
TPH
PCPA
5-OH tryptophan
AADC
5-HT
ALDH
5-HIAA
MAO
VMAT2
MAOA inhibitors (antidepressants)
5-HT
5-HT
reserpine
5-HT1B/D agonists (antimigraine triptans)
5-HT reuptake
(SERT)
5-HT1B/1D
NH2
antidepressant
SSRIs, SNRIs
tricyclics, cocaine
HO
N
H
Tegaserod (5-HT4 partial agonist)
Atypical antipsychotics
Buspirone (partial agonist)
Psychedelics
5-HT2A/C
5-HT4-7
5-HT1A
Na+
5HT3
Antiemetic agents (ondansetron et al.)
ion channel
Gq-PLC/IP3/Ca2+
PKC
Gs-AC-Gi
+
−
cAMP
βγ subunits of Gi can also activate K+ channels. See Table 15-4.
β-arrestin
Integrated cellular responses
POSTSYNAPTIC CELL

Figure 15–4 *A serotonergic synapse.* Presynaptic and postsynaptic molecular entities involved in the synthesis, release, signaling, and reuptake of serotonin are shown. MAO is in mitochondria within serotonergic nerve terminals.

stored in secretory granules by active transport, similar to the uptake and storage of serotonin by serotonergic nerve terminals. When platelets make contact with injured endothelium (see Chapter 36), they release substances that promote platelet aggregation; secondarily, they release 5HT (Figure 15–6). 5HT binds to platelet $5HT_{2A}$ receptors and elicits a weak aggregation response that is markedly augmented by the presence of collagen. If the damaged blood vessel is injured to a depth where vascular smooth muscle is exposed, 5HT exerts a direct vasoconstrictor effect, thereby contributing to hemostasis, which is enhanced by locally released autocoids (thromboxane A_2 [TxA_2], kinins, and vasoactive peptides). Conversely, 5HT may interact with endothelial cells to stimulate production of nitric oxide (NO) and antagonize its own vasoconstrictor action, as well as the vasoconstriction produced by other locally released agents.

Cardiovascular System

The classical response of blood vessels to 5HT is contraction, particularly in the splanchnic, renal, pulmonary, and cerebral vasculatures. 5HT also induces a variety of responses in the heart that are the result of activation of multiple 5HT receptor subtypes, stimulation or inhibition of autonomic nerve activity, or dominance of reflex responses to 5HT. Thus, 5HT has positive inotropic and chronotropic actions on the heart that may be blunted by simultaneous stimulation of afferent nerves from baroreceptors and chemoreceptors. Activation of $5HT_3$ receptors on vagal nerve endings elicits the Bezold-Jarisch reflex, causing extreme bradycardia and hypotension. The local response of arterial blood vessels to 5HT also may be inhibitory, the result of the stimulation of endothelial NO production and prostaglandin synthesis and blockade of NE release from sympathetic nerves. Conversely, 5HT amplifies the local constrictor actions of NE, angiotensin II, and histamine, which reinforce the hemostatic response to 5HT.

Gastrointestinal Tract

Enterochromaffin cells in the gastric mucosa are the site of the synthesis and most of the storage of 5HT in the body and are the source of circulating 5HT. Motility of gastric and intestinal smooth muscle may be either enhanced or inhibited via signaling mediated by at least five subtypes of 5HT receptors (Table 15–2).

TABLE 15–1 ■ SEROTONIN RECEPTOR SUBTYPES[a]

SUBTYPE	SIGNALING EFFECTOR	LOCALIZATION	FUNCTION	AGONISTS	ANTAGONISTS
5-HT$_{1A}$	↓ AC	Raphe nuclei, cortex, hippocampus	Somatodendritic autoreceptor in raphe Postsynaptic in cortex and hippocampus	8-OH-DPAT, buspirone	WAY 100135
5-HT$_{1B}$	↓ AC	Cortex, subiculum, globus pallidus, substantia nigra	Presynaptic autoreceptor	Sumatriptan, CP94253	GR-55562
5-HT$_{1D}$	↓ AC	Cranial vessels, globus pallidus, substantia nigra	Presynaptic autoreceptor, vasoconstriction	Sumatriptan, PNU142633	SB 714786
5-HT$_{1E}$	↓ AC	Cortex, striatum	—	—	—
5-HT$_{1F}$	↓ AC	Dorsal raphe, hippocampus, periphery	—	Lasmiditan	—
5-HT$_{2A}$	↑ PLC, PLA$_2$	Widespread, including platelets, smooth muscle, cerebral cortex, immune cells	Platelet aggregation, smooth muscle contraction, neuronal excitation	DOI, 25CN-NBOH	M100907, pimivanserin
5-HT$_{2B}$	↑ PLC	Stomach fundus	Smooth muscle contraction	BW723C86, DOI	LY266097
5-HT$_{2C}$	↑ PLC, PLA$_2$	Choroid plexus, substantia nigra, basal ganglia	CSF production, neuronal excitation	1-methylpsilocin, Ro-60-0175, DOI	RS102221,
5-HT$_3$	Cations	Parasympathetic nerves, solitary tract, area postrema, GI tract	Neuronal excitation	SR57227, quipazine	Ondansetron, palonosetron
5-HT$_4$	↑ AC	Hippocampus, striatum, GI tract	Neuronal excitation	BIMU8, tegaserod	GR 113808
5-HT$_{5A}$	↓ AC	Cortex, hippocampus	Unknown	—	SB-699551
5-HT$_{5B}$	Unknown	—	Pseudogene in humans	—	—
5-HT$_6$	↑ AC	Hippocampus, striatum, nucleus accumbens	Neuronal excitation	WAY-181187	SB-271046
5-HT$_7$	↑ AC	Hypothalamus, SCN, hippocampus, GI tract	Smooth muscle relaxation	AS-19, LP-12	SB-269970

[a] For further information on the pharmacological properties of the 5HT subtypes, see IUPHAR/BPS Guide to Pharmacology: http://www.guidetopharmacology.org/index.jsp. Abbreviations: AC, adenylyl cyclase; PLA$_2$, phospholipase A$_2$; PLC, phospholipase C; 5-CT, 5-carboxamino-tryptamine; DOI, 1-(2,5-dimethoxy-4-iodophenyl) isopropylamine; 8-OH-DPAT, 8-hydroxy-(2-*N*,*N*-dipropylamino)-tetraline; MCPP, metachlorphenylpiperazine; others are manufacturers' designations.

Mechanical stretching augments basal release of enteric 5HT, such as that caused by food and by efferent vagal stimulation. Released 5HT enters the portal vein and is metabolized by hepatic MAO-A. 5HT that survives hepatic oxidation may be captured by platelets or rapidly removed by the endothelium of lung capillaries and inactivated. 5HT released from enterochromaffin cells also acts locally to regulate GI function. 5HT$_3$ receptors in the GI tract and the vomiting center of the CNS participate in the emetic response, providing a basis for the antiemetic property of 5HT$_3$ receptor antagonists (see Figure 54–5 and Table 54–6). 5HT$_3$ receptor antagonists are highly efficacious in the treatment of nausea, with *ondansetron* being the most widely used as an antiemetic. The 5HT$_3$ receptor antagonist *alosetron* is approved for the treatment of inflammatory bowel syndrome in women whose main symptom is diarrhea.

Inflammation

In inflammation-related disease, 5HT exerts a proinflammatory influence primarily via activation of 5HT$_{2A}$ receptors. Activation of this receptor subtype contributes to maturation of T cells, recruitment of other types of immune-related cells to sites of inflammation, and secretion of proinflammatory molecules. Although 5HT itself is proinflammatory, activation of 5HT$_{2A}$ receptors with certain psychedelics can have potent

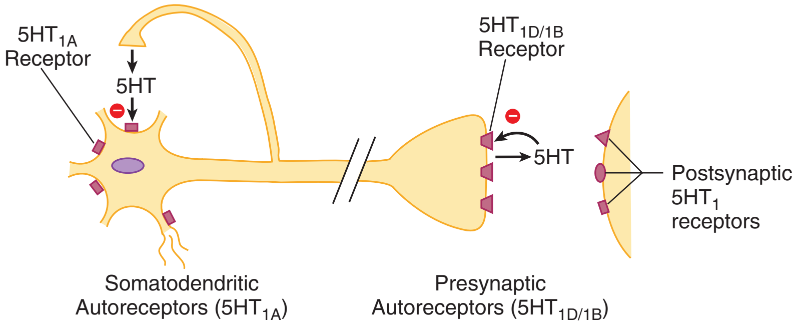

5HT$_{1A}$ Receptor
5HT
5HT$_{1D/1B}$ Receptor
5HT
Postsynaptic 5HT$_1$ receptors
Somatodendritic Autoreceptors (5HT$_{1A}$)
Presynaptic Autoreceptors (5HT$_{1D/1B}$)

Figure 15–5 *Two classes of 5HT autoreceptors with differential localizations.* Somatodendritic 5HT$_{1A}$ autoreceptors decrease raphe cell firing when activated by 5HT released from axon collaterals of the same or adjacent neurons. The receptor subtype of the presynaptic autoreceptor on axon terminals in the forebrain has different pharmacological properties and has been classified as 5HT$_{1D}$ (in humans) or 5HT$_{1B}$ (in rodents). This receptor modulates the release of 5HT. Postsynaptic 5HT$_1$ receptors are also indicated.

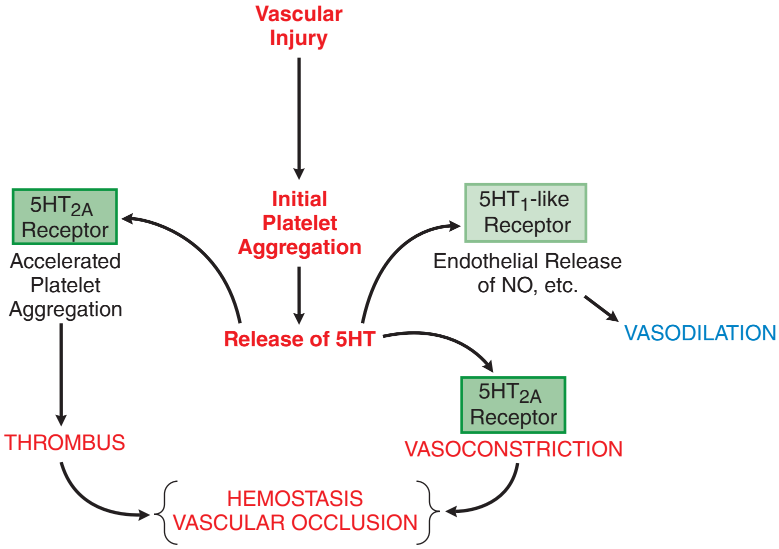


Figure 15–6 *The local influences of platelet 5HT.* The release of 5HT stored in platelets is triggered by aggregation. The local actions of 5HT include feedback actions on platelets (shape change and accelerated aggregation) mediated by interaction with platelet $5HT_{2A}$ receptors, stimulation of NO production mediated by $5HT_1$-like receptors on vascular endothelium, and contraction of vascular smooth muscle mediated by $5HT_{2A}$ receptors. These influences act in concert with many other mediators to promote thrombus formation and hemostasis. See Chapter 36 for details of adhesion and aggregation of platelets and factors contributing to thrombus formation and blood clotting.

anti-inflammatory effects in animal models of human disease (Flanagan and Nichols, 2018).

CNS

All 5HT receptor subtypes are expressed in the brain, where 5HT influences a multitude of functions, including sleep, cognition, sensory perception, motor activity, temperature regulation, nociception, mood, appetite, sexual behavior, and hormone secretion. The principal cell bodies of 5HT neurons are located in raphe nuclei of the brainstem and project throughout the brain and spinal cord (see Figure 15–3). In addition to release at discrete synapses, serotonin release also occurs at sites of axonal varicosities that do not form distinct synaptic contacts. 5HT released at nonsynaptic varicosities is thought to diffuse to outlying targets, rather than acting on discrete synaptic targets, perhaps acting as a neuromodulator as well as a neurotransmitter (see Chapter 16).

Sleep-Wake Cycle

Control of the sleep-wake cycle is one of the first behaviors in which a role for 5HT was identified. Depletion of 5HT with *p*-chlorophenylalanine, a tryptophan hydroxylase inhibitor, elicits insomnia that is reversed by the 5HT precursor, 5-hydroxytryptophan. Conversely, treatment with L-tryptophan or with nonselective 5HT agonists accelerates sleep onset and prolongs total sleep time. 5HT antagonists reportedly can increase and decrease slow-wave sleep, probably reflecting interacting or opposing roles for subtypes of 5HT receptors. One relatively consistent finding in humans and in laboratory animals is an increase in slow-wave sleep following administration of a selective $5HT_{2A/2C}$ receptor antagonist such as *ritanserin*. Selective $5HT_{2A}$ receptor antagonists like *pimavanserin* were found to have positive effects on sleep consolidation in late-stage clinical trials but have not been further developed for this indication.

TABLE 15–2 ■ ACTIONS OF 5HT IN ACT

SITE	RESPONSE	RECEPTOR
Enterochromaffin cells	Release of 5HT Inhibition of 5HT release	$5HT_3$ $5HT_4$
Enteric ganglion cells (presynaptic)	Release of ACh Inhibition of ACh release	$5HT_4$ $5HT_{1A}$
Enteric ganglion cells (postsynaptic)	Fast depolarization	$5HT_3$
Smooth muscle, intestinal	Contraction	$5HT_{2A}$
Smooth muscle, stomach fundus	Contraction	$5HT_{2B}$
Smooth muscle, esophagus	Contraction	$5HT_4$

ACh, acetylcholine.

Aggression and Impulsivity

Serotonin serves a critical role in aggression and impulsivity. Human studies reveal a correlation between low CSF 5-HIAA and violent impulsivity and aggression. A human genetic study identified a point mutation in the gene encoding MAO-A that was associated with extreme aggressiveness and mental impairment (Brunner et al., 1993); this has been confirmed in knockout mice lacking MAO-A (Cases et al., 1995). Pharmacological activation of $5HT_{1A}$ or $5HT_{1B}$ receptors has been demonstrated to have antiaggressive effects, and gene knockout of the $5HT_{1B}$ receptor results in enhanced aggression (Olivier and van Oorschot, 2005). Several serotonin receptors have been implicated in impulsivity, including $5HT_{1A}$, $5HT_{1B}$, and $5HT_{2C}$.

Appetite and Obesity

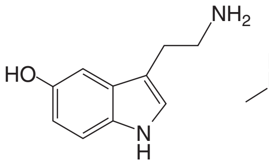
Lorcaserin is a preferential $5HT_{2C}$ receptor agonist that was approved for weight loss; however, it is no longer on the U.S. market due to the FDA's concerns over an increased risk of certain cancers. The drug is thought to decrease food consumption and promote satiety by activating $5HT_{2C}$ receptors on anorexigenic proopiomelanocortin neurons in the arcuate nucleus of the hypothalamus. Halogenated amphetamines, which are known to promote the release of 5HT and block its reuptake, are valuable experimental tools; two of them, *fenfluramine* and *dexfenfluramine*, were used clinically to reduce appetite. The once-popular diet drug regimen, "fen-phen," combined *fenfluramine* and *phentermine*. *Fenfluramine* and *dexfenfluramine* were withdrawn from the U.S. market in the late 1990s after reports of life-threatening heart valve disease and pulmonary hypertension associated with their use. This toxicity was the result of $5HT_{2B}$ receptor activation promoting hyperproliferation of cells within the heart valves (Hutcheson et al., 2011). Subsequent to this finding, other drugs (e.g., some ergot alkaloids) with demonstrated agonist activity at the $5HT_{2B}$ receptor have been withdrawn from the market.

Drugs Affecting 5HT Signaling

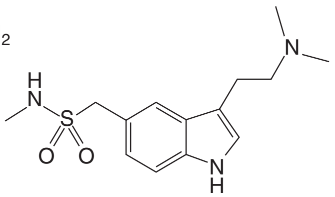
Direct-acting 5HT receptor agonists have widely different chemical structures and diverse pharmacological properties and are used in the pharmacotherapy of a number of disorders (Table 15–3), including anxiety, depression, nausea, disorders of GI motility, and migraine. 5HT is a key mediator in the pathogenesis of migraine. Consistent with the 5HT hypothesis of migraine, 5HT receptor agonists are a mainstay for acute treatment of migraine headaches, although the newer calcitonin gene-related peptide (CGRP) antagonist class of drug is quite effective in the treatment of migraine. The efficacy of serotonergic antimigraine drugs varies with the absence or presence of aura, duration of the headache, its severity and intensity, and as yet undefined environmental and genetic factors.

$5HT_{1B/1D}$ Receptor Agonists: The Triptans

The triptans are indole derivatives that are effective, acute antimigraine agents. Their capacity to decrease nausea and vomiting of migraine is an important advance in the treatment of this condition. Some examples include *almotriptan, eletriptan, frovatriptan, naratriptan, rizatriptan, sumatriptan*, and *zolmitriptan*. *Sumatriptan* for migraine headaches is also marketed in a fixed-dose combination with the nonsteroidal anti-inflammatory drug (NSAID) *naproxen*. The triptans are effective in the acute treatment of migraine (with or without aura) but are not intended for use in prophylaxis of migraine. Treatment with triptans should begin as soon as possible after the onset of a migraine attack. Oral dosage forms of the triptans are the most convenient to use, but they may not be practical in patients experiencing migraine-associated nausea and vomiting.



5HT



Sumatriptan

Migraine

Migraine headache afflicts 10% to 20% of the population. Although migraine is a specific neurological syndrome, the manifestations vary widely. The principal types are migraine without aura (common migraine), migraine with aura (classic migraine, which includes subclasses of migraine with typical aura, migraine with prolonged aura, migraine aura without headache, and migraine with acute-onset aura), and several rarer types. Premonitory aura may begin as long as 24 h before the onset of pain and often is accompanied by photophobia, hyperacusis, polyuria, and diarrhea and by disturbances of mood and appetite. A migraine attack may last for hours or days and be followed by prolonged pain-free intervals. The frequency of migraine attacks is extremely variable. Therapy of migraine headaches is complicated by the variable responses among and within individual patients and by the lack of a firm understanding of the pathophysiology of the syndrome. The efficacy of antimigraine drugs varies with the absence or presence of aura, duration of the headache, its severity and intensity, and possibly undefined environmental and genetic factors.

The pathogenesis of migraine headache is complex, involving both neural and vascular elements. Evidence suggesting that 5HT is a key mediator in the pathogenesis of migraine includes the following:

- Plasma and platelet concentrations of 5HT vary with the different phases of the migraine attack.
- Urinary concentrations of 5HT and its metabolites are elevated during most migraine attacks.
- Migraine may be precipitated by agents (e.g., *reserpine* and *fenfluramine*) that release 5HT from intracellular storage sites.

Consistent with the 5HT hypothesis, 5HT receptor agonists have become a mainstay for *acute* treatment of migraine headaches. Treatments for the *prevention* of migraines, such as β adrenergic antagonists and newer antiepileptic drugs, have mechanisms of action that are, presumably, unrelated to 5HT (Mehrotra et al., 2008).

Mechanism of Action

The pharmacological effects of the triptans appear to be limited to the $5HT_1$ family of receptors, providing evidence that this receptor subclass plays an important role in the acute relief of a migraine attack. The triptans interact potently with $5HT_{1B}$ and $5HT_{1D}$ receptors and have low or no affinity for other subtypes of 5HT receptors or for α_1 and α_2 adrenergic, β adrenergic, dopaminergic, muscarinic cholinergic, and benzodiazepine receptors. Clinically effective doses of the triptans correlate well with their affinities for both $5HT_{1B}$ and $5HT_{1D}$ receptors, supporting the hypothesis that $5HT_{1B}$ and $5HT_{1D}$ receptors are the most likely receptors involved in the mechanism of action of acute antimigraine drugs.

The mechanism of the efficacy of $5HT_{1B/1D}$ agonists in migraine is not resolved. One hypothesis of migraine suggests that unknown events lead to the abnormal dilation of carotid arteriovenous anastomoses in the head and shunting of carotid arterial blood flow, producing cerebral ischemia and hypoxia perceived as migraine pain; activation of $5HT_{1B/1D}$ receptors may cause constriction of intracranial blood vessels, including arteriovenous anastomoses, closing the shunts and restoring blood flow to the brain. An alternative hypothesis proposes that both $5HT_{1B}$ and $5HT_{1D}$ receptors serve as presynaptic autoreceptors that block the release of neurotransmitter or proinflammatory neuropeptides at nerve terminals in the perivascular space, which could account for the efficacy of agonists at those receptors in the acute treatment of migraine. Successful treatment of migraine with *sumatriptan* was shown to correlate with lower blood

TABLE 15–3 ■ EXAMPLES OF SEROTONERGIC DRUGS: PRIMARY ACTIONS AND CLINICAL INDICATIONS

RECEPTOR	ACTION	DRUG EXAMPLES	CLINICAL DISORDER
$5HT_{1A}$	Partial agonist	Buspirone, ipsaperone	Anxiety, depression
$5HT_{1D}$	Agonist	Sumatriptan	Migraine
$5HT_{2A/2C}$	Antagonist	Risperidone	Schizophrenia, depression
$5HT_{2A/2C}$	Agonist	Psilocybin	Depression
$5HT_3$	Antagonist	Ondansetron	Chemotherapy-induced emesis
$5HT_4$	Agonist	Tegaserod, cisapride	GI disorders
SERT (5HT transporter)	Inhibitor	Fluoxetine, sertraline	Depression, obsessive-compulsive disorder, panic disorder, social phobia, posttraumatic stress disorder

levels of a CGRP, a potent vasodilatory and proinflammatory neuropeptide. Along these lines, a new class of migraine drugs has been developed that blocks signaling by CGRP. These include the small-molecule CGRP receptor antagonist drug *rimegepant*, and the CGRP-blocking monoclonal antibodies *erenumab*, *fremanezumab*, and *galcanezumab*.

Clinical Use

The triptans are effective in the acute treatment of migraine (with or without aura) but are not intended for prophylaxis of migraine. Treatment with triptans should begin as soon as possible after the onset of a migraine attack. Oral dosage forms of the triptans are the most convenient to use but may not be practical in patients experiencing migraine-associated nausea and vomiting, for whom injectable and nasal spray formulations are useful. Approximately 70% of individuals report significant headache relief from a 6-mg subcutaneous dose of *sumatriptan*, a dose that may be repeated once within a 24-h period if the first dose does not relieve the headache. The other triptans have distinct dosing requirements as summarized on their FDA-approved package inserts. A recent meta-analysis concluded that *eletriptan* is the most likely triptan to produce a favorable outcome at the 2-h and 24-h time points after administration (Thorlund et al., 2014). No triptan should be used concurrently with (or within 24 h of) an ergot derivative (described in the next section) or another triptan.

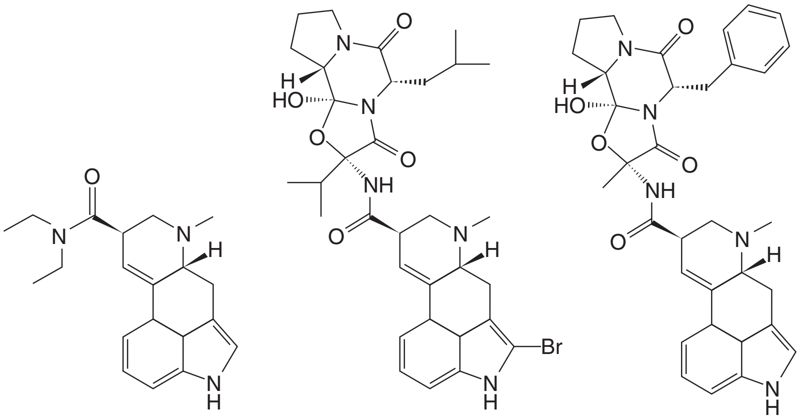
Adverse Effects and Contraindications

In general, only minor side effects are seen with the triptans in the acute treatment of migraine. After subcutaneous injection of *sumatriptan*, patients often experience irritation at the site of injection (transient mild pain, stinging, or burning sensations). The most common side effect of *sumatriptan* nasal spray is a bitter taste. Triptans can cause paresthesias; asthenia and fatigue; flushing; feelings of pressure, tightness, or pain in the chest, neck, and jaw; drowsiness; dizziness; nausea; and sweating. In the extreme, these agents can cause serotonin syndrome, a consequence of a generalized excess of 5HT receptor stimulation, especially when used in combination with SSRIs, SNRIs, TCAs, and MAO inhibitors.

Rare but serious cardiac events have been associated with the administration of triptans, including coronary artery vasospasm, transient myocardial ischemia, atrial and ventricular arrhythmias, and myocardial infarction, predominantly in patients with risk factors for coronary artery disease. The triptans are contraindicated in patients with a history of ischemic or vasospastic coronary artery disease (including history of stroke or transient ischemic attacks), cerebrovascular or peripheral vascular disease, hemiplegic or basilar migraines, other significant cardiovascular diseases, or ischemic bowel diseases. Because triptans may cause an acute, usually small, increase in blood pressure, they also are contraindicated in patients with uncontrolled hypertension. *Lasmiditan*, a nontriptan $5HT_{1F}$ receptor–selective agonist, has been approved for the treatment of migraine and has fewer adverse cardiovascular effects associated with its use than the $5HT_{1B/D}$ activating triptans and is particularly indicated in patients with preexisting cardiovascular risk factors. *Naratriptan* is contraindicated in patients with severe renal or hepatic impairment; *rizatriptan* should be used with caution in such patients. *Eletriptan* is contraindicated in hepatic disease. *Almotriptan, rizatriptan, sumatriptan*, and *zolmitriptan* are contraindicated in patients who have taken an MAO inhibitor within the preceding 2 weeks, and all triptans are contraindicated in patients with near-term prior exposure to ergot alkaloids, other triptans or 5HT agonists, SSRIs, and SNRIs. With respect to the use of triptans in pregnancy, there are no adequate and well-controlled studies in pregnant women. The FDA recommends the use of triptans during pregnancy only if the potential benefit justifies a potential risk to the fetus; evidence of safety in pregnancy is best with *sumatriptan*. These agents should also be used with caution in nursing mothers. Källén and Reis (2016) have reviewed drugs for managing pain, including migraine, during pregnancy.

The Ergot Alkaloids

Ergot is the product of a fungus (*Claviceps purpurea*) that grows on rye and other grains. The elucidation of the constituents of ergot and their complex actions was an important chapter in the evolution of modern pharmacology, even though the multiplicity of their actions limits their therapeutic uses. The pharmacological effects of the ergot alkaloids result from their actions as partial agonists or antagonists at serotonergic, dopaminergic, and adrenergic receptors. Ergot alkaloids can be subdivided into smaller molecular weight ergolines, such as *d-lysergic acid diethylamide* (LSD), a potent psychedelic, and larger molecular weight ergots that incorporate a cyclic peptide moiety, such as *bromocriptine*, which is used to control the secretion of prolactin, a property derived from its effect as a DA agonist. Other ergot alkaloids employed therapeutically include *methysergide* and *ergonovine* (and *methyl ergonovine*), which were historically used to treat migraine and postpartum hemorrhage, respectively.



LSD

Bromocriptine

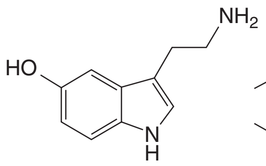
Ergotamine

Ergots in the Treatment of Migraine

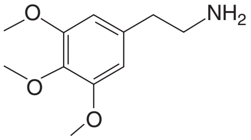
The multiple pharmacological effects of ergot alkaloids have complicated the determination of their precise mechanism of action in the acute treatment of migraine. The actions of ergot alkaloids at $5HT_{1B/1D}$ receptors likely mediate their *acute* antimigraine effects, and potentially at $5HT_{2A}$ receptors to mediate prophylactic effects. Due to their numerous adverse side effects including pleuropulmonary fibrosis and coronary and endocardial fibrosis, ergots such as *dihydroergotamine* and *methysergide* are no longer prescribed frequently and their use has for the most part been supplanted by triptans. The European Medicines Agency has recommended that ergot derivatives no longer be used to treat migraine. Ergot alkaloids with demonstrated agonist activity at the $5HT_{2B}$ receptor resulting in cardiac valvopathy have been withdrawn from the market.

Psychedelics

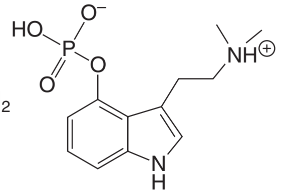
Psychedelics are a class of hallucinogenic drugs whose primary effects are mediated by activation of 5HT$_{2A}$ receptors. Although able to elicit somewhat similar behavioral effects, other hallucinogens like 3,4-Methyl enedioxy methamphetamine (MDMA), ketamine, phencyclidine (PCP), and cannabinoids are not pharmacologically considered psychedelics. Classic psychedelics include those that occur naturally in nature and fall into three structurally distinct superfluous families: phenethylamine (e.g., *mescaline*), tryptamine (e.g., *psilocybin*), and ergot alkaloid derivatives (e.g., LSD). Novel psychedelics include synthetic derivatives of the classic psychedelics such as 2C-B and 4-OH-DIPT. Some are highly selective for the 5HT$_2$ receptor family (e.g., 2,5-dimethoxy-4-iodoamphetamine; DOI), whereas others have affinity and activity at nearly all serotonin receptors (e.g., *psilocin*), and dopamine and adrenergic receptors as well (e.g., LSD). The shared effects of psychedelics, which have been shown in both animal models and humans to be mediated by activation of 5HT$_{2A}$ receptors, include profound sensory alterations and detachment from reality. One of the most potent psychedelics, LSD, is behaviorally active at doses as low as 25 µg in humans. Although ignored and little researched due to restrictive controlled substance scheduling and societal misperceptions for 30 years, psychedelics have recently shown promise in several areas as new therapeutics. Clinical trials using primarily *psilocybin*, but also others such as LSD, have shown promise in the treatment of depression, anxiety, substance use disorder, obsessive-compulsive disorder, and migraines (Bogenschutz et al., 2018; Davis et al., 2021; Moreno et al., 2006; Reiff et al., 2020; Schindler et al., 2021). Remarkably, these trials have shown that only a single treatment can have persistent effects lasting months to years. The physiological mechanisms underlying these effects are currently unknown but may involve increases in synaptic connectivity (Jones et al., 2009; Ly et al., 2018; Shao et al., 2021). Preclinical studies of psychedelics in animal models have also shown promise for treating inflammation-related disorders such as asthma and ocular and cardiovascular disease, with effects mediated through activation of 5HT$_{2A}$ receptors (Flanagan and Nichols, 2018).



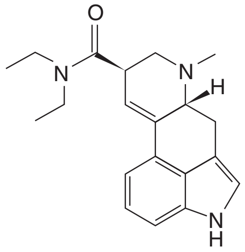
5HT



Mescaline



Psilocybin



LSD

Serotonin Receptor Agonists, Inverse Agonists, SSRIs, and MSAAs

Anxiolytic and Antidepressant Agents

Buspirone, *gepirone*, and *ipsapirone* are selective partial agonists at 5HT$_{1A}$ receptors. *Buspirone* has been effective in the treatment of anxiety (see Chapter 18). *Buspirone* mimics the antianxiety properties of benzodiazepines but does not interact with GABA$_A$ receptors or display the sedative and anticonvulsant properties of benzodiazepines. The effects of 5HT-active drugs in anxiety and depressive disorders, like the effects of SSRIs, strongly suggest a role for 5HT in the neurochemical mediation of these disorders. Inhibition of neuronal reuptake of 5HT via the 5HT transporter prolongs the dwell time of 5HT in the synapse. SSRIs, such as *citalopram*, potentiate and prolong the action of 5HT released by neuronal activity. When coadministered with L-5-hydroxytryptophan, SSRIs elicit profound activation of serotonergic responses. However, the capacity to enhance serotonergic neurotransmission alone does not explain the antidepressant effectiveness: Uptake inhibition occurs immediately, whereas weeks of treatment are required to achieve clinical efficacy. This has led to the proposal that long-term homeostatic adaptations in brain function underlie the therapeutic effects of this class of antidepressants. SSRIs (*citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine*, and *sertraline*) are the most widely used treatment of major depressive disorder (see Chapter 18).

Vilazodone is an SSRI and a partial agonist at the 5HT$_{1A}$ receptor, which has a faster time of therapeutic onset than traditional SSRIs due to direct activation of 5HT$_{1A}$ receptors.

Viloxazine is a medication that is used in the treatment of attention-deficit/hyperactivity disorder (ADHD) in children and depression. It was marketed for more than two decades as an antidepressant in Europe before being repurposed as a treatment for ADHD and recently launched in the United States. While its mechanism of action was originally thought to be blockade of NET, more recently, it has been determined to act as an antagonist of the 5HT$_{2B}$ receptor and as an agonist of the 5HT$_{2C}$ receptor, actions that may be involved in its therapeutic effects.

5HT and Sexual Dysfunction

One of the most common side effects of SSRIs and SNRIs is sexual dysfunction, such as anorgasmia, erectile dysfunction, diminished libido, and sexual anhedonia. Poor sexual function is one of the most common reasons that patients discontinue taking these medications. The mechanism by which SSRIs/SNRIs cause sexual side effects is not well understood. In contrast, the serotonergic drug *flibanserin*, which is an agonist of cortical 5HT$_{1A}$ receptors and an antagonist of 5HT$_{2A}$ receptors, is approved to treat hypoactive sexual desire disorder (HSDD) in premenopausal women. This disorder is also referred to as female sexual interest/arousal disorder. Clinical trials showed that *flibanserin* can increase the number of satisfying sexual events in some, but not all, women with this disorder by about one episode compared to placebo during the trial duration (Fisher et al., 2017). Administration of *flibanserin* leads to decreases of 5HT levels in the cortex and increases in DA and NE. This redistribution of monoamine levels has been speculated to be the mechanism of the observed response of increased sexual function.

Psychosis

Recently, *pimavanserin* has been introduced to treat hallucinations and delusions associated with psychosis experienced by some people with Parkinson's disease (PD). This drug is a potent and selective inverse agonist at 5HT$_{2A}$ with 40-fold selectivity over 5HT$_{2C}$ receptors and no appreciable affinity for the 5HT$_{2B}$ or other monoaminergic receptors. Typically, patients with PD cannot tolerate other antipsychotics on the market because this drug class functions through blocking dopamine receptors, which results in a worsening of the motor symptoms of this disease. The efficacy of *pimavanserin* in treating psychosis observed in other disease states, such as schizophrenia, has not yet been determined.

Clinical Manipulation of 5HT Levels: Serotonin Syndrome

Excessive elevation of 5HT levels in the body can cause *serotonin syndrome*, a constellation of symptoms sometimes observed in patients starting new or increased antidepressant therapy or combining an SSRI with an NE reuptake inhibitor or a triptan (for migraine). Serotonin syndrome is largely mediated by activation of $5HT_{2A}$ and $5HT_{1A}$ receptors, and symptoms may include restlessness, confusion, shivering, tachycardia, diarrhea, muscle twitches/rigidity, fever, seizures, loss of consciousness, and even death. Serotonin syndrome and its treatment are discussed in Chapter 18.

Dopamine

Dopamine consists of a catechol moiety linked to an ethyl amine, leading to its classification as a catecholamine (Figure 15–7). DA is a polar molecule that does not readily cross the BBB. It is closely related to melanin, a pigment that is formed by oxidation of DA, tyrosine, or 3,4-dihydroxyphenylalanine (L-DOPA). Melanin exists in the skin and cuticle and gives the substantia nigra brain region its namesake dark color. Both DA and L-DOPA are readily oxidized by nonenzymatic pathways to form cytotoxic reactive oxygen species and quinones. DA- and dopa-quinones form adducts with α-synuclein, a major constituent of Lewy bodies in PD (see Chapter 21).

HISTORICAL PERSPECTIVE

Dopamine was first synthesized in 1910. Later that year, Henry Dale characterized the biological properties of DA in the periphery and described it as a weak, adrenaline-like substance. In the 1930s, DA was recognized as a transitional compound in the synthesis of NE and EPI but was believed to be little more than a biosynthetic intermediate. Not until the early 1950s were stores of DA identified in tissues, suggesting that DA had a signaling function of its own. Soon thereafter, Oleh Hornykiewicz discovered the DA deficit in parkinsonian brains, fueling interest in the role of DA in neurological diseases and disorders (Hornykiewicz, 2010).

Synthesis and Metabolism

The biosynthesis and metabolism of DA are summarized in Figure 15–8. Phenylalanine and tyrosine are the precursors of DA. For the most part, mammals convert dietary phenylalanine to tyrosine by phenylalanine hydroxylase. Diminished levels of phenylalanine hydroxylase lead to high levels of phenylalanine, producing a condition known as phenylketonuria, which must be controlled by dietary restrictions to avoid intellectual impairment. Tyrosine crosses readily into the brain through uptake; normal brain levels of tyrosine are typically saturating. Conversion of tyrosine to L-DOPA by the tyrosine hydroxylase is the rate-limiting step in the synthesis of DA (as in NE synthesis; see Chapter 10). Once generated, L-DOPA is rapidly converted to DA by AADC, the same enzyme that generates 5HT from L-5-hydroxytryptophan. Unlike DA, L-DOPA readily crosses the BBB and is converted to DA in the brain, which explains its utility in therapy for PD (see Chapter 21).

Metabolism of DA occurs primarily by MAO in both pre- and postsynaptic elements. MAO acts on DA to generate an inactive aldehyde derivative by oxidative deamination; the aldehyde is subsequently metabolized by aldehyde dehydrogenase to form 3,4-dihydroxyphenylacetic acid (DOPAC). DOPAC can be further metabolized by catechol-*O*-methyl transferase (COMT) to form homovanillic acid (HVA). In humans, HVA is the principal metabolite of DA. DOPAC, HVA, and DA are excreted in the urine, where they are readily measured. Levels of DOPAC and HVA are reliable indicators of DA turnover; ratios of these metabolites to DA in CSF serve as accurate representations of brain dopaminergic activity. In addition to metabolizing DOPAC, COMT acts on DA to generate 3-methoxytyramine, which is subsequently converted to HVA by MAO. MAO-B–selective inhibitors, such as *selegiline*, *rasagiline*, or *safinamide*, can increase DA levels and are currently used to treat PD (see Chapter 21). COMT in the periphery also metabolizes L-DOPA to 3-*O*-methyldopa, which then competes with L-DOPA for uptake into the CNS (see Figure 21–4). Consequently, L-DOPA given in the treatment of PD must be coadministered with peripheral COMT inhibitors, such as *entacapone*, *tolcapone*, or *opicapone*, to preserve L-DOPA and allow sufficient entry into the CNS.

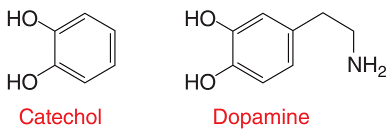


Figure 15–7 *The catechol nucleus of catecholamines.*

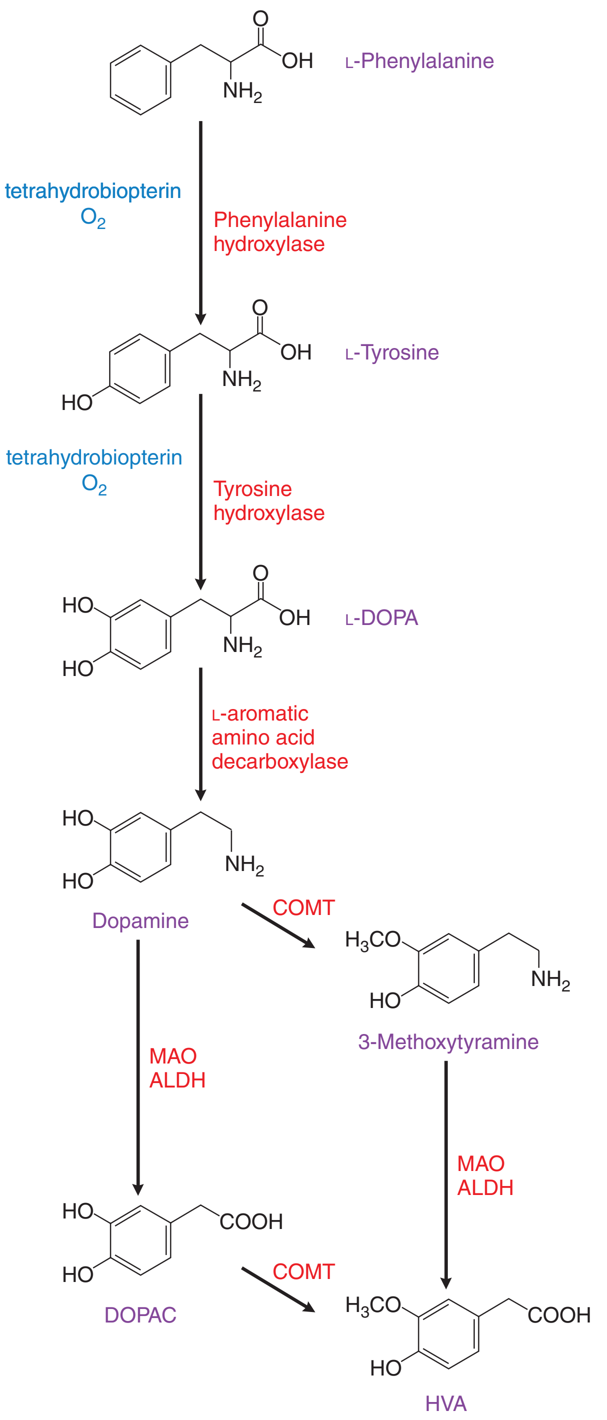


Figure 15–8 *Synthesis and inactivation of DA.* Enzymes are identified in red lettering, and cofactors are shown in blue letters.

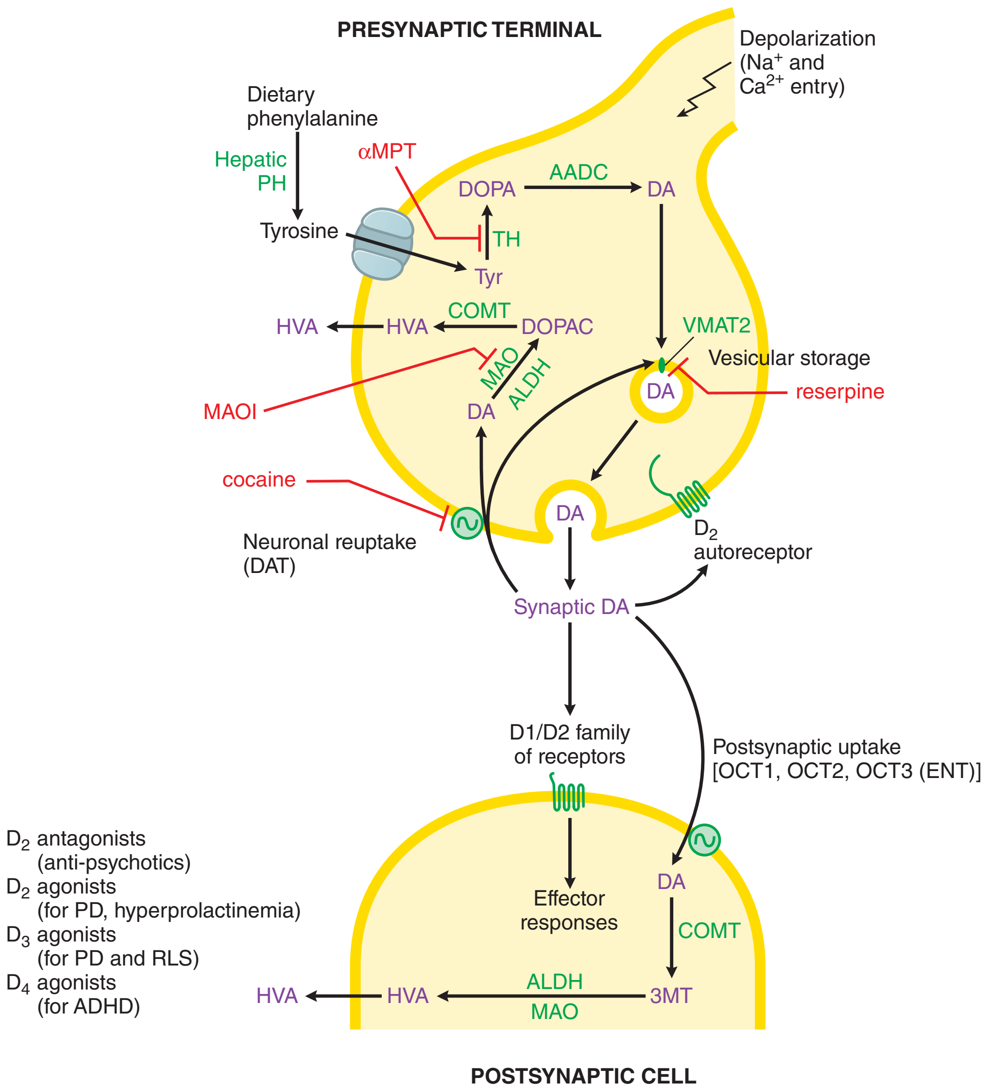

PRESYNAPTIC TERMINAL
Depolarization (Na+ and Ca2+ entry)
Dietary phenylalanine
Hepatic PH
αMPT
Tyrosine
DOPA
AADC
DA
TH
Tyr
HVA
HVA
COMT
DOPAC
VMAT2
Vesicular storage
MAOI
MAO
ALDH
DA
DA
reserpine
cocaine
DA
D2 autoreceptor
Neuronal reuptake (DAT)
Synaptic DA
D1/D2 family of receptors
Postsynaptic uptake [OCT1, OCT2, OCT3 (ENT)]
D2 antagonists (anti-psychotics)
D2 agonists (for PD, hyperprolactinemia)
D3 agonists (for PD and RLS)
D4 agonists (for ADHD)
Effector responses
DA
COMT
HVA
HVA
ALDH
MAO
3MT
POSTSYNAPTIC CELL

Figure 15–9 *A dopaminergic synapse.* DA is synthesized from tyrosine in the nerve terminal by the sequential actions of TH and AADC. DA is sequestered by VMAT2 in storage granules and released by exocytosis. Synaptic DA activates presynaptic autoreceptors and postsynaptic D_1-like and D_2-like receptors. Synaptic DA may be taken up into the neuron via the DA transporter (DAT) or removed by postsynaptic uptake via an organic cation transporter (OCT). Cytosolic DA is subject to degradation by MAO/ALDH in the neuron and by COMT in nonneuronal cells; the final metabolic product is HVA. See structures in Figure 15–8. ALDH, aldehyde dehydrogenase; MAOI, monoamine oxidase inhibitor; αMPT, α-methylphenyltyrosine; TH, tyrosine hydroxylase.

Figure 15–9 summarizes the neurochemical events that underlie DA neurotransmission. In dopaminergic neurons, synthesized DA is packaged into secretory vesicles by the vesicular monoamine transporter (VMAT2). Drugs such as *reserpine*, which inhibit VMATs and deplete DA levels, were once used to treat psychosis. Recently, a VMAT2-selective inhibitor, *valbenazine*, has been introduced for the treatment of tardive dyskinesia, a neurological disorder characterized by involuntary movements. Although the exact cause tardive dyskinesia is unknown, it is hypothesized to be due to DA hypersensitivity, and valbenazine causes a reversible reduction in DA release. Notably, the presynaptic vesicular packaging of DA allows it to be stored in readily releasable quanta and protects the transmitter from further anabolism or catabolism. By contrast, in adrenergic and noradrenergic cells, DA is not packaged; instead, it is converted to NE by DA β-hydroxylase and, in adrenergic cells, methylated to epinephrine (EPI) in cells expressing phenylethanolamine *N*-methyltransferase (see Chapter 10).

Synaptically released DA activates postsynaptic receptor subtypes, the expression of which is cell specific, leading to signal transduction via G protein-mediated pathways, although in some cases, G protein-independent signaling occurs (see further discussion). DA receptor subtypes are also the targets of many therapeutically employed drugs and pharmacological tool compounds. Specific receptor subtypes of the D_2-like category can also be expressed on the presynaptic nerve terminal, where they regulate the release of DA, or on the cell bodies and dendrites, where they regulate DA synthesis. Reuptake of released DA by the DA transporter is the primary mechanism for termination of DA action and allows for either vesicular repackaging of transmitter or metabolism.

The DA transporter, DAT, localizes to dendrites, axons, and soma of mesencephalic DA neurons and is also found peripherally in the stomach, pancreas, and lymphocytes. Psychostimulants, such as *cocaine*, *amphetamine*, and *methamphetamine*, induce euphoria and hyperactivity by increasing extracellular DA. *Cocaine* is not a substrate of the DAT but rather acts as an antagonist to block DA reuptake, thereby potentiating DA signaling. However, the actions of *amphetamines* are more complex: *Amphetamines* are competitive substrates for both the DATs and the VMATs. *Amphetamines* enter the cell through the DAT, where they displace DA from vesicular stores, causing an accumulation of DA within the neuronal cytoplasm. This resulting increase in cytosolic DA drives the release of DA by a nonvesicular mechanism that involves efflux through the DAT (Sitte et al., 2015). Newer studies also support the idea that *amphetamines* also target the trace amine-associated receptor 1 (TAAR1), an intracellular receptor within DA neurons, to activate cellular signaling pathways. *Amphetamines* activate G_s-dependent pathways coupled to increases in cyclic AMP and G_{13}-dependent pathways coupled to the activation of the small GTPase, RhoA (Underhill et al., 2021). Agonists for TAAR1 include *amphetamines*, DA, trace amines, and a variety of drugs. TAAR1 has been proposed to mediate some of the actions of *amphetamines* (Gainetdinov et al., 2018).

The DA transporter can also serve as a molecular entryway for some neurotoxins, including 6-OHDA (6-hydroxydopamine) and MPP^+ (1-methyl-4-phenylpyridinium), the neurotoxic metabolite of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). Following uptake into dopaminergic neurons, MPP^+ and 6-OHDA facilitate intra- and extracellular DA release and generate reactive oxygen species such as superoxide radicals (O_2^-) that cause neuronal death. This selective dopaminergic degeneration mimics PD and serves as an animal model for this disorder.

Dopamine Receptors

Early investigations found that DA increases cyclic AMP levels in both the brain and retina, presumably by activating a DA-sensitive adenylyl cyclase. Subsequent studies revealed the existence of DA receptors not linked to adenylyl cyclase activation, thus implying the existence of multiple DA receptor subtypes. It was then hypothesized that there were D_1 and D_2 receptors that could be distinguished on the basis of their pharmacological properties and signaling mechanisms. Subsequent molecular biological studies identified not only genes corresponding to the originally defined D_1 and D_2 receptor subtypes but also genes for additional DA receptors. We now recognize that there are five distinct DA receptors in mammals that are members of the GPCR superfamily and categorized into two D_1-like and D_2-like subfamilies (Sibley and Monsma, 1992). The D_1-like subfamily consists of the D_1 and D_5 receptors, whereas the D_2, D_3, and D_4 subtypes compose the D_2-like subfamily. Receptors in the D_1-like subfamily (D_1 and D_5) couple to heterotrimeric G_s or G_{olf} proteins to stimulate the adenylyl cyclase–cyclic AMP–protein kinase A (PKA) pathway. In contrast, the D_2-like receptors (D_2, D_3, and D_4) couple to G_i, G_o, or G_z proteins to inhibit adenylyl cyclase and diminish cyclic AMP production (Figure 15–10). Activation of $G_{i/o/z}$ proteins can also directly modulate the activity of certain K^+ and Ca^{2+} channels.

It is now recognized that GPCRs can signal independently from G proteins via interactions with β-arrestin proteins (Shukla et al., 2011). Agonist-activated GPCRs typically recruit β-arrestins to their intracellular surface as part of a homeostatic mechanism that turns off G protein-mediated signaling and facilitates receptor internalization. However, the GPCR-β-arrestin dimer can serve as a signaling complex that leads to the activation of downstream protein kinase–mediated signaling pathways. Among DA receptors, this process has been best described for the D_2 receptor subtype. When the agonist-activated D_2 receptor binds β-arrestin, two other proteins are recruited to this complex, namely protein phosphatase 2A (PP2A) and protein kinase B (also known as Akt). As a result, Akt is dephosphorylated and inactivated by PP2A. Since Akt constitutively phosphorylates and inhibits glycogen synthase kinase 3β (GSK-3β), D_2 receptor activation ultimately results in increased GSK-3β–mediated signaling (see Figure 15–10), which is thought to play a role in the actions of antipsychotics and mood stabilizers (Urs et al., 2012).

Pharmacological agents targeting DA receptors are used in the treatment of numerous neuropsychiatric disorders, including PD, schizophrenia, bipolar disorder, Huntington's disease, ADHD, and Tourette's syndrome. Like many GPCRs, DA receptors may form homo- and hetero-oligomers and also oligomerize with other GPCRs as well as ligand-gated ion channels (Fuxe et al., 2015). Notably, the D_2 and A_{2A} adenosine receptors form heteromers, and this complex provides a drug target for the treatment of PD (Chen and Cunha, 2020). Within a heteromeric complex, the D_2 and A_{2A} adenosine receptors mutually inhibit each other's signaling through allosteric mechanisms. However, the A_{2A} adenosine receptor antagonist *istradefylline* can enhance D_2 receptor signaling through blocking D_2 receptor inhibition by the A_{2A} receptor. *Istradefylline* has recently been approved as adjunctive therapy with L-DOPA for the treatment of PD (see Chapter 21). For further information on DA receptor signaling, see Beaulieu and Gainetdinov (2011) and Beaulieu et al. (2015).

The D_1 Receptor

- The D_1 receptor is the most highly expressed of the DA receptors; the highest levels of D_1 receptor protein are found within the CNS, but it is also located in the kidney, retina, and cardiovascular system.
- The neostriatum expresses the highest levels of D_1 receptor in the CNS but does not express any detectable G_s. In this region, the D_1 receptor appears to couple to G_{olf} to increase levels of cyclic AMP and its downstream effectors. In contrast, the D_1 receptor couples to G_s in the cerebral cortex to regulate cognition.
- In addition to activating G proteins, the D_1 receptor can form hetero-oligomers with ionotropic *N*-methyl-D-aspartate (NMDA) glutamate receptors (see Chapter 16) to modulate glutamatergic signaling.

The D_2 Receptor

- The D_2 receptor is the second most highly expressed DA receptor and consists of short (D_{2S}) and long (D_{2L}) isoforms that arise from alternative messenger RNA splicing. The D_{2S} isoform is missing 29 amino acids in the third intracellular loop that are present in the D_{2L} variant.
- The D_{2S} and D_{2L} receptors are pharmacologically identical; both couple to G_i, G_o, or G_z to decrease cyclic AMP production. The D_{2L} receptor is more prevalent and postulated to function postsynaptically. In contrast, the D_{2S} isoform functions as a putative presynaptic autoreceptor that regulates DA synthesis and release.

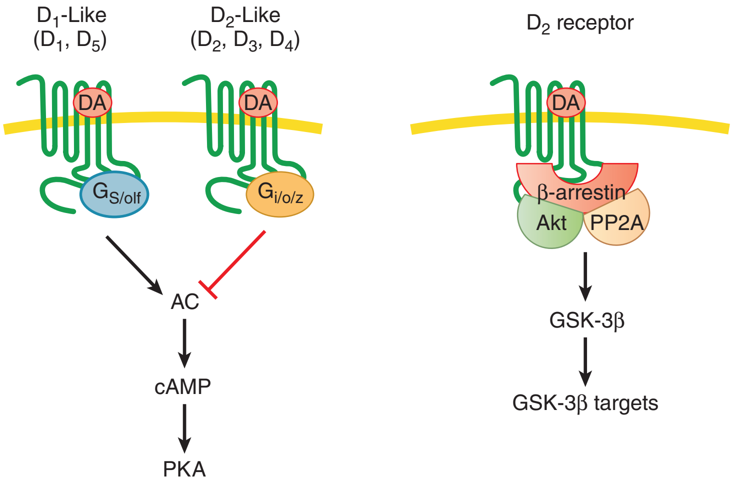


Figure 15–10 *The two subfamilies of DA receptors and their signaling pathways.* **Left:** D_1-like and D_2-like DA receptors differentially regulate G protein-mediated signaling pathways. **Right:** The D_2 receptor enhances GSK-3β signaling by recruiting β-arrestin and inactivating Akt. AC, adenylyl cyclase.

- The D_2 receptors can signal through $G_{\beta\gamma}$ subunits to regulate a variety of functions, including inwardly rectifying K^+ channels (GIRKs), N-type Ca^{2+} channels, and L-type Ca^{2+} channels.
- The D_2 receptor can signal through recruitment of the scaffolding protein, β-arrestin, thereby coupling to downstream signaling through the protein kinases B (Akt) and GSK-3β.

The D_3 Receptor

- The D_3 receptor is less abundant than the D_2 receptor and is mainly expressed in the limbic regions of the brain. The highest levels of the D_3 receptor are found in the islands of Calleja, nucleus accumbens, substantia nigra pars compacta, and ventral tegmental area.
- The D_3 receptor signals through pertussis toxin–sensitive $G_{i/o/z}$ proteins, although not as effectively as the D_2 receptor.
- The D_3 receptor may function as a drug target for the treatment of substance use disorders, particularly for those involving opioids (Galaj et al., 2020).

The D_4 Receptor

- The D_4 receptor is expressed in the retina, hypothalamus, prefrontal cortex (PFC), amygdala, and hippocampus.
- The D_4 receptor is highly polymorphic, containing variable number of tandem repeat (VNTR) encoding sequences within the third intracellular loop. In humans, the four-repeat variant is the most common. Association between a seven-repeat VNTR variant of the D_4 receptor and ADHD has been suggested.
- The D_4 receptor couples to $G_{i/o/z}$ to inhibit adenylyl cyclase activity and depress intracellular cyclic AMP levels.

The D_5 Receptor

- The D_5 receptor is the least abundant DA receptor but is found in the hippocampus, substantia nigra, hypothalamus, striatum, cerebral cortex, nucleus accumbens, and olfactory tubercle. It is also expressed in the kidney.
- The D_5 receptor activates G_s and G_{olf} to increase cyclic AMP production and can also modulate Na^+ currents and N-, P-, and L-type Ca^{2+} currents via PKA-dependent pathways. The D_5 receptor can also directly interact with $GABA_A$ receptors to decrease Cl^- flux.
- Along with the D_1 receptor, the D_5 receptor regulates neuronal circuitry controlling learning and memory.

The Dopamine Transporter

- DAT (SLC6A3) clears extracellular DA released during neurotransmission and is a major target for both therapeutic and addictive psychostimulant drugs.
- Like SERT, the DAT is a member of the NSS family (see Chapter 4, section on *Transporters and Pharmacodynamics: Drug Action in the Brain*), which couples neurotransmitter transport across the plasma membrane to the movement of Na^+ ions into the cell.
- The DAT has 12 membrane-spanning domains; a recent high-resolution X-ray structure of a *Drosophila* DAT has been determined (Penmatsa et al., 2015).
- The DAT protein is abundantly expressed in mesostriatal, mesolimbic, and mesocortical DA pathways, where it can be found on cell bodies, dendrites, and axons of DA neurons (Ciliax et al., 1999). However, the DAT is not readily detected within synapses, suggesting that rather than regulating synaptic neurotransmitter concentrations, it is poised to regulate spillover and diffusion of DA away from sites of release.
- The DAT is the therapeutic target of *methylphenidate* and *amphetamine*, the two major drugs used to treat attention-deficit disorders. The DAT inhibitor *bupropion* is used to treat depression and to support smoking cessation.

Actions of Dopamine in Physiological Systems

Heart and Vasculature

At low concentrations, circulating DA primarily stimulates vascular D_1 receptors (see below), causing vasodilation and reducing cardiac load. The net result is a decrease in blood pressure and an increase in cardiac contractility. As circulating DA concentrations rise, DA is able to activate β adrenergic receptors to further increase cardiac contractility. At very high concentrations, circulating DA activates α adrenergic receptors in the vasculature, thereby causing vasoconstriction; thus, high concentrations of DA increase blood pressure. Clinically, DA administration is used to treat severe congestive heart failure, sepsis, or cardiogenic shock. It is only administered intravenously and is not considered a long-term treatment.

Kidney

Dopamine is a paracrine/autocrine transmitter in the kidney and binds to receptors of both the D_1 and D_2 subfamilies. Renal DA primarily serves to increase natriuresis, although it can also increase renal blood flow and glomerular filtration. Under basal sodium conditions, DA regulates Na^+ excretion by inhibiting the activity of various Na^+ transporters, including the apical Na^+/H^+ exchanger and the basolateral Na^+/K^+-ATPase. Activation of D_1 receptors increases renin secretion, whereas DA, acting on D_3 receptors, reduces renin secretion. Abnormalities in the DA system and its receptors have been implicated in human hypertension.

Pituitary Gland

Dopamine is the primary regulator of prolactin secretion from the pituitary gland. DA released from the hypothalamus into the hypophyseal portal blood supply acts on lactotroph D_2 receptors to decrease prolactin secretion (see Chapter 46). The ergot-based DA agonists *bromocriptine* and *cabergoline* are used in the treatment of hyperprolactinemia. Both have a high affinity for D_2 receptors, with a lower affinity for D_1, 5HT, and adrenergic receptors; both activate D_2 receptors in the pituitary to reduce prolactin secretion. The risk of valvular heart disease in ergot therapy is not associated with the lower doses used in treating hyperprolactinemia. The use of *bromocriptine* and *cabergoline* in the management of hyperprolactinemia is described in Chapter 46.

Catecholamine Release

Both D_1 and D_2 receptors modulate the release of NE and EPI. The D_2 receptor provides tonic inhibition of EPI release from chromaffin cells of the adrenal medulla and of NE release from sympathetic nerve terminals. In contrast, activation of the D_1 receptor promotes the release of catecholamines from the adrenal medulla.

CNS

There are three major groups of DA projections in the brain (Figure 15–11): mesocortico/mesolimbic (originating in the ventral tegmental area), nigrostriatal (originating in the substantia nigra pars compacta), and tuberoinfundibular (originating in the hypothalamus). The physiological processes under dopaminergic control include reward, emotion, cognition, memory, and motor activity. Dysregulation of the dopaminergic system is critical in a number of disease states, including PD, Tourette's syndrome, bipolar depression, schizophrenia, ADHD, and addiction/substance abuse.

The mesolimbic pathway is associated with reward and, less so, with learned behaviors. Dysfunction in this pathway is associated with addiction, schizophrenia, and psychoses (including bipolar depression) and learning deficits. The mesocortical projections are important for "higher-order" cognitive functions, including motivation, reward, emotion, and impulse control; they are also implicated in psychoses, including schizophrenia, and in ADHD. The nigrostriatal pathway is a key regulator of movement. Impairments in this pathway are involved in PD and underlie detrimental motor side effects associated with dopaminergic

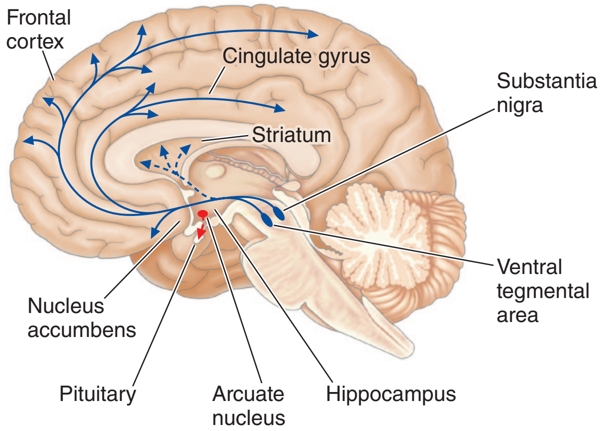


Figure 15–11 *Major dopaminergic projections in the CNS.*

- The nigrostriatal (or mesostriatal) pathway. Neurons in the substantia nigra compacta project to the dorsal striatum (*upward dashed blue arrows*); this is the pathway that degenerates in Parkinson's disease.
- The mesocortico/mesolimbic pathway. Neurons in the ventral tegmental area project to the ventral striatum (nucleus accumbens), olfactory bulb, amygdala, hippocampus, orbital and medial prefrontal cortex, and cingulate gyrus (*solid blue arrows*).
- The tuberoinfundibular pathway. Neurons in the arcuate nucleus of the hypothalamus project by the tuberoinfundibular pathway in the hypothalamus, from which DA is delivered to the anterior pituitary (*red arrows*).

therapy, including tardive dyskinesia (see Chapter 21). As noted previously, DA released in the tuberoinfundibular pathway is carried by the hypophyseal blood supply to the pituitary, where it regulates prolactin secretion.

Dopaminergic neurons are strongly influenced by excitatory glutamate and inhibitory GABA input. In general, glutamate inputs enable burst-like firing of dopaminergic neurons, resulting in high concentrations of synaptic DA. GABA inhibition of DA neurons causes a tonic, basal level of DA release into the synapse. DA release also modulates GABA and glutamate neurons, thus providing an additional level of interaction between DA and other neurotransmitters.

Motor Control and Parkinson's Disease

In the early 1980s, several young people in California developed rapid-onset parkinsonism. All of the affected individuals had injected a synthetic analogue of *meperidine* that was contaminated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP is metabolized by MAO-B to the neurotoxin 1-methyl-4-phenylpyridinium (MPP^+). Because of the high specificity of MPP^+ for the DA transporter, neuronal death is largely restricted to the substantia nigra and ventral tegmental area, resulting in a phenotype remarkably similar to PD. 6-OHDA is similar to MPTP in both mechanism of action and utility in animal models. Administration of MPTP or 6-OHDA to animals results in tremor, grossly diminished locomotor activity, and rigidity. As with PD, these motor deficits are alleviated with L-DOPA therapy or dopaminergic agonists.

Other pharmacological agents that act on the DAT are known to potentiate locomotor activity via dopaminergic actions, including *cocaine* and *amphetamine*. The accumulation of extracellular DA increases stimulation of DA receptors and results in heightened locomotor activity. Mice lacking DAT are hyperactive and do not display increased locomotion in response to *cocaine* or *amphetamine* treatment.

Reward: Implications for Addiction

In general, drugs of abuse increase DA levels in the *nucleus accumbens*, an area critical for rewarded behaviors. This role for mesolimbic DA in addiction has led to numerous studies of abused drugs in DA receptor "knockout" mice in which the genes expressing specific receptors have been disrupted. Studies of D_1 receptor knockout mice showed a reduction in the rewarding properties of ethanol, suggesting that the rewarding and reinforcing properties of ethanol are dependent, at least in part, on the D_1 receptor. D_2 receptor knockout mice also display reduced preference for ethanol consumption. *Morphine* lacks rewarding properties in D_2 knockout mice when measured by conditioned place-preference or self-administration paradigms. However, mice lacking the D_2 receptor exhibit enhanced self-administration of high doses of *cocaine*. These data suggest a complex and drug-specific role for the D_2 receptor in rewarding and reinforcing behaviors. The D_3 receptor, highly expressed in the limbic system, has also been implicated in the rewarding properties of several drugs of abuse. However, D_3 knockout mice display drug-associated place preference similar to wild-type mice following *amphetamine* or *morphine* administration. Recently developed D_3 receptor–preferring ligands implicate a role for the D_3 receptor in motivation for drug seeking and in drug relapse, rather than in the direct reinforcing effects of the drugs (Keck et al., 2015; Newman et al., 2021).

Cognition and Memory

Seminal work by Goldman-Rakic, Arnsten, and their colleagues (Arnsten et al., 2017) showed that an optimum level of D_1 receptor activity in the PFC is required for optimum performance in learning and memory tasks. Either too little or too much D_1 receptor stimulation, due to disease or aging, impairs PFC function in rats, monkeys, and humans. Thus, low doses of D_1 agonists improve working memory and attention, whereas high levels of DA release, such as during stress, impair PFC function. These observations have led to the "inverted U" hypothesis of the relationship between D_1 receptor stimulation and normal physiological functioning of the PFC (Figure 15–12). Interestingly, suboptimal levels of D_1 receptor stimulation have been suggested to underlie age-associated learning deficits and to contribute to the decreased cognition observed in various pathophysiological states, especially schizophrenia. Not surprisingly, the D_1 receptor provides an attractive drug target for the treatment of disorders involving cognitive decline, including schizophrenia, PD, and Alzheimer's disease. Unfortunately, agonists that directly stimulate the D_1 receptor have failed clinically as cognitive enhancers mainly due to hypotensive side effects originating from D_1 receptor stimulation in the kidney. Recently, it has been hypothesized that positive allosteric modulators (PAMs) of the D_1 receptor, which have more limited effects on D_1 receptor stimulation (Hall et al., 2019), may prove successful as cognitive enhancers in patients without hypotensive side effects (Hao et al., 2019). Another approach is to use noncatechol D_1-like partial agonists that have good brain penetrability and D_1-like receptor selectivity without the hypotensive side effects seen with full D_1 agonists (Hall et al., 2019).

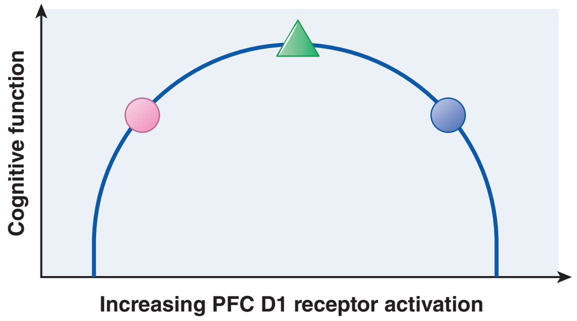


Figure 15–12 *Inverted U relationship between D_1 receptor signaling in the PFC and cognition.* The *green triangle* shows an optimum level of signaling/cognition, whereas the *pink ball* shows less than optimal levels due to disease/aging and the *blue ball* shows less than optimal levels due to stress.

Drugs Affecting Dopamine Signaling

Dopamine Receptor Agonists

Dopamine receptor agonists that target D_2-like receptors are mainly used in the treatment of PD, restless leg syndrome (RLS), and hyperprolactinemia. One limitation to the therapeutic use of dopaminergic agonists is the lack of receptor subtype selectivity. However, advances in receptor-ligand structure-function relationships have enabled the development of drugs that can fully distinguish between D_1-like and D_2-like receptor subfamilies and, in some cases, show selectivity for individual receptor subtypes, particularly for the D_3 and D_4 receptors. Many of these compounds have already proven to be useful experimental tools (Table 15–4), and this remains an active area of research.

Parkinson's Disease

Dopamine does not cross the BBB; thus, the principal pharmacotherapy for PD is to administer the precursor to DA, L-DOPA, which crosses the BBB and is converted to DA in the brain. Commonly, L-DOPA is formulated with a decarboxylase inhibitor to prevent the peripheral conversion of L-DOPA to DA, which can result in adverse side effects. While the response to L-DOPA by patients with PD is usually quite favorable, longer-term treatment can result in a loss of effectiveness and the emergence of dyskinetic syndromes referred to as L-DOPA–induced dyskinesias. These limitations to the therapeutic effects of L-DOPA have generated interest in developing alternative therapies for PD, with the intent of either delaying the use of L-DOPA or alleviating its side effects. DA receptor agonists can be used in conjunction with lower doses of L-DOPA in a combined therapy approach or as monotherapy. Historically, two general classes of dopaminergic agonists have been used in the treatment of PD: ergots and nonergots. The pharmacological management of PD is detailed in Chapter 21.

Ergot derivatives (see above) act on several different neurotransmitter systems, including DA, 5HT, and adrenergic receptors. *Bromocriptine* and *pergolide* have been used for the treatment of PD; however, their use is associated with risk for serious cardiac complications, specifically, the promotion of valvular heart disease due to $5HT_{2B}$ serotonin receptor stimulation (Hutcheson et al., 2011). *Bromocriptine* is a potent D_2 receptor agonist and a weak D_1 antagonist. *Pergolide* is a partial agonist of D_1 receptors and a strong D_2 family agonist with high affinity for both D_2 and D_3 receptor subtypes. *Pergolide* was removed from the U.S. market as therapy for PD after it was associated with an increased risk for valvular heart disease. *Bromocriptine* remains on the market primarily for the treatment of hyperprolactinemia or prolactin-secreting adenomas, where lower (D_2-selective) doses can be employed to avoid cardiac complications.

Several nonergot alkaloids are also employed in the management of PD. *Apomorphine* is a pan-DA receptor agonist most commonly used in the acute treatment of sudden "off" periods (bradykinesia, freezing) that can occur after long-term L-DOPA treatment. *Pramipexole* and *ropinirole*, widely used in the treatment of PD, are agonists at all D_2-like receptors but have the highest affinities for the D_3 receptor subtype. However, these agents are less effective than L-DOPA in the early stages of PD treatment, and both are associated with the development of impulse control disorders, such as compulsive gambling or hypersexuality; notably, fewer drug-induced dyskinesias are observed. The mechanisms underlying the impulse control disorders are currently unknown. *Rotigotine* is a DA agonist with preference for the D_2-like subfamily and is offered in a transdermal patch that is approved for the treatment of PD.

TABLE 15–4 ■ EXPERIMENTAL TOOLS AT DA RECEPTORS

RECEPTOR	AGONIST	ANTAGONIST
D_1-like[a]	SKF-81297 PF-06412562	SCH-23390 SCH-39166 (Ecopipam)
D_2-like[b]	Quinpirole	Spiperone Sulpiride
D_2	Sumanirole	L-741626 ML-321
D_3	PD128907 ML-417	SB-277011 VK4-40
D_4	PD168077 A412997	L-745870 L-741742

[a] These compounds are selective for D_1-like versus D_2-like receptors. There are no useful tool compounds that can differentiate D_1 from D_5 receptors.

[b] These compounds are selective for D_2-like versus D_1-like receptors.

Hyperprolactinemia

Despite the contraindications for PD, ergot-based DA agonists are still used in the treatment of hyperprolactinemia. Like *bromocriptine*, *cabergoline* is a strong agonist at D_2 receptors and has lower affinity for D_1, 5HT, and α adrenergic receptors. The therapeutic utility of *bromocriptine* and *cabergoline* in hyperprolactinemia is derived from their properties as DA receptor agonists: They activate D_2 receptors in the pituitary to reduce prolactin secretion. The risk of valvular heart disease from ergot therapy is associated with higher doses of drug (necessary for PD treatment) but not with the lower doses used in treating hyperprolactinemia (see Chapter 46).

Restless Leg Syndrome

Restless leg syndrome is a neurological deficit characterized by abnormal sensations in the legs that are alleviated by movement. Decreased DA receptor expression and mild dopaminergic hypofunction are noted in patients with RLS. *Rotigotine*, *ropinirole*, and *pramipexole* are FDA approved as pharmacotherapies for both PD and RLS.

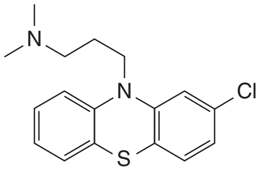
Dopamine Receptor Antagonists

Just as enhancing DA neurotransmission can be clinically important, so can inhibiting dopaminergic signaling be useful in certain disease states. Clinically, most DA antagonists are used to treat schizophrenia and bipolar disorders (see below). However, some antagonists, such as *metoclopramide* and *domperidone*, have been used to treat nausea and vomiting (see Chapter 54). As with the DA receptor agonists, a lack of subtype-specific antagonists has limited the therapeutic utility of this group of ligands. Recent advances in elucidating GPCR structures and modeling ligand binding have advanced drug design, and subtype-selective antagonists are beginning to emerge as experimental tools (see Table 15–4). Some receptor subtype-selective antagonists are in early stages of preclinical testing for therapeutic utility.

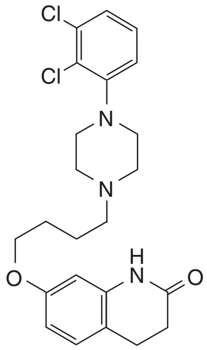
Schizophrenia

Dopamine receptor antagonists of the D_2-like subfamily are a mainstay in the pharmacotherapy of schizophrenia. While many neurotransmitter systems likely contribute to the complex pathology of schizophrenia (see Chapter 19), modulating DA signaling is considered the basis of treatment. The DA hypothesis of schizophrenia has its origins in the characteristics of the drugs used to treat this disorder: All antipsychotic compounds used clinically have high affinity for DA receptors, especially for the D_2 receptor subtype. Moreover, psychostimulants that increase extracellular DA levels can induce or worsen psychotic symptoms in schizophrenic patients. The advent of neuroimaging techniques for visualization of DA in human brain regions has led to new insights in the role of specific DA systems. DA hyperfunction in subcortical regions, most notably the striatum, has been associated with the positive symptoms of schizophrenia, which respond well to antipsychotic treatment. In contrast, the PFC of schizophrenic patients exhibits dopaminergic hypofunction, which has been associated with the more treatment-refractory negative/cognitive symptoms. The drugs currently used to treat schizophrenia are classified as either typical (or first-generation) or atypical (second-generation) antipsychotics. This nomenclature stems from the fewer extrapyramidal symptoms (EPS), or parkinsonian-like side effects, observed with atypical antipsychotics.

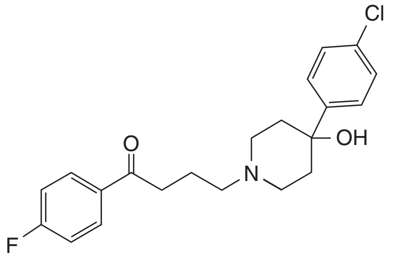
Typical Antipsychotics. The first antipsychotic drug used to treat schizophrenia was *chlorpromazine*. Its antipsychotic properties were attributed to its antagonism of DA receptors, especially the D_2 receptor. More D_2-selective ligands (e.g., *haloperidol*) were developed to improve the antipsychotic properties (see Chapter 19). Notably, drugs that are completely selective for the D_2 receptor subtype, without overlapping with affinity for the D_3 or D_4 receptor subtypes, are currently unavailable. While all typical antipsychotics markedly improve positive symptoms (e.g., hallucinations), they are not very beneficial in the treatment of negative or cognitive symptoms of this disease.



CHLORPROMAZINE



ARIPIPRAZOLE



HALOPERIDOL

Atypical Antipsychotics. This class of antipsychotic drugs originated with *clozapine* and is distinguished by lower EPS than typical antipsychotics. Atypical agents are also less likely to stimulate prolactin production. The lack of extrapyramidal side effects has been partly attributed to a much lower affinity for the D_2 receptor compared to typical antipsychotics. This frequently results from a slower association and faster dissociation of the drug to/from the D_2 receptor, resulting in a lower receptor occupancy time. It has been hypothesized that this allows for more receptor occupancy by dopamine and a higher dopaminergic "tone" leading to fewer EPS (Sykes et al., 2017). Although atypical antipsychotics are considered safer than many of the older drugs, they can also have serious metabolic side effects that include weight gain, type 2 diabetes, and hyperlipidemia, as well as cardiovascular actions that can result in arrhythmias and stroke. Most, but not all, atypical antipsychotics are also high-affinity antagonists or inverse agonists at the 5HT$_{2A}$ receptor. While the precise role of 5HT$_{2A}$ receptor blockade in the atypical effects of antipsychotics remains unclear, dual DA–5HT receptor blockade has contributed to the development of many atypical antipsychotics (see Chapter 19). Atypical antipsychotics have also proven useful as adjunctive therapeutics in both major depression and bipolar disorder (see Chapters 18 and 19).

Partial D_2-Like Receptor Agonists. *Aripiprazole* has even fewer side effects than earlier atypical antipsychotics. *Aripiprazole* diverges from the traditional atypical profile in several ways: First, it has higher affinity for D_2 receptors than for 5HT$_{2A}$ receptors; second, it is a partial agonist at D_2 receptors. As a partial agonist, *aripiprazole* may diminish subcortical (striatal) DA hyperfunction by competing with DA for receptor binding, while simultaneously enhancing dopaminergic neurotransmission in the PFC by acting as an agonist. The dual mechanism afforded by a partial agonist may thus treat both the positive and negative symptoms associated with schizophrenia. *Aripiprazole* also exhibits functional selectivity at the D_2 receptor in that it exhibits higher efficacy for β-arrestin–mediated signaling than for G protein-mediated signaling. How this property may contribute to the unique effects of *aripiprazole* is not yet clear.

Recently, a derivative of *aripiprazole*, *brexpiprazole*, has been approved for the treatment of schizophrenia and as an adjunctive treatment of depression. The pharmacological properties of *brexpiprazole* are similar to those of *aripiprazole* except that *brexpiprazole* has lower D_2 receptor agonist efficacy and high partial agonist effects at the 5HT$_{1A}$ receptor; perhaps this latter property underlies its effectiveness in treating depression.

Another partial agonist of the D_2 receptor, *cariprazine*, has recently been approved for treating schizophrenia and bipolar disorder. Interestingly, *cariprazine* is also a partial agonist at the D_3 receptor and exhibits higher affinity for the D_3 versus the D_2 receptor. In some studies, *cariprazine* has been shown to exhibit procognitive effects, suggesting that it may be useful for treating negative as well as positive symptoms of schizophrenia.

D_3 Receptor Antagonists and Drug Addiction

Although much work remains to determine their clinical utility, D_3-selective antagonists show promise in the treatment of addiction (Newman et al., 2012, 2021). This interest stems from the high expression of the D_3 receptor in the limbic system, the reward center of the brain, and from animal studies of D_3-selective antagonists that suggest a role for the D_3 receptor in the motivation to abuse drugs and in the potential for drug-abuse relapse (Keck et al., 2015). Preclinical research has suggested that D_3-selective antagonists may be particularly effective in treating opioid use disorders, and several such agents are currently under development (Galaj et al., 2020; Newman et al., 2021).

Drug Facts for Your Personal Formulary: *Serotonergic Ligands*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
5HT$_3$ Receptor Antagonists • Antiemetic agents • Additional detail in Chapters 54 and 55		
Ondansetron Dolasetron Granisetron Palonosetron	• Antiemetics • Treatment of nausea	• Associated with asymptomatic electrocardiogram changes, including prolongation of PT and QTc intervals
Alosetron	• Irritable bowel syndrome	• Most useful in women with irritable bowel syndrome when diarrhea is the principal symptom
5HT$_{2A}$ Receptor Agonists • Psychedelics		

Psilocybin
Mescaline
LSD
	• In clinical trials for:
	  • Depression
	  • Substance use disorder
	  • Migraine
	  • Obsessive-compulsive disorder
		• Produce profound hallucinations, which may be relevant to therapeutic mechanisms
		• Efficacy in preclinical models as an anti-inflammatory

Drug Facts for Your Personal Formulary: *Serotonergic Ligands (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
$5HT_{2A}$ Receptor Inverse Agonists • Psychosis		
Pimavanserin	• Treatment of psychosis	• Currently restricted to psychosis occurring within PD
The Triptans: $5HT_{1B/1D}$ Receptor Agonists • Migraine		
Almotriptan[a] Eletriptan Frovatriptan Naratriptan Rizatriptan Sumatriptan[b] Zolmitriptan	• Acute treatment of migraine	• Most effective in acute settings; should be used as soon as possible after onset of attack • Usually dosed orally; onset, 1–3 h • Use with caution in patients with cardiovascular issues; contraindicated in patients with ischemic heart disease and coronary artery vasospasm • Drug interactions: CYP3A4 inhibitors ↑ C_p and $t_{1/2}$ of eletriptan, naratriptan; MAO inhibitors ↑ levels of almo-, riza-, suma-, and zolmitriptan • Side effects: dizziness, somnolence, neck and chest pain • May cause fetal harm; not recommended during pregnancy and nursing; reduce dose in renal and hepatic impairment; do not administer within 24 h of other triptans, ergots, SSRIs/SNRIs • Beware serotonin syndrome, especially in combination with SSRIs and SNRIs
$5HT_{1F}$ Receptor Agonists • Migraine		
Lasmiditan	• Acute treatment of migraine	• Fewer adverse cardiovascular events than triptans
The Ergot Alkaloids • Interact with multiple 5HT receptor isoforms • Broad therapeutic utility		
LSD	• No longer employed clinically • Potent hallucinogen	• Positron emission tomographic imaging reveals similar activation patterns between schizophrenic patients experiencing hallucinations and LSD-induced hallucinations • $5HT_{2A}$ receptor activation is believed to mediate the hallucinogenic effect of LSD
Methysergide	• Acute treatment of migraine • Treatment of vascular headaches	• Restricted to use in patients with frequent, moderate, or infrequent, severe migraine attacks • Erratic drug absorption • Potential for inflammatory fibrosis with prolonged use, including pleuropulmonary and endocardial fibrosis
Ergonovine Methylergonovine	• Prevention of postpartum hemorrhage	• Increasing dose results in prolonged duration and increased force of uterine contraction • Sustained contracture can result at high doses • Hallucinations at supratherapeutic levels
$5HT_{1A}$ Receptor Partial Agonists and SSRIs • Anxiolytics and antidepressants • Additional detail in Chapter 18		
Buspirone	• Treatment of anxiety	• Mimics antianxiety effects of benzodiazepines but does not interact with $GABA_A$ receptors • Partial agonist of the $5HT_{1A}$ receptor
Fluoxetine Fluvoxamine Paroxetine Citalopram Escitalopram Sertraline Vilazodone	• Antidepressants • Also used to treat anxiety, panic disorder, obsessive-compulsive disorder, fibromyalgia, and neuropathic pain	• Selectively inhibit the serotonin transporter (SSRIs) • Most widely used treatments for major depressive disorder • Sexual dysfunction is a common side effect with SSRIs • Precaution: serotonin syndrome
Multifunctional Serotonin Agonists and Antagonists (MSAAs) • Treatment of sexual dysfunction • Activity at multiple receptor isoforms		
Flibanserin	• Treatment of HSDD/FSIAD[c] in premenopausal women	• Potent $5HT_{1A}$ receptor agonist and $5HT_2$ receptor family antagonist • Exerts both agonist and antagonist activity at 5HT receptors → MSAA designation
Dopamine Receptor Agonists • Some with subfamily specificity		
Dopamine	• Congestive heart failure • Sepsis • Cardiogenic shock	• Only used acutely via intravenous administration
Bromocriptine Cabergoline	• PD (see Chapter 21) • Hyperprolactinemia	• Ergot derivatives with D_2 agonist activity and D_1 antagonist activity • Limited utility for PD due to high potential for cardiac valvulopathies via $5HT_{2B}$ stimulation • Bromocriptine and cabergoline can be used at low doses to treat hyperprolactinemia
Apomorphine Pramipexole Ropinirole Rotigotine	• PD (see Chapter 21 for more details) • RLS	• Nonergot alkaloids with broader DA receptor agonist activity • Less efficacious than L-DOPA in PD; often used as adjunct therapy in advanced PD • Use in early PD can lead to poor impulse control • Pramipexole, ropinirole, and rotigotine are used to treat RLS

Drug Facts for Your Personal Formulary: *Serotonergic Ligands* (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Dopamine Receptor Antagonists • Antipsychotics • Emerging subtype specificity of ligands (see also Chapter 19)		
Chlorpromazine Haloperidol	• Schizophrenia (see Chapter 19)	• Classified as typical antipsychotics • Agents block D$_2$ receptors but are not completely selective • Improvements are most notable in positive symptoms of schizophrenia
Clozapine Risperidone Olanzapine	• Schizophrenia (see Chapter 19)	• Classified as atypical antipsychotics • Mixed 5HT$_{2A}$–D$_2$ receptor blockade • Fewer extrapyramidal side effects than typical antipsychotics, but can have greater effects on metabolism and weight gain
Aripiprazole Brexpiprazole Cariprazine	• Schizophrenia (see Chapter 19)	• D$_2$ partial agonists with varied profiles at 5HT receptors • Improved side effect profile over many other antipsychotics
DAT Ligands • High potential for abuse • Interact with the dopamine transporter		
Bupropion	• Depression • Smoking cessation	• Also inhibits NET • ↑ risk of suicidal ideation in pediatric/young adult patients taking this medication
Cocaine	• Rarely used therapeutically	• Schedule II classification • Limited clinical utility as a topical anesthetic in eye and nasal surgeries
Methylphenidate Methamphetamine Amphetamine	• ADHD, ADD[d] • Narcolepsy • Obesity	• Can worsen psychosis; use with extreme caution in patients with bipolar disorder • Schedule II drug classification due to psychostimulant properties if misused

[a] Fewest side effects.
[b] Has best evidence for safety in pregnancy.
[c] Female sexual interest/arousal disorder.
[d] Attention-deficit disorder.

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Chapter 16

Neurotransmission in the Central Nervous System

R. Benjamin Free, Suzanne M. Underhill, Susan G. Amara, and David R. Sibley

CELLULAR ORGANIZATION OF THE CNS
- Neurons
- Nonneuronal Cells
- Blood-Brain Barrier

NEURONAL EXCITABILITY AND ION CHANNELS

CHEMICAL COMMUNICATION IN THE CNS
- Identification of Central Neurotransmitters
- Cell Signaling and Synaptic Transmission
- Fast Neurotransmission
- Slow Neurotransmission

CENTRAL NEUROTRANSMITTERS
- Amino Acids
- Acetylcholine
- Monoamines
- Trace Amines

REGULATION OF NEUROTRANSMISSION
- Peptides
- Purines
- Cannabinoids
- Other Lipid Mediators
- Gases: Nitric Oxide and Carbon Monoxide
- Termination of Neurotransmitter Action

CNS DRUG DISCOVERY AND DEVELOPMENT

The brain is a complex assembly of interacting cells that regulate many of life's activities in a dynamic fashion, generally through a communication process termed neurotransmission. Because the CNS drives so many physiological responses, it stands to reason that centrally acting drugs are invaluable for a plethora of conditions. CNS-acting drugs are used not only to treat anxiety, depression, mania, and schizophrenia, but also to target diverse pathophysiological conditions such as pain, fever, movement disorders, insomnia, eating disorders, nausea, vomiting, and migraine. However, as the CNS dictates such diverse physiology, the recreational use of some CNS-acting drugs can and does lead to physical dependence (see Chapter 28) with enormous societal impacts. The sheer breadth of physiological and pathological activities mediated by drug molecules acting in the CNS makes this class of therapeutics both wide-ranging and important.

The identification of CNS targets and the development of drug molecules for those targets present extraordinary scientific challenges. While years of investigation have begun to dissect the cellular and molecular bases for many aspects of neuronal signaling, complete understanding of the functions of the human brain remains in its infancy. Complicating the effort is the fact that a CNS-active drug may act at multiple sites with disparate and even opposing effects. Furthermore, many CNS disorders likely involve multiple brain regions and pathways, which can frustrate efforts that focus on a single therapeutic agent.

The pharmacology of CNS-acting drugs is primarily driven by two broad and overlapping goals:

- To develop/use drugs as biochemical probes to both elucidate and manipulate the normal CNS; and
- To develop drugs to correct pathophysiological changes in the abnormal CNS.

Modern advances in molecular biology, neurophysiology, structural biology, epigenetics, biomarkers, immunology, and an array of other fields have facilitated both our understanding of the brain and the development of an ever-expanding repertoire of drugs that can selectively treat diseases of the CNS. An important goal in drug discovery is to determine receptor structure at the atomic level and understand how neurotransmitters and drug molecules interact with receptors to stimulate or inhibit receptor-mediated signaling. This has spurred the use of complex techniques such as x-ray crystallography and cryo-electron microscopy to solve receptor structures to a high degree of accuracy, followed by computer-aided molecular dynamic simulations and modeling as essential components to understand how neurotransmitters and drug molecules affect signaling. As more atomic level structures of receptors, transporters, ion channels, and other relevant drug targets are elucidated, such structural biology techniques will likely become even more prevalent in drug discovery and development.

This chapter introduces fundamental principles and guidelines for the comprehensive study of drugs that affect the CNS. Specific therapeutic approaches to neurological and psychiatric disorders are discussed in subsequent chapters. For further detail, see specialized texts such as Sibley et al. (2007), Brady et al. (2012), Nestler et al. (2020), and Kandel et al. (2021). Detailed information on nearly all specific receptors and ion channels can be found at the official databases of the International Union of Basic and Clinical Pharmacology (IUPHAR) Guide to Pharmacology (http://www.guidetopharmacology.org).

Cellular Organization of the CNS

The CNS is made up of several types of specialized cells that are physiologically integrated to form complex functional brain tissue. The primary communicating cell is the neuron, which is strongly influenced and sustained by a variety of important supporting cells. Specific connections between neurons, both within and across the macro-divisions of the brain, are essential for neurological function. Through patterns of neuronal circuitry, individual neurons form functional ensembles to regulate the flow of information within and between the regions of the brain. Under these guidelines, present understanding of the cellular organization of the CNS can be viewed from the perspective of the size, shape, location, and interconnections between neurons (Shepherd, 2004; Squire, 2013).

Neurons

Neurons are the highly polarized signaling cells of the brain and are subclassified into types based on factors including function (sensory, motor, or interneuron), location, morphology, neurotransmitter phenotype, or the class(es) of receptors expressed. Neurons are electrically active cells that express a variety of ion channels and ion transport proteins that

Abbreviations

ACh: acetylcholine
ADHD: attention-deficit/hyperactivity disorder
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
BBB: blood-brain barrier
CFTR channel: cystic fibrosis transmembrane conductance regulated channel
CGRP: calcitonin gene–related peptide
ClC: chloride channel
CNG channel: cyclic nucleotide–gated channel
CSF: cerebrospinal fluid
CYP: cytochrome P450
DA: dopamine
DAT: dopamine transporter
EAAT: excitatory amino acid transporter
EPI: epinephrine
GABA: γ-aminobutyric acid
GABA-T: GABA transaminase
GAT: GABA transporter
GluR: AMPA/kainate type of glutamate receptor
GPCR: G protein-coupled receptor
HCN channel: hyperpolarization-activated, cyclic nucleotide–gated channel
HP loop: hairpin loop
5HT: 5-hydroxytryptamine, serotonin
IP$_3$: inositol 1,4,5-trisphosphate
IUPHAR: International Union of Basic and Clinical Pharmacology
KA: kainic acid
L-DOPA: levodopa
MGL: monoacylglycerol lipase
mGluR: metabotropic glutamate receptor
NADA: *N*-arachidonoyl-dopamine
NAM: negative allosteric modulator
NE: norepinephrine
NET: norepinephrine transporter
NMDA: *N*-methyl-D-aspartate
NO: nitric oxide
OCT: organic cation transporter
PAM: positive allosteric modulator
PK_: protein kinase _, as in PKA, PKC
PL_ : phospholipase_, as in PLA, PLC
PMAT: plasma membrane monoamine transporter
SERT: serotonin transporter
TAAR: trace amine–associated receptors
TRP channel: transient receptor potential channel
VAChT: vesicular acetylcholine transporter
VGAT: vesicular GABA and glycine transporter
VGLUT: vesicular glutamate transporter
VMAT: vesicular monoamine transporter

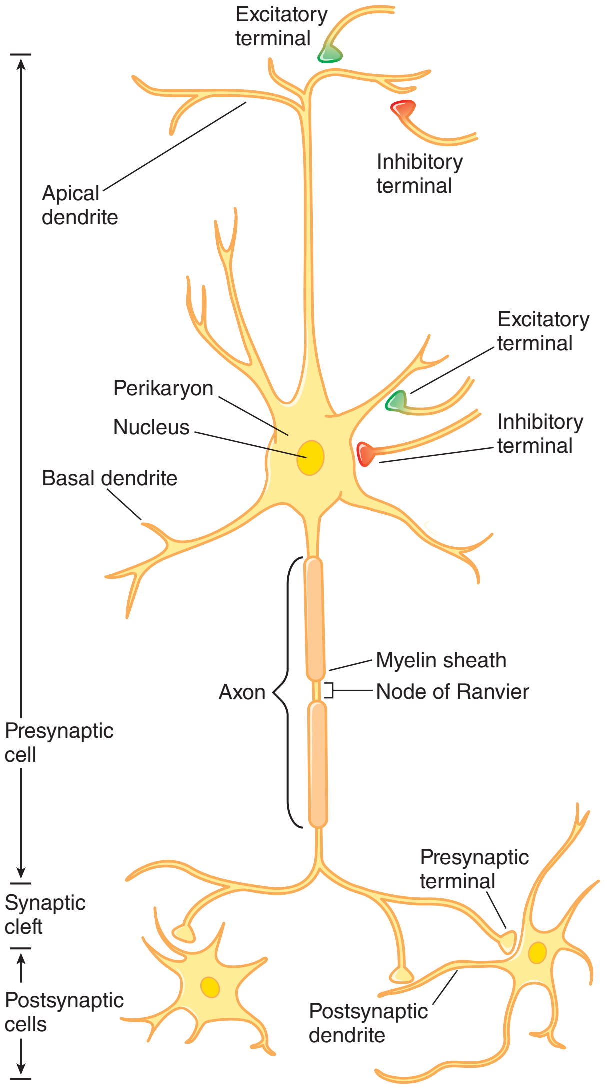


Figure 16–1 *Principal features of a neuron.* Dendrites, including apical dendrites, receive synapses from presynaptic terminals. The cell body (~50 μm in diameter) contains the nucleus and is the site of transcription and translation. The axon (0.2–20 μm wide, 100 μm to 2 m in length) carries information from the perikaryon to the presynaptic terminals, which form synapses (up to 1000) with the dendrites of other neurons. Axo-somatic synapses also occur. Many CNS-active pharmacological agents act at the presynaptic and postsynaptic membranes of the synaptic clefts and at areas of transmitter storage near the synapses. (Adapted with permission from Kandel ER, Schwartz JH, Jessell TM (eds). *Principles of Neural Science*, 4th ed. McGraw-Hill, New York, **2000**, p 22.)

allow them to conduct nerve impulses or action potentials that ultimately trigger release of neurotransmitters during chemical neurotransmission. Neurons also exhibit the cytological characteristics of highly active secretory cells with large nuclei, large amounts of smooth and rough endoplasmic reticulum, and frequent clusters of specialized smooth endoplasmic reticulum (Golgi complex) in which secretory products of the cell are packaged into membrane-bound organelles for transport from the perikaryon/soma to the axon or dendrites (Figure 16–1). The sites of interneuronal communication in the CNS are termed *synapses*. Although synapses are functionally analogous to “junctions” in the somatic motor and autonomic nervous systems, central synapses contain an array of specific proteins presumed to be the active zone for transmitter release and response. Like peripheral “junctions,” central synapses are denoted by accumulations of tiny (50–150 nm) *synaptic vesicles*. The proteins of these vesicles have specific roles in neurotransmitter storage, vesicle docking, secretion, and reaccumulation of neurotransmitter. The release of these neurotransmitters and their action on the neighboring cells via specific receptors, through mechanisms discussed below, underlie the ability of these specialized cells to communicate with each other to dictate complex physiological actions.

Nonneuronal Cells

A diverse cast of support cells outnumber neurons in the CNS. These include neuroglia, vascular elements, the cerebrospinal fluid (CSF)-forming cells found within the intracerebral ventricular system, and the meninges that cover the surface of the brain and comprise the CSF-containing envelope. *Neuroglia* (often referred to simply as glia) are the most abundant support cells. They are nonneuronal cells that maintain important brain functions such as holding neurons in place, supplying oxygen and nutrients to neurons, insulating signaling between neurons, and destroying potential pathogens. Traditionally it was thought that neuroglia acted only in a supporting role; however, newer studies have demonstrated that they may also be involved in some signaling processes.

Neuroglia are classified as either *micro-* or *macro*glia. In the CNS, the macroglia consist of *astrocytes, oligodendroglia, ependymal cells*, and *radial glia. Astrocytes* (cells interposed between the vasculature and the neurons) are the most abundant of these and often surround individual compartments of synaptic complexes. They play a variety of metabolic support roles including furnishing energy intermediates, anchoring neurons to their blood supply, and regulating the external environment of the neuron by active removal of neurotransmitters and excess ions following release. The *oligodendroglia* produce myelin—multilayer compacted membranes that electrically insulate segments of axons and permit nondecremental propagation of action potentials. Ependymal cells line the spinal cord and ventricular system and are involved in the creation of CSF, while radial cells act as neuroprogenitors and scaffolds. *Microglia* consist of specialized immune cells found within the CNS. Although the brain is immunologically protected by the blood-brain barrier (BBB), these microglia act as macrophages to protect the neurons and are therefore mediators of immune response in the CNS. Microglia respond to neuronal damage and inflammation, and many diseases are associated with deficient microglia. In some instances, such as in chronic neuroinflammation, the balance between the numbers of microglia and astrocytes can determine whether there will be resulting cell damage or protection. Thus, in addition to neurons, support cells such as glia are key players in facilitating most aspects of neuronal function and CNS signaling.

Blood-Brain Barrier

The BBB is an important boundary separating the periphery (capillaries carrying blood) from the CNS. This barrier consists of *endothelial cells, astrocytes,* and *pericytes* on a *noncellular basement membrane*. The BBB diminishes the rate of access of many chemicals in plasma, thus preventing unencumbered access to the brain by circulating blood components. The BBB evolved as a mechanism to protect the brain from toxins in the environment. In terms of CNS therapeutics, the BBB represents a substantial obstacle to overcome for drug delivery to the site of action. An exception exists for lipophilic molecules, which diffuse freely across the BBB and accumulate in the brain. In addition to its relative impermeability to charged molecules such as neurotransmitters, the BBB can be viewed as a combination of the partition of solute across the vasculature (which governs passage by definable properties such as molecular weight, charge, and lipophilicity) and the presence or absence of energy-dependent transport systems (see Chapter 4). However, the cells within the barrier also have the capacity to actively transport molecules such as glucose and amino acids that are critical for brain function (see Chapter 17). One of these transport systems that is selective for large amino acids catalyzes the movement across the BBB and thus contributes to the therapeutic utility of L-DOPA in the treatment of Parkinson's disease. Furthermore, for some compounds, including metabolites of neurotransmitters (e.g., homovanillic acid and 5-hydroxyindoleacetic acid), the acid transport system of the choroid plexus provides an important route for clearance from the brain. The BBB is covered in detail in Chapter 17.

Neuronal Excitability and Ion Channels

As noted above, neurons, the primary signaling cells of the brain, release neurotransmitters in response to a rapid rise and fall in membrane potential known as an action potential. Voltage-dependent ion channels within the plasma membrane open when the membrane potential increases to a threshold value, thus regulating the electrical excitability of neurons. Action potentials are the signals by which the brain and neurons receive and transmit information to one another through pathways determined by their connectivity.

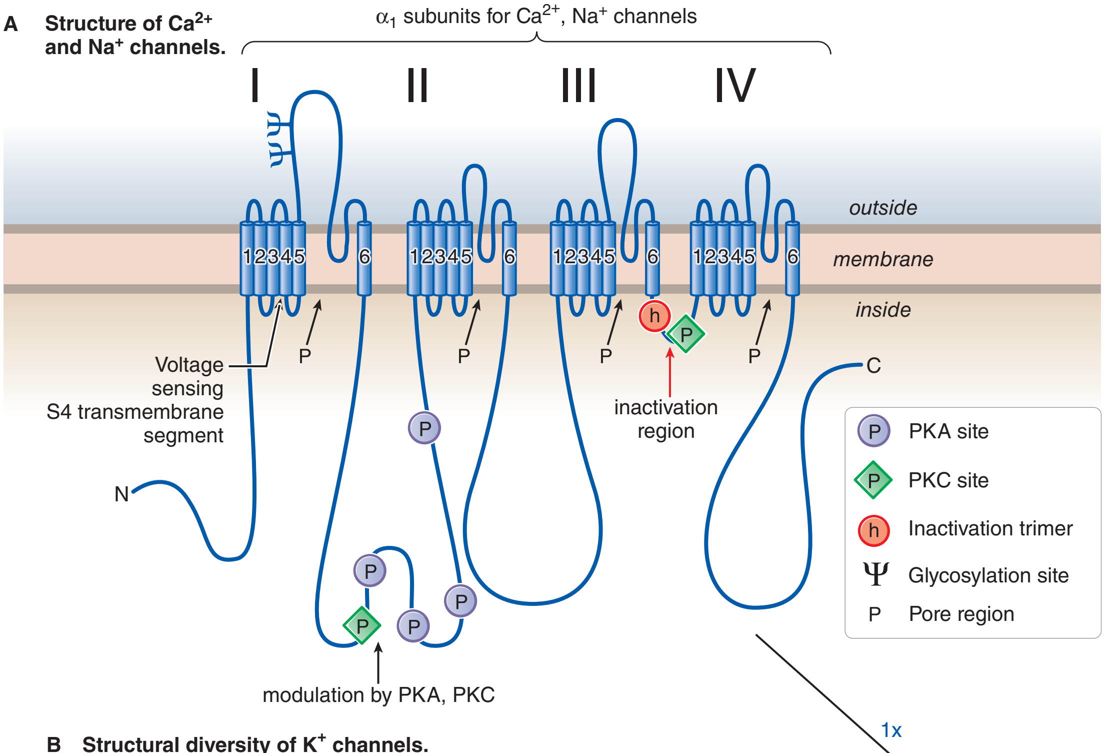
We now understand in considerable detail how three major cations, Na^+, K^+, and Ca^{2+}, as well as Cl^- anions, are regulated via their flow through highly discriminative ion channels (Figures 16–2 and 16–3). Active transmembrane countertransport of Na^+ and K^+ and the intracellular sequestration and mobilization of Ca^{2+} permit resting cells to maintain a steady-state concentration of ions that enable electrical depolarization. Voltage-sensitive ion channels permit the flow of Na^+ and K^+ ions that mediate depolarization and repolarization, while homeostatic mechanisms (e.g., Na^+/K^+-ATPase, Na^+/Ca^{2+} exchanger, Ca^{2+}-ATPases) restore the transmembrane ionic gradients to their resting conditions. The relatively high extracellular concentration of Na^+ (~140 mM) compared to its intracellular concentration (~14 mM) means that increases in permeability to Na^+ cause cellular depolarization, ultimately leading to the generation of an action potential. In contrast, the intracellular concentration of K^+ is relatively high (~120 mM, vs. 4 mM outside the cell), and increased permeability to K^+ results in hyperpolarization. Changes in the concentration of intracellular Ca^{2+} (100 nM to 1 μM) affect multiple processes in the cell and are critical in the release of neurotransmitters. Electrical excitability thus generates the action potential through changes in the distribution of charged ions across the neuronal cell membrane.

The principal anion involved in neuronal excitability is Cl^-, and its flux is also regulated by membrane channels and transporters. Cl^- channels are a superfamily of ion channels that are important for maintaining resting potential and are also responsible for the inhibitory postsynaptic potentials that dampen neuronal excitability. In most neurons, the Cl^- gradient across the plasma membrane is inwardly driven (~116 mM outside vs. 20 mM inside the cell), and as a result, inactivation of these Cl^- channels leads to hyperexcitability. There are several families of both voltage-gated and ligand-gated Cl^- channels (Figure 16–3). Ligand-gated Cl^- channels are linked to inhibitory transmitters including γ-aminobutyric acid (GABA) and glycine (discussed in detail below). A class of secondary active transporters, the cation-chloride cotransporters, plays an essential role in establishing the electrochemical Cl^- gradient that is required for the hyperpolarizing postsynaptic inhibition mediated by both GABA receptors and glycine receptors. During brain development, changes in the expression of neuronal cation-chloride cotransporter isoforms can result in shifts in the direction of the chloride gradient such that activation of a ligand-gated chloride channel becomes excitatory.

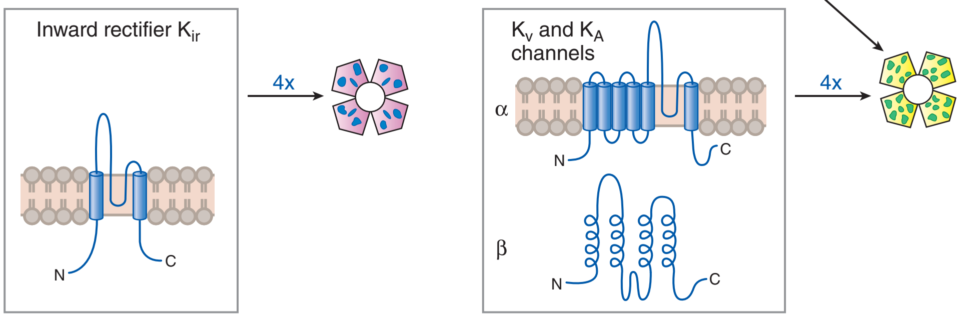
The chloride channel (ClC) family of Cl^- channels consists of plasma membrane channels that affect Cl^- flux and membrane potential as well as channels that function as Cl^-/H^+ antiporters. ClC Cl^- channels can also influence the pH of intracellular vesicles. Cystic fibrosis transmembrane conductance regulated (CFTR) channels are gated by ATP and activated by PKA; CFTR channels mediate the conductance of certain anions, mainly Cl^- and HCO_3^-. Overall, these channels are responsible for a variety of important neurophysiological roles including regulation of membrane potential, volume homeostasis, and regulation of pH on internal extracellular compartments.

Cyclic nucleotide–gated (CNG) channels are nonselective cation channels that regulate ion flux in neurons. CNG channels are activated by cyclic nucleotide binding, and their primary function involves sensory transduction, especially in the retina and olfactory neurons. Since CNG channels are nonselective and also allow alkali ions to flow, they can result in either depolarization or hyperpolarization. These channels consist of four subunits assembled around a central pore and are subclassified into α (four genes) and β (two genes) subunits. Another type of CNG channel is the *hyperpolarization-activated, cyclic nucleotide–gated (HCN)* channel. HCN channels are nonselective ligand-gated cation channels that are encoded by four genes and are expressed in the heart as well as in the brain, where they are thought to influence the way neurons respond to synaptic input. HCN channels have been implicated in epilepsy and neuropathic pain. These channels open with hyperpolarization and close with depolarization; upon direct binding of cyclic AMP or cyclic GMP, the activation curves for the channels are shifted to more hyperpolarized potentials. These channels play essential roles in cardiac pacemaker cells and in rhythmic and oscillatory activity as they are finely tuned to respond to activation thresholds in the CNS.

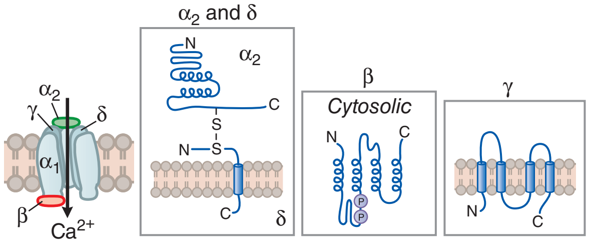
A Structure of Ca^{2+} and Na^+ channels.


α_1 subunits for Ca^{2+}, Na^+ channels
I
II
III
IV
outside
membrane
inside
12345
6
Voltage sensing S4 transmembrane segment
P
N
C
h
inactivation region
modulation by PKA, PKC
P PKA site
P PKC site
h Inactivation trimer
Ψ Glycosylation site
P Pore region
1x

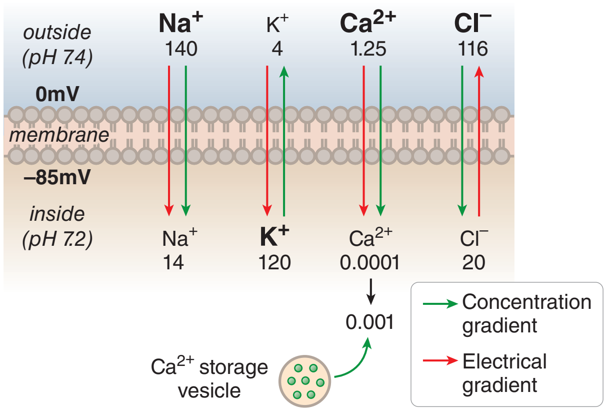
B Structural diversity of K^+ channels.


Inward rectifier K_{ir}
N
C
4x
K_v and K_A channels
α
β
N
C
4x

C Multisubunit assembly of Ca^{2+} channels.


α_2
γ
δ
α_1
β
Ca^{2+}
α_2 and δ
N
α_2
C
S
S
N
C
δ
β
Cytosolic
N
C
P
P
γ
N
C

D Ionic gradients across a mammalian nerve cell membrane


outside (pH 7.4)
0mV
membrane
–85mV
inside (pH 7.2)
Na^+ 140
K^+ 4
Ca^{2+} 1.25
Cl^- 116
Na^+ 14
K^+ 120
Ca^{2+} 0.0001
Cl^- 20
0.001
Ca^{2+} storage vesicle
Concentration gradient
Electrical gradient

Transient receptor potential (TRP) channels are a large family of around 28 ion channels that are nonselectively permeable to cations including Na^+, Ca^{2+}, and Mg^{2+}. The name *transient receptor potential channel* is unfortunate and does not describe the function of these channels, which is to respond to multiple types of stimuli to allow cation entry into the cell. They are broadly grouped into six receptor subfamilies with diverse structures and functions and possessing four subunits with a cation-permeable pore: ankyrin (TRPA), canonical (TRPC), melastatin (TRPM), mucolipin (TRPML), polycystin (TRPP), and vanilloid (TRPV). These channels can have diverse modes of activation and permeation and respond to multiple stimuli and function in sensory physiology, including thermosensation, osmosensation, and taste. Importantly some TRP channels are also mediators of pain as they function as detectors of thermal and chemical stimuli that activate sensory neurons. Spices such as garlic, chili powder, and wasabi activate certain subtypes. Others respond to such diverse chemicals as menthol, peppermint, and camphor. Mutations in TRP channels have been associated with neurodegenerative diseases as well as cancer. The diversity of their physiology has led to their investigation as drug targets. The capsaicin receptor, TRPV1, is the most thoroughly characterized TRP channel, and both agonists and antagonists are being investigated, particularly for the treatment of chronic pain. Reviews of TRP channels and drug development are available (Moran, 2018; Zhao et al., 2021). The 2021 Nobel Prize in Physiology or Medicine was awarded to David Julius for his work on TRP channels and to Ardem Patapoutian for his work on the mechanosensitive ion channels, piezos (https://www.nobelprize.org/prizes/medicine/2021/summary/).

Chemical Communication in the CNS

A central concept of neuropsychopharmacology is that drugs that improve the functional status of patients with neurological or psychiatric diseases typically act by enhancing or blunting neurotransmission in the CNS. Therapeutic targets typically include *ion channels* (discussed above) that mediate changes in excitability induced by neurotransmitters, *neurotransmitter receptors* that physiologically respond to activation by neurotransmitters, and *transport proteins* that clear released transmitter from the extracellular space.

Identification of Central Neurotransmitters

Neurotransmitters are endogenous chemicals in the brain that act to enable signaling across a chemical synapse. They carry, boost, and modulate signals between neurons or other cell types and act on a variety of targets to elicit a host of biological functions. An essential step in understanding the functional properties of neurotransmitters within the context of the circuitry of the brain is to identify substances that are transmitters at specific interneuronal connections. The precise number of transmitters is unknown, but more than 100 chemical messengers have been identified to date. The criteria for identification of central transmitters are similar to those used to establish the transmitters of the autonomic nervous system (see Chapter 14):

- The transmitter must be present in the presynaptic terminals of the synapse and in the neurons from which those presynaptic terminals arise.
- The transmitter must be released from the presynaptic nerve concomitantly with nerve activity in high enough quantity to have an effect.
- The effects of experimental application of the putative transmitter should mimic the effects of stimulating the presynaptic pathway.
- If available, specific pharmacological agonists and antagonists should stimulate and block, respectively, the measured functions of the putative transmitter.
- There should be a mechanism present (either reuptake or enzymatic) that stops the actions of the transmitter.

Many neurons contain multiple transmitter substances either packaged together in the same vesicles or in adjacent active zones that may act jointly on the postsynaptic membrane(s) or may act presynaptically to affect release of transmitter from the presynaptic terminal. In the setting of concurrent release of multiple signaling molecules, mimicking or fully antagonizing the action of a given transmitter with a single drug compound is difficult. This complexity in identifying signaling molecules has been partially overcome by use of defined *in vitro* cell culture systems and conditional transgenic animal models that can then be extrapolated to the human CNS.

Cell Signaling and Synaptic Transmission

Cellular signaling links neurotransmitter receptor activation to downstream biological effects. A number of mechanisms have been identified, and these can be broadly classified into two main types of signaling: fast and slow neurotransmission. The most common postreceptor events are fast transmission resulting from rapid changes in ion flux through ion channels. Slow neurotransmission is primarily the role of a second major group of receptors, the G protein-coupled receptors (GPCRs), which interact with heterotrimeric GTP-binding proteins. Additional mechanisms are seen with growth factor receptors and a distinct class of nuclear receptors that transduce steroid hormone signaling. Since the majority of cell-to-cell communication in the CNS involves chemical transmission, neurons require specialized cellular functions to mediate these actions including (Figure 16–4):

Figure 16–2 *Voltage-dependent Na^+, Ca^{2+} and K^+ channels.* Voltage-dependent channels provide for rapid changes in ion permeability along axons and within dendrites and for excitation-secretion coupling that causes neurotransmitter release from presynaptic sites. **A.** *Structure of Ca^{2+} and Na^+ channels.* The α subunit in both Ca^{2+} and Na^+ channels consists of four sub-subunits or segments (labeled **I** through **IV**), each with six transmembrane (TM) hydrophobic domains (blue cylinders). The hydrophobic regions that connect TM5 and TM6 in each segment associate to form the pore of the channel. Segment 4 in each domain includes the voltage sensor. (Adapted with permission from Catterall W. *Neuron* **2000**, *26*:13–25. © Elsevier.) **B.** *Structural diversity of K^+ channels. Inward rectifier, K_{ir}.* The basic subunit of the inwardly rectifying K^+ channel protein K_{ir} has the general configuration of TM5 and TM6 of a segment of the α subunit shown in panel **A**. Four of these subunits assemble to create the pore. *Voltage-sensitive K^+ channel, K_v.* The α subunits of the voltage-sensitive K^+ channel K_v and the rapidly activating K^+ channel K_A share a hexaspanning structure resembling in overall configuration a single segment of the Na^+ and Ca^{2+} channel structure, with six TM domains. Four of these assemble to form the pore. Regulatory β subunits (cytosolic) can alter K_v channel functions. **C.** *Multisubunit assembly of Ca^{2+} channels.* Ca^{2+} channels variably require several auxiliary small proteins ($α_2$, β, γ, and δ); $α_2$ and δ subunits are linked by a disulfide bond. Likewise, regulatory subunits also exist for Na^+ channels. **D.** *Ionic gradients across a mammalian nerve cell membrane.* The kidney is the primary regulator of the extracellular ionic environment. Active transport of cations and the relatively selective permeabilities of ion channels maintain the intracellular milieu. In this figure, the numbers below the various ions are resting state concentrations in mM; the large bold lettering of the elements indicates the location of the higher concentration of the ion; the red and green arrows indicate the direction of the electrical and concentration gradients. In the resting state, Na^+ channels are closed, K^+ channels are open, and the membrane potential approaches the Nernst potential for K^+. The opening of Na^+ channels results in depolarization. In contrast, the K^+ gradient is such that increased permeability to K^+ results in *hyperpolarization*. Changes in the concentration of intracellular Ca^{2+} (entry via Ca^{2+} channels and mobilization of Ca^{2+} sequestered in the cell) affect multiple cellular processes and are critical for the release of neurotransmitters. Cl^- flows through membrane channels, a large fraction of which are gated by GABA or glycine. Activation of neuronal $GABA_A$ receptors generally leads to a net influx of Cl^-, resulting in membrane hyperpolarization and inhibition of depolarization. The equilibrium potential for Cl^- is relatively close to the membrane resting potential, and small changes in cellular Cl^-, the membrane potential, and the activities of Cl^- transporters (e.g., KCC2 and NKCC1) can influence transmembrane Cl^- movements.

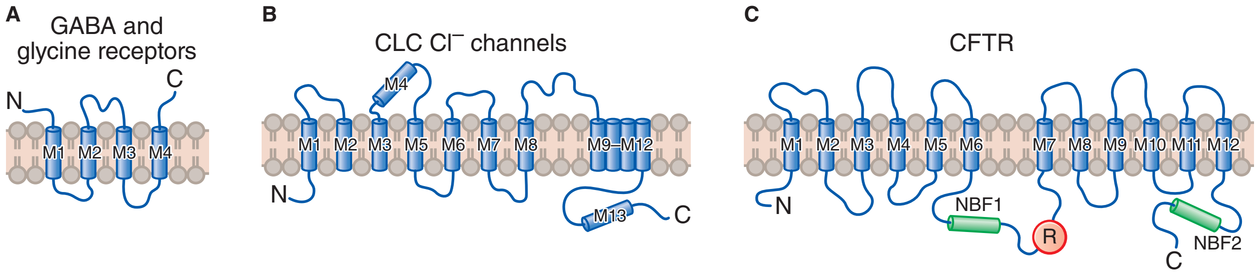


Figure 16–3 *Families of Cl^- channels.* Due to the Cl^- gradient across the plasma membrane (~116 mM outside vs. 20 mM inside the cell), activation of Cl^- channels causes an inhibitory postsynaptic potential (IPSP) that dampens neuronal excitability; inactivation of these channels can lead to hyperexcitability. There are three distinct types of Cl^- channels. **A**. *Ligand-gated channels* are linked to inhibitory transmitters, including GABA and glycine. These channels are pentamers that are composed of differing subunits that each have four transmembrane domains as shown (discussed below). **B.** *CLC Cl^- channels* affect Cl^- flux, membrane potential, and the pH of intracellular vesicles. **C.** *CFTR channels* bind ATP and are regulated by phosphorylation of serine residues. M, transmembrane domains; NBF, nucleotide-binding fold; R, regulatory (phosphorylation) domain. (Adapted with permission from Jentsch J. Chloride channels: a molecular perspective. *Curr Opin Neurobiol,* **1996**, *6*:303-310. Copyright Elsevier.)

- Neurotransmitter synthesis. Small-molecule neurotransmitters are synthesized in nerve terminals, whereas others, such as peptides, are synthesized in cell bodies and transported to nerve terminals.
- Neurotransmitter storage. Synaptic vesicles store transmitters, often in association with various proteins and frequently with ATP.
- Neurotransmitter release. Release of transmitter into the synaptic cleft occurs by exocytosis. Depolarization of the presynaptic neuron results in a complex initiation of stimulus-secretion coupling, which involves vesicle docking at the plasma membrane, followed by the formation of membrane fusion/release complexes, and culminates in the Ca^{2+}-dependent release of vesicle contents.
- Neurotransmitter recognition. Neurotransmitters diffuse from sites of release and bind selectively to receptor proteins to initiate intracellular signaling events within the postsynaptic cell.
- Termination of action. A variety of mechanisms terminate the action of synaptically released transmitters, including diffusion from the synapse, enzymatic inactivation (for acetylcholine and peptides), and uptake into neurons and/or glial cells by specific transporters.

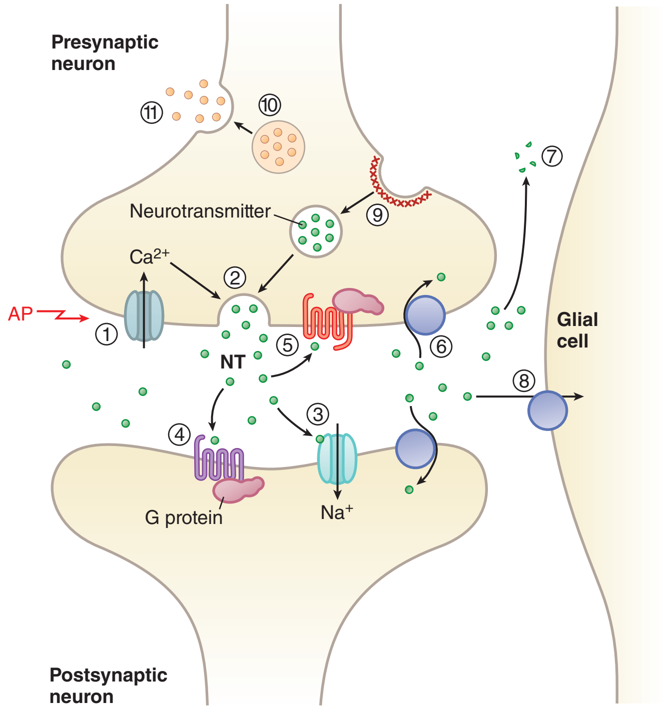


Figure 16–4 *Transmitter release, action, and inactivation.* Depolarization opens voltage-dependent Ca^{2+} channels in the presynaptic nerve terminal (1). The influx of Ca^{2+} during an action potential (AP) (2) triggers the exocytosis of small synaptic vesicles that store neurotransmitter (NT). Released neurotransmitter interacts with receptors in the postsynaptic membranes that either couple directly with ion channels (3) or act through second messengers, such as GPCRs (4). Neurotransmitter receptors in the presynaptic nerve terminal membrane (5) can inhibit or enhance subsequent exocytosis. Released neurotransmitter is inactivated by reuptake into the nerve terminal by (6) a transport protein coupled to the Na^+ gradient (e.g., for DA, NE, or GABA), which can also be found in the postsynaptic membrane, or by (7) degradation (ACh, peptides), or by (8) transporter-facilitated uptake and metabolism by glial cells (glutamate). The synaptic vesicle membrane is recycled by (9) clathrin-mediated endocytosis. Neuropeptides and proteins are sometimes stored in (10) larger, dense core granules within the nerve terminal. These dense core granules can be released from sites (11) distinct from active zones after repetitive stimulation.

Fast Neurotransmission

Responses to activation of receptors consisting of an ion channel as part of its structure tend to be very rapid (milliseconds) since the effects are direct and generally do not require multiple steps leading to second messenger activation. In fast neurotransmission (also called directly gated transmission), neurotransmitters bind directly to ligand-gated ion channels on the postsynaptic membrane to rapidly open the channel and change the permeability of the postsynaptic site leading to depolarization or hyperpolarization. Depolarization results in continuation of the nerve impulse, whereas hyperpolarization leads to diminished signaling (see Figure 13–5).

Ligand-gated ion channels mediating fast transmission (also called ionotropic receptors) are composed of multiple subunits, each usually having four transmembrane domains (Figure 16–5). Receptors with this structure include nicotinic cholinergic receptors, normally activated by acetylcholine (ACh; one subtype of which is responsible for muscle contraction via the neuromuscular junction); the receptors for the amino acids GABA, glycine, glutamate, and aspartate; and the serotonin $5HT_3$ receptor. The nicotinic ACh receptor provides a good example of receptor structure and how subunit composition varies with anatomic location and affects function (Figure 16–6).

Slow Neurotransmission

Slower transmission (although still relatively fast, often on a time scale of seconds) is mediated by neurotransmitters that do not bind to ion channels but to receptors with a very different architecture that generate second messengers upon activation (also called metabotropic receptors). This major group of receptors are the GPCRs that contain seven transmembrane spanning domains (Figure 16–7). GPCRs typically contain sites for N-linked glycosylation on their extracellular amino tail and sometimes on their second extracellular loop. There are also multiple potential sites for phosphorylation, often by PKA and PKC, on the third intracellular loop and the carboxyl tail. Phosphorylation can regulate GPCR–G protein-effector coupling and provide docking sites for arrestins and scaffolding proteins. Some GPCRs are palmitoylated on the carboxyl tail (see Figure 16–7). There are more

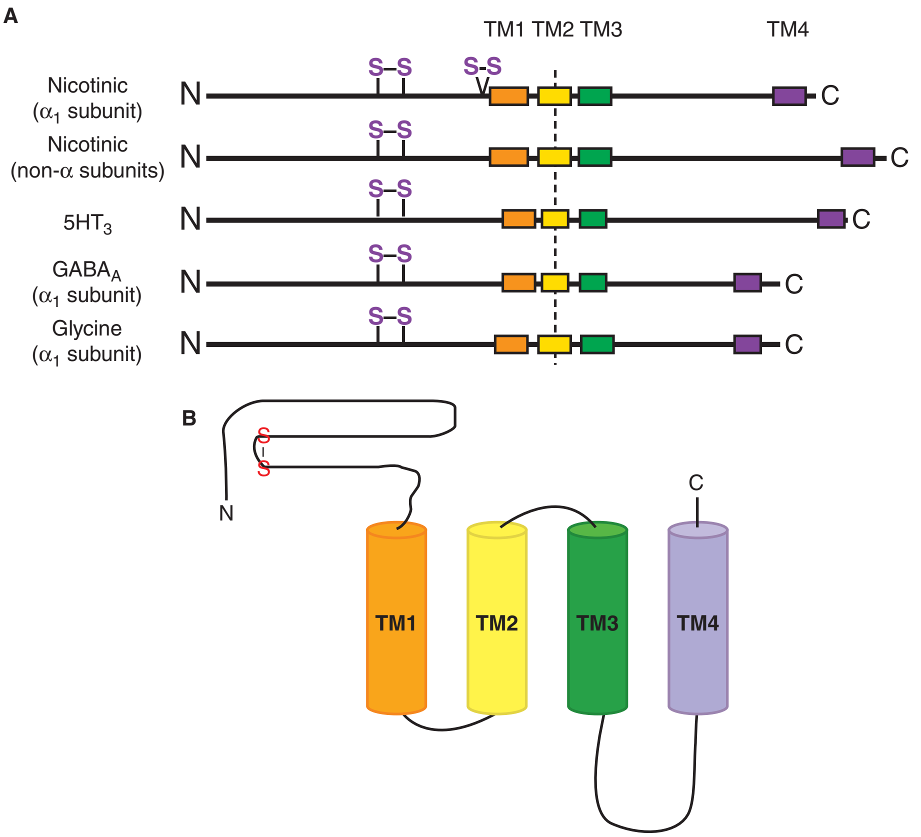

A
TM1 TM2 TM3
TM4
S–S
S–S
Nicotinic (α_1 subunit)
Nicotinic (non-α subunits)
$5HT_3$
$GABA_A$ (α_1 subunit)
Glycine (α_1 subunit)
N
C
B
S
S
N
C
TM1
TM2
TM3
TM4

Figure 16–5 *Pentameric ligand-gated ion channels.* The subunits of these channels, which mediate fast synaptic transmission, are embedded in the plasma membrane to form a roughly cylindrical structure with a central pore. In response to binding of transmitter, the receptor proteins change conformation; the channel gate opens, and ions diffuse along their concentration gradient across the membrane through a hydrophilic opening in the otherwise-hydrophobic membrane. **A.** *Subunit organization.* For each subunit of these pentameric receptors, the amino terminal region of approximately 210 amino acids is extracellular. It is followed by four hydrophobic regions that span the membrane (TM1–TM4); a small carboxyl terminus is on the extracellular surface. The TM2 region is α helical, and TM2 regions from each subunit line the internal pore of the pentameric receptor. Two disulfide loops at positions 128–142 and 192–193 are found in the α subunit of the nicotinic receptor. The 128–142 motif is conserved in the family of pentameric receptors; the vicinal cysteines at 192–193 occur only in α subunit of the nicotinic receptor. **B.** *Schematic rendering of a nicotinic ACh receptor α subunit.* Five such subunits form a pentameric receptor. See Figure 16–6 for an example.

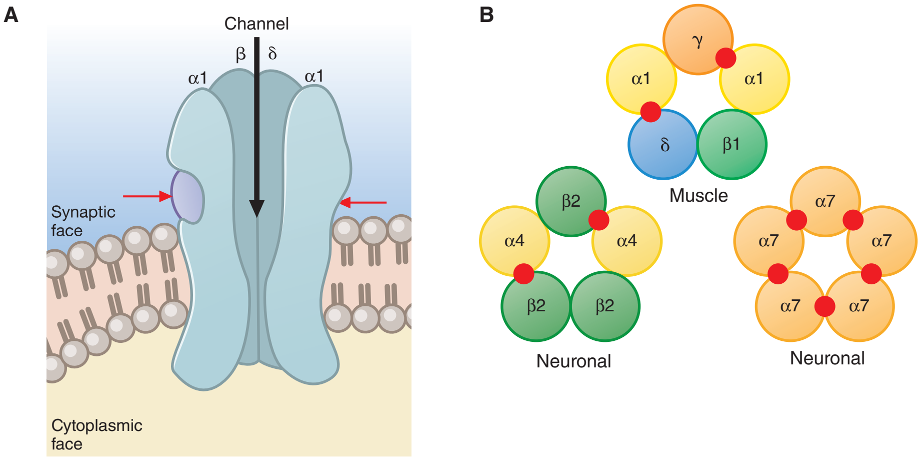


Figure 16–6 *Subunit arrangement: the nicotinic ACh receptor.* **A.** *Longitudinal view of receptor schematic with the γ subunit removed.* The remaining subunits, two copies of α, one of β, and one of δ, are shown to surround an internal channel with an outer vestibule and its constriction located deep in the membrane bilayer region. Spans of α helices with slightly bowed structures form the perimeter of the channel and come from the TM2 region of the linear sequence (see Figure 16–5). ACh-binding sites, indicated by red arrows, occur at the αγ and αδ (not visible) interfaces. **B.** *Nicotinic receptor subunit arrangements.* Agonist-binding sites (red circles) occur at α subunit–containing interfaces. At least 17 functional receptor isoforms have been observed *in vivo*, with different ligand specificity, relative Ca^{2+}/Na^{+} permeability, and physiological function determined by their subunit composition. The only isoform found at the neuromuscular junction is shown for comparison. The neuronal receptor isoforms found at autonomic ganglia and in the CNS are homomeric or heteromeric pentamers of α (α_2–α_{10}) and β (β_2–β_4) subunits.

AL GRAWANY

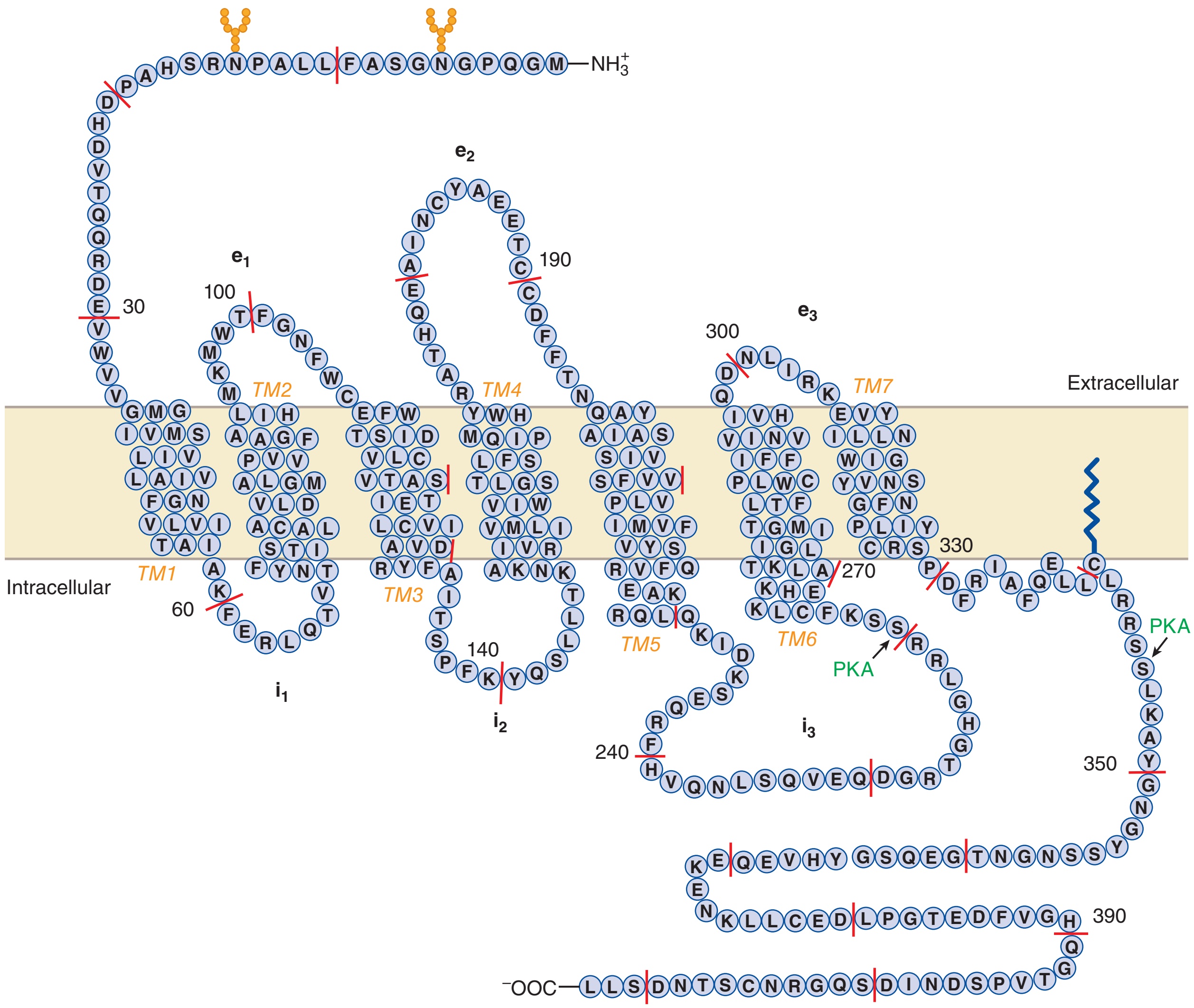


Figure 16–7 *The β adrenergic receptor as a model for GPCRs.* This two-dimensional model illustrates features common to most GPCRs. Red lines mark 10–amino acid regions. The amino terminus (N) is extracellular and the carboxyl terminus (C) is intracellular; in between are seven hydrophobic transmembrane (TM) domains and alternating intracellular and extracellular loops ($e_{1\text{-}3}$ and $i_{1\text{-}3}$). Glycosylation sites are found near the N terminus; consensus sites for phosphorylation by PKA (*arrows*) are found in the i_3 loop and the carboxyl terminal tail. An aspartate residue in TM_3 (Asp^{113}) interacts with the nitrogen of catecholamine agonists while two serines (Ser^{204}, Ser^{207}) in TM_5 interact with the hydroxyl groups on the phenyl ring of catecholamine agonists. A cysteine residue (Cys^{341}) is a substrate for palmitoylation. Interaction of the palmitoyl group with membrane lipids reduces the flexibility of the carboxyl tail. (Figure modified with permission from Rasmussen SGF et al. Crystal structure of the human $β_2$ adrenergic G-protein-coupled receptor. *Nature*, **2007**, *450*:383. Copyright © 2007.)

than 800 human GPCRs, which can be classified into five major families in vertebrates: rhodopsin (class A); secretin (class B); glutamate (class C); adhesion; and frizzled/taste receptor 2 (TAS2) (class F) (Stevens et al., 2013). These families can be further divided into subfamilies based on amino acid sequence similarities (see Figure 3–14).

GPCRs are associated with a broad spectrum of physiological effects including activation of K^+ channels, activation of phospholipase (PL) C–inositol 1,4,5-trisphosphate (IP_3)–Ca^{2+} pathways, and modulation of adenylyl cyclase activity and downstream systems affected by cyclic AMP. These effects are typically mediated through the activation of specific G proteins, each a heterotrimer of α, β, and γ subunits where the β and γ units are constitutively associated. The GTP-binding α subunits can modulate the activities of numerous effectors (e.g., adenylyl cyclase, PLC). The βγ subunits are also active in mediating signaling, especially in the regulation of ion channels. Table 16–1 shows examples of the variety of physiological functions mediated by G proteins. Notably, GPCRs can also signal to downstream pathways through other intermediary proteins, such as the β-arrestins (Shukla et al., 2011; van Gastel et al., 2018). Drugs targeting GPCRs represent a core of modern medicine and comprise over one-third of all pharmaceuticals.

Central Neurotransmitters

CNS transmitters are classified by chemical structure into various categories including amino acids, acetylcholine, monoamines, neuropeptides, purines, and gases. This section describes each category and examines some prominent members and their receptors.

Amino Acids

The CNS contains high concentrations of certain amino acids that potently alter neuronal firing. They are ubiquitously distributed within the brain and produce rapid and readily reversible effects on neurons. The dicarboxylic amino acids, glutamate and aspartate, produce excitation, while the monocarboxylic amino acids, γ-aminobutyric acid (GABA), glycine, β-alanine, and taurine, cause inhibition. The effects of glutamate and GABA are especially notable. Following the emergence of selective agonists and antagonists, the identification of pharmacologically distinct amino acid receptor subtypes became possible (see below). Figure 16–8 shows these amino acid transmitters and their drug congeners.

TABLE 16–1 ■ G PROTEIN-MEDIATED SIGNALING

FAMILY	α SUBUNITS	SIGNALS TRANSDUCED
Family Members		
G_s family		
G_s	α_s	Activation of AC
G_{olf}	α_{olf}	Activation of AC
G_i family		
$G_{i/}G_o$	α_i, α_o	Inhibition of AC
G_z	α_z	Inhibition of AC
G_{gust} (gustducin)	α_{gust}	Activation of PDE6
G_t (transducin)	α_t	Activation of PDE6
G_q family		
G_q	α_q, α_{11}, α_{14}, α_{15}, α_{16}	Activation of PLC
$G_{12/13}$ family		
$G_{12/13}$	α_{12}, α_{13}	Activation of Rho GTPases
βγ Subunits (acting as a heterodimer)		
G_β	β1, β2, β3, β4, β5	↓ AC, ↑ Ca^{2+} and K^+ channels, ↑ PI3K, ↑ PLC_β, ↑AC2 and AC4, ↑ Ras-dependent MAPK activation, ↑ recruitment of GRK2 and GRK3
G_γ	γ1, γ2, γ3, γ4, γ5, γ7, γ8, γ9, γ10, γ11, γ12, γ13	

Glutamate and Aspartate

Glutamate and aspartate are found in high concentrations in brain, and both amino acids have powerful excitatory effects on neurons in virtually every region of the CNS. Glutamate is the most abundant excitatory neurotransmitter and serves as the principal fast excitatory neurotransmitter. Glutamate acts through receptors that are classed as either *ligand-gated ion channels (ionotropic)* or *metabotropic GPCRs* (Table 16–2). A well-characterized phenomenon involving glutamate transmission is the induction of long-term potentiation and its converse, long-term depression. These phenomena are known for strengthening and weakening synapses and have long been hypothesized to be important mechanisms in learning and memory and other processes that depend on adaptive changes within the brain.

Ionotropic glutamate receptors are ligand-gated ion channels that were historically divided into three classes, including *N*-methyl-ᴅ-aspartate (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, and kainic acid (KA) receptors, which are named for their preferred synthetic ligands. With the discovery of an increasing number of subunits comprising these receptor categories, the classification has recently been refined (see Table 16–2).

NMDA receptors consist of heteromers that are made up of multiple subunit combinations (termed GluN*x*) with the minimal receptor being a dimer of the GluN1 subunit and at least one GluN2 subunit; more complex heteromeric complexes are generated by incorporating multiple subunits. The NMDA receptors have relatively high permeability to Ca^{2+} and are blocked by Mg^{2+} in a voltage-dependent manner. NMDA receptors are unique in that their activation requires the simultaneous binding of two different agonists; in addition to glutamate, glycine binding is necessary for activation (Figure 16–9). While NMDA receptors are involved in normal synaptic transmission, their activation is more closely associated with the induction of various forms of synaptic plasticity rather than fast point-to-point signaling in the brain.

Aspartate is also a selective NMDA receptor agonist. Other NMDA receptor ligands include open-channel blockers such as *phencyclidine* (PCP or "angel dust") and *ketamine*; antagonists include 5,7-dichlorokynurenic acid, which acts at an allosteric glycine-binding site, and *ifenprodil*, which selectively inhibits NMDA receptors containing GluN2B subunits. *Ketamine*, which is a racemic mixture of *R* and *S* enantiomers, has attracted interest for the treatment of drug-resistant depression. In fact, the *S* enantiomer of *ketamine* (*esketamine*) has recently been introduced to the marketplace, although controversy exists as to whether *esketamine* is clinically more efficacious than the *R* isomer of *ketamine* or the racemic mixture itself. The clinical effects of *ketamine* are thought to be due in part to its NMDA receptor antagonism; see Figure 18–2 for a schematic of possible effects of *ketamine* at a glutamatergic synapse.

The activity of NMDA receptors is sensitive to pH and to modulation by a variety of endogenous agents including Zn^{2+}, some neurosteroids, arachidonic acid, redox reagents, and polyamines such as spermine (see Figure 16–9).

AMPA receptors exist predominantly as heterotetramers and contain multiple subunits (termed GluA*x*) (see Table 16–2). In addition, there are transmembrane AMPA receptor regulatory proteins (TARPs), which, together with a variety of scaffolding and regulatory proteins, modulate channel properties and alter the trafficking of receptors to and from perisynaptic and postsynaptic regions. AMPA receptors open and close rapidly, making them well-suited to mediate the majority of excitatory synaptic transmission in the brain and, like NMDA receptors, they are involved in synaptic plasticity. AMPA receptors can be selectively antagonized by NBQX (2,3-dioxo-6-nitro-7-sulfamoyl-benzo[f]quinoxaline); and CNQX (6-cyano-7-nitroquinoxaline-2,3-dione), and similar antagonists are being explored as neuroprotective drugs for the treatment of stroke.

KA receptors are composed of a distinct array of subunits (termed GluK*x*) that assemble as homo- or heterotetramers to form functional receptors. An important difference between KA and AMPA receptors is that KA receptors require extracellular Na^+ and Cl^- for activation. KA receptors differ functionally from AMPA and NMDA receptors in other important ways. KA receptors do not reside predominantly within postsynaptic signaling complexes and are positioned to modulate neuronal excitability and synaptic transmission by altering the likelihood that the postsynaptic cell will fire in response to subsequent stimulation. Presynaptic KA receptors have also been implicated in modulating GABA release through presynaptic mechanisms.

Glutamate-mediated excitotoxicity may underlie the damage that occurs when ischemia or hypoglycemia in the brain leads to a massive release and impaired reuptake of glutamate resulting in excess stimulation of glutamate receptors and subsequent cell death. The cascade of events leading to neuronal death seems to be triggered by excessive activation of NMDA or AMPA/KA receptors, allowing an excessive influx and mobilization of Ca^{2+} in neurons. A general picture of glutamate-mediated excitotoxicity is emerging. In overview, the influx of Ca^{2+} is thought to promote oxidation within the cell (↑ nitric oxide/peroxynitrite, ↓ glutathione, ↑ reactive oxygen species), resulting in lipid peroxidation, destabilization of lysosomes, and damage to mitochondria and ultimately leading to cell death via apoptosis and by cathepsin- and calpain-dependent pathways (Kritis et al., 2015). NMDA receptor antagonists can attenuate neuronal cell death induced by activation of these receptors. Glutamate receptors have become targets for diverse therapeutic interventions. For example, a role for disordered glutamatergic transmission in the etiology of chronic neurodegenerative diseases has been postulated (see Chapter 21).

Metabotropic glutamate receptors (mGluRs) are GPCRs structurally defined by the presence of a large N-terminal domain that is linked via a cysteine-rich region to a seven-transmembrane spanning domain that is typical of other GPCRs (Nasrallah et al., 2021). Like all class C GPCRs, these receptors function as obligate dimers. Orthosteric agonists, including glutamate, and antagonists bind to the N-terminal domain, whereas allosteric modulators typically interact within the seven-transmembrane spanning domain. G proteins and β-arrestins engage the cytoplasmic surface of the activated mGluR (Figure 16–10). There are eight unique mGluRs organized into three subgroups (see Table 16-2). mGluRs bind glutamate and function to fine-tune excitatory and inhibitory transmission by presynaptic, postsynaptic, and glial mechanisms, including the

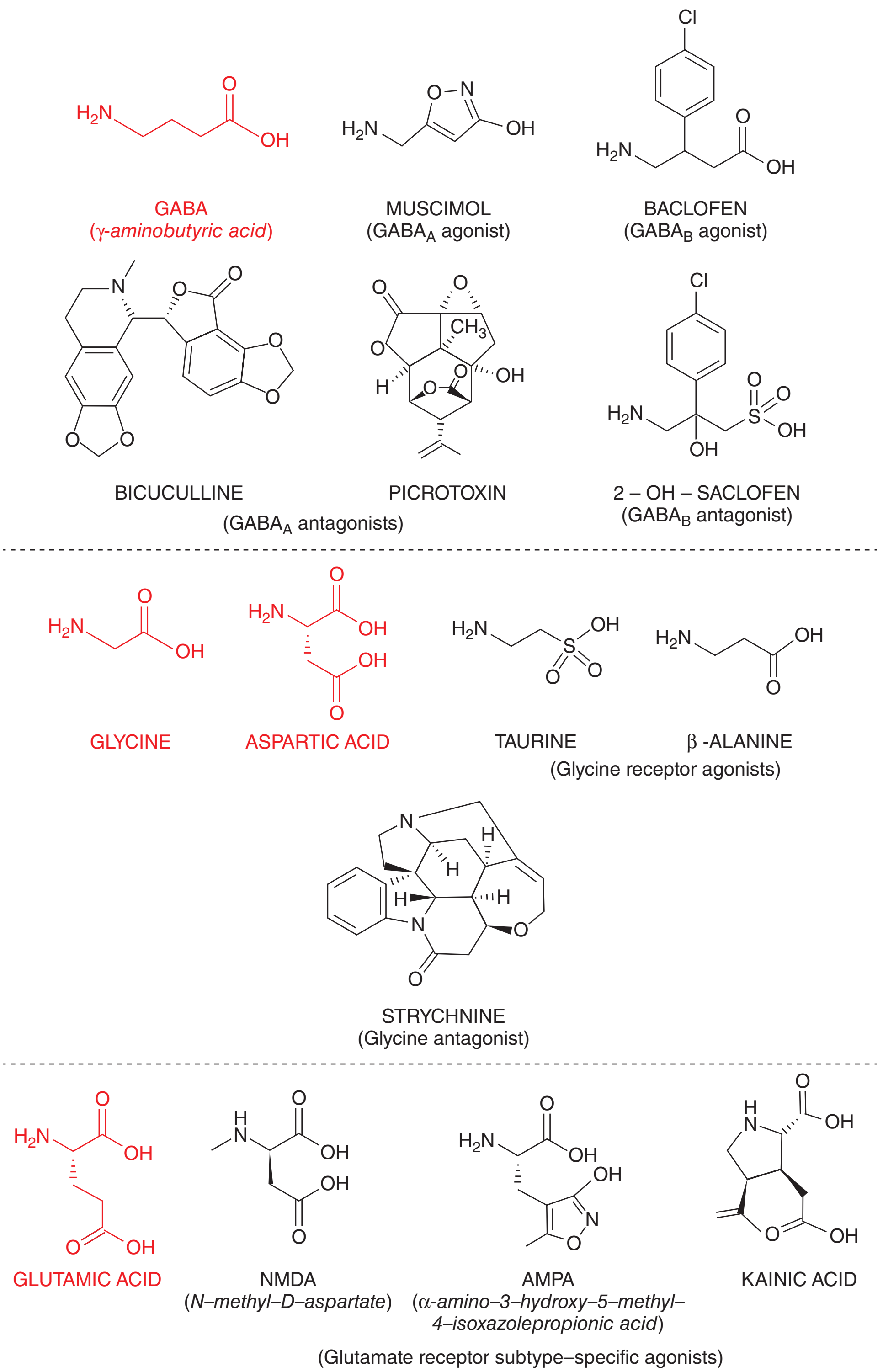

GABA
(γ-aminobutyric acid)
MUSCIMOL
(GABA$_A$ agonist)
BACLOFEN
(GABA$_B$ agonist)
BICUCULLINE
PICROTOXIN
(GABA$_A$ antagonists)
2 – OH – SACLOFEN
(GABA$_B$ antagonist)
GLYCINE
ASPARTIC ACID
TAURINE
β -ALANINE
(Glycine receptor agonists)
STRYCHNINE
(Glycine antagonist)
GLUTAMIC ACID
NMDA
(N–methyl–D–aspartate)
AMPA
(α-amino–3–hydroxy–5–methyl–4–isoxazolepropionic acid)
KAINIC ACID
(Glutamate receptor subtype–specific agonists)

Figure 16–8 *Amino acid transmitters (red) and congeners (black).*

modulation of release and signaling of other neurotransmitters including GABA, purines, dopamine, serotonin (5-hyroxytryptamine [5HT]), and neuropeptides. Group I mGluRs couple to G_q activation, while groups II and III are known to couple to G_i/G_o. mGluRs are located in a variety of brain regions and sometimes linked to opposing functional responses. In general, group I receptors increase neuronal excitability, whereas both group II and group III suppress excitability. They are known to play a role in the modulation of other receptors and to function in synaptic plasticity.

In the past few years, researchers have made significant progress in determining the mechanisms of mGluR activation and the proteins mGluRs interact with and in developing both orthosteric and allosteric ligands to modulate receptor activity. Widespread expression of mGluRs, their capacity to modulate presynaptic neurotransmission in a dynamic and activity-dependent manner, and the availability of subtype-selective and pharmacologically novel *negative* and *positive allosteric modulators* (NAMs and PAMs) make these receptors attractive targets for the treatment of cognitive impairment related to several psychiatric and neurodegenerative diseases. Studies now suggest that ligands for specific mGluR subtypes have potential for the treatment of multiple CNS disorders, including anxiety disorders, schizophrenia, Alzheimer's disease, and Parkinson's disease. For example, studies have shown high involvement of mGluR$_5$ in anxiety disorders as this receptor increases neuronal

TABLE 16–2 ■ CLASSIFICATION OF GLUTAMATE RECEPTORS

FAMILY	SUBTYPE	AGONISTS	ANTAGONISTS	
Ionotropic				
NMDA	GluN1, GluN2A, GluN2B, GluN2C, GluN2D, GluN3A, GluN3B	NMDA, aspartate	D-AP5, 2R-CPPene, MK-801, ketamine, phenycylidine, D-aspartate	
AMPA	GluA1, GluA2, GluA3, GluA4	AMPA, kainate, (s)-5-fluorowillardiine	CNQX, NBQX, GYK153655	
Kainate	GluK1, GluK2, GluK3, GluK4, GluK5	Kainate, ATPA, LY-339,434, SYM-2081, 5-iodowillardiine	CNQX, LY294486	
Metabotropic				
				SIGNALING
Group I	mGlu$_1$, mGlu$_5$	3,5-DHPG, quisqualate	AIDA S-(+)-CBPG	Activation of PLC (G$_q$)
Group II	mGlu$_2$, mGlu$_3$	APDC, MGS0028 DCG-IV, LY354740	EGLU PCCG-4	Inhibition of AC (G$_i$/G$_o$)
Group III	mGlu$_4$, mGlu$_6$, mGlu$_7$, mGlu$_8$	L-AP4, (RS)-PPG	CPPG, MPPG, MSOP, LY341495	Inhibition of AC (G$_i$/G$_o$)

AIDA, 1-aminoindan-1,5-dicarboxylic acid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; L-AP4, L-2-amino-4-phosphonobutiric acid; ATPA, 2-amino-3(3-hydroxy-5-tert-butylisoxa-zol-4-yl)propanoic acid; CBPG, (S)-(+)-2-(3-carboxybicyclo(1.1.1)pentyl)-glycine; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; D-AP5, D-2-amino-5-phosphonovaleric acid; DCG-IV, (2S,2′R,3′R)-2-(2′,3′-Dicarboxycyclopropyl)glycine; (S)-3,4-DCPG, (S)-3,4-dicarboxyphenylglycine; 3,5-DHPG, 3,5-dihydroxyphenylglycine; EGLU, (2S)-α-ethylglutamic acid; MPPG, (RS)-α-methyl-4-phosphonophenylglycine; MSOP, (RS)-α-methylserine-O-phosphate; NBQX, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide; NMDA, *N*-methyl-D-aspartate; PCCG-4, phenylcarboxycyclopropylglycine; (RS)-PPG, (RS)-4-phosphonophenylglycine.
Glutamate is the principal agonist at both ionotropic and metabotropic receptors for glutamate and aspartate.

excitability and NMDA receptor currents, leading to the hypothesis that an antagonist of mGluR$_5$ might dampen the hyperactivity. Consistent with this, mGluR$_5$ NAMs have robust efficacy in several animal models of anxiolytic activity, and the clinically validated anxiolytic agent *fenobam* acts as a selective mGluR$_5$ NAM. In addition, group III mGluRs (i.e., mGluR$_4$, mGluR$_7$, and mGluR$_8$) are expressed in GABAergic and glutamatergic terminals, and agonists and PAMs for these receptors can inhibit the release of both glutamate and GABA in Parkinson's disease, thereby opposing glutamate hyperactivity in this disorder. mGluR$_4$ PAMs reduce motor symptoms in animal models of Parkinson's disease and may play a role in neuroprotection and enrichment of motor responses to low doses of L-DOPA. Similarly, inhibition of presynaptic glutamate release has been implicated as a target for other excitation-toxicity–associated disorders such as Huntington's disease. There is also growing evidence that group II mGluRs may play a role in the cognitive decline seen with schizophrenia. Together these findings highlight the diverse roles that this receptor family can play in the modulation of CNS signaling while providing an opportunity for the development of novel therapeutics for the treatment of neurodegenerative and neuropsychiatric conditions (Crupi et al., 2019).

Gamma-Aminobutyric Acid

GABA (see Figure 16–8) is the main inhibitory neurotransmitter in the CNS. GABA is synthesized in the brain beginning with the Krebs cycle at the point where α-ketoglutarate is transaminated to glutamate by GABA transaminase (GABA-T). GABA is subsequently formed from glutamate by glutamic acid decarboxylase; the presence of glutamic acid decarboxylase in a neuron therefore delineates a neuron that uses GABA as a transmitter. Interestingly, intraneuronal GABA is also inactivated by GABA-T to succinic semialdehyde, but only in the presence of α-ketoglutarate. This GABA shunt serves to maintain levels of GABA, making GABA-T both a synthesis and degradative enzyme (Brady et al., 2012). There is a vesicular GABA transporter (VGAT) that is involved in vesicular storage of GABA for release into the synaptic cleft. The action of GABA is primarily terminated by reuptake by one of four different GABA transporters (GATs) present on both neurons and glia. GABA acts by binding to and activating specific ionotropic or metabotropic receptors on both pre- and postsynaptic membranes. *GABA$_A$ receptors,* the most prominent GABA receptor subtype, are ionotropic ligand-gated Cl$^-$ channels. The *GABA$_B$ receptors* are metabotropic GPCRs. One subtype formerly known

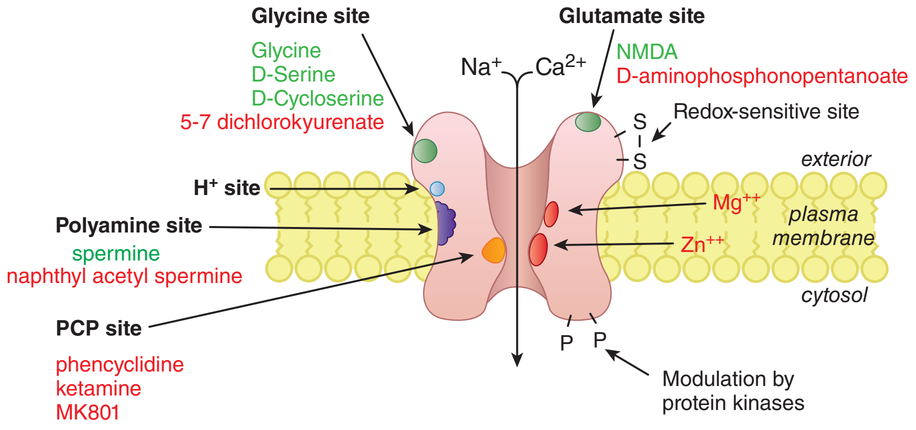


Figure 16–9 *Pharmacological binding sites on the NMDA receptor.* Agents that promote receptor function are shown in green. Those that inhibit receptor function appear in red. Binding of both glutamate and glycine is necessary for activation.

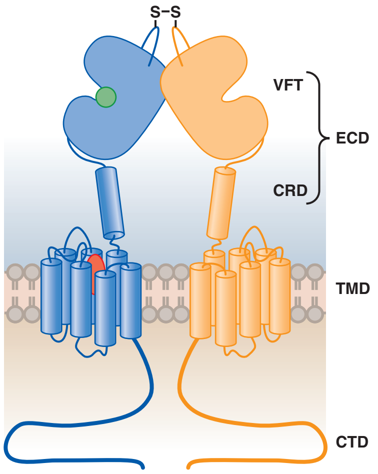


Figure 16–10 *General structure of metabotropic glutamate receptors.* Functional metabotropic glutamate receptors (mGluRs) are GPCRs that exist as obligate dimers, both homodimers and heterodimers. Each monomer possesses a large extracellular N-terminal domain (ECD) comprising the Venus flytrap domain (VFT) that contains the orthosteric binding site for glutamate (green dot in the cleft of the VFT) and a cysteine-rich domain (CRD) that links the VFT to the seven-transmembrane domain (TMD). Disulfide bonds between regions of adjacent VFTs facilitate dimerization. The TMD contains binding sites for allosteric modulators (red dot). The TMD and the C-terminal domain (CTD) together regulate G protein coupling. The long CTD (40–380 amino acids) may also facilitate regulatory interactions with protein kinases and scaffolding proteins. (Reproduced with permission from McCullock TW, Kammermeier PJ. The evidence for and consequences of metabotropic glutamate receptor heterodimerization. *Neuropharmacology,* **2021**, *199*:108801. Copyright © Elsevier Ltd.)

as the *GABA$_C$ receptor* is now classified as a type of GABA$_A$ receptor based on structural and functional criteria.

GABA$_A$ receptors have been extensively characterized as important drug targets and are the site of action of many neuroactive drugs, notably benzodiazepines (such as *diazepam*), barbiturates, ethanol, anesthetic steroids, and volatile anesthetics among others (Figure 16–11). These drugs are used to treat various neuropsychiatric disorders including epilepsy, Huntington's disease, addictions, sleep disorders, and more. The GABA$_A$ receptors are pentamers of subunits that each contain four transmembrane domains and assemble around a central ion-permeable pore (Figures 16–3 and 16–5), which is selective for Cl$^-$ in the case of the GABA$_A$ receptor. The major forms of the GABA$_A$ receptor contain at least three different types of subunits: α, β, and γ, with a likely stoichiometry of 2α, 2β, 1γ. The IUPHAR/British Pharmacological Society recognizes 19 unique subunits that are known to form 11 native GABA$_A$ receptors that can be pharmacologically differentiated. Of interest is that the particular combination of the α and γ subunits can affect the efficacy of benzodiazepine binding and channel modulation. Many drugs, such as benzodiazepines and volatile anesthetics, act as positive allosteric modulators of the GABA$_A$ receptor (i.e., act at a site distinct from the GABA binding site to positively modulate the function of the receptor). The high-resolution cryo-electron microscopy structures of the α1β3γ2L GABA$_A$ receptor bound to the agonist GABA and the classical benzodiazepines, *alprazolam* and *diazepam*, have been solved and utilized to further deduce signaling mechanisms of this group of receptors (Masiulis et al., 2019). *Brexanolone* is a neuroactive steroid that is a PAM at GABA$_A$ receptors and is approved to treat postpartum depression. The interaction of various drugs with the GABA$_A$ receptor and their therapeutic uses are presented in Chapter 18.

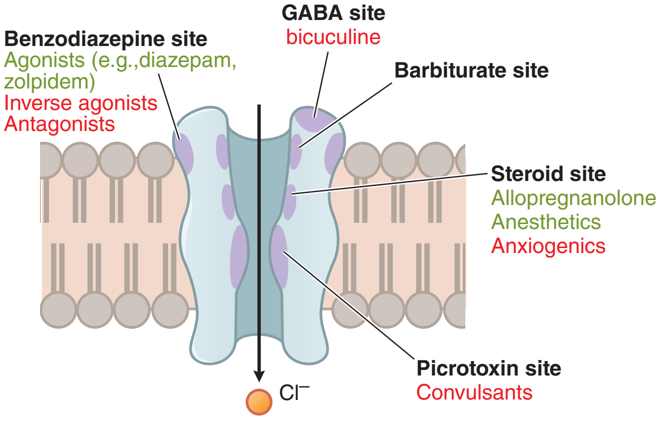


Figure 16–11 *Pharmacologic binding sites on the GABA$_A$ receptor.* GABA binds at the orthosteric site on the GABA$_A$ receptor. Other sites noted are modulatory in nature, at which allosteric agonists or antagonists may promote (green) or inhibit (red) receptor function. (Reproduced with permission from Nestler EJ, Hyman SE, Holtzman DM, Malenka RC, eds. *Molecular Neuropharmacology: A Foundation for Clinical Neuroscience,* 3rd ed. McGraw Hill, New York, **2015**.)

GABA$_B$ receptors are metabotropic GPCRs that function as obligate heterodimers of two subunits named GABA$_{B1}$ and GABA$_{B2}$. GABA$_B$ receptors are widespread in the CNS and regulate both pre- and postsynaptic activity. These receptors interact with G$_i$ to inhibit adenylyl cyclase, activate K$^+$ channels, and reduce Ca^{2+} conductance, and with G$_q$ to enhance PLC activity. Presynaptic GABA$_B$ receptors function as autoreceptors, inhibiting GABA release, and may play the same role on neurons releasing other transmitters. A number of GABA$_B$ agonists have been identified, including *baclofen* (see Figure 16–8), a skeletal muscle relaxant, and the psychoactive drug *gamma-hydroxybutyric acid* (GHB), which is sometimes used to treat narcolepsy and also used recreationally as an intoxicant.

Glycine

Glycine (see Figure 16–8) is an amino acid normally incorporated into proteins that can also act as an inhibitory neurotransmitter, particularly in the spinal cord and brainstem. Glycine is synthesized primarily from serine by the enzyme serine hydroxymethyltransferase. Glycine enters synaptic vesicles via VGAT, the same vesicular transport system used by GABA. The action of glycine in the synaptic cleft is terminated by reuptake through specific transporters (GLYT1 and GLYT2) located on presynaptic nerve terminals and glial cells. These transporters can be pharmacologically differentiated and present an attractive therapeutic target for the modulation of glycine levels; this is an active area of research, especially considering that there are glycine binding sites on NMDA receptors. Glycine acts as a co-agonist at NMDA receptors such that both glutamate and glycine must be present for activation to occur (see above). In addition to the NMDA receptor site, there are specific ionotropic glycine receptors that contain many of the structural features described for other ligand-gated ion channels (pentamers of subunits containing four transmembrane domains). These function as hyperpolarizing Cl$^-$ channels and are prominent in the brainstem and spinal cord. There are four known α subunits and a single β subunit that assemble into a variety of glycine receptor subtypes. Taurine and β-alanine are agonists of glycine receptors; strychnine, a potent neurotoxin, is a selective antagonist (see Figure 16–8).

Acetylcholine

Acetylcholine (ACh), the first neurotransmitter discovered, occurs throughout the nervous system and functions as a neurotransmitter. It plays a primary role in the autonomic nervous system in ganglionic transmission and in innervation of autonomic effector cells by parasympathetic postganglionic fibers; ACh is also the neurotransmitter of the somatic motor nerves that innervate skeletal muscle in vertebrates (see Figure 10–2). In the CNS, ACh is found primarily in interneurons. ACh is synthesized by choline acetyltransferase and stored in vesicles in the nerve endings (see Figure 10–6). Following release and receptor activation, it is degraded by acetylcholinesterase (see Chapter 12). The effects of ACh result from interaction with two broad classes of receptors: ionotropic ligand-gated ion channels termed nicotinic receptors and metabotropic

TABLE 16–3 ■ SUBTYPES OF MUSCARINIC RECEPTORS IN THE CNS

SUBTYPE	TRANSDUCER EFFECTOR	AGONISTS (EXAMPLES)	ANTAGONISTS (EXAMPLES)
M_1	G_q Activation of PLC	Acetylcholine, carbachol, oxotremorine, pilocarpine, McN-A-343	Pirenzepine, telenzepine, 4-DAMP, xanomeline
M_2	$G_{i/}G_o$ Inhibition of AC	Acetylcholine, carbachol, oxotremorine	AF-DX 116, AF-DX 384, AQ-RA 741, tolterodine, (S)-(+)-dimethindene maleate, methoctramine
M_3	G_q Activation of PLC	Acetylcholine, carbachol, oxotremorine, pilocarpine, cevimeline	Darifenacin, 4-DAMP, DAU 5884, J-104129, tropicamide, tolterodine
M_4	$G_{i/}G_o$ Inhibition of AC	Acetylcholine, carbachol oxotremorine	AF-DX384, 4-DAMP, PD 102807, xanomeline
M_5	G_q Activation of PLC	Acetylcholine, carbachol, oxotremorine, pilocarpine	4-DAMP, xanomeline, VU-0488130 (ML381)

Acetylcholine is the endogenous transmitter for all muscarinic receptors. Nonselective antagonists include atropine, scopolamine, and ipratropium. 4-DAMP, 1,1-dimethyl-4-diphenylacetoxypiperidinium iodide.

GPCRs called muscarinic receptors. In the CNS, the degeneration of cholinergic pathways is a hallmark of Alzheimer's disease.

Nicotinic ACh receptors are found in skeletal muscle (see Figure 16–6) as well as in autonomic ganglia, the adrenal gland, and the CNS. Their activation by ACh results in a rapid increase in the influx of Na^+, depolarization, and the influx of Ca^{2+}. Nicotinic receptors are pentamers consisting of various combinations of five of the 17 known subunits [$\alpha_{(1-10)}$ and $\beta_{(1-4)}$, γ, δ, ε] that can form the ion channel. In the CNS, nicotinic receptors are assembled as combinations of $\alpha_{(2-10)}$ and $\beta_{(2-4)}$ subunits. Pairwise combinations of α and β (e.g., $\alpha_3\beta_4$ and $\alpha_4\beta_2$) and, in at least one case, a homomeric α_7 are sufficient to form a functional receptor *in vitro*; however, more complex isoforms have been identified *in vivo* (see Figure 13–1). The subunit composition strongly influences the biophysical and pharmacological properties of the receptor. Comprehensive listings of nicotinic receptor subunit combinations and brain locations can be found in Zoli et al. (2015). These receptors have high therapeutic value, not only in the treatment of smoking cessation (as they are the primary receptors for nicotine; see Chapter 13), but also for other neurological pathologies (Papke and Horenstein, 2021).

Muscarinic ACh receptors are GPCRs consisting of five subtypes, all of which are expressed in the brain. M_1, M_3, and M_5 couple to G_q, while the M_2 and M_4 receptors couple to G_i/G_o (Table 16–3). Chapter 11 covers the physiology and pharmacology of muscarinic receptors and their agonist and antagonist ligands in detail.

Monoamines

Monoamines are neurotransmitters whose structure contains an amino group connected to an aromatic ring by a two-carbon chain. All are derived from aromatic amino acids and regulate neurotransmission that underlies cognitive processes, including emotion. Drugs that affect monoamine receptors and signaling are used to treat a variety of conditions such as depression, schizophrenia, and anxiety, as well as movement disorders like Parkinson's disease. Monoamines include dopamine (DA), norepinephrine (NE), epinephrine (EPI), histamine (H), serotonin (5HT), and the trace amines. Each system is anatomically distinct and serves separate functional roles within its field of innervation.

Dopamine

DA along with NE and EPI are catecholamine neurotransmitters (see Chapters 14 and 15). In contrast to its presence in the periphery, DA is the predominant catecholamine in the CNS. Its synthesis and degradation are discussed in Chapter 15.

There are three major DA-containing pathways in the CNS: the nigrostriatal, the mesocortical/mesolimbic, and the tuberoinfundibular (see Figure 15–3). These pathways mediate DA signaling and play a role in motivation and reward (most drugs of abuse increase DA signaling), motor control, and the release of various hormones. These effects are mediated by five distinct GPCRs grouped into two subfamilies: D1-like receptors (D_1 and D_5) that stimulate adenylyl cyclase activity via coupling to G_s or G_{olf} and D2-like receptors (D_2, D_3, and D_4) that couple to G_i/G_o to inhibit adenylyl cyclase activity and modulate various voltage-gated ion channels (see Figure 15–10). Dopamine receptor subtypes are discussed extensively in Chapter 15. DA-containing pathways and receptors have been implicated in the pathophysiology of schizophrenia and Parkinson's disease and in the side effects following the pharmacotherapy of these disorders (see Chapters 19 and 21).

Norepinephrine

Both α and β adrenergic receptor subtypes are present in the CNS; all are GPCRs (Table 16–4; see also Chapter 14). β Adrenergic receptors couple to G_s to activate adenylyl cyclase. α_1 Adrenergic receptors are coupled

TABLE 16–4 ■ ADRENERGIC RECEPTORS IN THE CNS

FAMILY	SUBTYPES	TRANSDUCER	AGONIST	ANTAGONIST
α_1 Adrenergic	α_{1A} α_{1B} α_{1D}	$G_{q/11}$	Epinephrine, phenylephrine, oxymetazoline, dabuzalgron (α_{1A}), A61603 (α_{1B})	Prazosin, doxazosin, terazosin, tamsulosin, alfuzosin, S(+)-niguldipine (α_{1A}), L-765314 (α_{1B}), BMY-7378 (α_{1D})
α_2 Adrenergic	α_{2A} α_{2B} α_{2C}	G_i/G_o	Epinephrine, norepinephrine, dexmedetomidine, clonidine, guanfacine	Yohimbine, rauwolscine
β Adrenergic	β_1 β_2 β_3	G_s	Epinephrine, norepinephrine, prenalterol (β_1), fenoterol (β_2), salbutamol (β_2), mirabegron (β_3), BRL37344 (β_3)	Carvedilol, bupranolol, levobunolol, metoprolol, propranolol, betaxolol (β_1), ICI118554 (β_2), SR 59230A (β_3)

to G_q, resulting in stimulation of the PLC-IP_3/diacylglycerol-Ca^{2+}-PKC pathway, and are associated predominantly with neurons. α_1 Adrenergic receptors on noradrenergic target neurons respond to NE with *depolarizing responses* because of decreases in K^+ conductance. α_2 Adrenergic receptors are found on glial and vascular elements, as well as on neurons. They are prominent on noradrenergic neurons, where they couple to G_i, inhibit adenylyl cyclase, and mediate a *hyperpolarizing response* due to enhancement of an inwardly rectifying K^+ channel (via βγ heterodimer). α_2 Adrenergic receptors are also located presynaptically where they function as inhibitory autoreceptors to diminish the release of NE. The antihypertensive effects of *clonidine* may result from stimulation of such autoreceptors.

There are relatively large amounts of NE within the hypothalamus and in certain parts of the limbic system, such as the central nucleus of the amygdala and the dentate gyrus of the hippocampus. NE also is present in significant amounts in most brain regions. Mapping studies indicate that noradrenergic neurons of the locus coeruleus innervate specific target cells broadly throughout cortical, subcortical, and spinomedullary fields.

Epinephrine

Most EPI in the brain is contained in vascular elements. Neurons in the CNS that contain EPI were recognized only after the development of sensitive enzymatic assays and immunocytochemical staining techniques for phenylethanolamine-*N*-methyltransferase, the enzyme that converts NE into EPI. EPI-containing neurons are found in the medullary reticular formation and make restricted connections to pontine and diencephalic nuclei, eventually coursing as far rostrally as the paraventricular nucleus of the thalamus. Their physiological properties have not been unambiguously identified.

Histamine

In addition to its well-known physiological functions in immune and digestive responses, histamine is a monoamine neurotransmitter in the CNS. Histaminergic neurons are located in the ventral posterior hypothalamus; they give rise to long ascending and descending tracts similar to other monoaminergic systems. The histaminergic system is thought to affect arousal, body temperature, and vascular dynamics. The biosynthesis of histamine is described in Chapter 43. Vesicular monoamine transporter (VMAT) 2 facilitates vesicular histamine storage. There does not appear to be a specific transport process for the reuptake of histamine after its release. Rather, histamine reuptake can occur via the facilitative organic cation transporters OCT2 (SLC22A2) and OCT3 (SLC22A3) and, to a lesser degree, by the plasma membrane monoamine transporter (PMAT) (SLC24A4). Histamine is metabolized by histamine-*N*-methyltransferase and, in the periphery, also by diamine oxidase. Histamine signals through four GPCR subtypes (H_1–H_4) that regulate either adenylyl cyclase or PLC (Figure 16–12).

H_1 receptors are widely distributed in the brain where high densities are found in regions linked to neuroendocrine, behavioral, and nutritional state control. H_1 receptor activation excites neurons in most brain regions, and genetic knockout of the H_1 receptor results in behavioral state abnormalities, consistent with a major role for H_1 receptors in cortical control of the sleep-wake cycle. This is evident in the well-known sedative actions of H_1 receptor blockers that are used as antihistamines in the treatment of allergies. The development of H_1 antagonists with low CNS penetration has reduced the incidence of sedation in the treatment of allergy-related disorders (see Chapter 43), although in some

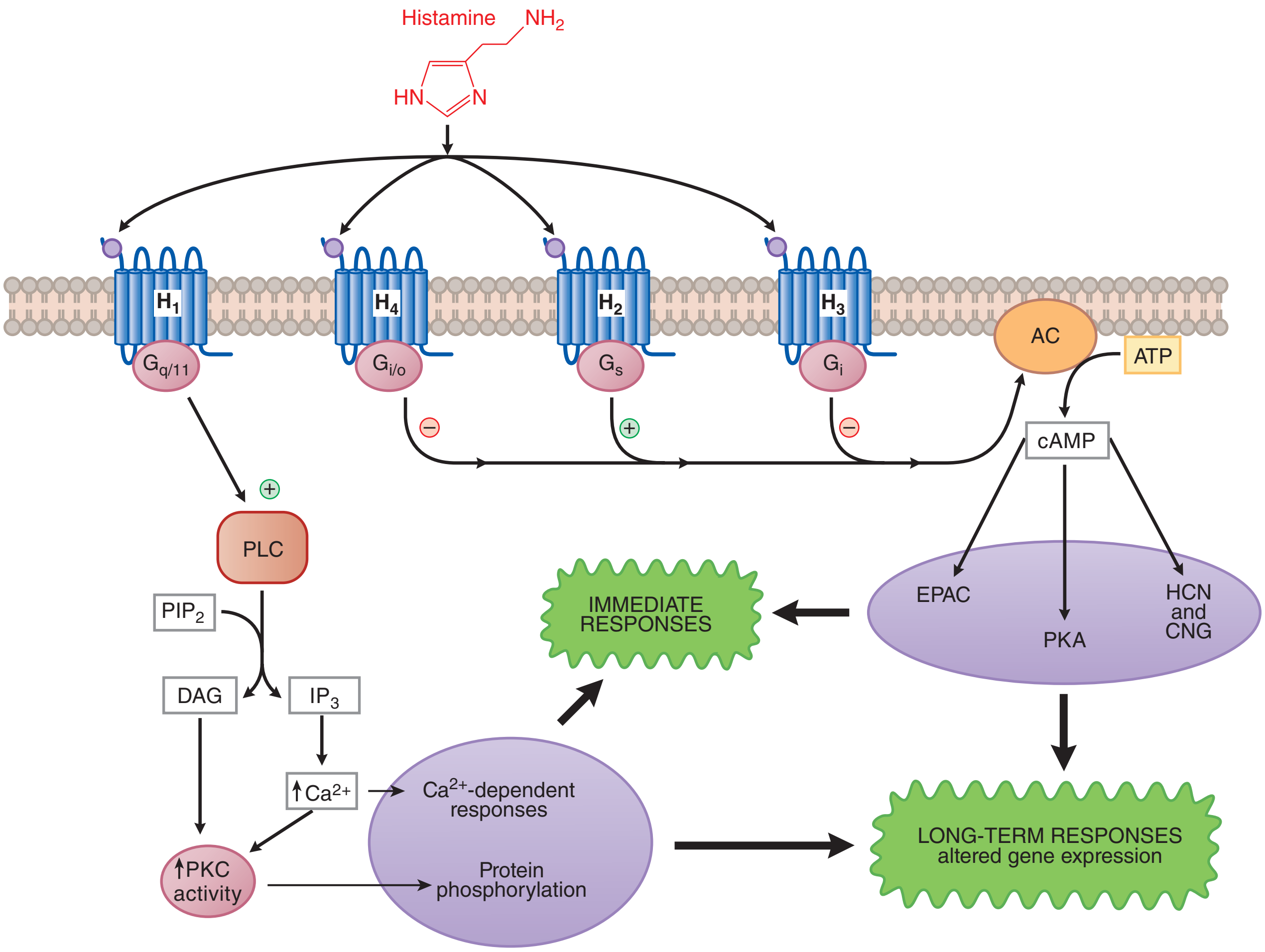


Figure 16–12 *Main signal transduction pathways for histamine receptors.* Histamine can couple to a variety of G protein-linked signal transduction pathways via four different receptors. The H_1 receptors activate phosphatidylinositol turnover via $G_{q/11}$. The other receptors couple either positively (H_2 receptor) or negatively (H_3 and H_4 receptor) to adenylyl cyclase activity via G_s and $G_{i/o}$, respectively.

conditions, the sedative effect of antihistamines can be beneficial in inducing sleep.

H_2 receptors activate adenylyl cyclase and are primarily involved in gastric acid secretion and smooth muscle relaxation. H_2 receptor antagonists are a mainstay of treatment for dyspepsia and acid reflux, as discussed in detail in Chapter 43. H_2 receptors are also highly expressed in the brain where they regulate neuronal physiology and plasticity. Mice lacking H_2 receptors show cognitive defects and impaired hippocampal long-term potentiation along with abnormalities in nociception. Systemic inhibition of the H_2 receptor has antinociceptive effects. To date, no H_2 receptor (or other histamine antagonist) has been approved for pain management.

H_3 receptors are also present in the CNS and can act as autoreceptors on histaminergic neurons to inhibit histamine synthesis and release. These receptors act to inhibit adenylyl cyclase as well as modulate N-type voltage-gated calcium channels. While it is known that H_3 receptors function as autoreceptors for histamine, they are not confined to histaminergic neurons and have been found to regulate serotonergic, cholinergic, noradrenergic, and dopaminergic neurotransmitter release. As a consequence of its ability to modulate other neurotransmitters, the H_3 receptor has become a therapeutic target for treating various conditions such as obesity, movement disorders, schizophrenia, attention-deficit/hyperactivity disorder (ADHD), and wakefulness. A wide array of compounds have been developed that interact with the H_3 receptor. They are useful pharmacological tools both *in vitro* and *in vivo*, and one compound, *pitolisant*, an inverse agonist at the H_3 receptor, is FDA approved for the treatment of narcolepsy.

H_4 receptors are expressed on cells of hematopoietic origin—eosinophils, T cells, mast cells, basophils, and dendritic cells—and are involved in eosinophil shape and mast cell chemotaxis. More recent evidence suggests that they have limited expression in the CNS and on microglia, where they may indirectly affect neurons. Regardless, most of the information about this subtype is related to allergy, asthma, and the antipruritic properties of H_4 antagonists. These functions are discussed further in Chapter 43.

Serotonin (5HT)

The synthesis and metabolism of 5HT are summarized in Figure 15–2. There are diverse pathways mediating serotonin signaling that play a role in modulating mood, depression, anxiety, and phobia, as well as GI motility (Tables 15–1 and 15–2). These effects are mediated by 13 distinct GPCRs and one ligand-gated ion channel, which have characteristic ligand-binding profiles, couple to different intracellular signaling systems, and exhibit subtype-specific distribution within the CNS. The 5HT receptors, their pharmacology, and their therapeutic utility are discussed in detail in Chapter 15.

Trace Amines

Trace amines have only recently been appreciated as neurotransmitters. As the name implies, these compounds are detected only at trace levels; they have very short half-lives due to their rapid metabolism by monoamine oxidase. Some trace amines act as neuromodulators/neurotransmitters interacting with specific receptors. Trace amines are structurally related to catecholamines and consist of the phenethylamines (phenethylamine, *N*-methylphenethylamine [an endogenous amphetamine isomer]), phenylethanolamine, tyramine, tryptamine, *N*-methyltyramine, octopamine, synephrine, and 3-methoxytyramine. These trace amines are thought to act through GPCRs that were originally termed *trace amine receptors* but are now called *trace amine–associated receptors* (TAARs) since not all members have very high affinity for trace amines. The first receptor was identified in 2001 (Borowsky et al., 2001; Bunzow et al., 2001), and to date, six human genes (*TAAR1, TAAR2, TAAR5, TAAR6, TAAR8*, and *TAAR9*) have been identified along with several potential pseudo-genes. Multiple TAAR-related receptor genes have been identified in other species, and several are expressed prominently in the olfactory epithelium, where they may serve as olfactory receptors for volatile amines. Only one TAAR (TAAR1) has been recognized by IUPHAR as a trace amine receptor, TA_1. TA_1 is a GPCR that couples to G_s and G_{13} and possibly to other G protein α-subunits, depending on the cell type and cellular location. TA_1 has the highest affinity for the trace amines tyramine, β-phenylephrine, and octopamine. In neurons, TA_1 is predominantly intracellular and activates downstream signaling pathways from an intracellular membrane compartment. TA_1 modulates monoaminergic activity in the CNS and is activated by amphetamine and related psychostimulants, as well as the endogenous thyronamines (Gainetdinov et al., 2018). Agonists for the TAAR1 receptors have been shown to have favorable effects in animal models of addiction and schizophrenia, though human therapeutics targeting TAAR1 have yet to be further developed.

Regulation of Neurotransmission

Peptides

Neuropeptides in the CNS typically behave as modulators of neurotransmission rather than direct agents of excitation or inhibition (Mains and Epper, 2012). The growing number of neuropeptides (Table 16–5) are involved in a wide array of brain functions, ranging from analgesia to social behaviors, learning, and memory. In contrast to the biosynthesis of monoamines and amino acids, peptide synthesis requires transcription of DNA into mRNA and translation of mRNA into protein. This takes place primarily in perikarya, after which the resulting peptide is transported to nerve terminals. Single genes can, through transcriptional and posttranslational modifications, give rise to multiple neuropeptides. For example, proteolytic processing of proopiomelanocortin (POMC) gives rise to, among other peptides, adrenocorticotropic hormone (ACTH; corticotropin), α-, γ-, and β-melanocyte-stimulating hormones (MSH), and β-endorphin (Figure 16–13). In addition, alternative splicing of RNA transcripts in different tissues may produce distinct mRNA species (e.g., calcitonin and calcitonin gene–related peptide [CGRP]). Furthermore, while some CNS peptides function independently, most are thought to act in concert with coexisting neurotransmitters. They are often packaged into vesicles and released along with other neurotransmitters to modulate their actions. While classical neurotransmitters generally signal to neurons by depolarizing or hyperpolarizing, neuropeptides have more diverse mechanisms of action and can also affect gene expression. Their action is not terminated by rapid reuptake into the presynaptic cell, but rather they are enzymatically inactivated by extracellular peptidases. As a result, their effects on neuronal signaling can be prolonged in nature (Mains and Epper, 2012).

Most neuropeptide receptors are GPCRs in which the extracellular domains of the receptors play a primary role in peptide ligand binding. As with other transmitter systems, there are often multiple receptor subtypes for the same peptide transmitter (Table 16–6). Neuropeptide receptors can exhibit different affinities for the nascent neuropeptide and for peptide analogues. As peptides are typically inefficient as drugs, particularly at CNS targets behind the BBB due to difficulties with their delivery, major efforts have been made to develop small-molecule drugs that are effective as either agonists or antagonists. Through a combination of structural biology, chemistry, high-throughput screening, and drug development, there are now small-molecule ligands for many neuropeptide receptors. Some of these compounds are listed in Table 16–6. Natural products have not typically been good sources of drugs that affect peptidergic transmission. One notable exception is the plant alkaloid *morphine*, which acts selectively at opioid receptor subtypes. The actions of neuropeptides have been targeted in other ways. Several approaches have been used to disrupt the actions of CGRP, a neuropeptide that plays a key role in the development of migraine. Small-molecule antagonists, antibodies against CGRP itself, and antibodies against the CGRP receptor have all been approved as effective treatments for the prevention of migraine. The use of antibody-based therapeutics provides a new avenue

TABLE 16–5 ■ EXAMPLES OF NEUROPEPTIDES

Calcitonin Family
- Calcitonin
- Calcitonin gene-related peptide (CGRP)

Hypothalamic Hormones
- Oxytocin, vasopressin

Hypothalamic Releasing and Inhibitory Hormones
- Corticotropin-releasing factor (CRF or CRH)
- Gonadotropin-releasing hormone (GnRH)
- Growth hormone-releasing hormone (GHRH)
- Somatostatin (SST)
- Thyrotropin-releasing hormone (TRH)

Neuropeptide Y Family
- Neuropeptide Y (NPY)
- Neuropeptide YY (PYY)
- Pancreatic polypeptide (PP)

Opioid Peptides
- β-Endorphin (also pituitary hormone)
- Dynorphin peptides
- *Leu*-enkephalin
- *Met*-enkephalin

Pituitary Hormones
- Corticotropin (formerly adrenocorticotropic hormone; ACTH)
- α-Melanocyte-stimulating hormone (α-MSH)
- Growth hormone (GH)
- Follicle-stimulating hormone (FSH)
- β-Lipotropin (β-LPH), luteinizing hormone (LH)

Tachykinins
- Neurokinins A and B
- Neuropeptide K, substance P

VIP-Glucagon Family
- Glucagon, glucagon-like peptide (GLP-1)
- Pituitary adenylyl cyclase–activating peptide (PACAP)
- Vasoactive intestinal polypeptide (VIP)

Other Peptides
- Agouti-related peptide (ARP)
- Bombesin, bradykinin (BK)
- Cholecystokinin (CCK)
- Cocaine/amphetamine-regulated transcript (CART)
- Galanin, ghrelin
- Melanin-concentrating hormone (MCH)
- Neurotensin, nerve growth factor (NGF)
- Orexins, orphanin FQ (nociceptin)
- Hemopressin (CB$_1$ inverse agonist)

Source: Modified with permission from Nestler EJ, et al., eds. *Molecular Neuropharmacology: A Foundation for Clinical Neuroscience,* 2nd ed. McGraw Hill, New York, **2009**.

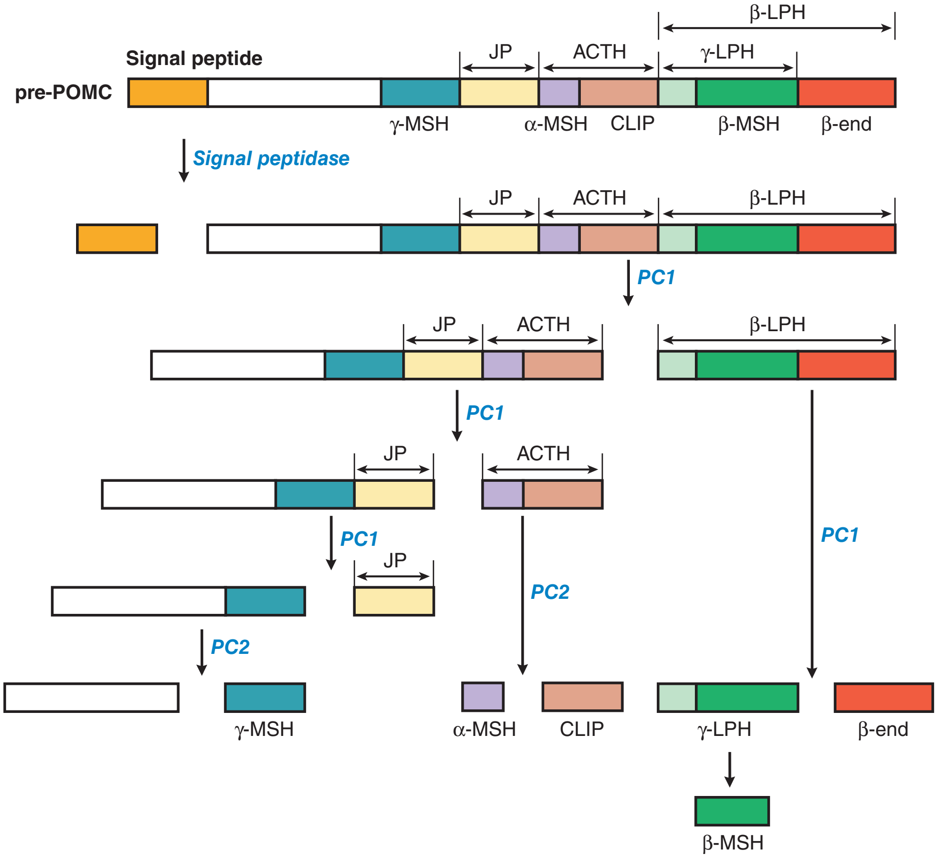


Figure 16–13 *Proteolytic processing of proopiomelanocortin (POMC).* After removal of the signal peptide from pre-POMC, the remaining propeptide undergoes endoproteolysis by prohormone convertases 1 and 2 (PC1 and PC2) at dibasic residues. PC1 liberates the bioactive peptides adrenocorticotropic hormone (ACTH), β-endorphin (β-end), and γ-LPH (lipotrophic hormone). PC2 cleaves ACTH into CLIP (corticotropin-like intermediate peptide) and α-melanocyte-stimulating hormones (MSH) and also releases γ-MSH from the N-terminal portion of the propeptide. The JP (joining peptide) is the region between ACTH and γ-MSH. β-MSH is formed by cleavage of γ-LPH. Some of the resulting peptides are amidated or acetylated before they become fully active.

TABLE 16–6 ■ PEPTIDE TRANSMITTERS AND RECEPTORS

FAMILY	SUBTYPE	TRANSDUCER	AGONISTS	ANTAGONISTS
Opioid	δ κ μ NOP	G_i/G_o	β-Endorphin, dynorphin, DPDPE (δ), salvinorin A(κ), hydromorphone (μ), fentanyl (μ), codeine (μ), methadone (μ), DAMGO (μ), etorphine, Ro64-6198 (NOP)	Naltrexone, naloxone, SB612111
Somatostatin	SST_1, SST_2 SST_3, SST_4 SST_5	G_i	SST-14, SST-18, pasireotide, cortistatin, BIM23059, BIM23066, BIM23313, CGP23996, octreotide ($sst_{2,3,5}$)	SRA880 (sst_1), D-Tyr8-CYN154806 (sst_2), NVPACQ090 (sst_3)
Neurotensin	NTS_1 NTS_2	$G_{q/11}$	EISAI-1, JMV431, JMV449 (NTS_1), levocabastine (NTS_2)	SR142948A, meclinertant (NTS_1)
Orexin	OX_1 OX_2	$G_{q/11,}$ G_s, G_i	Orexin-A, Orexin-B	Suvorexant, filorexant, SB-649868, almorexant, SB-410220, JNJ 10397049
Tachykinin	NK_1 NK_2 NK_3	$G_{q/11}$	Neurokinin A, neurokinin B, substance P, GR 73632 (NK_1), GR 64349 (NK_2), senktide	Aprepitant (NK_1), GR 159897 (NK_2), SB218795 (NK_3)
Cholecystokinin	CCK_1 CCK_2	$G_{q/11}$ (CCK_1), G_s	Cholecystokinin-8, CCK-33, CCK-58, gastrin, A-71623 (CCK_1)	Proglumide, FK-480, lintitript, PD-149164, devazepide (CCK_1), CL988 (CCK_2)
Neuropeptide Y	Y_1 Y_2 Y_4 Y_5	G_i/G_o	Neuropeptide Y, BWX 46	BIBO 3304 (Y_1), BIIE0246 (Y_2), UR-AK49, CGP 71683A GW438014A (Y_5)
Neuropeptide FF	NPFF1 NPFF2	$G_{q/11}$, G_i/G_o	Neuropeptide FF, RFRP-3 (NPFF1)	RF9

for targeting peptidergic systems within both central and peripheral nervous systems (Lu et al., 2020).

Purines

Adenosine, ATP, UDP, and UTP have roles as extracellular signaling molecules. ATP is also a component of many neurotransmitter storage vesicles and is released along with transmitters. Intracellular nucleotides may also reach the cell surface by other means, and extracellular adenosine can result from cellular release and metabolism of ATP. These released nucleotides can be hydrolyzed extracellularly by ectonucleotidases. Adenosine is reaccumulated by transport into neighboring cells unless extracellular adenosine deaminase is present. Extracellular nucleotides and adenosine can act on a family of diverse purinergic receptors, which have been implicated in a variety of functions including memory and learning, locomotor behavior, and feeding.

There are three classes of *purinergic receptors*: adenosine receptors (also called P1), P2Y, and P2X (Table 16–7). *Adenosine receptors* are GPCRs that consist of four subtypes (A_1, A_{2A}, A_{2B}, and A_3) activated endogenously by adenosine. A_1 and A_3 couple to G_i, whereas A_2 receptors couple to G_s. Activation of A_1 receptors is associated with inhibition of adenylyl cyclase, activation of K^+ currents, and in some instances, activation of PLC; stimulation of A_2 receptors activates adenylyl cyclase. In the CNS, both A_1 and A_{2A} receptors are involved in regulating the release of other neurotransmitters such as glutamate and dopamine, making the A_{2A} receptor a potential therapeutic target for disorders such as Parkinson's disease. Recently, the A_{2A} receptor antagonist istradefylline was approved as adjunctive therapy with L-DOPA for the treatment of Parkinson's disease (see Chapters 15 and 21).

P2Y receptors are also GPCRs and are activated by ATP, ADP, UTP, UDP, and UDP-glucose. There are eight known subtypes of P2Y receptors that couple to a variety of G proteins as indicated in Table 16–7. The P2Y receptor is expressed in the CNS, where it is stimulated by UDP-glucose and may play a role in neuroimmune functions. The $P2Y_{12}$ receptor is important clinically: Antagonism of this receptor in platelets inhibits platelet aggregation.

ATP-sensitive *P2X receptors* are ligand-gated cation channels that are expressed throughout the CNS on presynaptic and postsynaptic nerve terminals and on glial cells. P2X receptors have been found on nociceptive sensory neurons, where they primarily gate Na^+, K^+, and Ca^{2+}, and have been implicated in mediating sensory transduction. There are seven subtypes of P2X receptors with varying sensitivities to their endogenous agonist, ATP (see Table 16–7). Functional P2X receptors have a trimeric topology, existing either as homopolymers or heteropolymers with other P2X receptors. Following the first crystal structure that confirmed this arrangement in 2009 (Kawate et al., 2009), more than 20 crystal structures of P2X receptors from different subtypes and in different states have been described and have added to our understanding of how these receptors interact with agonists and antagonists (Schmid and Evans, 2019). The study of compounds that are selective for P2X subtypes suggests that targeting these receptors may be useful in the therapy of neurological disorders such as neuropathic and inflammatory pain, epilepsy, memory, and alcohol use disorders. The first P2X drug to come to clinical fruition (approved in Japan in 2022) is *gefapixant,* a $P2X_3$ receptor antagonist for refractory cough. The release of ATP from irritated bronchial cells can stimulate $P2X_3$ receptors on sensory nerve fibers in the airway, initiating an action potential that can lead to coughing. *Gefapixant* inhibits ATP binding to $P2X_3$ receptors, thereby reducing the stimulation that leads to coughing. Among the notable features of *gefapixant* is that it is named after the late Geof Burnstock, a pioneer of purinergic signaling (North and Costa, 2021).

Cannabinoids

Delta-9-tetrahydrocannabinol (THC) was identified as a psychoactive substance in marijuana in the 1960s (Figure 16–14). This led to the

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TABLE 16–7 ■ CHARACTERISTICS OF PURINERGIC RECEPTORS

CLASS	RECEPTOR							
Adenosine (P1)[a]	**A_1**	**A_{2A}**	**A_{2B}**	**A_3**				
Transducer	$G_{i/o}$	G_s	G_s	$G_{i/o}$				
Agonists	CPA	CGS21680	BAY 60-6583	1B-MECA				
Antagonists	CPX	SCH58261	MRS1754	VUF5574				
P2X (ionotropic)	**$P2X_1$**	**$P2X_2$**	**$P2X_3$**	**$P2X_4$**	**$P2X_5$**	**$P2X_6$**	**$P2X_7$**	
Substrate specificity	ATP	ATP	ATP	ATP>CTP	ATP	ATP	ATP	
Antagonist	NF449, TNP-ATP	NF770	TNP-ATP	5-BDBD, paroxetine	PPADS, suramin		AZ10606120	
P2Y (metabotropic)	**$P2Y_1$**	**$P2Y_2$**	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{11}$**	**$P2Y_{12}$**	**$P2Y_{13}$**	**$P2Y_{14}$**
Transducer	$G_{q/11}$	$G_{q/11}$	$G_{q/11}$	$G_{q/11}$	G_s, $G_{q/11}$	$G_{i/o}$	$G_{i/o}$	$G_{i/o}$
Substrate specificity	ADP>ATP	ATP>UTP	UTP>ATP	UDP>>UTP>ADP	ATP=UTP	ADP	ADP>>ATP	UDP-glucose[b]
Agonists	MRS2365	MRS2698, PSB1114	MRS4062	MRS2957	AR-C67085	2MeSADP	2MeSADP	MRS2690
Antagonists	MRS2279	ARC118925X		MRS2578	NF157	ticagrelor, clopidogrel	MRS2211, cangrelor	PPTN

CPA, N6-cyclopentyladenosine; CPX, 8-cyclopentyl-1,3-dipropylxanthine; 1B-MECA, N6-(3-iodobenzyl)-adenosine-5α-*N*-methylcarboxamide; NECA, 1-(6-amino-9H-purin-9-yl)-1-deoxy-*N*-ethyl-β-D-ribofuronamide; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid; TNP-ATP, 2′,3′-O-(2,4,6-trinitrophenyl)adenosine-5′-triphosphate. For further details, consult information about the three classes of purinergic receptors at http://www.guidetopharmacology.org.

[a] NECA is a nonselective agonist of P1 receptors.

[b] $P2Y_{14}$ binds UDP-glucose, UDP-galactose, or UDP-acetylglucosamine.

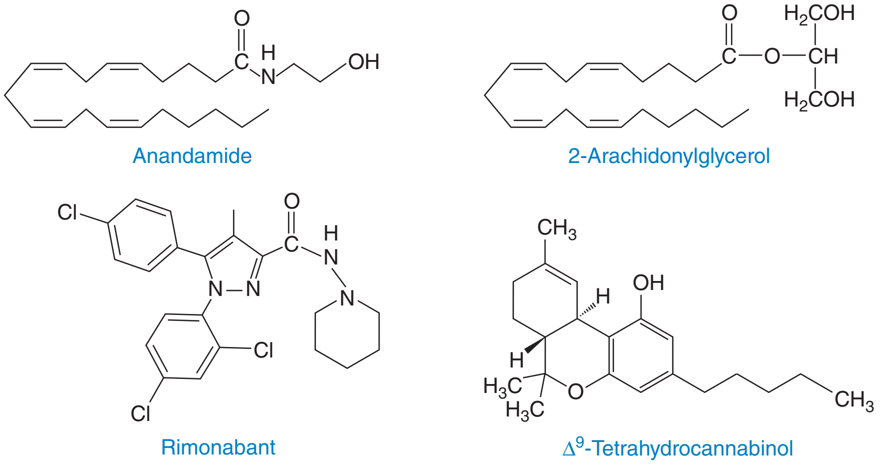

Figure 16–14 *Cannabinoid (CB) receptor ligands.* Anandamide and 2-arachidonylglycerol are endogenous agonists. *Rimonabant* is a synthetic CB receptor antagonist. Δ^9-Tetrahydrocannabinol is a CB agonist derived from marijuana.

discovery of two cannabinoid receptors and the identification of endogenous compounds that modulate them. The two receptor subtypes (CB_1 and CB_2) are GPCRs that couple to G_i/G_o to inhibit adenylyl cyclase and, in some cell types, inhibit voltage-gated Ca^+ channels or stimulate K^+ channels. The receptors share relatively low overall homology and are found in differing locations, although both are found in the CNS. CB_1 receptors are found in high levels throughout the brain, whereas CB_2 receptors are prominent in immune cells. Within the CNS, CB_2 receptors are less abundant than CB_1 receptors and are thought to occur primarily on microglia. The finding of endogenous cannabinoids responsible for signaling to these receptors, along with a host of clinical data from marijuana use, has fueled interest in this signaling system and has greatly expanded our understanding of its physiology.

The endogenous cannabinoid system consists of the cannabinoid receptors, endogenous cannabinoids, and the enzymes that synthesize and degrade endocannabinoids (see Figure 26–5). The endocannabinoids are lipid molecules and include anandamide (*N*-arachidonoylethanolamine) and 2-arachidonoylglycerol (2-AG), as well as other compounds that have been putatively identified to serve as endogenous endocannabinoids, including *O*-arachidonoylethanolamine (virodhamine), *N*-dihomo-γ-linolenoylethanolamine, *N*-docosatetraenoic-ethanolamine, oleamide, 2-arachidonyl-glyceryl-ether (2-AGE), *N*-arachidonoyl-dopamine (NADA), and *N*-oleoyl-dopamine. The actions of endocannabinoids are terminated by their uptake into cells, followed by hydrolysis. Two enzymes known to break down anandamide and 2-AG are fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL), respectively. Although a few studies suggested the existence of a specific transport system for endocannabinoids, no molecular entity that mediates such a carrier-mediated process has been identified. Obviously, drugs that inhibit the transport or degradation of endocannabinoids would prolong their physiological actions. Chapter 26 reviews the pharmacology of endo- and over-the-counter cannabinoids, cannabinoid receptor signaling, and associated therapeutic developments.

Other Lipid Mediators

Arachidonic acid, normally stored within the cell membrane as a glycerol ester, can be liberated during phospholipid hydrolysis (by pathways involving phospholipases A_2, C, and D). Arachidonic acid can be converted to highly reactive modulators by three major enzymatic pathways (see Chapter 41): *cyclooxygenases* (leading to prostaglandins and thromboxane), *lipoxygenases* (leading to the leukotrienes and other transient catabolites of eicosatetraenoic acid), and *CYP450s* (which are inducible and expressed at low levels in brain). Arachidonic acid metabolites have been implicated as diffusible modulators in the CNS, possibly involved with the formation of long-term potentiation and other forms of neuronal plasticity.

Gases: Nitric Oxide and Carbon Monoxide

Both constitutive and inducible forms of nitric oxide (NO) synthase are expressed in the brain. The application of inhibitors of NO synthase (e.g., *methylarginine*) and of NO donors (e.g., *nitroprusside*) suggests the involvement of NO in a host of CNS phenomena, including long-term potentiation, activation of soluble guanylyl cyclase, neurotransmitter release, and enhancement of glutamate (NMDA)-mediated neurotoxicity. Another diffusible gas, carbon monoxide, is generated in neurons or glia and may act as an intracellular messenger stimulating soluble guanylyl cyclase through nonsynaptic actions.

Termination of Neurotransmitter Action

It is essential that there are mechanisms to terminate the actions of released neurotransmitters to maintain the balance of neuronal signaling. There are two primary mechanisms for terminating the signaling of released transmitters. One is the conversion of the transmitter into an inactive compound via an enzymatic reaction. The best example of enzymatic inactivation is for the transmitter ACh, the action of which is terminated via hydrolysis by acetylcholinesterase to choline and acetate (see Chapter 12). A second mechanism involves the clearance of the neurotransmitter by transport proteins present on neuronal or glial membranes, so that it can no longer act on the target receptors; reuptake of DA by the dopamine transporter (DAT) is a good example. In addition to the above, slow diffusion of the transmitter away from the synapse and subsequent degradation also play a role for both conventional neurotransmitters and neuropeptides.

Neurons and glial cells express *specific transporter proteins* such as those for the monoamines norepinephrine (NE transporter [NET]), serotonin (5HT transporter [SERT]), and dopamine (DAT), which remove NE, 5HT, and DA from the extracellular space by transporting it back into the presynaptic neuron (see Chapters 10 and 15). These plasma membrane carriers serve as a major mechanism for limiting the extent and duration of neurotransmitter signaling. To accomplish this task, they couple the movement of neurotransmitters to the influx of multiple sodium ions, which provides a strong thermodynamic driving force for inward transport. The carriers for NE, 5HT, DA, GABA, and glycine have 12 hydrophobic membrane-spanning domains with their amino- and carboxyl-termini located within the cytoplasm (Figure 16–15). These transporters are generally glycosylated within an extracellular loop and possess sites for phosphorylation and binding to intracellular regulatory proteins, primarily on their amino- and carboxyl-termini.

A second family of plasma membrane neurotransmitter transporters mediates the clearance of glutamate and aspartate released during synaptic transmission. In humans, five subtypes of glutamate transporters (referred to as excitatory amino acid transporters [EAATs] 1–5) clear

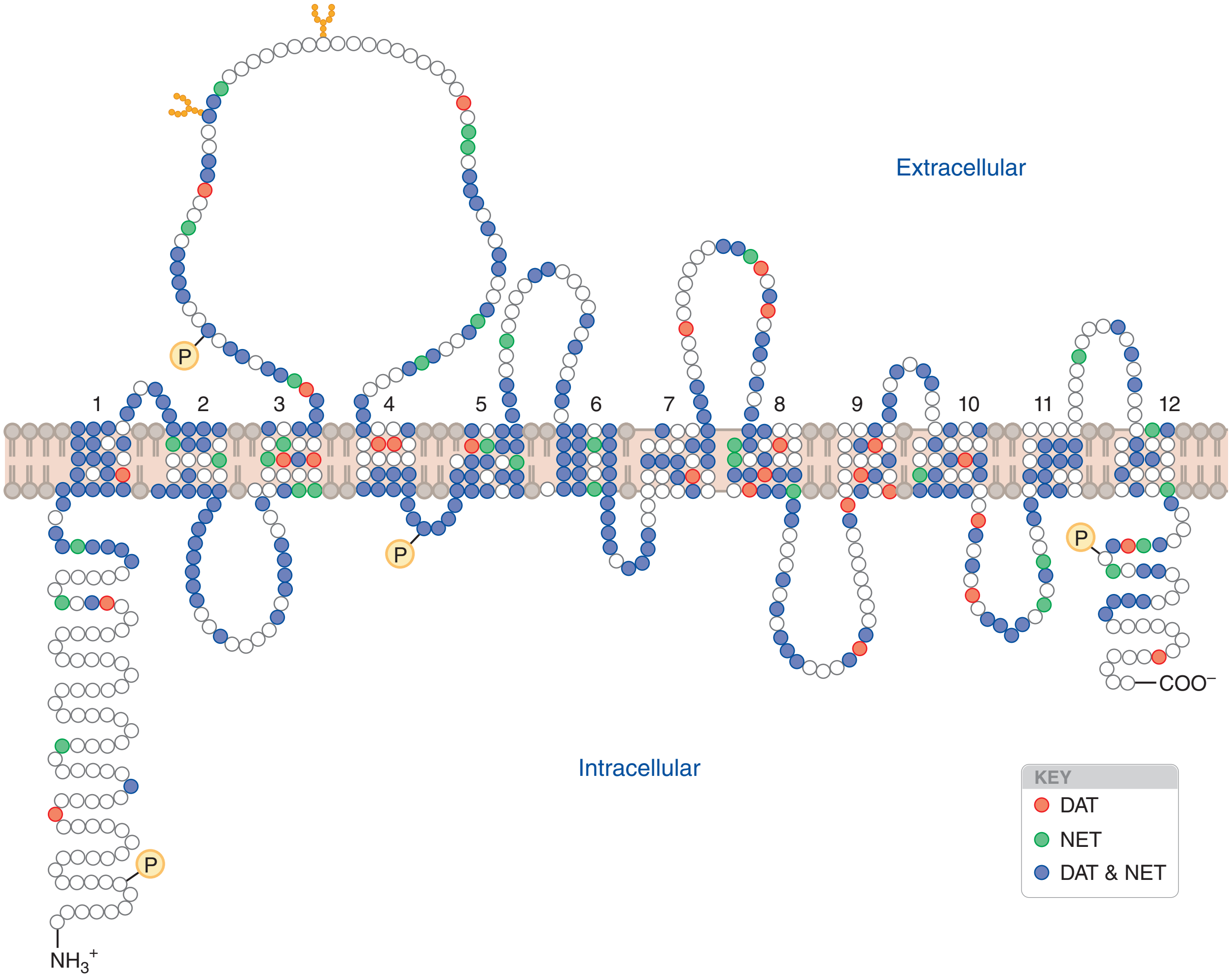

Figure 16–15 *Structure of the rat 5HT transport protein.* Both the amino and carboxyl termini are intracellular. These transport proteins typically have 12 hydrophobic, membrane-spanning domains with intervening extracellular and intracellular loops. The second extracellular loop is the largest and contains several potential glycosylation sites (indicated with tree-like symbols). Amino acid residues that are homologous to those in the DAT and the NET are colored, as noted. The most highly conserved regions of these transporters are located in the transmembrane domains; the most divergent areas occur in the N and C termini. (Used with permission from Dr. Beth J. Hoffman, San Diego, CA.)

glutamate into neurons and glial cells. The two glial carriers, EAAT1 and EAAT2, are responsible for the bulk of glutamate transport activity in the CNS and are critical for limiting excitotoxic actions of glutamate. These transporters assemble as homotrimers within the membrane with each protomer possessing eight transmembrane domains (TM1–8) and two re-entrant hairpin loops (HP1 and HP2) that appear to serve as intracellular and extracellular gates during the transport process (Jiang and Amara, 2011; Yernool et al., 2004) (Figure 16–16).

There are also at least three distinct gene families of vesicular neurotransmitter transporters that sequester the neurotransmitters within synaptic vesicles for storage and, ultimately, for release during neuronal signaling. These include the vesicular monoamine and ACh transporters (VMAT1, VMAT2, and VAChT), a vesicular carrier for both GABA and glycine (VGAT), and three vesicular glutamate carriers, VGLUT1, VGLUT2, and VGLUT3. These transporters ensure that vesicles fill rapidly during neurotransmission and provide a means for reducing cytoplasmic concentrations of neurotransmitter in areas where rates of reuptake are high. Three structurally related VMAT2 inhibitors, *tetrabenazine*, *deutetrabenazine*, and *valbenazine*, have been shown to effectively lower the vesicular storage and release of DA and are now used clinically in the treatment of involuntary movement disorders such as Huntington's chorea and tardive dyskinesia (Koch et al., 2020).

The monoamine transporters, DAT, NET, and SERT, are well-established targets for therapeutic antidepressants and for addictive drugs including *cocaine* and amphetamines. Selective inhibitors of these carriers can increase the duration and spatial extent of the actions of neurotransmitters. Inhibitors of the uptake of NE and/or 5HT are used to treat depression and other behavioral disorders, as described in Chapter 18. The psychostimulants *methylphenidate* and *amphetamine* are the major drugs used to treat ADHD in children and in adults. Although the two drugs have stimulant actions in healthy individuals, in ADHD patients, they reduce hyperactivity and increase attention by inhibiting DAT and NET and enhancing DA and NE neurotransmission. These transporters are discussed in further detail in Chapters 4, 10, and 15.

CNS Drug Discovery and Development

After determining a CNS target site for which modulation would prove helpful for treating a disorder, the next step involves the discovery of a compound suitable for targeting that site that can be developed into a drug. Four core approaches are typically used to identify and develop small-molecule compounds for targeting neuronal signaling:

- Synthesis of structural analogues of known neurotransmitters or modulators of receptors/channels to generate structure-activity relationship information for determining how chemical structure can lead to greater target specificity.
- Use of natural products that exist in nature (e.g., toxins or plant extracts) that have shown efficacy for treating a particular disease, either as drugs themselves or as templates for drug design.

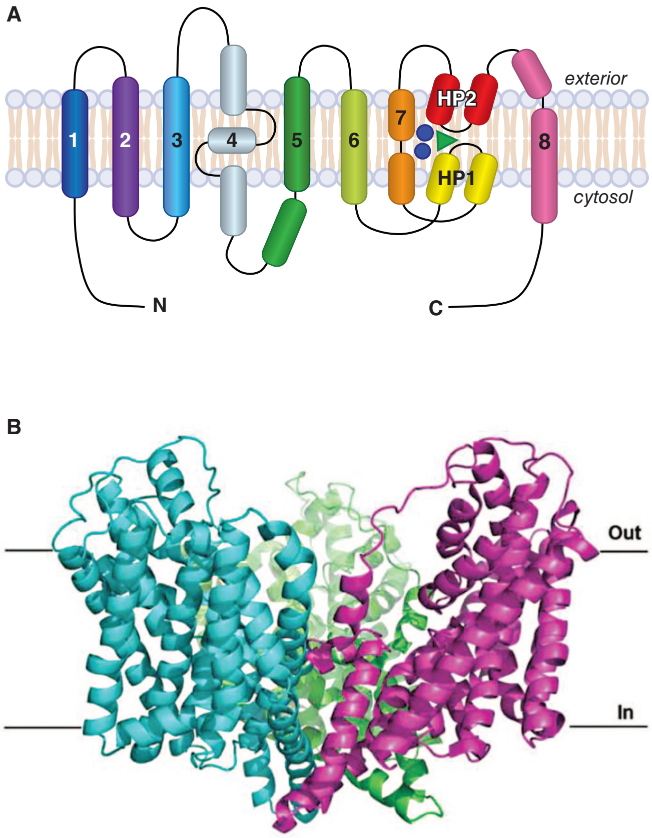

Figure 16–16 *Structure of the excitatory amino acid transporters.* The mammalian EAAT family includes the plasma membrane transporters EAAT1 to EAAT5. **A.** Membrane topology of a single EAAT family subunit: transmembrane domains (colored oblongs) are labeled 1–8. Approximate binding sites occupied by Na$^+$ (blue dots) and substrate (green triangle) are formed by the nonhelical segments at the tips of two hairpin loops, HP1 and HP2. **B.** Crystal structure (protein data base ID: 1XFH) of the archaeal glutamate transporter ortholog, Glt_{Ph}, from *Pyrococcus horikoshii*. Individual subunits have been colored distinctively to illustrate their trimeric organization within the membrane. (Reproduced, with permission, from Jiang J, Amara SG. New views of glutamate transporter structure and function: advances and challenges. *Neuropharmacology*, **2011**, *60*:172–181. Copyright © Elsevier Ltd.)

- The development of a signaling assay that is amenable for the screening of large (thousands to millions) compound libraries with diverse chemical structures to identify novel scaffolds with promising functional profiles for drug development.
- Use of atomic-level (x-ray or cryo-electron microscopy) structures of targets to rationally design new compounds or use structure-based virtual (*in silico*) screening to identify chemical scaffolds that likely bind to the target.

Each approach has benefits and pitfalls, but together, these types of approaches have been successful in discovering and developing many new drugs for targeting CNS signaling processes.

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Chapter 17

The Blood-Brain Barrier and Its Influence on Drug Transport to the Brain

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BRAIN BARRIERS

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SUMMARY

Brain Barriers

For a drug to be active, it must reach a certain concentration in the target tissue. The central nervous system (CNS) possesses a series of barriers that separate the neural tissue from the periphery. These barriers act to stringently regulate the movement of ions, molecules, and cells between peripheral fluids (i.e., blood) and the CNS, thus tightly regulating the extracellular environment of the CNS, which is critical to maintain homeostasis. The barriers not only control the influx of glucose and essential nutrients but also greatly limit the entry of many exogenous compounds, including drugs. The pharmaceutical industry has struggled with developing drugs that can cross these barriers and enter the brain without requiring high doses that give unwanted peripheral side effects or are too costly. For large-molecule drugs like antibodies, this problem is greater since larger molecules have an even lower ability to cross brain barriers.

The brain barriers include the blood vessels that vascularize the CNS parenchyma, the meningeal covering of the brain, and the choroid plexus within the ventricles (Figure 17–1).

The barriers are especially important to insulate the neurons from ionic fluctuations, such that the neurons can maintain appropriate ion gradients required for neural circuit function. The brain barriers also protect the CNS from toxins, pathogens, and even the body's own immune system, which is crucial because the CNS fails to regenerate after many injuries and diseases. The importance of the barriers is highlighted by the severe pathology of diseases in which the barriers are disrupted, such as multiple sclerosis, stroke, traumatic brain injury, meningitis, and other neurological injuries and disease. When intact, these barriers also create obstacles for drug delivery, as they greatly impede the entry of most blood-borne molecules from entering the brain.

The most studied barrier is the BBB, an endothelial barrier formed by the blood vessels that vascularize the parenchyma of the CNS. The BBB makes up most of the surface area of the brain barriers and hence is most important for drug delivery to the CNS. The meningeal coverings of the brain have two distinct barriers that restrict the movement of solutes from the periphery into the brain. The first barrier is the arachnoid barrier, which stringently regulates the passage of molecules and cells between the outer dura mater, which contains fenestrated "leaky" blood vessels, and the inner subarachnoid space, which contains the cerebrospinal fluid (CSF). This barrier is an epithelial barrier made up of arachnoid barrier cells that physically separate the dura mater and the subarachnoid space. The second barrier is an endothelial barrier possessed by the blood vessels within the subarachnoid space, which restrict the movement of molecules and cells between the blood and the CSF within the subarachnoid space. The choroid plexuses, which secrete CSF into the ventricles, also contain a barrier that is formed by choroid plexus epithelial cells that surround fenestrated "leaky" blood vessels within the plexuses. These epithelial cells act to tightly regulate the composition of the CSF that they are secreting into the ventricles, forming what is termed the blood-CSF barrier (BCSFB).

This chapter focuses on describing the cellular and molecular composition of the BBB, how the barrier properties are regulated, the role of the BBB in CNS pharmacology, and how the BBB is being targeted for CNS drug delivery.

The Blood-Brain Barrier

Cellular Composition of the BBB: The Neurovascular Unit

The BBB is a term used to describe the unique properties of the blood vessels that vascularize the CNS, which allows them to tightly regulate the movement of ion molecules and cells between the blood and the brain. Endothelial cells that form the walls of the blood vessels provide most the BBB properties, greatly restricting the passage of nonspecific molecules, while transporting specific nutrients into the CNS. Critical to the function of the BBB is also the interaction of the endothelial cells with other cells within the neurovascular unit including mural cells, fibroblasts, astrocytes, and immune cells (Figure 17–2).

Endothelial cells are thin cells that form the inner walls of all blood vessels, generating a lumen for blood to flow. In larger vessels, arteries and veins, the circumference of the vessel can be made up of dozens of endothelial cells, whereas the smallest capillaries can consist of a single endothelial cell folding upon itself to form a lumen of 6 to 8 μm in diameter.

Abbreviations

Apo: apolipoprotein
ATP: adenosine triphosphate
AUC: area under the concentration-time curve
BBB: blood-brain barrier
BCRP: breast cancer resistance protein
BCSFB: blood–cerebrospinal fluid barrier
CSF: cerebrospinal fluid
$f_{u,brain}$: unbound fraction of drug in brain homogenate
$f_{u,plasma}$: unbound fraction of drug in plasma
FUS: focused ultrasound
IR: insulin receptor
ISF: interstitial fluid
$K_{p,uu,brain}$: partition coefficient of unbound drug in brain interstitial fluid to that in plasma
$K_{p,uu,cell}$: partition coefficient of unbound drug between intracellular and interstitial fluids
LDLRf: low-density lipoprotein receptor family
MRP: multidrug resistance protein
PET: positron emission tomography
PS: permeability surface area product
RMT: receptor-mediated transcytosis
Tf: transferrin
TfR: transferrin receptor
$V_{u,brain}$: unbound volume of distribution in the brain; i.e., partitioning of total drug to that unbound in the brain interstitial fluid

The vascular network of the human brain is roughly 600 km long, with a thickness of 200 to 400 nm of the endothelial cells and a surface area of 15 to 25 square meters (Wong et al., 2013). Thus, the endothelial cells make up the primary cellular interface between the blood and the tissue and thus regulate permeability, transport, coagulation, and immune cell infiltration.

Mural cells are vascular support cells that sit on the abluminal surface of the endothelial cells, embedded in the vascular basement membrane, and regulate vascular parameters. On large vessels, *vascular smooth muscle cells* form concentric rings around the vessels and, through their contraction, control vessel diameter and thus blood flow. On capillaries and postcapillary venules, *pericytes* form incomplete layers, extending processes that interact with endothelial cells through peg-and-socket junctions. Pericytes regulate vascular permeability, endothelial immune activation, and blood flow (Armulik et al., 2011).

Fibroblasts are found embedded in the vascular smooth muscle layer of large vessels (Vanlandewijck et al., 2018b), sensing vascular stretch and regulating wound healing through the deposition of extracellular matrix.

A *basement membrane* consisting of extracellular matrix proteins including type IV collagens, laminins, nidogen, and heparan sulfate proteoglycans surrounds the CNS blood vessels. The basement membrane can be divided into two layers, the inner vascular basement membrane secreted by endothelial cells, mural cells, and fibroblasts, and the outer parenchymal basement membrane secreted primarily by astrocytes. These two basement membranes are separated in larger vessels but fuse around capillaries (Xu et al., 2019).

Astrocytes are a major glial cell population that extends polarized cellular processes; one set of processes ensheathes either synapses in the gray matter or nodes of Ranvier in the white matter, whereas the other process extends end feet that ensheathe more than 95% of the abluminal surface of the blood vessels, separated from the vessels by the basement membrane (Sofroniew and Vinters, 2010). Therefore, astrocytes are situated to sense and respond to both neural activity and vascular function and have been shown to regulate blood flow, vascular permeability, and CNS fluid dynamics as part of the glymphatic system (Hablitz and Nedergaard, 2021). In addition, neural progenitors, neurons, oligodendrocyte progenitors, and oligodendrocytes have been shown to interact with endothelial cells, regulating different aspects of vascular function.

Immune cells, both within the brain and within the blood, interact with CNS blood vessels. *Perivascular macrophages* are found in the perivascular spaces of draining venules where the glial lamina separates from the vascular basement membrane and survey this Virchow-Robin space as the first line of immunity in the CNS. *Microglia*, the CNS resident myeloid cells, extend highly motile ramified processes that survey the parenchyma and also poke between astrocyte end feet to inspect the vascular space (Li and Barres, 2018). These microglia have been shown to regulate vascular repair following injury (Lou et al., 2016).

Properties of the BBB

The BBB is not one entity, but a series of properties that allow the blood vessels to limit the passage of nonspecific molecules while delivering specific nutrients to the underlying neural tissue. Many of these properties are possessed by the endothelial cells that form the walls of the blood vesssels, such that CNS endothelial cells have distinct properties compared to endothelial cells in other tissues.

Glycocalyx

The *glycocalyx* is the carbohydrate-rich matrix that lines the luminal surface (blood side) of the endothelial cells and forms the first barrier to blood-borne molecules and cells. The vascular glycocalyx is made up of glycoproteins, proteoglycans, and glycolipids that can protrude several microns into the lumen of the vessel. Two-photon imaging in rodents has shown that this acts as the first barrier to large molecules, limiting their diffusion and ability to reach the endothelial cell (Kutuzov et al., 2018). It is currently unclear how the composition of the glycocalyx is different in CNS vessels compared to peripheral vessels and how the sieve-like barrier properties of the vascular glycocalyx are different in different tissues.

Tight Junctions

CNS endothelial cells are held together by *tight junctions* that form a tight paracellular barrier that polarizes the cell into distinct luminal and abluminal membrane compartments. Tight junctions, primarily studied in epithelial cells, are intercellular adhesions formed by transmembrane proteins including claudin family members, occludin, and junctional adhesion molecules that are linked to the cytoskeleton by adapters including zona occludens (Kniesel and Wolburg, 2000). The composition of claudins appears to determine the permeability of the paracellular pore, with claudin 5 being the most prominent claudin in CNS endothelial cells, creating a barrier that is greatly restrictive to ions with an electrical resistance of 1000 to 4000 ohms/cm^2. Genetic deletion of claudin 5 in mice results in a size-specific leakiness of the BBB to molecules less than 1000 Da (Nitta et al., 2003).

Transcellular Permeability

CNS endothelial cells restrict the transcellular movement of solutes with a *lack of fenestra* (pores in the membranes) and *low rates of transcytosis* (transcellular vesicle trafficking) compared to other vascular beds. Transcytosis in the endothelial cells can be divided into two mechanisms: (1) nonspecific transcytosis mediated through caveolin-coated vesicles and (2) receptor-mediated transcytosis of specific substrates through clathrin-coated vesicles. The tight paracellular barrier and low amounts of nonspecific transcytosis allow CNS endothelial cells to control the passage of molecules through transport. The receptor-mediated transport allows for the uptake of specific molecules including transferrin, insulin, and leptin (Yang et al., 2020).

Efflux Transport

The physical barrier properties restrict the passage of hydrophilic molecules across the endothelial cells; however, many small lipophilic molecules can passively diffuse across the endothelial cell membrane into the parenchyma. To regulate movement of lipophilic molecules across the BBB, CNS endothelial cells express a variety of *efflux transporters*

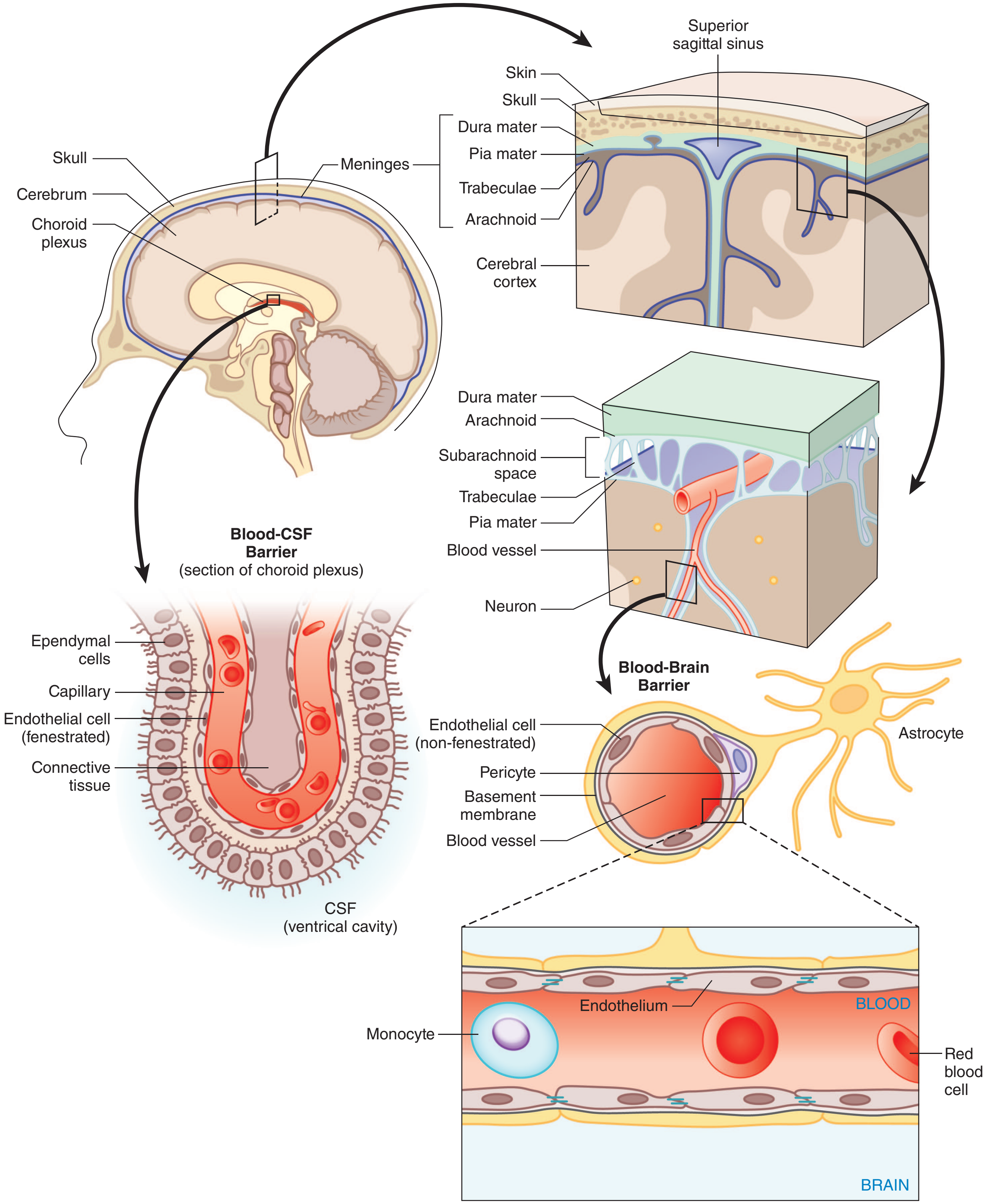
Superior sagittal sinus
Skin
Skull
Dura mater
Pia mater
Trabeculae
Arachnoid
Cerebral cortex
Skull
Cerebrum
Choroid plexus
Meninges
Dura mater
Arachnoid
Subarachnoid space
Trabeculae
Pia mater
Blood vessel
Neuron
Blood-CSF Barrier
(section of choroid plexus)
Ependymal cells
Capillary
Endothelial cell (fenestrated)
Connective tissue
CSF (ventrical cavity)
Blood-Brain Barrier
Endothelial cell (non-fenestrated)
Pericyte
Basement membrane
Blood vessel
Astrocyte
Endothelium
BLOOD
Monocyte
Red blood cell
BRAIN

Figure 17–1 *Schematic representation of the major brain barriers.* Top right is a schematic of a cross-section through the meningeal coverings of the brain depicting the major meningeal barrier sites including the arachnoid barrier between the dura and the subarachnoid space and the vascular barrier possessed by the blood vessels within the subarachnoid space. Bottom left is a schematic of a cross-section through the choroid plexus that sits within the brain ventricles, depicting the choroid plexus epithelial cells that form the blood-CSF barrier between the leaky fenestrated vessels within the choroid plexus and the CSF within the ventricles. Bottom right depicts a cross-section of a parenchymal capillary that forms the BBB.

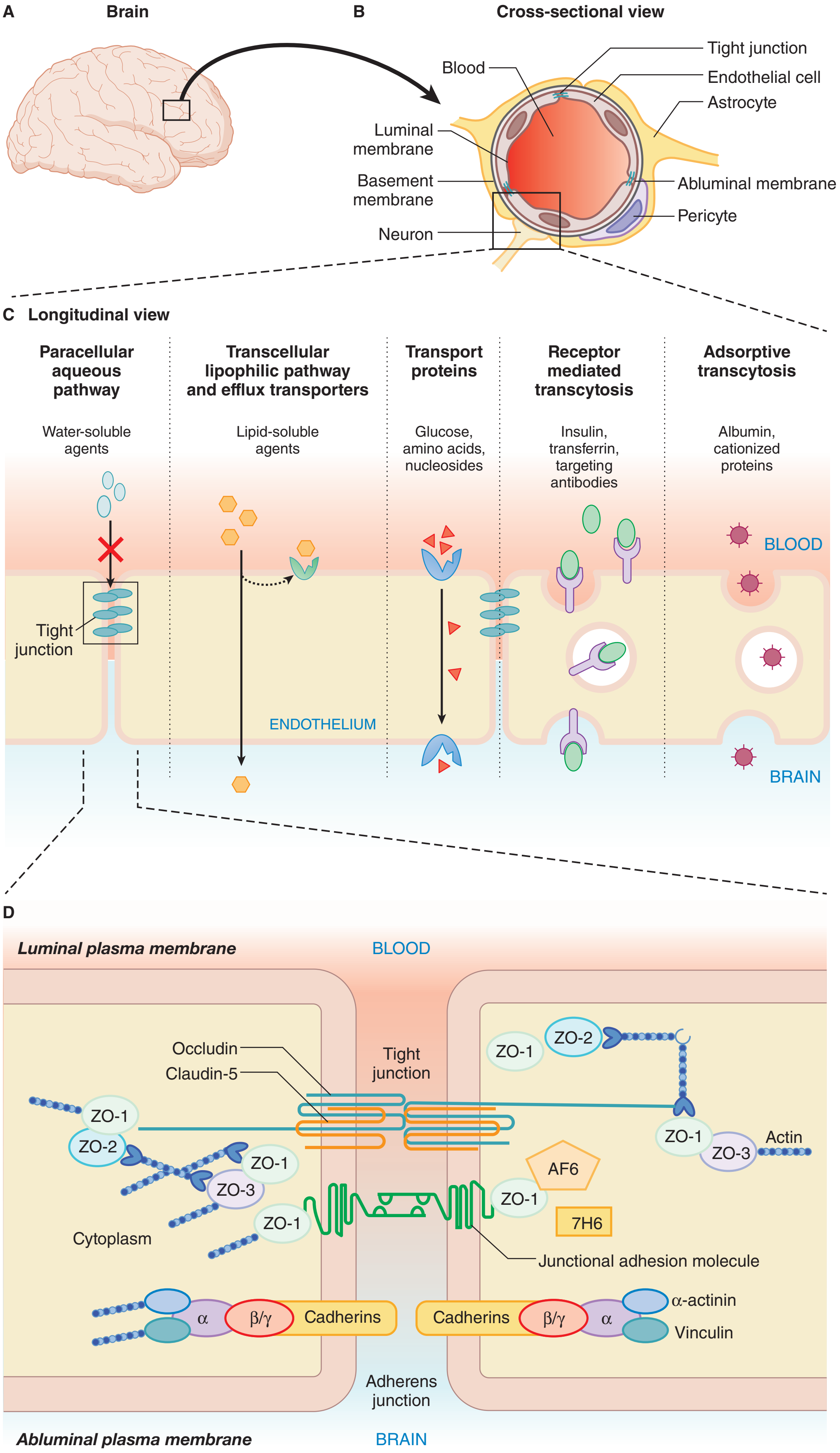
A
Brain
B
Cross-sectional view
Blood
Tight junction
Endothelial cell
Astrocyte
Luminal membrane
Basement membrane
Neuron
Abluminal membrane
Pericyte
C Longitudinal view
Paracellular aqueous pathway
Transcellular lipophilic pathway and efflux transporters
Transport proteins
Receptor mediated transcytosis
Adsorptive transcytosis
Water-soluble agents
Lipid-soluble agents
Glucose, amino acids, nucleosides
Insulin, transferrin, targeting antibodies
Albumin, cationized proteins
BLOOD
Tight junction
ENDOTHELIUM
BRAIN
D
Luminal plasma membrane
BLOOD
Occludin
Claudin-5
Tight junction
ZO-1
ZO-2
ZO-1
ZO-2
ZO-1
ZO-3
ZO-1
Cytoplasm
ZO-1
ZO-3
Actin
AF6
ZO-1
7H6
Junctional adhesion molecule
α
β/γ
Cadherins
Cadherins
β/γ
α
α-actinin
Vinculin
Adherens junction
Abluminal plasma membrane
BRAIN

Figure 17–2 *Cellular and molecular anatomy of the BBB.* The vasculature of the brain (**A**) possesses specialized barrier properties that are induced and maintained by perivascular cells of the neurovascular unit (**B**). The BBB has a specialized network of transporters and properties to support selective transport of nutrients and metabolites into and out of the CNS (**C**). Brain endothelial cell adherens junctions and tight junctions are comprised of a complex network of cytoskeletal and transmembrane proteins (**D**).

including P-glycoprotein (ABCB1/MDR1) and breast cancer resistance protein (ABCG2/BRCP) (Loscher and Potschka, 2005) (Figure 17–2). These ABC transporters are present on the luminal membrane and use energy derived from the hydrolysis of adenosine triphosphate (ATP) to pump a wide array of substrates that would otherwise passively diffuse across the membrane, up their concentration gradient, and back into the blood. These transporters are critical modulators of drug delivery as they limit the entry of many small-molecule therapeutics into the brain.

Solute Transporters

The paracellular and transcellular barrier properties allow CNS endothelial cells to greatly restrict the movement of nonspecific molecules; however, the underlying neural tissue still requires specific nutrients for cell survival and physiological function. Therefore, CNS endothelial cells express a variety of *solute transporters* that facilitate the transport of specific substrates into the brain, including GLUT1 (glucose), LAT1 (amino acids), MCT1 (lactate), MCT8 (thyroid hormone), and others. Although peripheral tissues also require these nutrients, these transporters are largely lacking on the surface of peripheral vascular beds since these small molecules can easily access the tissues through paracellular routes. Lack of specific transporters in the BBB can lead to distinct neurological syndromes including De Vivo disease (GLUT1 deficiency) (De Vivo et al., 1991), an autistic syndrome (LAT1 deficiency) (Tarlungeanu et al., 2016), and psychomotor retardation (MCT8 deficiency) (Vatine et al., 2017). In addition, CNS endothelial cells express a variety of unique metabolic and signaling systems that act to regulate the extracellular composition of the CNS.

Immune Modulation

CNS endothelial cells also have much lower levels of *leukocyte adhesion molecules* compared to endothelial cells in other tissues. Binding of immune cells to leukocyte adhesion molecules is a critical step in their entry into a tissue. This is a multiple-step process that involves initial binding to the endothelium through selectins (e.g., E-selectin, P-selectin), rolling on the endothelium, firm adhesion through binding of immunoglobulin superfamily members (e.g., ICAM1, VCAM1), followed by transendothelial migration (see Figure 38–5). The low levels of these adhesion molecules on CNS endothelial cells correlate with very low amounts of immune cell infiltration across a healthy BBB, as most CNS immune surveillance occurs in CSF compartments including the ventricles and subarachnoid space. However, in many neuroinflammatory diseases, such as stroke, encephalitis, and multiple sclerosis, the endothelial cells can become activated and upregulate these adhesion molecules, leading to immune cell infiltration into the CNS. Inhibition of immune cell interactions with VCAM1 using *natalizumab*, an anti-VlA4 antibody, is effective at limiting new neuroinflammatory lesion formation in patients with relapsing-remitting multiple sclerosis (Polman et al., 2006).

Regulation of the BBB

To improve CNS drug delivery, it is critical to understand how the properties of the BBB are regulated within brain endothelial cells. Although many properties of the BBB are possessed by the endothelial cells, these properties can be induced and maintained by interactions with other cells within the neurovascular unit. This was first identified in transplantation studies, where blood vessels gained barrier properties upon vascularizing transplanted brain tissue, whereas brain blood vessels were found to lose barrier properties upon vascularizing the gut (Stewart and Wiley, 1981).

BBB Induction

Genetic manipulations in rodent models have identified that Wnt/β-catenin signaling drives CNS angiogenesis, but not angiogenesis in peripheral tissues, as well as the induction of tight junctions and solute transporter expression in the newly formed CNS vessels, indicating that specific barrier properties are induced as part of the CNS-specific angiogenic program (Daneman et al., 2009; Liebner et al., 2008; Stenman et al., 2008). Wnt/β-catenin signaling is also required for maintenance of the BBB, as genetic inhibition of this pathway in endothelial cells in adults leads to a cell-intrinsic reduction in tight junction proteins and leakage of blood vessels to nonspecific molecules (Wang et al., 2012). The cellular source of Wnt signals appears to be neural stem cells and progenitors during the initial invasion of blood vessels into the CNS and other cells, including astrocytes and oligodendrocytes, later in development and through adulthood.

Pericytes and Astrocytes

Pericytes are also critical regulators of BBB properties, acting to limit the activated state of the endothelial cells. Genetic manipulations in mice that reduce pericyte coverage of CNS vessels lead to BBB leakage through an increase in nonspecific caveolin-mediated transcytosis as well as an increase in the expression of leukocyte adhesion molecules and thus the inflammatory state of the vessels (Armulik et al., 2010; Daneman et al., 2010). Astrocytes are also critical regulators of the barrier properties in endothelial cells: astrocyte-conditioned media can decrease paracellular permeability and increase endothelial efflux, and reactive astrocytes can regulate BBB repair following disease (Abbott et al., 2006; Bush et al., 1999). Therefore, BBB function is induced and maintained through a series of complex and coordinated cellular signals derived from neural progenitors, astrocytes and pericytes.

Heterogeneity of the BBB

Although the BBB is largely a property of the capillaries in the CNS, barrier properties are present throughout the vascular trees from the invading arterioles to the draining venules. It is critical that each branch possesses barrier properties, as leakage at any level of the vascular tree would disrupt CNS homeostasis. There is both molecular and functional heterogeneity along the different segments of the vascular tree with regulation of blood flow most prominent in the arterioles, transport properties most prominent in the capillaries, and immune cell interactions most prominent in the postcapillary venules (Aird, 2007a, 2007b; Vanlandewijck et al., 2018a).

In addition, there is regional heterogeneity of the BBB to differentially control the local environment of different brain regions. For example, the area postrema, the subfornical organ, the vascular organ of lamina terminalis, the median eminence, the pituitary neural lobe, and the pineal gland, together termed the circumventricular organs, lack BBB properties. Instead, the fenestrated vessels in these regions allow for diffusion of molecules between the blood and the neural tissue, which is critical to the neurosensory and neurosecretory function of these brain regions (Kaur and Ling, 2017). Tanycytes are cells that create borders around these regions providing a cellular barrier restricting the blood-derived molecules from entering neighboring brain regions. Less is known about whether there is heterogeneity of the BBB in brain regions that contain a functional BBB to meet the unique requirements of different brain regions. Interestingly, there are 6-fold differences in the uptake of *paliperidone*, a strong P-glycoprotein substrate, among different brain regions, indicating that regional heterogeneity of the BBB may be a critical modulator of drug uptake (Loryan et al., 2016).

Plasticity of the BBB in Health

Blood-brain barrier function is not static but can change at different stages of life and in response to different stimuli. During embryonic development, the earliest invading vessels have immature junctions, large amounts of transcytosis, and high levels of leukocyte adhesion molecules, and thus are leakier than mature blood vessels (Siegenthaler et al., 2013). In aging, there is a decrease in specific receptor-mediated transcytosis and an increase in nonspecific caveolin-mediated transcytosis; thus, the BBB less stringently regulates the extracellular environment of aged brains (Yang et al., 2020). The properties of the BBB can also be dynamically regulated by signals coming from both the brain and periphery. For example, neural activity can decrease barrier efflux (Pulido et al., 2020), likely coordinating detoxification of the brain during rest periods with other brain exit routes including the glymphatic system. Furthermore, diet has been shown to alter the components of brain endothelial cell tight junctions, thus modulating the paracellular permeability (Salameh et al., 2019). Hence, the BBB is not simply a static wall that protects that brain, but a dynamic component of the neural circuitry, changing in response to different stimuli. These are important principles for understanding how the BBB regulates brain homeostasis and acts as an obstacle for drug delivery.

AL GRAWANY

BBB in Disease

Blood-brain barrier dysfunction is observed in many neurological diseases, including stroke, multiple sclerosis, traumatic brain injury, epilepsy, and other neuroinflammatory and neurodegenerative diseases. This dysfunction can involve an increase in transcytosis, a loss of tight junction integrity, alterations in transporter expression, and an increase in the inflammatory state of the endothelial cells, which can lead to massive leakage of the BBB (Profaci et al., 2020). This leakage is often localized spatially to the region insult and temporally to specific pathophysiological stages of the injury and disease. For instance, in patients with multiple sclerosis, there is a leakage of the BBB, as measured by gadolinium enhancement on magnetic resonance imaging, at the location of neuroinflammatory lesions specifically at the onset of lesion formation. This leakage can be harnessed to target drug delivery to specific injured regions of the CNS within the specific time window of leakage (Miller et al., 1988).

Drug Delivery Across the BBB

Given the unique properties the BBB possesses, drug delivery across the BBB is a challenge. A circulating drug first encounters the endothelial glycocalyx, which reduces drug penetration by binding and sequestering molecules as they diffuse to the endothelial cell surface. If a drug molecule can penetrate the glycocalyx, it next needs to cross the barrier-forming endothelial cells of the BBB. There are several possible modalities for the trans-BBB passage of drug molecules (see Figure 17–2).

1. *Paracellular transport by diffusion between adjacent endothelial cells:* This process works for water-soluble molecules in peripheral vascular beds but is negligible at the BBB because of the high resistance tight junctions. Indeed, the introduction of small hydrophilic tracers into the bloodstream is often used as a measure of BBB integrity given their extremely low brain penetration. As a result of this physical barrier, drug penetration through the endothelial tight junctions does not result in therapeutic concentrations in the brain.
2. *Crossing plasma membranes:* Drug molecules can diffuse serially through the endothelial cell plasma membranes. For this process, drugs need to be small (<500–1200 Da) and rather lipophilic. In many cases, however, drugs having more lipophilic physiochemical properties are substrates for the efflux transporters P-glycoprotein, breast cancer resistance protein (BCRP), or members of the multidrug resistance protein (MRP) family. Thus, despite the cell-penetrable physiochemical properties, lipophilic drugs may not reach the brain because the active barrier of efflux transporters pumps drugs back to the bloodstream.
3. *Co-opting nutrient transporters:* For drugs that are large and/or hydrophilic, other delivery routes must be identified. One strategy takes advantage of the nutrient transporters expressed at the BBB. If a drug molecule is structurally similar to a nutrient, it may be possible for it to transport across the BBB using the endogenous transporter. For instance, the prodrug *levodopa* is structurally similar to phenylalanine, allowing it to enter brain endothelial cells via the large neutral amino acid transporter, after which it is subsequently metabolically converted to dopamine (Wade and Katzman, 1975). Unfortunately, steric limitations on such membrane carriers prevent their use with many drug cargoes. It is also important to note that since brain endothelial cells are polarized, the influx and efflux transporters can be found with differential abundance on the blood and brain side endothelial membranes, and this polarization may also impact the efficiency of trans-BBB delivery.
4. *Co-opting receptor-mediated transporters:* The endosomal trafficking network of brain endothelial cells can be targeted for drug delivery. Nonspecific fluid-phase endocytosis or pinocytosis and subsequent transcytosis are very limited at the BBB in healthy conditions, so drug uptake solely through this pathway is insufficient. However, the endosomal trafficking network of brain endothelial cells can be co-opted with carefully designed drug delivery constructs. For instance, protein cationization can lead to interactions with the brain endothelial cell surface and trigger the process known as adsorptive-mediated transcytosis. Alternatively, endothelial cell surface receptors that interact with endogenous large-molecule ligands like transferrin can be targeted using a variety of strategies, allowing conjugated drug molecules to cross the BBB and enter the brain.
5. *BBB opening:* Another option for crossing the BBB involves chemical or physical disruption of the tight junctions such that paracellular diffusion through the spaces between endothelial cells is enhanced. While this can increase drug uptake into the brain, it is inherently nonspecific in that other blood components can also enter the brain upon BBB disruption, potentially resulting in detrimental side effects.

Diffusion Into Brain

Once a drug leaves the brain endothelial cell, it also encounters the vascular basement membrane that surrounds endothelial cells and their associated pericytes and the glial limitans, an additional barrier formed by the extracellular matrix proteins secreted by astrocyte foot processes that wrap the brain vasculature. To reach neuronal or glial therapeutic targets, drugs must therefore diffuse through both the basal lamina and glial limitans before they can diffuse through the brain extracellular space. The brain extracellular space is estimated to have an average pore size of about 40 to 60 nm (Thorne and Nicholson, 2006), which could limit the transport of antibodies and nanoparticulate therapeutics.

Taken together, several routes exist for drug entry into the brain. The delivery of small-molecule therapeutics to the CNS by crossing plasma membranes is by far the most clinically advanced approach. With the continued development of protein and gene medicines (biologics) that are too large to transport through the endothelial cell membranes, there have also been significant recent efforts in the co-opting of receptor-mediated transport systems and leveraging of BBB opening to deliver biologics. The principles governing small-molecule transport into the brain are discussed below, followed by parallel efforts in developing brain delivery paradigms for biologics.

Small Molecules

When a drug is administered, independent of being given orally, intravenously, subcutaneously, or intramuscularly, it will first distribute into the bloodstream and from there out in the whole body. In all tissues and organs, there is further a distribution across the capillaries into the tissue and further into cells. As the brain constitutes around 2% of whole-body weight, most of the administered drug will distribute to the rest of the body, although clinically relevant concentrations can be obtained in the brain.

To enter the brain, the drug must cross the BBB. It is only the unbound drug molecules that can transverse the BBB. The molecules that are bound to plasma proteins cannot. Once inside the brain, it is only the unbound molecules that can interact with the target. Thus, what is nonspecifically bound to tissue components or to plasma proteins in the blood can be considered as an inactive reservoir in rapid equilibrium with the unbound, freely moving molecules. Therefore, measuring the unbound concentration is the most important way to understand membrane transport and drug action, without confounding the measurement with binding in plasma or to tissue components.

Drug distribution can be considered as a series of equilibria across barriers and between bound and unbound moieties of the drug. There is also an equilibrium between unionized and ionized drugs that is dependent on the pK_a of the drug and the pH at the specific site. In Figure 17–3, the equilibria relevant for drug transport across the BBB are depicted.

Rate and Extent of Transport Across the BBB

Transport across the BBB can have different rates depending on the drug's physicochemical properties in relation to those of the membrane. Also, different relationships can exist between the unbound concentrations in brain interstitial fluid (ISF) and plasma, due to the efflux and uptake transporter's efficiency. Thus, the rate and the extent of transport are two important aspects of transport across the BBB. These are two independent properties governing the pharmacodynamics of drug action in the brain. For anesthesia before surgery, drugs with rapid transport are sought after. It is also optimal that the anesthesia goes away quickly after surgery. For

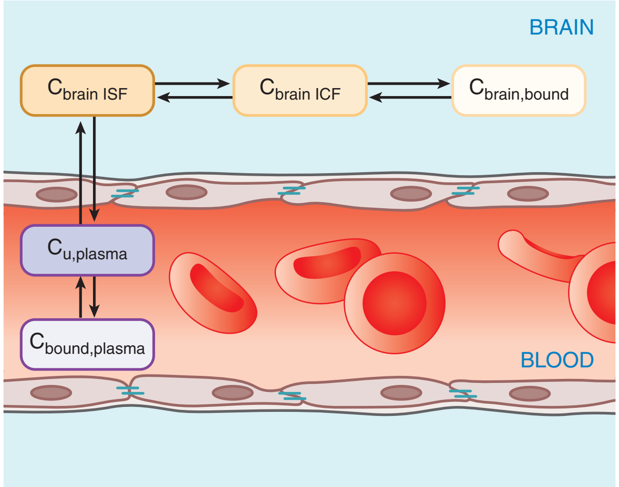

Figure 17–3 *Drug transport and equilibration across the BBB and into the brain parenchyma.* Drugs distribute in blood plasma between what is free and what is bound to plasma proteins. Only the free, unbound molecules can transverse the endothelial cell layer of the brain capillaries and reach the interstitial fluid (ISF) and go back into blood. Once inside the brain parenchyma, the drug equilibrates into cell intracellular fluid (ICF) and binds to cellular components. C describes the concentration.

other drugs that are taken daily for chronic diseases, the rate is not as important as the extent (i.e., how much drug enters the brain). Instead, we want high enough concentrations in the brain for action over time. Therefore, even though the transport across the BBB may be slow, a drug may still enter in enough quantities to be valuable. The extent of transport is therefore the most relevant property for drugs given chronically.

From Figure 17–3, it is clear that if a drug has a constant concentration in plasma and the passage across the BBB is passive (i.e., not influenced by any transporters either hindering or helping uptake into the brain [influx clearance = efflux clearance]), the unbound concentrations will be the same on both sides of the BBB at steady state. If transporters at the BBB hinder uptake (influx clearance < efflux clearance), the concentration in brain ISF will never reach as high levels as those in plasma. Conversely, if transporters in the BBB pick up drug from plasma (influx clearance > efflux clearance), the brain ISF concentration will become higher than those in plasma. This is illustrated in Figure 17–4. The ratio between brain ISF and unbound plasma concentrations is named $K_{p,uu,brain}$ (the partition coefficient of unbound drug across the BBB or, expressed differently, the concentration ratio of unbound drug between brain ISF and plasma) (Gupta et al., 2006; Hammarlund-Udenaes et al., 2008). Depending on the efficiency of an efflux transporter to hinder uptake, the ratio can be somewhat or much smaller than unity. The most potent efflux transporter for small molecular drugs is P-glycoprotein. Conversely, if a drug is actively taken up into the brain at the BBB, the higher the efficiency of uptake transport, the higher is the brain ISF concentration. Glucose enters the brain using the glucose uptake transporter GLUT1, but as glucose is rapidly consumed in the brain, the concentration is not generally higher than in plasma. Drugs are normally not metabolized in brain tissue. Therefore, their transport properties will more directly influence their concentration in the brain versus that in plasma.

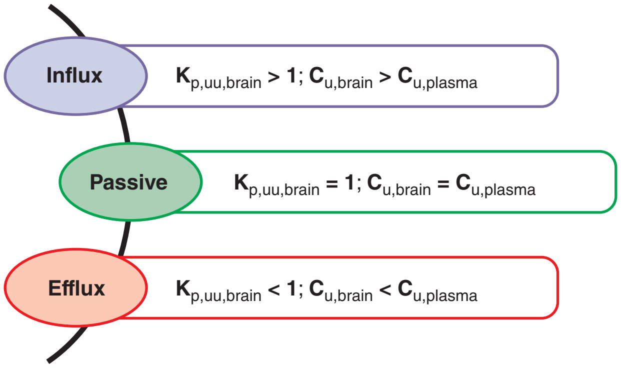
Influx
$K_{p,uu,brain} > 1$; $C_{u,brain} > C_{u,plasma}$
Passive
$K_{p,uu,brain} = 1$; $C_{u,brain} = C_{u,plasma}$
Efflux
$K_{p,uu,brain} < 1$; $C_{u,brain} < C_{u,plasma}$

Figure 17–4 *Drugs equilibrate across the BBB in three ways depending on the activity of transporters, as described with $K_{p,uu,brain}$, the partition coefficient of drug concentrations across the BBB between unbound in brain ISF and unbound in plasma at steady state.* When efflux transporters like P-glycoprotein hinder transport into the brain, the unbound brain ISF concentration ($C_{u,brain}$) will never reach the same concentration as that in plasma ($C_{u,plasma}$) and $K_{p,uu,brain}$ will be lower than unity. When drug can transverse the BBB freely, the unbound concentrations will be similar in brain ISF and plasma, and when there are active uptake transporters acting on the drug, the concentration in brain ISF will be higher than that in plasma, and $K_{p,uu,brain}$ will be above unity.

$K_{p,uu,brain}$ can be expressed as a concentration ratio and as a ratio of clearances across the BBB:

$$K_{p,uu,brain} = \frac{C_{u,brain}}{C_{u,plasma}} = \frac{CL_{in}}{CL_{out}} \qquad \text{(Equation 17–1)}$$

where CL_{in} is the net influx clearance at the BBB from plasma to brain ISF and CL_{out} is the net efflux clearance from brain ISF to plasma (see Figure 17–4). The unit for clearance is μL/min/g brain.

In general, many more drugs are effluxed at the BBB than taken up actively. Examples of drugs on the market and their $K_{p,uu,brain}$ values are depicted in Figure 17–5. Measuring $K_{p,uu,brain}$ is so far done only in preclinical studies (Loryan et al., 2014). The values given in Figure 17–5 are therefore mainly from rat studies. Humans have a less "efficient" P-glycoprotein function at the BBB; thus, drug delivery to the brain may be higher in humans than in rodents (Syvanen et al., 2009; Uchida et al., 2020).

$K_{p,uu,brain}$ values of drugs provide important information for determining drug action in the CNS. For therapeutic targets in the CNS, drug developers should aim at higher $K_{p,uu,brain}$ values. To minimize CNS side effects, it should be low. At the same time, drug potency needs to be considered.

$K_{p,uu,brain}$ values can vary drastically within a drug class and can determine their therapeutic actions. Opioids are a good example: *Loperamide* is a strong P-glycoprotein substrate that is very efficiently effluxed. Its $K_{p,uu,brain}$ is less than 0.01, indicating that less than 1% equilibrates across the BBB. In other words, more than 99% is kept out of the brain by the BBB. On the other hand, *morphine* is only mildly effluxed out of the brain ($K_{p,uu,brain} = 0.3$), and *oxycodone* is actively taken up at the BBB ($K_{p,uu,brain} = 3$), reaching 3-fold higher concentration in the brain than plasma. Hence, the BBB actions determine the clinical use of these opioids: *Loperamide* can be used for diarrhea without effects from the CNS, while *morphine* and *oxycodone* are centrally acting analgesics. *Oxycodone* also shows much faster uptake into the brain than *morphine* (Bostrom et al., 2006, 2008). Although *morphine* and *oxycodone* have a 10-fold difference in their extent of BBB transport, they are both active in the CNS, as other pharmacokinetic factors are also contributing, including the dose that is used to give a clinically relevant effect.

Another class of drugs with varying brain delivery is the antihistamines. The newer generation antihistamines, like *cetirizine* or *loratadine*, are all significantly effluxed, thereby causing much less central side effects such as sedation. On the other hand, *diphenhydramine* is actively taken up at the BBB with a $K_{p,uu,brain}$ of 5 (i.e., five times higher concentrations in brain ISF than unbound in blood), explaining why it causes much more sedation. *Diphenhydramine* in fact has the highest active brain uptake of all drugs measured thus far.

Intrabrain Distribution

After traversing the BBB, the drug enters the brain parenchyma and distributes in the ISF and cells (Figure 17–6). The concentration in brain ISF is the driving force for further distribution. The distribution can involve passive diffusion and binding as well as active uptake into or efflux from cells. Intrabrain distribution can be described as the ratio of total amount of drug in the brain parenchyma to unbound drug concentration in brain

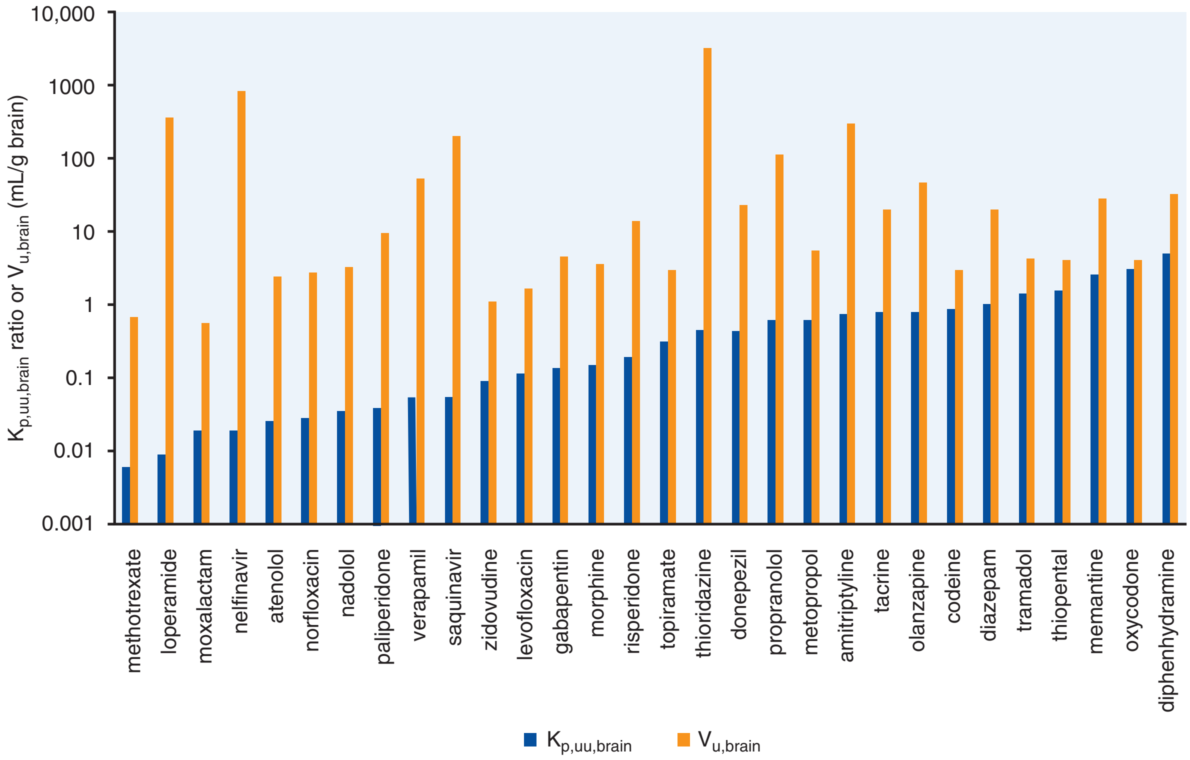

Figure 17–5 *Examples of how different drugs partition across the BBB and how they distribute in the brain parenchyma, showing that the two properties are not at all related.* This is described by the partition coefficient of unbound drug in brain ISF to that in plasma $K_{p,uu,brain}$ (blue) and the intrabrain distribution parameter $V_{u,brain}$ (orange), the unbound volume of distribution of drug in the brain, estimated as the ratio of the total amount of drug per gram of brain divided by the unbound concentration in brain ISF (mL/g) (describing both binding and/or intracellular distribution).

ISF, also called $V_{u,brain}$ (the unbound volume of distribution in the brain, expressed in mL/g brain). Approximating that 1 g brain equals 1 mL, this can be considered as a ratio, where the unbound volume of distribution in the brain $V_{u,brain}$ (mL/g brain) is described as:

$$V_{u,brain} = \frac{A_{tot,brain} - V_{blood\ in\ brain} \bullet C_{tot,blood}}{C_{u,brainISF}} \qquad \text{(Equation 17–2)}$$

$A_{tot,brain}$ is the total amount of drug in the brain per gram of brain. As there is also blood in the brain capillaries, this amount needs to be subtracted from the total amount in the brain to obtain a correct value. Therefore, $V_{blood\ in\ brain}$ is the physiological volume of blood in the brain, and $C_{tot,blood}$ is the total concentration of drug in the blood present in the brain. The blood volume in brain is 3% or less, depending on the sampling technique. $C_{u,brainISF}$ is the unbound concentration of drug in the brain ISF.

The values found so far of $V_{u,brain}$ range from 0.6 mL/g brain for *moxalactam* to 3300 mL/g brain for *thioridazine* (see Figure 17–5). The higher the value, the more the drug is bound to or distributed into cells than what is present in brain ISF. Some drugs, generally the more lipophilic ones, distribute and bind extensively to brain tissue components, as the brain parenchyma is also lipophilic in nature. Thus, BBB transport and brain drug binding are two independent parameters. A drug can have a very low BBB transport but quite extensive distribution and binding in the brain parenchyma (see Figure 17–5). The opposite is also possible (i.e., a drug can have a rather high BBB transport but not so extensive binding in brain). For example, the antiarrhythmic agent *verapamil* has a $K_{p,uu,brain}$ of 0.05 and a $V_{u,brain}$ of 54 mL/g brain, the antiviral drug *nelfinavir* has a $K_{p,uu,brain}$ of 0.02 and a $V_{u,brain}$ of 860 mL/g brain, while *diazepam* has a $K_{p,uu,brain}$ of unity, thus being mainly passively transported, and a $V_{u,brain}$ of 20 mL/g brain (i.e., 20 times more bound and/or distributed than unbound in brain). Drugs that do not enter cells to any significant degree have $V_{u,brain}$ values below unity. This is the case for *methotrexate* due to its high hydrophilicity.

Thus, when only total brain concentrations are measured, a candidate for further development may erroneously be selected that has high total brain concentrations but much lower unbound concentrations. Thus, if the high concentration is due to high binding, the part that is active (i.e., the unbound part) is much lower. Over the last decades, this has resulted in selection of many unsuccessful candidate drugs.

Intracellular Distribution

Drugs are taken up into cells due to pH partitioning as well as active uptake or efflux from the brain parenchymal cells (see Figure 17–6). In this context, cells of different types are averaged to a "typical" cell, independent of whether they are neuronal cells or glial cells. Of course, the distribution into these different cell types may differ, something that can be studied in cell cultures. The purpose here is to obtain an overview of how drugs are generally distributed.

Due to a lower intracellular pH, especially in lysosomes and other acidic organelles, basic drugs tend to accumulate in brain parenchymal cells, whereas acidic drugs do not. Basic drugs therefore tend to cause more side effects due to their accumulation in lysosomes, so-called lysosomotropism.

The ratio of unbound drug between intracellular and interstitial fluids is called $K_{p,uu,cell}$ (see Equation 17–3). As for the BBB, a ratio around unity indicates mainly passive transport, values below unity indicate reduced uptake into cells, and values above unity indicate accumulation into the brain parenchymal cell.

$$V_{p,uu,cell} = \frac{C_{u,cell}}{C_{u,brainISF}} \qquad \text{(Equation 17–3)}$$

where $C_{u,cell}$ is the average unbound concentration in cells and $C_{u,brainISF}$ is the concentration in brain ISF.

The equilibration of drug between the brain ISF and the average brain intracellular compartment, $K_{p,uu,cell}$, is a separate property from the overall binding and distribution to brain parenchyma ($V_{u,brain}$) (Figure 17–7).

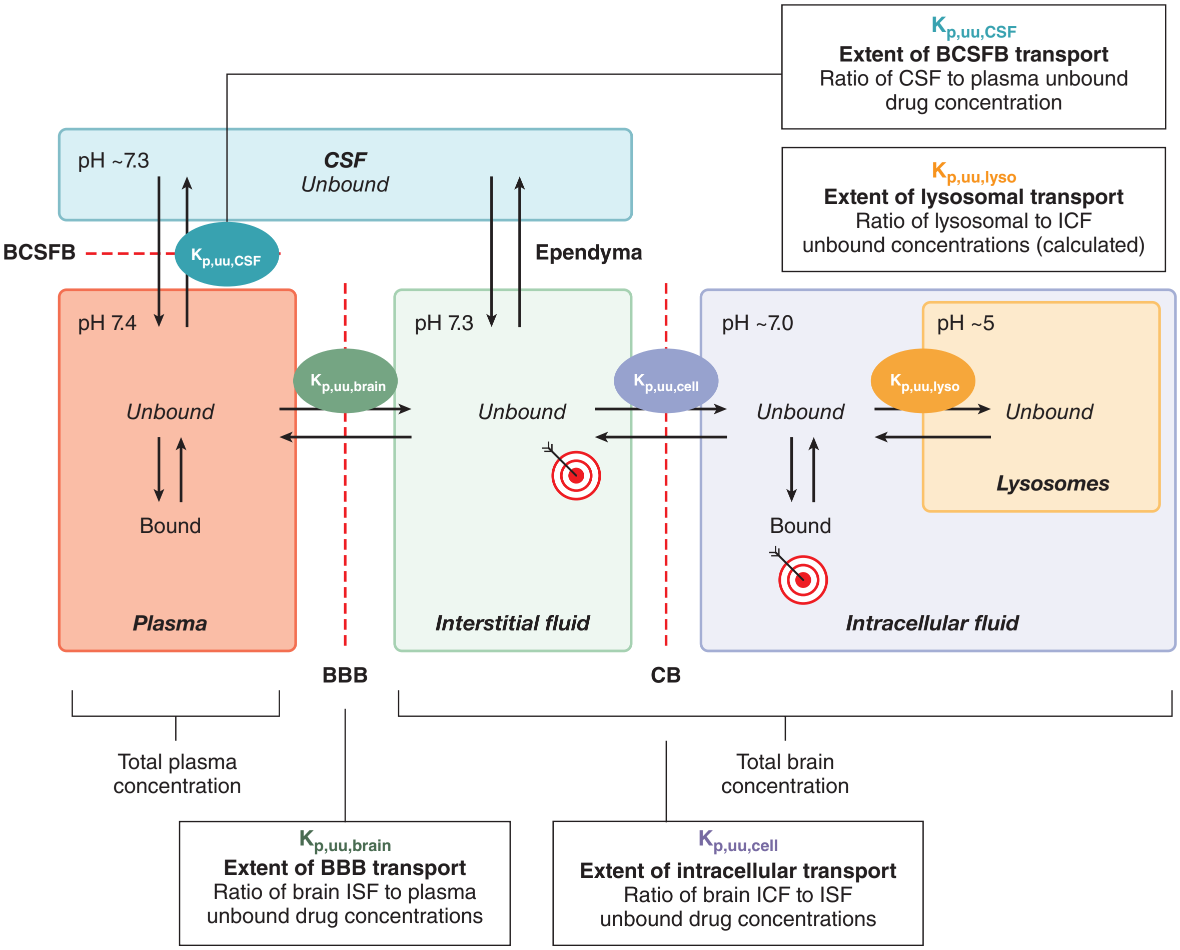
$K_{p,uu,CSF}$
Extent of BCSFB transport
Ratio of CSF to plasma unbound drug concentration
pH ~7.3
CSF
Unbound
BCSFB
$K_{p,uu,CSF}$
Ependyma
$K_{p,uu,lyso}$
Extent of lysosomal transport
Ratio of lysosomal to ICF unbound concentrations (calculated)
pH 7.4
Unbound
Bound
Plasma
$K_{p,uu,brain}$
pH 7.3
Unbound
Interstitial fluid
$K_{p,uu,cell}$
pH ~7.0
Unbound
Bound
Intracellular fluid
pH ~5
$K_{p,uu,lyso}$
Unbound
Lysosomes
BBB
CB
Total plasma concentration
Total brain concentration
$K_{p,uu,brain}$
Extent of BBB transport
Ratio of brain ISF to plasma unbound drug concentrations
$K_{p,uu,cell}$
Extent of intracellular transport
Ratio of brain ICF to ISF unbound drug concentrations

Figure 17–6 *Schematic representation of the equilibration of drug across the brain and cellular barriers (CB) and their representative parameters.* To be noted is the pH difference between the different compartments, influencing basic and acidic drugs to distribute differently, with basic drugs tending to accumulate into acidic lysosomes. ICF, intracellular fluid.

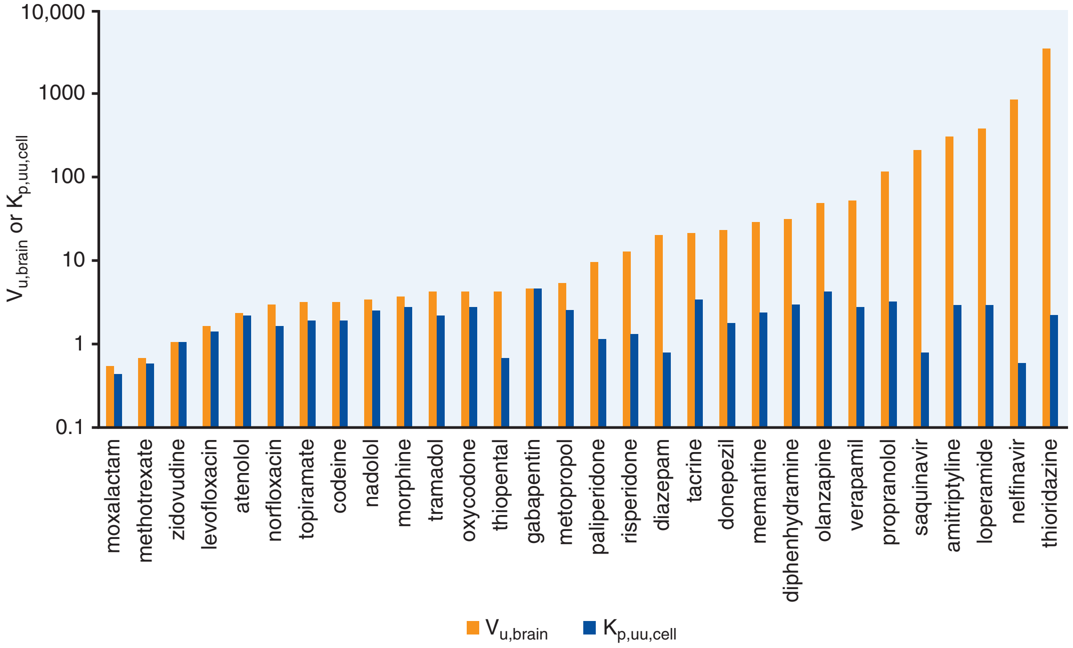

Figure 17–7 *Examples of how drugs distribute and bind in the brain parenchyma on average ($V_{u,brain}$) and their cellular partitioning ($K_{p,uu,cell}$), showing no relationship between the two.* This indicates that they are independent properties, where some drugs are accumulating into cells ($K_{p,uu,cell} > 1$) and others are not ($K_{p,uu,cell} = 1$ or < 1).

The binding is more governed by lipophilicity, while the cellular distribution, as described with $K_{p,uu,cell}$, is governed by other processes including pH partitioning. For example, compared to *methotrexate* with a $K_{p,uu,cell}$ of 0.6, the antidepressant *amitriptyline* with a $K_{p,uu,cell}$ of 2.9 accumulates intracellularly to much higher levels (Figure 17–7). A special case is *gabapentin*, which does not bind to cellular components, as shown by a $V_{u,brain}$ of close to unity, but exhibits a $K_{p,uu,cell}$ of 5. The likely explanation is the presence of an active uptake mechanism for *gabapentin* at the cellular barrier.

Whether drug concentrations in a given brain compartment are relevant for drug effects is dependent on the potency of the drug together with where the target is situated, be it toward brain ISF or cytosol or in the nucleus (see Figure 17–6). The same goes for side effects, as mentioned earlier with lysosomotropism.

CSF Versus Brain ISF Concentrations of Drugs

Cerebrospinal fluid is used as a surrogate site for measuring unbound drug brain concentrations in humans, as there is no other way of measuring unbound brain concentrations directly. Lumbar puncture is used to collect CSF. CSF has very little protein in healthy individuals, meaning that CSF drug concentrations are largely unbound. However, in disease, the protein concentration in CSF may increase. To understand the relationship of drug concentrations in CSF versus brain ISF, anatomical and physiological comparison of CSF versus brain ISF production and relation to plasma concentrations of the drugs must be considered. As discussed earlier, CSF is mainly produced at the BCSFB, in the choroid plexus (see Figure 17–1), while the main origin of brain ISF comes from the BBB. Transporter expression and hence active transport at the BBB are somewhat different than at the BCSFB. Thus, CSF drug concentrations may differ from those in brain ISF. For example, the transporter MRP2 is present at the BCSFB but not at the BBB (Gazzin et al., 2008). Nevertheless, the few studies that have been performed *in vivo* indicate that CSF concentrations are a reasonable estimate of ISF concentrations for most drugs (Friden et al., 2009).

Drug Interactions at the BBB

Drug-drug interactions could be expected since the transporter activity is high at the BBB. One might envision that two drugs that are both substrates for P-glycoprotein may compete with each other, resulting in higher brain concentrations of both drugs. However, to date, no such interaction has been reported. A likely explanation is that the plasma concentrations that the BBB "sees" are quite low in relation to the capacity of the transporters to handle the drug transport (i.e., the transport is not saturated). This contrasts to the liver and the gastrointestinal tract, where often much higher drug concentrations are present that can saturate transport systems (see Chapters 2 and 5 for details). In that regard, *in vitro* cell studies using much higher concentrations than those attained *in vivo* may suggest potential drug-drug interactions that are not observed *in vivo*.

Methods to Study BBB Transport

To measure BBB transport of drugs, the gold standard is microdialysis, where a microdialysis catheter is placed in a specific brain region and concentrations there are compared with unbound plasma concentrations (Chaurasia et al., 2007). Unfortunately, the method cannot be used for many drugs that bind avidly to plastic material in the catheter. Useful for those drugs is a combination of several methods, called the "combinatory mapping approach" (Loryan et al., 2014). Here, the total brain and plasma concentrations are measured at steady state, combined with plasma protein binding and brain tissue binding with the brain slice and brain homogenate techniques. These three measurements together will give the $K_{p,uu,brain}$:

$$K_{p,uu,brain} = \frac{C_{tot,brain}}{C_{tot,plasma} \cdot V_{u,brain} \cdot f_{u,plasma}} \qquad \text{(Equation 17–4)}$$

$C_{tot,brain}$ and $C_{tot,plasma}$ are the corresponding total concentrations at steady state, and $f_{u,plasma}$ is the unbound fraction of drug in plasma, measured with equilibrium dialysis of plasma versus buffer at pH 7.4. $V_{u,brain}$ is measured with the brain slice technique (Loryan et al., 2013), alternatively as a surrogate with the brain homogenate method, where $f_{u,brain}$ (unbound fraction of drug in brain homogenate) ≈ $1/V_{u,brain}$. However, the brain homogenate lacks pH partitioning and active membrane transport due to the homogenization of the tissue cells, which reduce the quality of the measurement.

To measure intrabrain distribution, the brain slice and the brain homogenate methods can be combined, resulting in $K_{p,uu,cell}$ (Equation 17–5). If the pK_a of a drug is known, it is also possible to estimate the partitioning between lysosomal and intracellular compartments, resulting in $K_{p,uu,lyso}$ (Figure 17–6).

$$K_{p,uu,cell} = \frac{C_{u,cell}}{C_{u,brain\ ISF}} = V_{u,brain} \cdot f_{u,brain} \qquad \text{(Equation 17–5)}$$

Positron emission tomography (PET) is a noninvasive method that can measure brain concentrations in humans. However, it measures total radioactivity and thereby both bound and unbound drug, as well as metabolites. Ongoing research attempts to translate PET measurements to unbound drug concentrations, which would give much more information on how humans are handling drugs at the BBB (Gustafsson et al., 2019).

Biologics

The BBB properties of tight junctions and limited pinocytosis severely restrict the uptake of large-molecule biologics into the brain. For example, only 0.03% to 0.1% of monoclonal antibodies administered intravenously reach the brain (Jones and Shusta, 2009), and thus, therapeutic concentrations are rarely achievable even under high dosing regimens (e.g., 20–50 mg/kg). Hence, biologics such as protein, DNA, and nanoparticles must use alternative routes for brain entry. Such routes include targeting the endosomal trafficking network of brain endothelial cells via adsorptive or receptor-mediated transcytosis where appropriately targeted drug cargo can engage with an endothelial cell receptor and piggyback across the BBB into the brain. For adsorptive or receptor-mediated processes, the steps for crossing the BBB include (1) binding to the endothelial cell surface via nonspecific charge interactions (adsorptive) or by targeting a specific BBB receptor (receptor-mediated); (2) inducing the formation and endocytosis of vesicles; (3) trafficking through the endothelial cell; (4) exocytosis; and (5) therapeutic release at the basolateral membrane (Figure 17–8). Alternatively, pathological BBB disruption or BBB disruption through chemical or physical means can be leveraged to increase brain entry of blood-borne biologics.

Quantifying Brain Uptake of Biologics

The critical measurement to understand potential therapeutic efficacy is the drug concentration that can be achieved in brain tissue. Initial rate pharmacokinetic analyses can allow the comparison of biologic delivery strategies as they relate to BBB permeability, the property that ultimately determines drug concentrations reached in brain. As is the situation for biologics, where the BBB permeability is relatively low compared with blood flow (Bickel, 2005), initial rate pharmacokinetics indicate that the brain uptake (%ID/g) is a function of the BBB permeability surface area (PS) product and the area under the plasma concentration curve (AUC):

$$\% = \frac{ID}{g} = PSxAUC \qquad \text{(Equation 17–6)}$$

where %ID/g is the percentage of the injected dose delivered to brain per gram of brain, PS is the BBB permeability surface area product (μL/min•g), and AUC is the area under the plasma concentration curve (%ID•min/μL).

As seen from Equation 17–6, the brain uptake will be directly proportional to the BBB permeability of the biologic through the PS product.

Thus, the PS product is instructive for comparing various strategies for crossing the BBB. The PS product is a lumped parameter describing the entire BBB transport process from binding to the brain endothelial cell to release into the brain (steps 1–5 in Figure 17–8). Importantly, the PS products for different targeting approaches and transport systems are not equivalent due to differences in receptor density and the percentage of internalized receptors that undergo transendothelial transport rather than recycling or being targeted to the lysosome (see Figure 17–8). In addition, brain uptake depends on the dose and systemic clearance properties of the molecule, quantified as the area under the brain concentration curve (AUC). Importantly, the AUC can be influenced by the brain specificity of the biologic targeting strategy. An ideal biologic delivery system would combine high trans-BBB transport that approaches or exceeds the natural ligand (Table 17–1) with high brain specificity and reduced clearance

TABLE 17–1 ■ TRANSPORT OF ANTIBODIES AND NATURAL LIGANDS AT THE BBB

	PS PRODUCT [mL/g-s × 10^6] (% ID/brain)	TRANSPORTER (MECHANISM)	ANIMAL	REFERENCE
Human IgG	0.062 (ND)		Rat	[1]
Albumin[a]	0.097 {0.062}[b] (ND)		Rat	[1]
Tf[a]	2.432 {0.062}[b] (ND)	TfR (RMT)	Rat	[1]
Insulin[a]	18.50 {0.062}[b] (ND)	IR (RMT)	Rat	[1]
Cationized IgG	9.5 (ND)	NS (AMT)	Rat	[2]
Cationized D146 MAb	ND (0.07) {0}	NS (AMT)	Mouse	[3]
MAb OX26	27 (0.44) {0}[c]	Rat TfR (RMT)	Rat	[4], [5]
MAb OX26	0.77 (0.03)[c]	Rat TfR (RMT)	Mouse	[6]
Mab RI7-127	20 (0.8)[c]	Mouse TfR (RMT)	Mouse	[6]
MAb 8D3	25 (1.5)[c]	Mouse TfR (RMT)	Mouse	[6]
MAb 128.1	ND (0.3) {0.06}	Human TfR (RMT)	Monkey	[7]
MAb Z35.2	ND (0.2) {0.03}	Human TfR (RMT)	Monkey	[7]
MAb 83-14	88–90 (2.5–3.8) {0.06}	HIR (RMT)	Monkey	[8, 9]
Chimeric MAb 83-14	28 (2)	HIR (RMT)	Monkey	[9]
Humanized MAb 83-14	ND (1)	HIR (RMT)	Monkey	[10]

MAb, monoclonal antibody; ND, not determined or not available in reference; NS, nonspecific; Tf, transferrin; TfR, transferrin receptor; HIR, human insulin receptor; RMT, receptor-mediated transcytosis; AMT, adsorptive-mediated transcytosis.

[a] Average PS product from various brain regions, determined in referenced article.

[b] Numbers in {} are values for isotype control antibodies and are indicators of general antibody permeability.

[c] Values were calculated from reported %ID/g, assuming representative mass of animal brain (100 g for monkey, 1 g for rat, and 0.5 g for mouse).

Source: Reproduced with permission from Jones AR, Shusta EV. Antibodies and the blood-brain barrier. In: An Z, ed. *Therapeutic Monoclonal Antibodies: From Bench to Clinic.* John Wiley & Sons, New York, **2009**. Copyright © 2009 by John Wiley & Sons, Inc. All rights reserved.

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(increased AUC). Thus, throughout the sections below, PS product and AUC are used as means for comparison of different strategies for biologic delivery to brain.

Confirming Brain Exposure of Biologics

Although kinetic approaches are accurate measures for brain delivery, they are based on whole-brain uptake assays that include both the vasculature and the brain components. Hence, any biologic that is bound to or internalized into brain endothelial cells (e.g., Figure 17–8) but not yet transported fully into brain will be included in these measures. Importantly, when using transcytosis-based delivery approaches, the fraction of delivered therapeutic that can be "trapped" in the vasculature and not available for brain exposure can be substantial. Several measurement methods have been introduced to circumvent this challenge.

A method known as capillary depletion has been developed to separate the vascular component from the parenchymal component. Since the BBB is surrounded by a robust basement membrane, it is possible to separate the microvasculature from brain using mechanical homogenization and density centrifugation techniques. In this way, antibody that remains associated with the vasculature can be distinguished from that which has transcytosed fully into the parenchymal fraction. However, homogenization techniques can lead to a large fraction of negative control antibodies appearing to accumulate in the parenchymal fraction. To help circumvent this issue, parenchymal uptake time course measurements can be performed (Pardridge et al., 1991).

Immunocytochemical techniques have been used to qualitatively demonstrate parenchymal uptake of biologics into brain at microscopic resolution. However, when a biologic leaves the concentrated trafficking vesicles in endothelial cells and enters brain, it undergoes a roughly 1000-fold dilution. Thus, unless the biologic also subsequently targets a receptor on brain cells that allows it to reconcentrate, it often escapes detection. Autoradiographic techniques can be more sensitive and allow for semiquantitative uptake analyses, but at much lower resolution, such that distinguishing between vascular and parenchymal contributions can be difficult. The final strategy for measuring the quantity of biologic that transcytoses and enters brain is to monitor its accumulation in the CSF (Haqqani et al., 2013). While sampling of CSF has long been used as a surrogate measure of brain uptake, one has to distinguish the BBB and choroid plexus routes of entry into the CSF to avoid overestimating the amount of biologic crossing the BBB and leading to brain exposure.

Researchers have also developed pharmacodynamic approaches to quantify brain uptake of biologics that rely on the functional output of the biologic. For example, putative transcytosing antibodies have been connected to drug cargo that allows for straightforward phenotypic readouts in animal models. A widely used model involves the conjugation of a BBB-crossing antibody with an antiamyloid therapeutic; a decrease in the amyloid burden in a transgenic mouse is used as a surrogate measure of full BBB crossing of the therapeutic at pharmacologically relevant concentrations. Another strategy is based on delivery of the neuropeptide neurotensin. Neurotensin causes transient hypothermia, changes in locomotion, and changes in pain response if directly administered into the CNS, whereas blood-borne neurotensin cannot cross the BBB to elicit these effects. However, if neurotensin is conjugated to a BBB-crossing domain, it can accumulate in the CNS, causing pharmacodynamic responses, again demonstrating full transcytosis of the biologic at pharmacologically relevant doses (Demeule et al., 2014).

Adsorptive-Mediated Strategies

One approach for delivery of biologics is to modify the molecules themselves such that they can access the adsorptive-mediated transport network of brain endothelial cells. Cationization of a biologic can allow for its interaction with negatively charged moieties on the endothelial surface such as sialylated proteins and heparan sulfate proteoglycans. Such engagement can lead to membrane invagination and entry into the endosomal trafficking system of brain endothelial cells. In this way, biologics can transport into and across brain endothelial cells, leading to increased brain uptake. Cationization is often achieved by modifying carboxyl residues on the protein surface, such as through the conjugation of putrescine or spermine (Poduslo and Curran, 1996). To rate the efficiency of various BBB-crossing approaches for biologics, the resulting PS product can be compared. For instance, cationized antibodies had 4- to 100-fold higher PS products compared with control antibodies (see Table 17–1). While cationization can increase brain uptake, cationization will also increase interactions with vascular beds and cells throughout the body, leading to off-target drug accumulation. In addition, broad organ uptake can also reduce the AUC of circulating biologics, thereby limiting brain uptake (%ID/g) despite improved PS attributes.

Receptor-Mediated Strategies

Since adsorptive strategies are inherently nonspecific, alternative strategies have been developed that target specific receptor-mediated transcytosis (RMT) receptors at the BBB. Brain endothelial cells express a host of RMT receptors that bring large-molecule cargo into the brain. These include the transferrin receptor, insulin receptor, and low-density lipoprotein family receptors. Conceptually, RMT systems can be co-opted for brain drug delivery by using a natural or artificial targeting ligand conjugated to the drug of interest. The drug-ligand conjugate can bind to RMT receptors on the blood side of the brain endothelial cells and trigger endocytosis and trafficking, and a subset of the trafficked material can undergo full transcytosis. In this way, conjugated drug cargo can be shuttled across the BBB and into brain (see Figure 17–8). The transport properties of the targeting ligands can be further optimized through the modulation of targeting ligand binding affinity and the number of binding sites per ligand (valency). Several RMT-based strategies have recently moved to the clinic but are not yet at the point of FDA approval, as will be described below.

Transferrin Receptor

The transferrin receptor (TfR) was the first RMT system explored for delivery of biologics across the BBB (Pardridge et al., 1991), and it is still the most commonly used system. The TfR is highly abundant in the brain vasculature. TfR is responsible for transporting iron into the brain by mediating the trafficking of the iron-binding protein transferrin (Tf). The TfR has been targeted by conjugating therapeutic cargo to Tf itself. For example, Tf has been conjugated to monoclonal antibodies and various forms of nanoparticulate cargo, including pegylated albumin nanoparticles and pegylated liposomes, with the potential to increase brain uptake severalfold. Alternative strategies have employed iron-mimicking peptides that bind to Tf and ferry across the BBB with conjugated drug cargo. Targeting RMT receptors with the natural ligand-drug conjugate has a substantial drawback because to achieve transport of the desired drug conjugate, it needs to compete successfully with the endogenous natural ligands. For example, endogenous Tf is present at high concentrations in the bloodstream; hence, any Tf-drug conjugates will need to compete with this Tf pool for RMT binding and transport, necessitating high doses. Thus, research teams have instead been developing antibodies capable of targeting RMT receptors using epitopes that do not overlap with the natural ligand. In this way, the antibodies will not compete with endogenous ligand, which can help with delivery efficiency and potentially reduce side effects by not interfering with normal nutrient transport. Such antibodies raised against the TfR have elevated PS products by 20- to 30-fold that meet or exceed those of the natural ligand, leading to %ID in the 0.5% to 2% range (see Table 17–1). Targeted antibody strategies yield PS products that can exceed those of cationized antibodies by severalfold. Brain exposure of anti-TfR antibodies can reach actual brain concentrations as high as 50 nM with antibody optimization and elevated doses of 20 to 50 mg/kg (Yu et al., 2011). As such, TfR antibodies have been used to deliver a wide range of therapeutic cargo to animal models (e.g., delivery of antiamyloid antibodies in mouse and primate models of Alzheimer's disease). Anti-TfR–based biologics have already moved into clinical development. For example, conjugates of anti-TfR antibodies with lysosomal enzymes are being tested in patients with lysosomal storage diseases like Hunter syndrome with early evidence of efficacy (Okuyama et al., 2019) (JCR Pharmaceuticals NCT04573023 and Denali NCT04251026).

Insulin Receptor

The insulin receptor (IR) has also been targeted for biologics delivery to brain. The IR at the BBB mediates brain import of insulin by an RMT mechanism. Targeting this transport system using the endogenous insulin ligand has not been pursued owing to the very short serum half-life of insulin and concerns that insulin-linked drugs could cause hypoglycemia. Thus, monoclonal antibodies against IR have been tested for their potential as RMT-targeting agents. The most widely used anti-IR antibody is a mouse antibody known as 83-14 that targets the human IR (Pardridge et al., 1995). This antibody has a PS product that is 4-fold higher than the native insulin ligand, suggesting it is an efficient BBB-targeting moiety. Uptake in primate brain can reach nearly 4%ID. For clinical translation, the 83-14 antibody has been humanized to help prevent unwanted immunogenicity as a result of the murine antibody framework. As with the TfR, conjugation of therapeutic cargo with the 83-14 antibody can lead to increased brain uptake. For example, in primates, pegylated liposomes carrying transgene-encoding DNA could lead to selective expression of transgene within targeted neuronal populations. Also, 83-14 conjugation with neurotrophic cargo led to 10-fold increases in brain levels. Moreover, when the lysosomal enzyme that is deficient in patients with mucopolysaccharidosis type I is conjugated to 83-14, it could also enter primate brain. In a clinical trial, the humanized 83-14 antibody-enzyme construct stabilized cognitive function in patients with severe forms of mucopolysaccharidosis (Giugliani et al., 2018) (Armagen NCT03053089, NCT03071341).

The therapeutic drug cargo needs to be carefully mated to the antibody-RMT system for successful deployment. Given that the total concentrations of antibody uptake in brain remains in the 1 to 50 nM range even under high dosages, the conjugated therapeutic needs to be efficacious at these concentrations. For this reason, early clinical trials with anti-TfR and anti-IR antibodies have focused on delivery of enzymes that catalytically process many substrate molecules and, hence, operate within the modest concentration ranges afforded by targeted RMT systems.

Low-Density Lipoprotein Family Receptors

Low-density lipoprotein receptor family (LDLRf) receptors such as LRP1, LRP2, and LDLR are expressed at the BBB. These RMT receptors mediate the transport of lipoproteins and other ligands across the BBB. They may have significant potential for trans-BBB transport as the brain uptake rates of LDLRf ligands such as receptor-associated protein and melanotransferrin (P97) exceed that observed for Tf, suggesting a high-capacity RMT pathway (Demeule et al., 2002; Pan et al., 2004). Only ligand-based LDLRf approaches have been described thus far. Apolipoprotein (Apo) B and ApoE are protein constituents of lipoproteins that mediate interactions with LDLRf receptors. Fusion of therapeutic cargo to ApoB and ApoE receptor binding domains can lead to brain uptake and pharmacological effects in rodent models including the delivery of an Aβ-degrading enzyme and lysosomal enzymes. Similarly, a peptide based on a conserved binding motif of several LDLRf ligands known as the Kunitz protease inhibitor domain has been used for trans-BBB delivery. Known as Angiopep-2, this peptide enters the brain via LRP1 RMT receptor, and when conjugated to neurotensin, it can elicit pharmacodynamic responses. Subsequently Angiopep-2 has used to deliver genes, peptides, and small-molecule P-glycoprotein substrates to the brain. Angiopep-2 was tested in phase I and II clinical trials for the delivery of *paclitaxel* for primary and metastatic brain tumors (e.g., Angiochem, NCT00539383, NCT01967810). These trials have demonstrated brain uptake of the conjugate and have suggested therapeutic efficacy (Kurzrock et al., 2012).

Improving BBB Transport of RMT-Targeted Systems

Transferrin receptor, IR, and LDLRf RMT systems have significant drawbacks despite early success in clinical studies. They target RMT systems that are not exclusive in their BBB expression, but rather are expressed all throughout the body both at the vascular and tissue levels. For molecules targeting these RMT systems, this leads to a decreased plasma AUC because of peripheral organ uptake, which in turn can lead to a decrease in brain uptake. Moreover, side effects can be caused by conjugated drug cargo accumulation in nonbrain tissues. Identification of other RMT systems with more brain specificity would be desirable. In addition, the commonly used RMT systems may not have the optimal capacity for trans-BBB delivery either because of receptor abundance or differential trafficking dynamics. For instance, antibodies targeting the TfR can be sequestered and degraded within the brain endothelial cells, limiting full transcytosis across the BBB. As such, the PS products for all RMT systems are not necessarily the same. Therefore, to increase the brain uptake of RMT-targeted biologics, one needs to increase the AUC or PS products of the targeting molecules. Two strategies for improving these parameters are to search for more BBB-specific RMT systems, which could increase the AUC due to less uptake in the periphery, or to optimize the current antibody-RMT systems by increasing their BBB PS product. Hence, ongoing research is focused on finding better BBB RMT systems and optimization of existing antibody-RMT systems.

BBB Opening

The BBB can be disrupted by pathological conditions such as stroke and brain cancer, but the timing and extent of disruption are often not adequate for efficient therapeutic delivery. On the other hand, chemical and physical methods can enhance the BBB opening and have been employed for drug delivery to the brain.

Chemical Methods

Chemical methods include the use of intra-arterial infusion of hyperosmolar *mannitol* that can transiently and reversibly disrupt the BBB in one brain hemisphere by opening the tight junctions in brain endothelial cells. *Mannitol* disruption has been deployed in the clinic for the treatment of glioblastoma and used in combination with therapeutic antibodies and small molecules (e.g., *bevacizumab*, NCT00968240; *cetuximab*, NCT02861898; and *temozolomide*, NCT01180816) and may enhance chemotherapy delivery, although the efficacy needs to be further evaluated (Neuwelt et al., 1983). Unfortunately, the method is inherently nonspecific and causes blood vessel opening throughout the entire targeted brain hemisphere and not just in the diseased region. The mechanism of opening allows not only the therapeutic to enter, but also any other blood-borne substances, which can lead to significant side effects such as seizures and brain edema. The patient also needs to be anesthetized for the procedure.

To identify agents that could be more selective in their regional modulation of BBB permeability, several biochemical agents have been used. These include ATP-sensitive and calcium-activated potassium channel activators that appear to selectively increase tumor BBB permeability by increasing the number of transport vesicles at the tumor BBB. Another approach is to activate bradykinin type 2 receptors. Bradykinin receptor activation can selectively increase tumor BBB permeability without breakdown of the healthy BBB. The mechanisms driving BBB opening are complex and may include both tight junction disruption and increased nonspecific transcytosis. In clinical studies, bradykinin analogues increased chemotherapeutic uptake into glioma tissue (Emerich et al., 2001). Region-specific BBB modulation with chemical approaches usually relies on the selective expression of brain endothelial targets in the diseased region. Hence, treatment paradigms aimed at preferentially increasing BBB permeability in the diseased brain region tend to be disease specific and are not generalizable.

Physical Methods

Physical methods, unlike biochemical methods, can be targeted to specific brain regions regardless of pathology. The most advanced physical BBB opening approach is focused ultrasound (FUS) (Figure 17–9). Microbubbles administered systemically are excited by focused ultrasonic

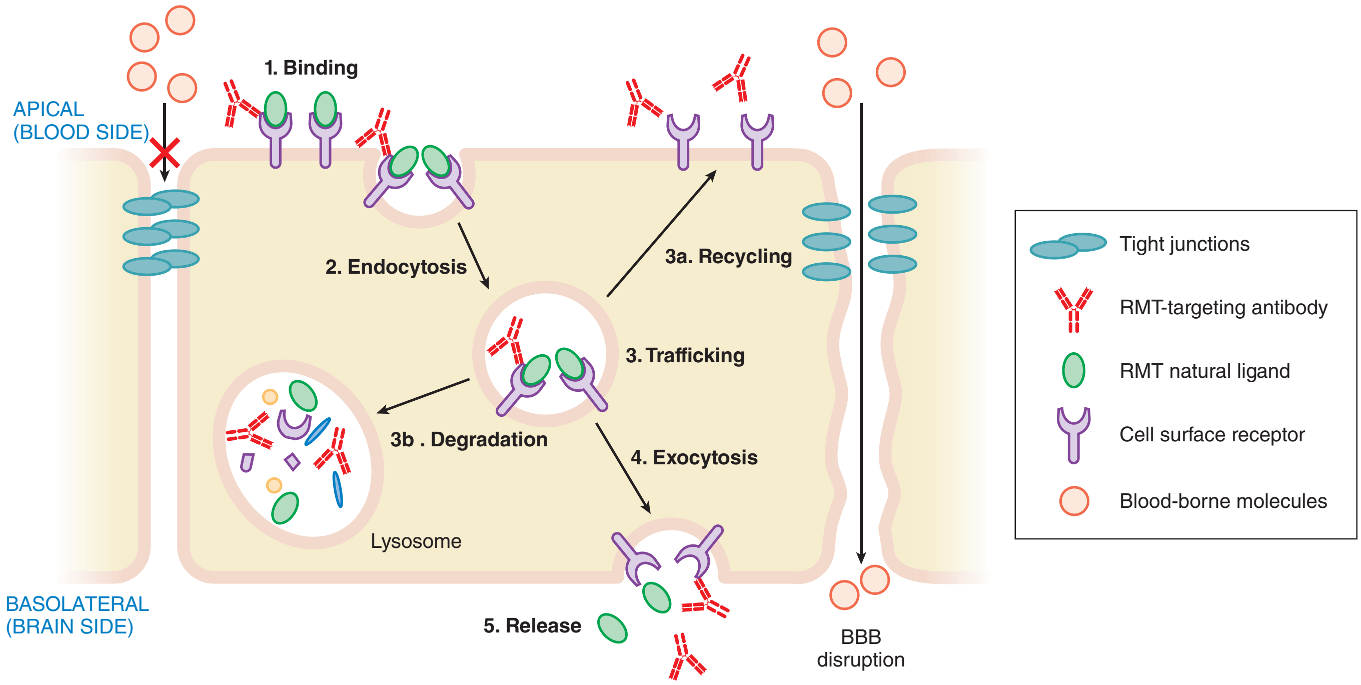
1. Binding
APICAL
(BLOOD SIDE)
2. Endocytosis
3a. Recycling
3. Trafficking
3b . Degradation
4. Exocytosis
Lysosome
BASOLATERAL
(BRAIN SIDE)
5. Release
BBB
disruption
Tight junctions
RMT-targeting antibody
RMT natural ligand
Cell surface receptor
Blood-borne molecules

Figure 17–8 *Schematic representation of the BBB RMT mechanism.* A natural ligand or antibody targeting an RMT receptor traffics through the brain endothelial cell vesicle transport network. Transcytosis across the BBB and into brain would constitute a molecule passing through steps 1 to 5.

waves, which in turn transiently and reversibly disrupt the BBB in FUS-treated regions.

The FUS procedure generates mechanical shear, which is thought to downregulate tight junction proteins and upregulate transcellular transport machinery, thereby increasing BBB permeability. After FUS, uptake of small molecules and biologics is increased in targeted regions. As with chemical methods, FUS-mediated BBB opening is not selective, and blood-borne substances can enter brain tissue, causing unwanted side effects. The mechanical shear forces may also cause immune activation and hemorrhage. FUS is being evaluated in several clinical trials for patients with glioma, neuropathic pain, Parkinson's and Alzheimer's disease (glioma, NCT03322813; neuropathic pain, NCT03309813; Parkinson's disease, NCT02347254; and Alzheimer's disease, NCT03671889). BBB-opening methods are increasingly studied for enhancing therapeutic uptake in the CNS. Given potential side effects, they may be best suited for conditions that do not require chronic treatment and repeated opening of the BBB.

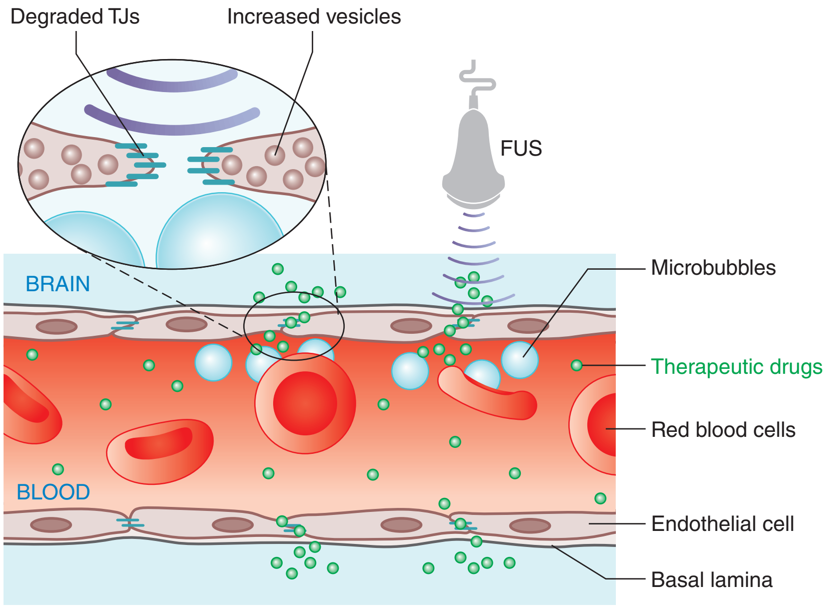
Degraded TJs
Increased vesicles
FUS
BRAIN
Microbubbles
Therapeutic drugs
Red blood cells
BLOOD
Endothelial cell
Basal lamina

Figure 17–9 *Schematic representation of BBB disruption by focused ultrasound (FUS).* Upon FUS application, microbubbles apply mechanical forces on brain endothelial cells leading to increased transport vesicles and disrupted tight junctions (TJs).

Summary

The BBB poses specific hurdles for brain disease drug development. Different components of the neurovascular unit cooperate to maintain the BBB in brain endothelial cells. The BBB is necessary for healthy brain function but challenges drug delivery. Some small nonpolar molecular drugs easily cross the BBB, but many drugs do not, due to the presence of active efflux transporters at the BBB. On the other hand, efflux transporters can minimize CNS side effects, such as for antihistamine sedation. Some drugs co-opt the active uptake systems that are expressed at the BBB. The function of the BBB limits the entry of antibodies and other biologics even more severely. Strategies to circumvent the BBB using adsorptive-mediated or receptor-mediated transcytosis are currently in development, and chemical or mechanical BBB disruption are additional possibilities for enhancing brain drug uptake.

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Chapter 18

Drug Therapy of Depression and Anxiety Disorders

James M. O'Donnell, Robert R. Bies, and Aislinn J. Williams

CHARACTERIZATION OF DEPRESSIVE AND ANXIETY DISORDERS
- Symptoms of Depression
- Symptoms of Anxiety

PHARMACOTHERAPY FOR DEPRESSION AND ANXIETY
- Clinical Considerations With Antidepressant Drugs
- Classes of Antidepressant and Antianxiety Agents
- ADME
- Adverse Effects
- Drug Interactions

ANXIOLYTIC DRUGS
- Clinical Considerations With Anxiolytic Drugs

Depression and anxiety disorders are the most common mental illnesses, each affecting in excess of 15% of the population at some point in the life span. With the advent of more selective and safer drugs, the use of antidepressants and anxiolytics has moved from the exclusive domain of psychiatry to primary care and other medical specialties. *The relative safety of the majority of commonly used antidepressants and anxiolytics notwithstanding, their optimal use requires a clear understanding of their mechanisms of action, pharmacokinetics, adverse effects, potential drug interactions, and the differential diagnosis of psychiatric illnesses* (Thronson and Pagalilauan, 2014).

Both depression and anxiety can affect an individual patient simultaneously; some of the drugs discussed here are effective in treating both types of disorders, suggesting common underlying mechanisms of pathophysiology and responses to pharmacotherapy. In large measure, our current understanding of pathophysiological mechanisms underlying depression and anxiety has been inferred from the mechanisms of action of psychopharmacological compounds, notably their actions on neurotransmission involving serotonin (5HT, 5-hydroxytryptamine), norepinephrine (NE), and γ-aminobutyric acid (GABA) (see Chapter 16). While depression and anxiety disorders comprise a wide range of symptoms, including changes in mood, behavior, somatic function, and cognition, progress has been made in developing animal models that respond with some sensitivity and selectivity to antidepressant or anxiolytic drugs (Cryan and Holmes, 2005; Xu et al., 2012). The last half-century has seen notable advances in the discovery and development of drugs for treating depression and anxiety (Hillhouse and Porter, 2015).

Characterization of Depressive and Anxiety Disorders

Symptoms of Depression

Depression can occur in major depressive disorder (i.e., unipolar depression), persistent depressive disorder (dysthymia), or bipolar I and II disorders (i.e., manic-depressive illness). Bipolar depression and its treatment are discussed in Chapter 19. Lifetime risk of unipolar major depression is approximately 15%. Females are affected with major depression twice as frequently as males (Brody et al., 2018). There also is some evidence for sex-based, differential response to pharmacotherapy. Depressive episodes are characterized by sad mood, pessimistic worry, diminished interest in normal activities, mental slowing and poor concentration, insomnia or increased sleep, significant weight loss or gain due to altered eating and activity patterns, psychomotor agitation or retardation, feelings of guilt and worthlessness, decreased energy and libido, and suicidal ideation. In depressive episodes, these symptoms occur most days for a period of at least 2 weeks. In some cases, the primary complaint of patients with depression involves somatic pain or other physical symptoms, which can present a diagnostic challenge for primary care physicians. Depressive symptoms also can occur secondary to other illnesses, such as hypothyroidism, Parkinson's disease, and inflammatory conditions, among others. In addition, depression often complicates the management of other medical conditions (e.g., severe trauma, cancer, diabetes, and cardiovascular disease, especially myocardial infarction).

Depression is underdiagnosed and undertreated (Johansson et al., 2013). Given that approximately 10% to 15% of those with severe depression attempt suicide at some time, it is important that symptoms of depression be recognized and treated in a timely manner. Furthermore, the response to treatment must be assessed and decisions made regarding continued treatment with the initial drug, dose adjustment, adjunctive psychotherapy, or alternative medication.

Symptoms of Anxiety

Anxiety is a normal human emotion that serves an adaptive function from a psychobiological perspective. Symptoms of anxiety include feelings of worry, muscle tension, restlessness, poor concentration, and panic (shortness of breath, heart palpitations, sweating, fear of impending doom). Anxiety disorders occur when symptoms of anxiety interfere significantly with normal function and are classified as generalized anxiety disorder, obsessive-compulsive disorder, panic disorder, acute stress disorder, posttraumatic stress disorder (PTSD), separation anxiety disorder, social phobia, and specific phobias (Atack, 2003). Although anxiety is not abnormal in itself, anxiety disorders are common and often require treatment. All of the anxiety disorders, with the exception of specific phobias, can be treated with antidepressant medications, particularly selective serotonin reuptake inhibitors (SSRIs). Drug treatment includes both acute drug administration to manage episodes of anxiety and chronic treatment to manage unrelieved and continuing anxiety disorders. Symptoms of anxiety also are often associated with depression and other medical conditions.

Pharmacotherapy for Depression and Anxiety

Most antidepressants enhance serotonergic or noradrenergic transmission, or both. Sites of interaction of antidepressant drugs with serotonergic and noradrenergic neurons are depicted in Figure 18–1. Table 18–1 summarizes the actions of the most widely used antidepressants. The most commonly used medications, often referred to as second-generation

Abbreviations

ADHD: attention-deficit/hyperactivity disorder
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, an exogenous ligand for the GluA subtype of glutamate receptors
BDNF: brain-derived neurotrophic factor
CREB: cyclic AMP response element binding protein
CYP: cytochrome P450
DA: dopamine
DAT: dopamine transporter
GABA: γ-aminobutyric acid
GI: gastrointestinal
MAO: monoamine oxidase
MAOI: monoamine oxidase inhibitor
NE: norepinephrine
NET: neuronal NE transporter
NMDA: *N*-methyl-D-aspartate
PTSD: posttraumatic stress disorder
SERT: neuronal serotonin/5HT transporter
SNRI: serotonin-norepinephrine reuptake inhibitor
SSRI: selective serotonin reuptake inhibitor
TCA: tricyclic antidepressant

antidepressants, are the SSRIs and the serotonin-norepinephrine reuptake inhibitors (SNRIs), which have less toxicity and improved safety compared to the first-generation drugs, which include monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs) (Millan, 2006; Rush et al., 2006).

In monoamine systems, termination of neurotransmitter action occurs via neuronal reuptake of the neurotransmitter from the synaptic cleft. This reuptake occurs via presynaptic high-affinity transporters; inhibition of these transporters enhances neurotransmission by slowing clearance of the transmitter and prolonging its dwell time in the synapse (Shelton and Lester, 2006). Reuptake inhibitors block the neuronal serotonin/5HT transporter (SERT), the neuronal NE transporter (NET), or both. Similarly, TCAs and MAOIs enhance monoaminergic neurotransmission—the TCAs by inhibiting 5HT or NE reuptake via SERT or NET and the MAOIs by inhibiting monoamine metabolism and thereby increasing the levels of neurotransmitter in storage granules available for later release.

Long-term effects of antidepressant drugs evoke regulatory mechanisms that might contribute to the effectiveness of therapy (Shelton, 2000). These responses include altered adrenergic or serotonergic receptor density or sensitivity, altered receptor–G protein coupling and cyclic nucleotide signaling, induction of neurotrophic factors, and increased neurogenesis in the hippocampus (Schmidt and Duman, 2007). Persistent antidepressant effects depend on the continued inhibition of SERT or NET or enhanced serotonergic or noradrenergic neurotransmission achieved by an alternative pharmacological mechanism (Delgado et al., 1991). Compelling evidence suggests that sustained signaling via NE or 5HT increases the expression of specific downstream gene products, particularly brain-derived neurotrophic factor (BDNF), which appears to influence dendritic spine formation, synaptogenesis, and neurogenesis (Duman and Duman, 2015).

Genome-wide association studies have suggested novel pathways that might be exploited for the discovery of antidepressants (Cannon and Keller, 2006; Lin and Lane, 2015). These include systems and pathways outside of monoaminergic neurotransmission, such as glutamatergic signaling (*ketamine, esketamine*), the endogenous opioid system (*naltrexone*), and GABAergic signaling (*brexanolone*) (Sanches et al., 2021). Other novel approaches involve enhancing neurogenesis (Pascual-Brazo et al., 2014) or cyclic nucleotide signaling (O'Donnell and Zhang, 2004), which may be impaired in depressed patients (Fujita et al., 2012). There also are emerging data suggesting a role for inhibition of somatosensory feedback loops with botulinum toxin for treatment of depression (Schulze et al., 2021).

Clinical Considerations With Antidepressant Drugs

The response to antidepressant drug treatment generally has a "therapeutic lag" lasting 3 to 4 weeks before a clinically relevant response is evident; however, symptoms respond differentially, with sleep disturbances improving sooner and mood and cognitive deficits later (Katz et al., 2004). Some of the lag is pharmacokinetic in nature, i.e., effective plasma concentrations are not achieved initially. However, it is likely that a component of the lag is related to delayed pre- and postsynaptic changes. After the successful initial treatment phase, a 6- to 12-month maintenance treatment phase is typical, after which the drug can be gradually withdrawn. If a patient is chronically depressed (i.e., has been depressed for >2 years), lifelong treatment with an antidepressant is advisable. Approximately two-thirds of patients show a marked decrease in depressive symptoms with an initial course of treatment, with one-third showing complete remission (Rush et al., 2006).

Antidepressants are not recommended as monotherapy for bipolar disorder. These drugs, notably TCAs, SNRIs, and, to a lesser extent, SSRIs, can induce a switch from a depressed episode to a manic or hypomanic episode in some patients (Gijsman et al., 2004; Williams et al., 2018).

A controversial issue regarding the use of all antidepressants is their relationship to suicide (Mann et al., 2006). Data establishing a clear link between antidepressant treatment and death by suicide are lacking. However, the U.S. Food and Drug Administration (FDA) has issued a "black-box" warning regarding the use of SSRIs and a number of other antidepressants in children and adolescents due to the possibility of an association between antidepressant treatment and suicidal ideation or behavior (Boaden et al., 2020; Isacsson and Rich, 2014). For seriously depressed patients, the risk of not being on an effective antidepressant drug outweighs the risk of being treated with one (Gibbons et al., 2007). However, it is important to monitor patients closely, particularly children and adolescents, especially during initial periods of treatment.

Classes of Antidepressant and Antianxiety Agents

Selective Serotonin Reuptake Inhibitors

The SSRIs are effective in treating major depression. SSRIs also are anxiolytics with demonstrated efficacy in the treatment of generalized anxiety, panic, social anxiety, and obsessive-compulsive disorders (Rush et al., 2006). *Sertraline* and *paroxetine* are approved for the treatment of PTSD. SSRIs also are used for treatment of premenstrual dysphoric syndrome and for preventing vasovagal symptoms in postmenopausal women, with *fluoxetine* specifically FDA-approved for treatment of bulimia nervosa.

As noted above, neuronal uptake by SERT is the primary process by which neurotransmission via 5HT is terminated (see Figure 18–1). SSRIs block reuptake and enhance and prolong serotonergic neurotransmission. SSRIs used clinically are relatively selective for inhibition of SERT over NET (Table 18–2).

Treatment with an SSRI causes stimulation of $5HT_{1A}$ and $5HT_{7}$ autoreceptors on cell bodies in the raphe nucleus and of $5HT_{1D}$ autoreceptors on serotonergic terminals; this initially reduces net synthesis and release of 5HT. With repeated treatment with SSRIs, there is a gradual downregulation and desensitization of these autoreceptor mechanisms, and with reuptake inhibited, net 5HT transmission is enhanced. In addition, downregulation of postsynaptic $5HT_{2A}$ receptors may contribute to antidepressant efficacy directly or by influencing the function of noradrenergic and other neurons via serotonergic heteroreceptors. Other postsynaptic 5HT receptors likely remain responsive to increased synaptic concentrations of 5HT and contribute to the therapeutic effects of the SSRIs.

Later-developing effects of SSRI treatment also may be important in mediating ultimate therapeutic responses. These include sustained increases in cyclic AMP signaling and phosphorylation of the cyclic AMP

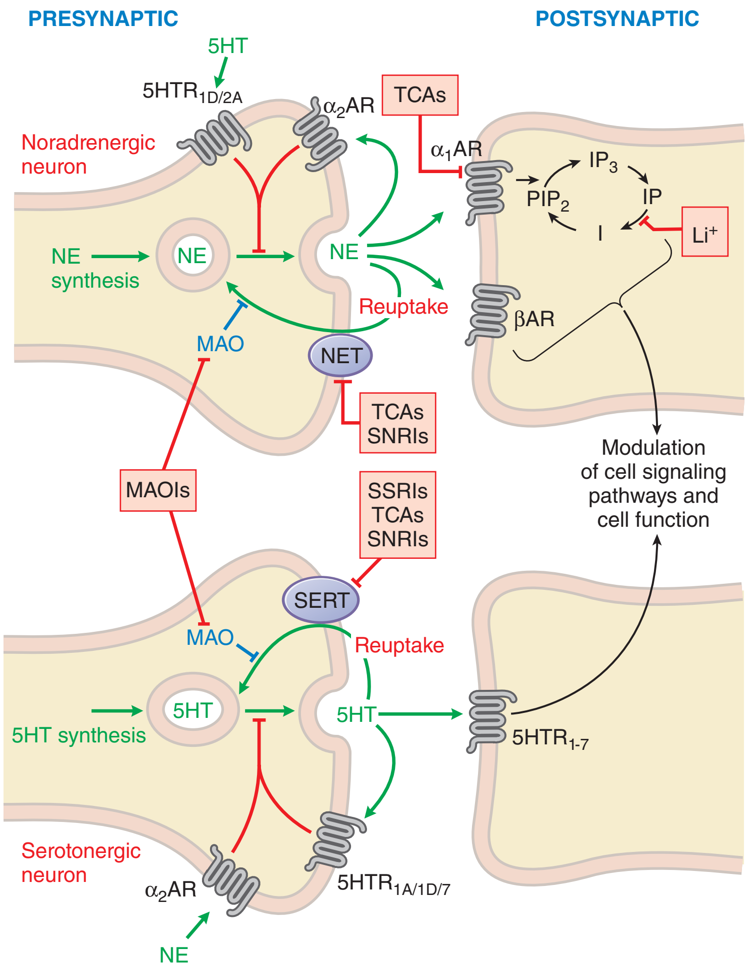


Figure 18–1 *Sites of action of antidepressants at noradrenergic (top) and serotonergic (bottom) nerve terminals.* SSRIs, SNRIs, and TCAs increase noradrenergic or serotonergic neurotransmission by blocking the NE or 5HT transporter (NET or SERT) at presynaptic terminals. MAOIs inhibit the catabolism of NE and 5HT. *Trazodone* and related drugs have direct effects on 5HT receptors (5HTRs) that contribute to their clinical effects. Chronic treatment with a number of antidepressants desensitizes presynaptic autoreceptors and heteroreceptors, producing long-lasting changes in monoaminergic neurotransmission. Postreceptor effects of antidepressant treatment, including modulation of G protein-coupled receptor (GPCR) signaling and activation of protein kinases and ion channels, are involved in the mediation of the long-term effects of antidepressant drugs. Li^+ inhibits IP breakdown and thereby enhances its accumulation and sequelae (Ca^{2+} mobilization, protein kinase C activation, depletion of cellular inositol). Li^+ may also alter release of neurotransmitters by a variety of putative mechanisms (see Chapter 19: Hypotheses for the Mechanism of Action of Lithium and Relationship to Anticonvulsants). Note that NE and 5HT may also affect each other's neurons by activating presynaptic receptors that couple to signaling pathways that reduce transmitter release. I, inositol; IP, inositol monophosphate; IP_3, inositol 1,4,5-trisphosphate; PIP2, phosphatidylinositol 4,5-bisphosphate.

response element binding protein (CREB), as well as increased expression of trophic factors such as BDNF and increased neurogenesis from progenitor cells in the hippocampus and subventricular zone (Licznerski and Duman, 2013; Santarelli et al., 2003). Repeated treatment with SSRIs reduces the expression of SERT, resulting in reduced clearance of released 5HT and increased serotonergic neurotransmission (Kittler et al., 2010; Matthaus et al., 2016).

Serotonin-Norepinephrine Reuptake Inhibitors

Five medications with a nontricyclic structure that inhibit the reuptake of both 5HT and NE have been approved for use in the U.S. and Europe for treatment of depression, anxiety disorders, pain, or other specific conditions: *venlafaxine* and its demethylated metabolite *desvenlafaxine*, *duloxetine*, *milnacipran*, and *levomilnacipran*.

The SNRIs inhibit both SERT and NET (see Table 18–2) and cause enhanced serotonergic and noradrenergic neurotransmission. Similar to the action of SSRIs, the initial inhibition of SERT induces activation of $5HT_{1A}$ and $5HT_{1D}$ autoreceptors, resulting in a decrease in serotonergic neurotransmission by a negative-feedback mechanism until these serotonergic autoreceptors are desensitized. Then, the enhanced 5HT concentration in the synapse can interact with postsynaptic 5HT receptors.

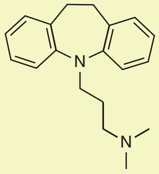
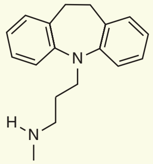
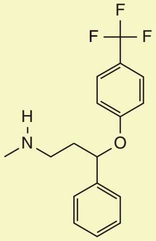
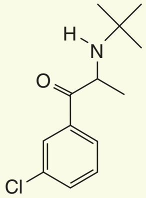
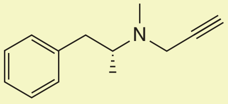
The noradrenergic action of these drugs may contribute to downstream gene expression changes affecting BDNF, Trk-B, and other neurotrophic factors and their signaling pathways (Shelton, 2000). Repeated treatment with SNRIs reduces the expression of SERT or NET, resulting in reduced neurotransmitter clearance and increased serotonergic or noradrenergic neurotransmission (Zhao et al., 2009).

The SNRIs were developed with the rationale that they might improve overall treatment response compared to SSRIs (Entsuah et al., 2001). The remission rate for *venlafaxine* appears slightly better than for SSRIs in head-to-head trials (Cipriani et al., 2018). *Duloxetine*, in addition to being approved for use in the treatment of depression and anxiety, is used for treatment of fibromyalgia and neuropathic pain associated with peripheral neuropathy (Finnerup et al., 2015). Off-label uses include stress urinary incontinence (*duloxetine*), autism, binge-eating disorders, hot flashes, pain syndromes, premenstrual dysphoric disorders, and PTSD (*venlafaxine*).

Serotonin Receptor Antagonists

Antagonists of the $5HT_2$ family of receptors are effective antidepressants. These include two close structural analogues, *trazodone* and *nefazodone*, as well as *mirtazapine* and *mianserin* (not marketed in the U.S.)

TABLE 18–1 ■ PROFILES OF REPRESENTATIVE ANTIDEPRESSANTS

CLASS Agent	DOSE[a] mg/day	BIOGENIC AMINE	SIDE EFFECTS									REPRESENTATIVE DRUG
			AGITATION	SEIZURES	SEDATION	HYPOTENSION	ANTI-ACh EFFECTS	GI EFFECTS	WEIGHT GAIN	SEXUAL EFFECTS	CARDIAC EFFECTS	
NE reuptake inhibitors: 3° amine tricyclics												 Imipramine
Amitriptyline	100–200	NE, 5HT	0	2+	3+	3+	3+	0/+	2+	2+	3+	
Clomipramine	100–200	NE, 5HT	0	3+	2+	2+	3+	+	2+	3+	3+	
Doxepin	100–200	NE, 5HT	0	2+	3+	2+	2+	0/+	2+	2+	3+	
Imipramine	100–200	NE, 5HT	0/+	2+	2+	2+	2+	0/+	2+	2+	3+	
(+)-Trimipramine	75–200	NE, 5HT	0	2+	3+	2+	3+	0/+	2+	2+	3+	
NE reuptake inhibitors: 2° amine tricyclics												 Desipramine
Amoxapine	200–300	NE, DA	0	2+	+	2+	+	0/+	+	2+	2+	
Desipramine	100–200	NE	+	+	0/+	+	+	0/+	+	2+	2+	
Maprotiline	100–150	NE	0/+	3+	2+	2+	2+	0/+	+	2+	2+	
Nortriptyline	75–150	NE	0	+	+	+	+	0/+	+	2+	2+	
Protriptyline	15–40	NE	2+	2+	0/+	+	2+	0/+	+	2+	3+	
SSRIs												 Fluoxetine
(±)-Citalopram	20–40	5HT	0/+	0	0/+	0	0	3+	0	3+	0	
(+)-Escitalopram	10–20	5HT	0/+	0	0/+	0	0	3+	0	3+	0	
(±)-Fluoxetine	20–80	5HT	+	0/+	0/+	0	0	3+	0/+	3+	0/+	
Fluvoxamine	100–200	5HT	0	0	0/+	0	0	3+	0	3+	0	
(−)-Paroxetine	20–40	5HT	+	0	0/+	0	0/+	3+	0	3+	0	
(+)-Sertraline	100–150	5HT	+	0	0/+	0	0	3+	0	3+	0	
(±)-Venlafaxine	75–225	5HT, NE	0/+	0	0	0	0	3+	0	3+	0/+	
Atypical antidepressants												 Bupropion
(−)-Atomoxetine	40–80[b]	NE	0	0	0	0	0	0/+	0	0	0	
Bupropion	200–300	DA, ?NE	3+	4+	0	0	0	2+	0	0	0	
(+)-Duloxetine	80–100	NE, 5HT	+	0	0/+	0/+	0	0/+	0/+	0/+	0/+	
(±)-Mirtazapine	15–45	5HT, NE	0	0	4+	0/+	0	0/+	0/+	0	0	
Nefazodone	200–400	5HT	0	0	3+	0	0	2+	0/+	0/+	0/+	
Trazodone	150–200	5HT	0	0	3+	0	0	2+	+	+	0/+	
MAO inhibitors												 Selegiline
Phenelzine	30–60	NE, 5HT, DA	0/+	0	+	+	0	0/+	+	3+	0	
Tranylcypromine	20–30	NE, 5HT, DA	2+	0	0	+	0	0/+	+	2+	0	
(−)-Selegiline	10	DA, ?NE, ?5HT	0	0	0	0	0	0	0	+	0	

0, negligible; 0/+, minimal; +, mild; 2+, moderate; 3+, moderately severe; 4+, severe. Other significant side effects for individual drugs are described in the text. Selegiline transdermal patch approved for depression.

[a] Higher and lower doses are sometimes used, depending on patient's needs and response to the drug; see the literature and FDA recommendations.

[b] Children, 0.5–1 mg/kg, up to 70 kg; see black-box warning.

TABLE 18–2 ■ SELECTIVITY OF ANTIDEPRESSANTS AT THE HUMAN BIOGENIC AMINE TRANSPORTERS

DRUG	SELECTIVITY	DRUG	SELECTIVITY
NE SELECTIVE	**NET vs. SERT**	**5HT SELECTIVE**	**SERT vs. NET**
Oxaprotiline	800	*S*-Citalopram	7127
Maprotiline	532	*R,S*-Citalopram	3643
Viloxazine	109	Sertraline	1390
Nomifensine	64	Fluvoxamine	591
Desipramine	22	Paroxetine	400
Protriptyline	14	Fluoxetine	305
Atomoxetine	12	Clomipramine	123
Reboxetine	8.3	Venlafaxine	116
Nortriptyline	4.2	Zimelidine	60
Amoxapine	3.6	Trazodone	52
Doxepin	2.3	Imipramine	26
		Amitriptyline	8.0
DA SELECTIVE	**DAT vs. NET**	Duloxetine	7.0
Bupropion	1000	Dothiepin	5.5
		Milnacipran	1.6

Selectivity is defined as the ratio of the relevant K_i values (SERT/NET, NET/SERT, NET/DAT). Bupropion is selective for the DAT relative to the NET and SERT. Data from Frazer, 1997; Owens et al., 1997; and Leonard and Richelson, 2000.

The efficacy of *trazodone* may be somewhat more limited than that of the SSRIs; however, low doses of *trazodone* (50–200 mg) have been used widely, both alone and concurrently with SSRIs or SNRIs, to treat insomnia. Both *mianserin* and *mirtazapine* are sedating and are treatments of choice for some depressed patients with insomnia. *Trazodone* blocks $5HT_2$ and α_1 adrenergic receptors. *Trazodone* also inhibits SERT but is markedly less potent for this action relative to its blockade of $5HT_{2A}$ receptors. Similarly, the most potent pharmacological action of *nefazodone* also is the blockade of the $5HT_2$ receptors. Both *mirtazapine* and *mianserin* potently block histamine H_1 receptors. They also have some affinity for α_2 adrenergic receptors. Their affinities for $5HT_{2A}$, $5HT_{2C}$, and $5HT_3$ receptors are high, although less than for histamine H_1 receptors. Both of these drugs increase the antidepressant response when combined with SSRIs compared to the action of the SSRIs alone. *Vortioxetine* is a potent SERT inhibitor and binds to a number of serotonergic receptors; the drug is a partial agonist at $5HT_{1A}$ and $5HT_{1B}$ receptors and an antagonist at $5HT_{1D}$, $5HT_3$, and $5HT_7$ receptors.

Bupropion

Bupropion has the chemical backbone of β-phenethylamine; it is discussed separately because it acts via multiple mechanisms that differ somewhat from the mechanisms of SSRIs and SNRIs (Foley et al., 2006; Gobbi et al., 2003). It enhances both noradrenergic and dopaminergic neurotransmission via inhibition of reuptake by the norepinephrine transporter (NET) and the dopamine transporter (DAT) (although its effects on DAT are not potent in animal studies) (see Table 18–2). *Bupropion*'s mechanism of action also may involve the presynaptic release of NE and DA and effects on the vesicular monoamine transporter (VMAT2; see Figure 10–8). The metabolite, hydroxybupropion, may contribute to the therapeutic effects of the parent compound: this metabolite has a similar pharmacology and is present at substantial levels. *Bupropion* is indicated for the treatment of depression, prevention of seasonal depressive disorder, and as a smoking cessation treatment (Carroll et al., 2014). *Bupropion* has effects on sleep electroencephalograms that are opposite those of most antidepressant drugs and has fewer sexual side effects and results in less weight gain than other antidepressants (Patel et al., 2016). *Bupropion* may improve symptoms of attention-deficit/hyperactivity disorder (ADHD) and has been used off-label for neuropathic pain and weight loss. Clinically, *bupropion* is widely used in combination with SSRIs with the intent of obtaining a greater antidepressant response; however, there are limited clinical data providing support for this practice.

Atypical Antipsychotics

In addition to their use in schizophrenia, bipolar depression, and major depression with psychotic features, atypical antipsychotics are used in the treatment of major depression without psychotic features, typically as an adjunct along with more conventional treatments (Jarema, 2007). The combination of *aripiprazole* or *quetiapine* with SSRIs and SNRIs and a combination of *olanzapine* and the SSRI *fluoxetine* have been FDA approved for treatment-resistant major depression (i.e., following an inadequate response to at least two different antidepressants). Additionally, *brexpiprazole* is FDA-approved as an adjunct medication for major depressive disorder in adults. *Quetiapine* may have either primary antidepressant actions on its own or adjunctive benefit for treatment-resistant depression. The mechanisms of action and adverse effects of the atypical antipsychotics are described in Chapter 19. The major risks of these agents are weight gain and metabolic syndrome, a greater problem for *quetiapine* and *olanzapine* than for *aripiprazole*. The mechanism by which atypical antipsychotics enhance the efficacy of antidepressant medications is not fully understood but may be due in part to $5HT_{2A}$ receptor blockade.

Tricyclic Antidepressants

While TCAs have long-established efficacy, they exhibit serious side effects and generally are not used as first-line drugs for the treatment of depression. TCAs and first-generation antipsychotics are synergistic for the treatment of psychotic depression. Tertiary amine TCAs (e.g., *doxepin, amitriptyline*) have been used for many years in relatively low doses for treating insomnia. In addition, because of the roles of NE and 5HT in nociception, these drugs are commonly used to treat a variety of pain conditions, typically at doses lower than those used psychiatric disorders (Finnerup et al., 2015).

The pharmacological action of TCAs is antagonism of SERT and NET (see Table 18–2). In addition to inhibiting NET somewhat selectively (*desipramine, nortriptyline, protriptyline, amoxapine*) or both SERT and NET (*imipramine, amitriptyline*), these drugs block other receptors (H_1 histamine, $5HT_2$, α_1 adrenergic, and muscarinic cholinergic receptors). Given the comparable activities of *clomipramine* and SSRIs (see Tables 18–2 and 18–4; see also Decloedt and Stein, 2010), it is possible that some combination of these additional pharmacological actions contributes to the therapeutic effects of TCAs and possibly SNRIs. One TCA, *amoxapine*, also is a DA receptor antagonist; its use, unlike that of other TCAs, poses some risk for the development of extrapyramidal side effects such as tardive dyskinesia.

Monoamine Oxidase Inhibitors

Monoamine oxidases A and B (MAO-A, MAO-B) are widely distributed mitochondrial enzymes. MAO activities in the gastrointestinal (GI) tract and liver, mainly MAO_A, protect the body from biogenic amines in the diet. In presynaptic nerve terminals, MAO metabolizes monoamine neurotransmitters via oxidative deamination. MAO_A preferentially metabolizes 5HT and NE and can metabolize DA; MAO_B is effective against 5HT and DA (see Chapters 10 and 15; see also Nestler et al., 2020). MAOIs have efficacy equivalent to that of the TCAs but are rarely used because of their toxicity and major interactions with some drugs (e.g., sympathomimetics and some opioids). The MAOIs first approved in the U.S. for treatment of depression—*tranylcypromine, phenelzine*, and *isocarboxazid*—irreversibly inhibit both MAO_A and MAO_B. These agents inhibit the body's capacity to metabolize not only endogenous monoamines such as NE and 5HT but also exogenous biogenic amines such as tyramine. This is problematic because some foods contain high amounts of tyramine. Global inhibition of MAOs will increase the bioavailability of dietary tyramine, and tyramine-induced NE release can cause marked increases in blood pressure (hypertensive crisis) (see Chapter 10).

This potential to exacerbate the effects of indirectly acting sympathomimetic amines seems to relate mainly to inhibition of MAO_A. *Selegiline* is an irreversible MAO inhibitor but with selectivity for MAO_B. Administration of *selegiline* at low doses spares MAO_A activity in the GI tract and elsewhere, reducing the risk of deleterious interactions with sympathomimetics (at higher doses, *selegiline* will also inhibit MAO_A). *Selegiline* is available as a transdermal patch for the treatment of depression; transdermal delivery may reduce the risk for diet-associated hypertensive reactions. Some MAOIs, such as *moclobemide* and *eprobemide*, are reversible, competitive inhibitors of MAO_A, and thus dietary substrates will still be metabolized to the extent that they compete with the inhibitor for binding to MAO_A (i.e., tyramine will still be metabolized and will compete better as a substrate for MAO_A as its concentration rises, dampening negative interactions). These reversible inhibitors of MAO_A are used elsewhere but are not approved for use in the U.S. (Finberg, 2014).

Mechanistically Distinctive Compounds: NMDA Receptor Antagonists and Neurosteroids

Antidepressant medications have traditionally focused their actions on the monoamine neurotransmitters NE, DA, and 5HT, and those medications generally require several weeks to produce clinical effects. In contrast, *ketamine*, acting on *N*-methyl-D-aspartate (NMDA) receptors to antagonize glutamate binding, and secondarily affecting AMPA receptors, produces rapid and lasting antidepressant effects. *Brexanolone* (*allopregnanolone*) acts at a peripheral site on the $GABA_A$ receptor to modulate Cl^- flux: It is approved for treatment of postpartum depression. These agents are the first mechanistically distinct antidepressants approved in decades and represent novel approaches to the pharmacotherapy of depression.

NMDA Receptor Antagonists. *Ketamine* is an NMDA receptor antagonist that is effective for depression as well as suicidality (Xiong et al., 2021; Xu et al., 2016). Its antidepressant effects are thought to be due to blockade of NMDA receptors on GABAergic interneurons, which leads to disinhibition of glutamatergic neurons and subsequent increases in glutamate release, ultimately affecting AMPA receptors and BDNF in the postsynaptic cell (Figure 18-2). *Ketamine* might also have a role in the treatment of bipolar disorder; more data are required to draw firm conclusions (Joseph et al., 2021). Although *ketamine* appears effective, its use in depression treatment is off-label; only *esketamine*, its *S*-enantiomer, is FDA-approved. It is available as a nasal spray, under a Risk Evaluation and Mitigation Strategy, for treatment-resistant depression. There is some debate as to whether the antidepressant effects of *ketamine* are due to the parent compound or its bioactive metabolites, such as (2R,6R)-hydroxynorketamine (HNK). A number of other glutamatergic modulators currently are under investigation (Sanches et al., 2021).

Neurosteroids. *Brexanolone* (*allopregnanolone*) is a neurosteroidal antidepressant with a novel mechanism of action: it is a positive allosteric modulator of the $GABA_A$ receptor (see Figure 16–11) (Sanches et al., 2021). Hypothetically, *brexanolone* may restore reduced levels of this neurosteroid found to be associated with depression, as well as altering $GABA_A$ receptor function. Currently, *brexanolone* is the only medication that is specifically FDA-approved for the treatment of postpartum depression. It is administered by continuous intravenous infusion over 2.5 days and subject to a Risk Evaluation and Mitigation Strategy; its use is somewhat limited due to the high cost of treatment. It is unknown whether neurosteroids, including *brexanolone*, are effective for depression treatment outside of postpartum depression.

ADME

These agents conveniently fit into groups with similarities of basic pharmacologic properties. Thus, information on the pharmacokinetics, clinical use, drug interactions, and adverse responses are presented sequentially, class by class.

Metabolism

The metabolism of most antidepressants is mediated by hepatic cytochromes P450 (CYPs); the half-lives and predominant CYPs responsible for metabolism are summarized in Table 18–3. Some antidepressants inhibit the clearance of other drugs by the CYP system and vice versa, and the possibility of drug interactions should be a significant factor in considering the choice of agents. Likewise, dose considerations have to include awareness of hepatic function (Mauri et al., 2014). CYPs 2D6 and 2C19 are highly polymorphic, and for antidepressants metabolized primarily by those CYPs, a range of metabolic rates can be expected. Patients at the extremes of CYP 2C19 and 2D6 activities, ultra-rapid and ultra-slow metabolizers, would seem most likely to exhibit adverse responses, from failure of therapy (ultra-rapid metabolism) to toxicity (ultra-slow metabolism). For example, in a fraction of patients, TCAs are metabolized slowly due to a variant CYP2D6 (poor metabolizers), and this necessitates a dose reduction (see Tricyclic Antidepressants, below). Probst-Schendzielorz et al. (2015) have reviewed the pertinent literature in the case of SSRIs and provide dosing recommendations for *fluvoxamine*, *paroxetine*, *citalopram*, *escitalopram*, and *sertraline* based on *CYP2D6* and/or *CYP2C19* genotype. Data on this subject are accumulating and recommendations may change; updated information on many compounds is available at the following site: http://www.pharmgkb.org.

Although genetic polymorphisms among CYPs can influence antidepressant metabolism, a number of other factors, such as disease processes, diet, environmental factors, and drug-drug interactions, influence CYP activities and drug disposition. Polymorphisms of target proteins (e.g., a specific 5HT receptor) may influence drug response (see Table 7–2). Dubovsky and Dubovsky (2015) have argued that CYP genotyping alone may be insufficient and also is not yet proven to have a practical influence on choice of drug treatment in clinical settings. In any event, there is no substitute for clinical judgement and careful monitoring.

Selective Serotonin Reuptake Inhibitors

All of the SSRIs are orally active and possess elimination half-lives consistent with once-daily dosing (Hiemke and Hartter, 2000). In the case of *fluoxetine*, the combined action of the parent and the demethylated metabolite norfluoxetine allows for a once-weekly formulation. CYP2D6 is involved in the metabolism of most SSRIs, and the SSRIs are at least moderately potent inhibitors of this isoenzyme. This creates a significant potential for drug interaction for postmenopausal women taking the breast cancer drug and estrogen antagonist *tamoxifen* (see Chapter 73). Because *venlafaxine* and *desvenlafaxine* are weak inhibitors of CYP2D6, these antidepressants are not contraindicated in this clinical situation. However, care should be used in combining SSRIs with drugs that are metabolized by CYPs. SSRIs such as *escitalopram* and *citalopram* should be dosed with care in elderly patients due to an age-dependent decrease in CYP2C19 metabolism. There is some evidence that the clearance of the *R*-enantiomer of *citalopram* is slower in women than in men (13 vs. 9 L/h, respectively), a difference that would result in higher exposures to the *R*-enantiomer in women (Akil et al., 2016).

Serotonin-Norepinephrine Reuptake Inhibitors

Both immediate-release and extended-release (tablet or capsule) preparations of *venlafaxine* result in steady-state levels of drug in plasma within 3 days. The elimination half-lives for the parent *venlafaxine* and its active and major metabolite *desmethylvenlafaxine* are 5 and 11 h, respectively. *Desmethylvenlafaxine* is eliminated by hepatic metabolism and by renal excretion. *Venlafaxine* dose reductions are suggested for patients with renal or hepatic impairment. *Duloxetine* has a $t_{1/2}$ of 12 h. *Duloxetine* is not recommended for those with end-stage renal disease or hepatic insufficiency.

Serotonin Receptor Antagonists

Mirtazapine has an elimination $t_{1/2}$ of 16 to 30 h. Thus, reaching a new steady-state blood level after a change in daily dose would take 90 to 150 h (five half-lives, 4–6+ days); dose changes are suggested no more often than 1 to 2 weeks. The recommended initial dosing of *mirtazapine* is 15 mg/day, with a maximal recommended dose of 45 mg/day. Clearance of *mirtazapine* is decreased in the elderly and in patients with moderate-to-severe renal or hepatic impairment. Pharmacokinetics and adverse effects of *mirtazapine* may have an enantiomer-selective

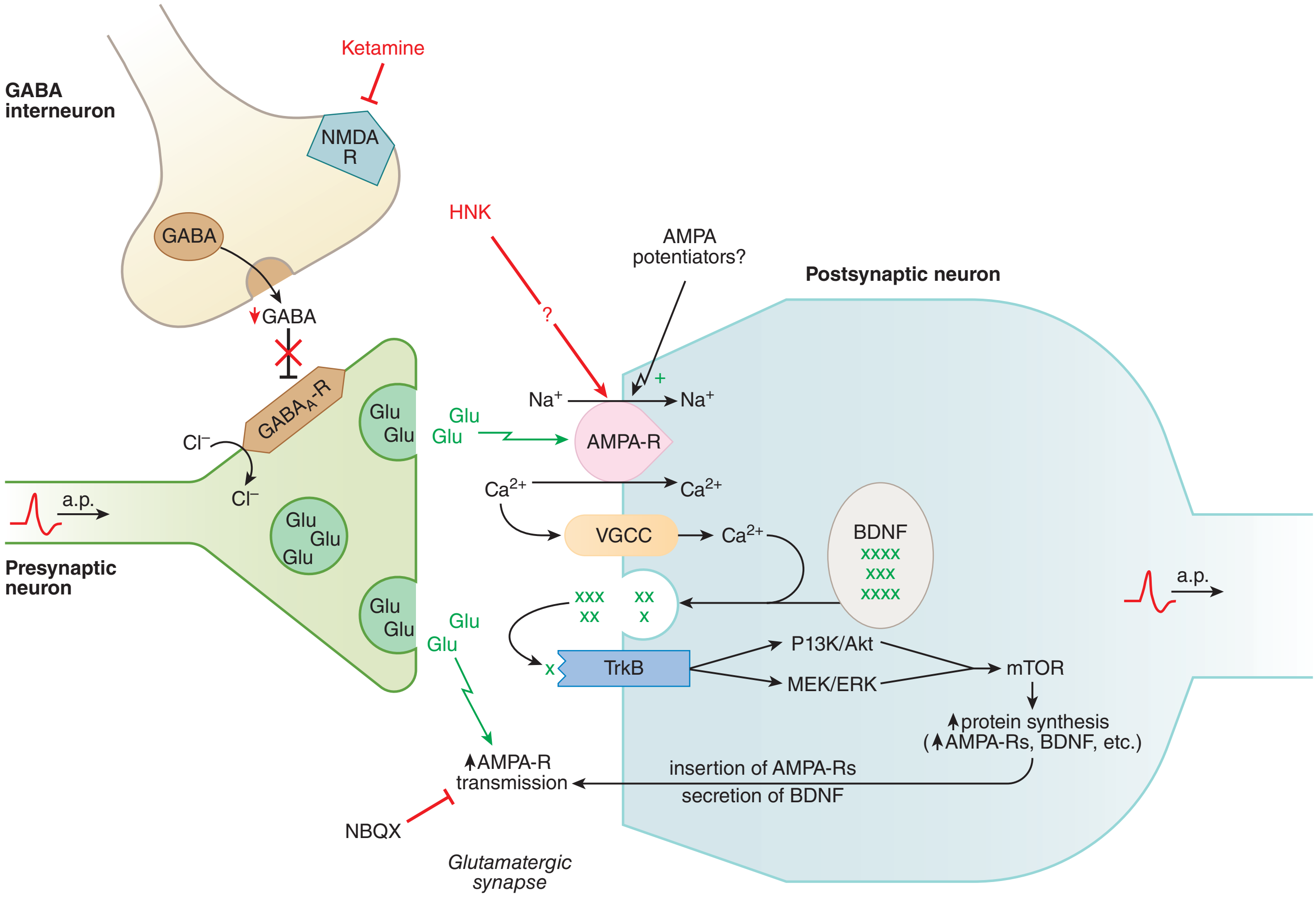


Figure 18–2 *Proposed mechanisms for the antidepressant effects of ketamine.* The figure depicts an interneuron interacting with the presynaptic fiber of a glutamatergic synapse, such as might occur in the prefrontal cortex. *Ketamine* interacts with and inhibits the function of NMDA receptors on the GABAergic interneuron, thereby inhibiting cation flux through the receptor channel and reducing excitability of the interneuron. The downstream effect is a reduction in GABA release onto the $GABA_A$ receptor of the presynaptic neuron, causing a disinhibition of that glutamatergic neuron (a reduction in the hyperpolarizing flow of chloride ions that stimulation of the $GABA_A$ receptor would normally cause), thereby promoting depolarization and glutamate release into the synaptic space. Released glutamate activates AMPA receptors (GluA1/GluA2 receptors) on the postsynaptic cell, resulting in depolarization of that cell and a number of sequelae, including Ca^{2+} entry (via voltage-gated calcium channels [VGCCs]) and release of brain-derived neurotrophic factor (BDNF). BDNF activates a membrane receptor tyrosine kinase, tropomyosin receptor kinase B (TrkB), initiating cellular responses that include stimulation of neuronal protein synthesis (including synthesis of BDNF and AMPA receptors), leading to further enhancement of glutamatergic neurotransmission (restoration of normal transmission). Hypothetically, these events account for the antidepressant activity of *ketamine*. Blockade of *ketamine*'s antidepressant effects by the AMPA receptor antagonist NBQX (see Table 16–2) is consistent with this scheme. The FDA-approved antidepressant is *esketamine*, the *S*-enantiomer of *ketamine*, but there is also interest in the therapeutic potential of other enantiomers and metabolites of ketamine. *R*-ketamine has antidepressant properties, and, as indicated in the figure, a ketamine metabolite, (2R,6R)-hydroxynorketamine (HNK), can also facilitate (enhance) neurotransmission mediated by the AMPA receptor, suggesting that this metabolite may play a role in the postsynaptic responses depicted above and raising the possibility of designing antidepressants that are direct potentiators of AMPA-mediated neurotransmission. For further details, see Sanacora and Schatzberg (2015), Zanos and Gould (2018), and Hashimoto (2020). a.p., action potential; mTOR, mechanistic target of rapamycin.

component (Brockmöller et al., 2007). Steady-state *trazodone* is observed within 3 days following initiation of a dosing regimen. *Trazodone* has a $t_{1/2}$ of 6 h; a metabolite, m-chlorophenylpiperazine, is a tryptaminergic agonist. *Trazodone* typically is started at 150 mg/day in divided doses, with 50-mg increments every 3 to 4 days. The maximally recommended dose is 400 mg/day for outpatients and 600 mg/day for inpatients. *Nefazodone* has a $t_{1/2}$ of only 2 to 4 h; its major metabolite hydroxynefazodone has a $t_{1/2}$ of 1.5 to 4 h.

Bupropion

Bupropion elimination has a $t_{1/2}$ of 21 h and involves both hepatic and renal routes. Patients with severe hepatic cirrhosis should receive a maximum dose of 150 mg every other day; consideration for a decreased dose should also be made in cases of renal impairment.

Tricyclic Antidepressants

The TCAs, or their active metabolites, have plasma half-lives of 8 to 80 h; this makes once-daily dosing possible for most of the compounds (Rudorfer and Potter, 1999). Steady-state concentrations occur within several days to several weeks of beginning treatment, as a function of the $t_{1/2}$. TCAs are largely eliminated by hepatic CYPs (see Table 18–3). Dosage adjustments of TCAs are typically made according to a patient's clinical response, not based on plasma levels. Nonetheless, monitoring the plasma exposure has an important relationship to treatment response; there is a relatively narrow therapeutic window. About 7% of patients metabolize TCAs slowly due to a variant CYP2D6 isoenzyme, causing a 30-fold difference in plasma concentrations among different patients given the same TCA dose. To avoid toxicity in "slow metabolizers," plasma levels should be monitored and doses adjusted downward.

Monoamine Oxidase Inhibitors

The MAOIs are metabolized by acetylation. A significant portion of the population (50% of the population of European descent and an even higher percentage among those of Asian ancestry) are "slow acetylators" (see Figure 65–4) and will exhibit elevated plasma levels. The nonselective

TABLE 18–3 ■ DISPOSITION OF ANTIDEPRESSANTS

DRUG	ELIMINATION $t_{1/2}$ (h) OF PARENT DRUG (*$t_{1/2}$ of active metabolite*)	TYPICAL C_p (ng/mL)	PREDOMINANT CYP INVOLVED IN METABOLISM
Tricyclic antidepressants			
Amitriptyline	16 *(30)*	100–250	2D6, 2C19, 3A3/4, 1A2
Amoxapine	8 *(30)*	200–500	
Clomipramine	32 *(70)*	150–500	
Desipramine	30	125–300	
Doxepin	18 *(30)*	150–250	
Imipramine	12 *(30)*	175–300	
Maprotiline	48	200–400	
Nortriptyline	31	60–150	
Protriptyline	80	100–250	
Trimipramine	16 *(30)*	100–300	
Selective serotonin reuptake inhibitors			
R,*S*-Citalopram	36	75–150	3A4, 2C19
S-Citalopram	30	40–80	3A4, 2C19
Fluoxetine	53 *(240)*	100–500	2D6, 2C9
Fluvoxamine	18	100–200	2D6, 1A2, 3A4, 2C9
Paroxetine	17	30–100	2D6
Sertraline	23 *(66)*	25–50	2D6
Serotonin-norepinephrine reuptake inhibitors			
Duloxetine	11	47–110	2D6
Venlafaxine	5 *(11)*	2–3200	2D6, 3A4
Other antidepressants			
Atomoxetine	5–20; child, 3	—	2D6, 3A3/4
Brexanolone	9	1800–7500 (ng*h/mL with 60 µg/h to 90 µg/h infusion range; 10–140 breast milk concentrations)	AKRs, UGTs, SULTs
Bupropion	11	75–100	2B6
Esketamine	7–12 *(8)*	5–300	2B6, 3A4 (primary) 2C9, 2C19 (minor)
Mirtazapine	16	5–100	2D6
Nefazodone	2–4	80–2300	3A3/4
Reboxetine	12	25–203	3A4
Trazodone	6	800–1600	2D6

Values shown are elimination $t_{1/2}$ values for a number of clinically used antidepressant drugs; numbers in parentheses are $t_{1/2}$ values of active metabolites. Fluoxetine (2D6), fluvoxamine (1A2, 2C8, 3A3/4), paroxetine (2D6), and nefazodone (3A3/4) are potent inhibitors of CYPs; sertraline (2D6), citalopram (2C19), and venlafaxine are less-potent inhibitors. Plasma concentrations are those observed at typical clinical doses.

Information sources: FDA-approved package inserts and Appendix I of this book.

MAOIs used in the treatment of depression are irreversible inhibitors; thus, it takes up to 2 weeks for MAO activity to recover, even though the parent drug is excreted within 24 h (Livingston and Livingston, 1996). Recovery of normal enzyme function is dependent on synthesis and transport of new MAO to monoaminergic nerve terminals.

Esketamine

Esketamine, the *S*-enantiomer of the NMDA antagonist *ketamine*, is administered as a nasal spray. The absolute bioavailability after intranasal administration is approximately 50% for the 56- and 84-mg intranasal doses. Approximately 46% of the inhaled dose is swallowed; 19% of this swallowed dose reaches the systemic circulation. This NMDA antagonist is noncompetitive and has a very slow off rate from its allosteric binding site on the NMDA receptor (see Figure 16–9), resulting in a much longer dosage interval for effective control of depression than would be predicted from the terminal plasma $t_{1/2}$ of 7 to 12 h. The maximal plasma concentration (C_{max}) occurs 20 to 40 min after intranasal administration. Elderly individuals and those with mild hepatic impairment have slower clearance of the drug, which results in higher exposure to the drug, as evidenced by increased C_{max} (67% higher); noresketamine's apparent clearance is 19% lower in this subgroup. Interindividual variability in concentration exposure to *ketamine* has been reported to be as high as 66% (FDA, 2019; Perez-Ruixo, 2021).

Brexanolone

Brexanolone acts at $GABA_A$ receptors to enhance Cl^- current. After administration by IV infusion, the terminal plasma concentration $t_{1/2}$ of *brexanolone* is 9 h (Scott, 2019). The primary metabolic pathways are α-keto reduction, glucuronidation, and sulfation; the disposition of *brexanolone* is not affected by renal function. However, the solubilizing agent used in the IV formulation (betadex sulfobutyl ether sodium) can accumulate in individuals with severe renal impairment (<5 mL/min glomerular filtration rate). There are no known pharmacokinetic drug interactions. *Brexanolone* exhibits linear pharmacokinetics across the range of infusions from 30 µg/kg/h through 270 µg/kg/h with low intersubject variability in concentration exposure (<21%) (Leader et al., 2019).

Adverse Effects

Selective Serotonin Reuptake Inhibitors

The SSRIs have no major cardiovascular side effects, are generally free of antimuscarinic side effects (dry mouth, urinary retention, confusion), and do not block α adrenergic receptors. Most SSRIs, with the exception of *paroxetine*, do not block histamine receptors and usually are nonsedating (Table 18–4).

Adverse side effects of SSRIs from excessive stimulation of brain $5HT_2$ receptors may result in insomnia, increased anxiety, irritability, and decreased libido, effectively worsening prominent depressive symptoms. Excess activity at spinal $5HT_2$ receptors causes sexual side effects, including erectile dysfunction, anorgasmia, and ejaculatory delay (Clayton et al., 2014). These effects may be more prominent with *paroxetine*. Aspects of sexual dysfunction can be treated with the PDE5 inhibitor *sildenafil* (Nurnberg, 2001; Nurnberg et al., 2008; see also Figure 49–6 and Table 49–2). Stimulation of $5HT_3$ receptors in the central nervous system (CNS) and periphery contributes to GI effects, which are usually limited to nausea but may include diarrhea and emesis. Some patients experience an increase in anxiety, especially with the initial dosing of SSRIs. With continued treatment, some patients also report a dullness of intellectual abilities and concentration. In general, there is not a strong relationship between SSRI serum concentrations and therapeutic efficacy. Thus, dosage adjustments are based more on evaluation of clinical response and management of side effects.

Sudden withdrawal of antidepressants can precipitate a discontinuation syndrome (Harvey and Slabbert, 2014). For SSRIs and SNRIs, the symptoms of withdrawal may include dizziness, headache, nervousness, nausea, and insomnia. This withdrawal syndrome appears most intense for *paroxetine*, *venlafaxine*, and *desvenlafaxine*, due to their relatively

TABLE 18–4 ■ POTENCIES OF SELECTED ANTIDEPRESSANTS AT MUSCARINIC, HISTAMINE H_1, AND α_1 ADRENERGIC RECEPTORS

	RECEPTOR TYPE		
DRUG	**MUSCARINIC CHOLINERGIC**	**HISTAMINE H_1**	**α_1 ADRENERGIC**
Amitriptyline	18	1.1	27
Amoxapine	1000	25	50
Atomoxetine	≥1000	≥1000	≥1000
Bupropion	40,000	6700	4550
R,*S*-Citalopram	1800	380	1550
S-Citalopram	1240	1970	3870
Clomipramine	37	31.2	39
Desipramine	196	110	130
Doxepin	83.3	0.24	24
Duloxetine	3000	2300	8300
Fluoxetine	2000	6250	5900
Fluvoxamine	24,000	>100,000	7700
Imipramine	91	11.0	91
Maprotiline	560	2.0	91
Mirtazapine	670	0.1	500
Nefazodone	11,000	21	25.6
Nortriptyline	149	10	58.8
Paroxetine	108	22,000	>100,000
Protriptyline	25	25	130
Reboxetine	6700	312	11,900
Sertraline	625	24,000	370
Trazodone	>100,000	345	35.7
Trimipramine	59	0.3	23.8
Venlafaxine	>100,000	>100,000	>100,000

Values are experimentally determined potencies (K_i values, nM) for binding to receptors that contribute to common side effects of clinically used antidepressant drugs: muscarinic cholinergic receptors (e.g., dry mouth, urinary retention, confusion); histamine H_1 receptors (sedation); and α_1 adrenergic receptors (orthostatic hypotension, sedation). Data from Leonard and Richelson, 2000.

short half-lives and, in the case of *paroxetine*, lack of active metabolites. Conversely, the active metabolite of *fluoxetine*, norfluoxetine, has such a long $t_{1/2}$ (1–2 weeks) that few patients experience any withdrawal symptoms with discontinuation of *fluoxetine*.

Unlike the other SSRIs, *paroxetine* is associated with an increased risk of congenital cardiac malformations when administered in the first trimester of pregnancy (Gadot and Koren, 2015). There has been substantial debate regarding the potential for SSRI administration to pregnant women to cause persistent pulmonary hypertension in newborns. While the data support this association, the absolute increase in risk to newborns is small compared to the high likelihood of harm to women whose antidepressants are discontinued during pregnancy (Ng et al., 2019).

Serotonin-Norepinephrine Reuptake Inhibitors

The SNRIs have a side effect profile similar to that of the SSRIs, including nausea, constipation, insomnia, headaches, and sexual dysfunction. The immediate-release formulation of *venlafaxine* can induce sustained diastolic hypertension (diastolic blood pressure >90 mmHg at consecutive weekly visits) in 10% to 15% of patients at higher doses; this risk is reduced with the extended-release form. This effect of *venlafaxine* may not be associated simply with inhibition of NET because *duloxetine* does not share this side effect. *Venlafaxine* also is associated with an increased risk of perinatal complications.

Serotonin Receptor Antagonists

The main side effects of *mirtazapine*, seen in more than 10% of the patients, are somnolence, increased appetite, and weight gain. A rare side effect of *mirtazapine* is agranulocytosis. *Trazodone* use is associated with priapism in rare instances. *Nefazodone* was voluntarily withdrawn from the market in several countries after rare cases of liver failure were associated with its use. In the U.S., *nefazodone* is marketed with a black-box warning regarding hepatotoxicity.

Bupropion

Typical side effects associated with *bupropion* include anxiety, mild tachycardia and hypertension, irritability, and tremor. Other side effects include headache, nausea, dry mouth, constipation, appetite suppression, insomnia, and, rarely, aggression, impulsivity, and agitation. Seizures are dependent on dose and plasma concentration, with seizures occurring rarely within the recommended dose range. *Bupropion* should be avoided in patients with seizure disorders as well as those with bulimia, due to an increased risk of seizures (Horne et al., 1988; Noe et al., 2011). At doses higher than that recommended for depression (450 mg/day), the risk of seizures increases significantly. The use of extended-release formulations often blunts the maximum concentration observed after dosing and minimizes the chance of reaching drug levels associated with an increased risk of seizures.

Tricyclic Antidepressants

The TCAs are potent antagonists at histamine H_1 receptors, and this antagonism contributes to the sedative effects of TCAs (see Table 18–4). Antagonism of muscarinic acetylcholine receptors contributes to cognitive dulling as well as a range of adverse effects mediated by the parasympathetic nervous system (blurred vision, dry mouth, tachycardia, constipation, difficulty urinating). Some tolerance does occur for these anticholinergic effects. Antagonism of α_1 adrenergic receptors contributes to orthostatic hypotension and sedation. Weight gain is another side effect of this class of antidepressants.

The TCAs have quinidine-like effects on cardiac conduction that can be life threatening with overdose and that limit the use of TCAs in patients with heart disease. This is the primary reason that only a limited supply should be available to the patient at any given time. Like other antidepressant drugs, TCAs also lower the seizure threshold.

Monoamine Oxidase Inhibitors

Hypertensive crisis resulting from food or drug interactions is one of the life-threatening toxicities associated with the use of the MAOIs (Rapaport, 2007). Foods containing tyramine are a contributing factor. MAO_A within the intestinal wall and MAO_A and MAO_B in the liver normally degrade dietary tyramine. When MAO_A is inhibited, tyramine can enter the systemic circulation and be taken up into adrenergic nerve endings, where it causes release of catecholamines from storage vesicles resulting in stimulation of postsynaptic receptors and an increase in blood pressure to dangerous levels. The concurrent use of MAOIs and medications that contain sympathomimetic compounds also results in a potentially life-threatening elevation of blood pressure. In comparison to *tranylcypromine* and *isocarboxazid*, the *selegiline* transdermal patch (*selegiline* is selective for MAO_B) is better tolerated and safer, as are the reversible, competitive inhibitors *moclobemide* and *eprobemide*. Another serious, life-threatening issue with chronic administration of MAOIs is hepatotoxicity.

Esketamine

Esketamine increases blood pressure for several hours following administration. It is reported to be sedating and to disrupt sleep and to impair

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memory and concentration. It has abuse potential; partly because of this, it is administered under a Risk Evaluation and Mitigation Strategy.

Brexanolone

Brexanolone is sedating and can cause dizziness. Because of its association with sudden loss of consciousness, it is administered under a Risk Evaluation and Mitigation Strategy. Additionally, *brexanolone* should not be used in patients with end-stage renal disease because of concern that the solubilizing agent, betadex sulfobutyl ether sodium, may accumulate in the kidney and liver and cause adverse effects.

Drug Interactions

Many antidepressant drugs are metabolized by hepatic CYPs, especially by CYP2D6. Thus, other agents that are substrates (e.g., *hydrocodone, diphenhydramine*) or inhibitors (e.g., *imatinib, fluoxetine, paroxetine, mirabegron, quinidine*) of CYP2D6 can increase plasma concentrations of the primary drug. The combination of other classes of antidepressant agents with MAOIs is inadvisable and can lead to *serotonin syndrome*, a serious triad of abnormalities consisting of cognitive, autonomic, and somatic effects due to excess serotonin. Symptoms of serotonin syndrome include hyperthermia, muscle rigidity, myoclonus, tremors, autonomic instability, confusion, irritability, and agitation; this can progress to coma and death.

Selective Serotonin Reuptake Inhibitors

Paroxetine and, to a lesser degree, *fluoxetine* are potent inhibitors of CYP2D6 (Hiemke and Hartter, 2000). The other SSRIs, outside of *fluvoxamine*, are at least moderate inhibitors of CYP2D6. This inhibition can result in disproportionate increases in plasma concentrations of drugs metabolized by CYP2D6 when doses of these drugs are increased. *Fluvoxamine* directly inhibits CYP1A2 and CYP2C19; *fluoxetine* and *fluvoxamine* also inhibit CYP3A4. A prominent interaction is the increase in TCA exposure that may be observed during coadministration of TCAs and SSRIs.

The MAOIs enhance the effects of SSRIs due to inhibition of 5HT metabolism. Administration of these drugs together can produce synergistic increases in extracellular brain 5HT, leading to serotonin syndrome (see previous discussion). Other drugs that may induce serotonin syndrome include substituted amphetamines such as *methylenedioxymethamphetamine* (MDMA; Ecstasy) and synthetic cathinones (i.e., "bath salts"), which directly release 5HT from nerve terminals.

The SSRIs should not be started until at least 14 days following discontinuation of treatment with an irreversible inhibitor of MAO; this time period allows for synthesis of new MAO. For all SSRIs but *fluoxetine*, at least 14 days should pass prior to beginning treatment with an MAOI following the end of treatment with an SSRI. Because the active metabolite norfluoxetine has a $t_{1/2}$ of 1 to 2 weeks, at least 5 weeks should pass between stopping *fluoxetine* and beginning an MAOI.

Serotonin-Norepinephrine Reuptake Inhibitors

While a 14-day period is recommended between ending MAOI therapy and starting *venlafaxine* treatment, an interval of 7 days is considered safe. *Duloxetine* has a similar interval for initiation following MAOI therapy; conversely, only a 5-day waiting period is needed before beginning MAOI treatment after ending *duloxetine*. Failure to observe these required waiting periods can result in serotonin syndrome.

Serotonin Receptor Antagonists

Trazodone dosing may need to be lowered when given together with drugs that inhibit CYP3A4. *Mirtazapine* is metabolized by CYPs 2D6, 1A2, and 3A4 and may interact with drugs that share these CYP pathways, requiring mutual dose reductions. *Trazodone* and *nefazodone* are weak inhibitors of 5HT uptake and should not be administered with MAOIs due to concerns about serotonin syndrome.

Bupropion

The major route of metabolism for *bupropion* is via CYP2B6. *Bupropion* and its metabolite hydroxybupropion can inhibit CYP2D6, the CYP responsible for metabolism of several SSRIs (see Table 18–3) as well *propranolol* and *haloperidol*, among others. Thus, the potential for interactions of *bupropion* with SSRIs and other drugs metabolized by CYP2D6 should be kept in mind until the safety of the combination is firmly established.

Tricyclic Antidepressants

Drugs that inhibit CYP2D6, such as *bupropion* and SSRIs, may increase plasma exposures of TCAs. TCAs can potentiate the actions of sympathomimetic amines and should not be used concurrently with MAOIs or within 14 days of stopping MAOIs. A *number of other drugs have similar side effect profiles as TCAs, and concurrent use risks enhanced side effects* (see previous discussion in Adverse Effects); this includes phenothiazine antipsychotic agents, type 1C antiarrhythmic agents, and other drugs with antimuscarinic, antihistaminic, and α adrenergic antagonistic effects.

Monoamine Oxidase Inhibitors

Serotonin syndrome is the most serious drug interaction for the MAOIs (see Adverse Effects). The most common cause of serotonin syndrome in patients taking MAOIs is the accidental coadministration of a 5HT reuptake-inhibiting antidepressant or *tryptophan*. Other serious drug interactions include those with *meperidine* and *tramadol*. MAOIs also interact with sympathomimetics such as *pseudoephedrine*, *phenylephrine*, *oxymetazoline*, *phenylpropanolamine*, and *amphetamine*; these are commonly found in cold and allergy medications and diet aids and should be avoided by patients taking MAOIs. Likewise, patients on MAOIs must avoid foods containing high levels of tyramine: soy products, dried meats and sausages, dried fruits, home-brewed and tap beers, red wine, pickled or fermented foods, and aged cheeses.

Esketamine

No clinically significant pharmacokinetic drug interactions have been reported for *esketamine*. Some reduction in plasma concentrations occurs with coadministration of *rifampin* (Perez Ruixo et al., 2021). *Esketamine* is primarily metabolized via CYPs 2B6 and 3A4 with minor contributions from CYPs 2C19 and 2C9 (FDA, 2019).

Brexanolone

No pharmacokinetic drug interactions have been reported to date for *brexanolone*. *Brexanolone* is primarily metabolized via aldo-keto reduction (AKR), glucuronidation, and sulfation (FDA, 2018).

Anxiolytic Drugs

Primary treatments for anxiety-related disorders include the SSRIs, SNRIs, benzodiazepines, *buspirone*, and β adrenergic antagonists (Atack, 2003). The SSRIs and the SNRI *venlafaxine* are well tolerated with a reasonable side effect profile; in addition to their documented antidepressant activity, they have anxiolytic activity with chronic treatment. The benzodiazepines are effective anxiolytics as both acute and chronic treatment. There is concern regarding their use because of their potential for dependence and abuse as well as negative effects on cognition and memory. *Buspirone*, like the SSRIs, is effective following chronic treatment. It acts, at least in part, via the serotonergic system, where it is a partial agonist at $5HT_{1A}$ receptors. *Buspirone* also has antagonistic effects at DA D_2 receptors, but the relationship between this effect and its clinical actions is uncertain. β Adrenergic antagonists, particularly those with higher lipophilicity (e.g., *propranolol* and *nadolol*), are occasionally used for performance anxiety such as fear of public speaking; their use is limited due to significant side effects, such as hypotension.

Antihistamines and sedative-hypnotic agents have been tried as anxiolytics but are generally not recommended because of their side effect profiles and the availability of superior drugs. *Hydroxyzine*, which produces short-term sedation, is used in patients who cannot use other types of anxiolytics (e.g., those with a history of drug or alcohol abuse where benzodiazepines would be avoided). *Chloral hydrate* has been used for situational anxiety, but there is a narrow dose range where anxiolytic effects are observed in the absence of significant sedation; therefore, the use of *chloral hydrate* is not recommended.

Clinical Considerations With Anxiolytic Drugs

The choice of pharmacological treatment of anxiety is dictated by the specific anxiety-related disorders and the clinical need for acute anxiolytic effects (Millan, 2003). Among the commonly used anxiolytics, only the benzodiazepines and β adrenergic antagonists are effective acutely; the use of β adrenergic antagonists is generally limited to treatment of situational anxiety. While SSRIs, SNRIs, and *buspirone* are the first-line medications for anxiety, chronic treatment is required to produce and sustain anxiolytic effects; these agents are not effective acutely. When an immediate anxiolytic effect is clinically indicated, benzodiazepines are typically selected.

Benzodiazepines, such as *alprazolam*, *chlordiazepoxide*, *clonazepam*, *clorazepate*, *diazepam*, *lorazepam*, and *oxazepam*, are effective in the treatment of generalized anxiety disorder, panic disorder, and situational anxiety. In addition to their anxiolytic effects, benzodiazepines produce sedative, hypnotic, anesthetic, anticonvulsant, and muscle relaxant effects. The benzodiazepines also impair cognitive performance and memory, adversely affect motor control, and potentiate the effects of other sedatives, including alcohol and opioids. The anxiolytic effects of this class of drugs are mediated by allosteric interactions with the pentameric benzodiazepine-$GABA_A$ receptor complex, in particular $GABA_A$ receptors comprising α2, α3, and α5 subunits (see Chapters 16 and 22). The primary effect of the anxiolytic benzodiazepines is to enhance the inhibitory effects of the neurotransmitter GABA.

One area of concern regarding the use of benzodiazepines in the treatment of anxiety is the potential for habituation, dependence, and abuse. Patients with certain personality disorders or a history of drug or alcohol abuse are particularly susceptible. In the context of the opioid abuse crisis, the rates of accidental overdose and death secondary to combining benzodiazepines and opioids have increased substantially, even in patients not previously identified as being at high risk of drug abuse (Dowell et al., 2016; Sun et al., 2017). While benzodiazepines are effective in both short- and long-term treatment of patients with sustained or recurring bouts of anxiety, caution is advised in initiating benzodiazepine treatment, with a focus on minimizing dose and co-initiation of long-term treatment with first-line medications, such as SSRIs or SNRIs, and/or psychotherapy. Further, premature discontinuation of benzodiazepines, in the absence of other pharmacological treatment, results in a high rate of relapse. Withdrawal of benzodiazepines after chronic treatment, particularly with benzodiazepines with short durations of action, can cause increased anxiety and seizures. For this reason, it is important that discontinuation be carried out in a gradual manner.

Benzodiazepines cause many adverse effects, including sedation, mild memory impairments, decreased alertness, and slowed reaction time (which may lead to accidents). Memory problems can include visual-spatial deficits but will manifest clinically in a variety of ways, including difficulty in word finding. Occasionally, paradoxical reactions can occur with benzodiazepines, such as increases in anxiety, sometimes reaching panic attack proportions. Other pathological reactions can include irritability, aggression, or behavioral disinhibition. Amnesic reactions (i.e., loss of memory for particular periods) can also occur. Benzodiazepines should not be used in pregnant women; there have been rare reports of fetal craniofacial defects. In addition, benzodiazepines taken prior to delivery may result in sedated, underresponsive newborns and prolonged withdrawal reactions. In the elderly, benzodiazepines increase the risk for falls and must be used cautiously. These drugs are safer than classical sedative-hypnotics in overdosage and typically are fatal only if combined with other CNS depressants.

Benzodiazepines have abuse potential, although their capacity for abuse is considerably below that of other classical sedative-hypnotic agents such as barbiturates. When these agents are abused, it is generally in a multidrug abuse pattern, frequently connected with failed attempts to control anxiety. Tolerance to the anxiolytic effects develops with chronic administration, with the result that some patients escalate the dose of benzodiazepines over time, contributing to increased adverse effects and morbidity. Ideally, benzodiazepines should be used for short periods of time and in conjunction with other medications (e.g., SSRIs) or evidence-based psychotherapies (e.g., cognitive behavioral therapy for anxiety disorders).

The SSRIs and the SNRI *venlafaxine* are first-line treatments for most types of anxiety disorders, except when an acute drug effect is desired; *fluvoxamine* is approved only for obsessive-compulsive disorder. As for their antidepressant actions, the anxiolytic effects of these drugs become manifest following chronic treatment. Other drugs with actions on serotonergic neurotransmission, including *trazodone*, *nefazodone*, and *mirtazapine*, also are used in the treatment of anxiety disorders. Details regarding the pharmacology of these classes were presented previously in this chapter.

Both SSRIs and SNRIs are beneficial in specific anxiety conditions, such as generalized anxiety disorder, social phobias, obsessive-compulsive disorder, and panic disorder. These effects appear to be related to the capacity of serotonin to regulate the activity of brain structures such as the amygdala and locus coeruleus that are thought to be involved in the genesis of anxiety. Interestingly, the SSRIs and SNRIs often will produce some increases in anxiety in the short term; these dissipate with time. Therefore, the maxim “start low and go slow” is indicated with anxious patients; however, many patients with anxiety disorders ultimately will require doses that are about the same as those required for the treatment of depression, and sometimes higher, especially in the treatment of obsessive-compulsive disorder. Anxious patients appear to be particularly prone to severe discontinuation reactions with certain medications such as *venlafaxine* and *paroxetine*; therefore, slow off-tapering is required.

Buspirone is used in the treatment of generalized anxiety disorder (Goodman, 2004). Like the SSRIs, *buspirone* requires chronic treatment for effectiveness. Also, like the SSRIs, *buspirone* lacks many of the other pharmacological effects of the benzodiazepines: It is not an anticonvulsant, muscle relaxant, or sedative, and it does not impair psychomotor performance or result in dependence. *Buspirone* is primarily effective in the treatment of generalized anxiety disorder, but not for other anxiety disorders. In fact, patients with panic disorder often note an increase in anxiety acutely following initiation of *buspirone* treatment; this may be the result of the fact that *buspirone* causes increased firing rates of the locus coeruleus, which is thought to underlie part of the pathophysiology of panic disorder.

Drug Facts for Your Personal Formulary: *Depression and Anxiety Disorders*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Selective Serotonin Reuptake Inhibitors		
Citalopram Escitalopram Fluoxetine Fluvoxamine Paroxetine Sertraline Vilazodone	• Anxiety and depression disorders • Obsessive-compulsive disorder, PTSD • SERT selective; little effect on NET • Vilazodone also acts as $5HT_{1A}$ partial agonist	• Risks of bleeding, mania/hypomania, seizures • May increase risk of suicidal thoughts or behavior • Serotonin syndrome with MAOIs • Numerous drug-drug interactions mediated by CYPs • May cause sexual dysfunctions, weight gain (less with vilazodone) • GI disturbances
Serotonin-Norepinephrine Reuptake Inhibitors		
Venlafaxine Desvenlafaxine Duloxetine Milnacipran Levomilnacipran	• Anxiety and depression, ADHD, autism, fibromyalgia, PTSD, menopause symptoms • Inhibitors of SERT and NET	• Side effects include nausea and dizziness • May increase risk of suicidal thoughts or behavior • Risk of bleeding, hyponatremia, sexual dysfunction • Duloxetine and milnacipran contraindicated in uncontrolled narrow-angle or angle-closure glaucoma
Tricyclic Antidepressants		
Amitriptyline Clomipramine Doxepin Imipramine Trimipramine Nortriptyline Maprotiline Protriptyline Desipramine Amoxapine	• Block SERT, NET, α_1, H_1, and M_1 receptors • Major depression	• Generally replaced by newer antidepressants with fewer side effects • Numerous side effects: orthostatic hypertension, weight gain, GI disturbances, sexual dysfunction, seizures, irregular heart beats • Should not be used within 14 days of taking MAOIs, linezolid, or methylene blue • May increase suicidal thoughts or behavior
Atypical Antipsychotics		
Aripiprazole Brexpiprazole Lurasidone Olanzapine Quetiapine Risperidone	• Treatment-resistant major depression and depression with psychotic features • Schizophrenia • Bipolar depression	• See Chapter 19 for details • Metabolic syndrome and weight gain
Monoamine Oxidase Inhibitors		
Isocarboxazid Phenelzine Selegiline Tranylcypromine	• Inhibit MAO_A and MAO_B to prevent NE, DA, and 5HT breakdown • Major depression disorders resistant to other antidepressants	• Many side effects, including weight gain and sexual dysfunction; replaced by newer antidepressants • May increase suicidal thoughts or behavior • Slow elimination • May cause hypertensive crisis if taken with tyramine-containing foods/beverages • At lower doses, selective for MAO_B (serotonergic neurons) • A transdermal patch is approved for treatment of depression
Atypical Antidepressants		
Bupropion (DAT inhibitor) Trazodone ($5HT_2$ antagonist) Nefazodone ($5HT_2$ antagonist) Mirtazapine ($5HT_2$ antagonist) Mianserin (not marketed in the U.S.) Vortioxetine (SERT inhibitor, $5HT_{1A}$ agonist, and $5HT_3$ antagonist)	• Depression • Smoking cessation (bupropion) • Insomnia (low-dose trazodone)	• Risk of adverse cerebrovascular events in elderly with dementia • Bupropion, no weight gain effect • Mirtazapine, trazodone, and nefazodone may cause drowsiness; take at bedtime • Risk of hepatic failure with nefazodone • May increase suicidal thoughts or behavior • Do not use within 14 days of taking MAOI
Esketamine	• Treatment-resistant depression	• Administered as a nasal spray under a Risk Evaluation and Mitigation Strategy
Brexanolone	• Postpartum depression	• Administered as an intravenous infusion over 2.5 days under a Risk Evaluation and Mitigation Strategy • Avoid in patients with end-stage renal disease

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Chapter 19

Pharmacotherapy of Psychosis and Mania

Jonathan M. Meyer

TREATMENT OF PSYCHOSIS
- Neurotransmitter Bases for Psychosis
- Mechanisms of Action at D_2 Receptors
- Review of Relevant Pathophysiology
- Review of Psychosis Pathology and the General Goals of Pharmacotherapy
- Short-Term Antipsychotic Treatment
- Long-Term Treatment
- Pharmacology of Antipsychotic Agents
- Other Therapeutic Uses
- Adverse Effects and Drug Interactions
- Major Drugs Available in the Class

TREATMENT OF MANIA
- Pharmacological Properties of Agents for Mania
- Lithium

Treatment of Psychosis

Psychosis is a symptom of numerous psychiatric illnesses, characterized by a distorted or nonexistent sense of reality or by disorganized behavior or speech. Psychotic disorders have different etiologies, each of which demands a unique treatment approach. Common psychotic disorders include mood disorders (major depression or mania) with psychotic features, substance-induced psychosis, dementia with psychotic features, delirium with psychotic features, brief psychotic disorder, delusional disorder, schizoaffective disorders, and schizophrenia.

The worldwide schizophrenia point prevalence has remained relatively constant, with the global estimate of 0.28% remaining unchanged from 1990 to 2016; however, the number of individuals suffering from schizophrenia rose nearly 60% due to population increases (Charlson et al., 2018). Older sources cite schizophrenia prevalence rates of 1%, but a 2018 analysis of 101 studies noted that higher study quality was associated with a lower estimated prevalence of psychotic disorders ($P < .001$) (Moreno-Kustner et al., 2018). Schizophrenia is considered the prototypic disorder for understanding chronic psychosis, but patients with schizophrenia exhibit features not seen in other psychotic illnesses. The positive symptoms of psychosis include hallucinations, delusions, disorganized speech, and disorganized or agitated behavior. These positive psychotic symptoms are found individually, and occasionally together, in all psychotic disorders and are typically responsive to pharmacotherapy. Schizophrenia patients also suffer from negative symptoms (apathy, avolition, diminished expression, reduced social drive) and cognitive deficits.

Neurotransmitter Bases for Psychosis

Dysfunction in cholinergic, glutamatergic, serotonergic, GABA-ergic, and dopaminergic systems is implicated in psychosis (McCutcheon et al., 2019). That excessive dopamine (DA) neurotransmission is linked to positive psychosis symptoms has been known for over 60 years since Carlsson deduced that postsynaptic DA receptor antagonism was the common mechanism for *haloperidol* and *chlorpromazine*'s therapeutic effects and for their ability to induce parkinsonism. This insight informed the development of antipsychotics that act at D_2 receptors including first-generation antipsychotics (FGAs) and second-generation antipsychotics (SGAs) that possess D_2 antagonism and newer agents with D_2 partial agonism (McCutcheon et al., 2019). DA dysfunction is no longer considered a hypothesis but an established fact now that imaging can demonstrate a direct correlation between positive psychotic symptoms and excessive D_2-mediated DA activity in the associative striatum and adjacent sensorimotor striatum of schizophrenia patients, and the association between antipsychotic exposure, reduced postsynaptic DA activity in these regions, and improved positive psychosis symptoms (McCutcheon et al., 2019).

Involvement of other neurotransmitters is based on the psychotomimetic effects of *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonists (e.g., *phencyclidine*, *ketamine*), serotonin $5HT_{2a}$ agonists (e.g., LSD [*lysergic acid diethylamide*]), and muscarinic anticholinergic agents. Combining the understanding of LSD's mechanism with the finding that Parkinson's disease psychosis (PDP) is associated with upregulation of cortical $5HT_{2A}$ receptors led to the approval of *pimavanserin*, a potent $5HT_{2a}$ inverse agonist with no DA binding, for treatment of PDP (Cummings et al., 2014). Recognition that muscarinic M_4 knockout mice exhibit the phenotype of psychosis spurred ongoing trials of the M_4 and M_1 agonist *xanomeline* for schizophrenia, with a positive phase II study published in 2021 (Brannan et al., 2021). Phase III trials of glutamate modulators (e.g., glycine reuptake inhibitors, metabotropic receptor agonists) have not been successful to date, but the importance of glutamatergic pathways means that these circuits will remain a focus of antipsychotic drug development (Figure 19–1).

Mechanisms of Action at D_2 Receptors

All DA receptors are G protein-coupled receptors (GPCRs), and D_2 receptors share common properties with D_3 and D_4 receptors in that each is linked to an inhibitory G protein G_i. D_2 receptor stimulation results in decreased cyclic AMP production and reduction in intracellular cyclic AMP (see Figure 19–1), whereas agonists at D_1 and D_5 receptors stimulate the G_s–adenylyl cyclase–cyclic AMP pathway. Antipsychotic actions at D_2 receptors are also mediated through a non-G protein pathway, particularly via modulation of glycogen synthase kinase-3β (GSK-3β) activity through the β-arrestin-2/PKB/PP2A signaling complex (see Chapter 3). SGAs antagonize D_2 receptor/β-arrestin-2 interactions more than GPCR-dependent signaling, but FGAs inhibit both pathways with similar efficacy (Urs et al., 2016). While antipsychotics reverse cognitive disruption induced by NMDA antagonists (e.g., MK-801), they are not effective at improving the cognitive dysfunction of schizophrenia thought to be associated with deficient D_1-mediated cortical signaling. However, experimental β-arrestin-2 biased D_2 partial agonists with limited GPCR effects exhibit a distinctly different profile as they are capable of the striatal D_2 antagonism needed for antipsychotic effects, but also possess cortical agonism as seen by enhanced firing of fast-spiking interneurons (Urs et al., 2016).

Abbreviations

ACEI: angiotensin-converting enzyme inhibitor
ARB: angiotensin receptor blocker
COX-2: cyclooxygenase 2
CV: cardiovascular
DA: dopamine
DRP: dementia-related psychosis
ECG: electrocardiogram
ECT: electroconvulsive therapy
eGFR: estimated glomerular filtration rate
ENaC: epithelial Na^+ channel
EPS: extrapyramidal symptom
FGA: first-generation antipsychotic
GFR: glomerular filtration rate
GI: gastrointestinal
GPCR: G protein-coupled receptor
GSK: glycogen synthase kinase
5HT: serotonin
I_{kr}: inwardly rectifying K^+ channels
LAI: long-acting injectable
Li^+: lithium
LSD: lysergic acid diethylamide
MCM: major congenital malformations
NDI: nephrogenic diabetes insipidus
NMDA: *N*-methyl-ᴅ-aspartate
NMS: neuroleptic malignant syndrome
ODT: oral dissolving tablet
PDP: Parkinson's disease psychosis
Pgp: P-glycoprotein
PI: phosphatidylinositol
PK_: protein kinase _, as in PKA, PKB, PKC
PP2A: protein phosphatase 2A
SCD: sudden cardiac death
SGA: second-generation antipsychotic
TAAR1: trace amine-associated receptor 1
TD: tardive dyskinesia
TSH: thyrotropin (thyroid-stimulating hormone)
VMAT2: vesicular monoamine transporter 2
VPA: valproic acid

Review of Relevant Pathophysiology

Not all psychosis is schizophrenia, and the pathophysiology relevant to effective schizophrenia treatment may not apply to other psychotic disorders as evidenced by the efficacy of *pimavanserin*, a $5HT_{2A}$ inverse agonist devoid of any DA receptor binding used to treat PDP. The effectiveness of DA D_2 modulators for the positive symptoms of psychosis suggests a common etiology related to excessive dopaminergic neurotransmission in striatal DA pathways. Prior studies ascribed the presence of positive psychotic symptoms to increased DA activity in mesolimbic pathways terminating in the ventral striatum, but this view has been revised. Improved imaging techniques indicate that the DA dysfunction associated with positive psychosis symptoms in schizophrenia patients is localized to the associative striatum and adjacent sensorimotor striatum (McCutcheon et al., 2019).

Delirium, Dementia, and Parkinson's Disease Psychosis

The psychoses related to delirium and dementia, particularly dementia of the Alzheimer type, may have cholinergic and noncholinergic etiologies: deficiency in muscarinic cholinergic neurotransmission due to medications, age- or disease-related neuronal loss (delirium and dementia), and excessive activity at cortical $5HT_{2a}$ receptors (dementia). Delirium can have numerous precipitants, including medication, infection, electrolyte imbalance, inflammation, or metabolic derangement, all of which require specific treatment in addition to removing offending anticholinergic and sedating medications. While PDP is related to Lewy body–associated loss of serotonin raphe neurons and subsequent upregulation of cortical postsynaptic $5HT_{2a}$ receptors, there is evidence for this process in other dementia syndromes. *Pimavanserin* is now approved for PDP and has one positive phase II study for dementia-related psychosis (DRP) in Alzheimer's disease patients (Ballard et al., 2018).

Schizophrenia

Schizophrenia is a neurodevelopmental disorder with complex genetics and incompletely understood pathophysiology. In addition to environmental exposures such as maternal infections (e.g., *Toxoplasma gondii*), fetal second-trimester infectious or nutritional insults, birth complications, and substance abuse in the late teen or early adult years, over 150 genes appear to contribute to schizophrenia risk. Implicated are genes that regulate neuronal migration, synaptogenesis, cellular adhesion, neurite outgrowth, synaptic DA availability, and glutamate, nicotinic, and DA neurotransmission. The impact of common single nucleotide polymorphisms can be quantified in a polygenic risk score that sums the genetic burden and its impact on schizophrenia risk and disease phenotype. Patients with schizophrenia also have increased rates of genome-wide DNA microduplications, termed *copy number variants* (Bergen et al., 2019) and *epigenetic changes*, including disruptions in DNA methylation patterns in various brain regions (Chen et al., 2020). This genetic variability is consistent with the heterogeneity of the clinical disease and suggests that multiple mechanisms account for disease risk and its manifestations.

Review of Psychosis Pathology and the General Goals of Pharmacotherapy

Common to all psychotic disorders are positive symptoms, which may include hallucinations, delusions, and behavioral dysfunction. All approved schizophrenia treatments to date have a direct impact on D_2-mediated DA neurotransmission, but research into muscarinic M_4 agonists (Brannan et al., 2021) and newly discovered trace amine-associated receptor 1 (TAAR1) agonists may drastically alter the established dogma that direct D_2 binding is necessary for an effective schizophrenia medication (Koblan et al., 2020) (see Figure 19–1).

Short-Term Antipsychotic Treatment

For some psychotic disorders the symptoms are transient, and antipsychotic drugs are only administered during and shortly after periods of symptom exacerbation. Patients with psychosis due to delirium, dementia, major depressive disorder, mania, substance-induced psychoses, and brief psychotic disorder will typically receive short-term antipsychotic treatment that is discontinued after resolution of psychotic symptoms, although the duration may vary considerably based on the etiology. Bipolar I disorder patients in particular may have antipsychotic treatment extended after resolution of mania and psychotic symptoms, as those on combined mood stabilizer and antipsychotic therapy have lower rates of manic relapse; moreover, several antipsychotics (*cariprazine, lumateperone, lurasidone, quetiapine*) are approved for bipolar I depression, and ongoing use may be dictated by treatment of that mood state (Wingård et al., 2019). Chronic psychotic symptoms in patients with dementia may also be amenable to longer term drug therapy, but potential benefits must be balanced by increased mortality risk associated with antipsychotics in this patient population (Maust et al., 2015).

Long-Term Antipsychotic Treatment

Delusional disorder, schizophrenia, and schizoaffective disorders are chronic diseases that require long-term antipsychotic treatment. For DRP and PDP, ongoing assessment of antipsychotic treatment appears reasonable given the paucity of long-term data, bearing in mind that the underlying disease processes are progressive and the prospect of remission remote. For schizophrenia and the schizoaffective disorders, the goal of antipsychotic treatment is to maximize functional recovery by decreasing the severity of positive symptoms and their behavioral influence and possibly improving negative symptoms and remediating cognitive

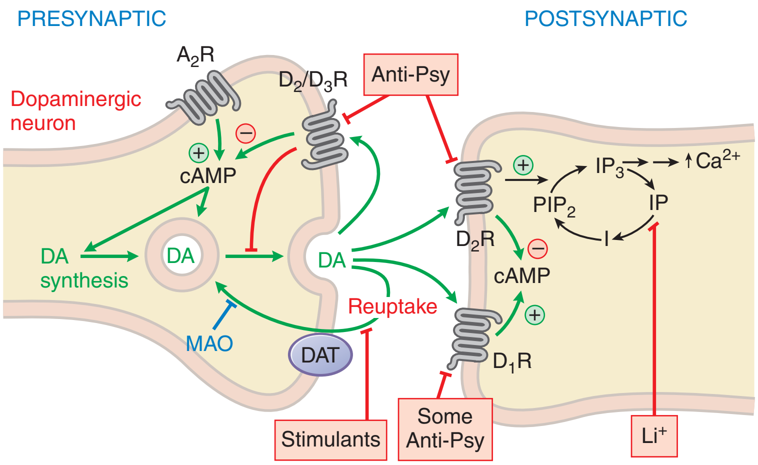


Figure 19–1 *Sites of action of antipsychotic agents and Li^+.* Following exocytotic release, DA interacts with postsynaptic receptors (R) of D_1 and D_2 types and presynaptic D_2 and D_3 autoreceptors. Termination of DA action occurs primarily by active transport of DA into presynaptic terminals via the DA transporter (DAT), with secondary deamination by mitochondrial monoamine oxidase (MAO). Stimulation of postsynaptic D_1 receptors activates the G_s–adenylyl cyclase–cAMP pathway. D_2 receptors couple through G_i to inhibit adenylyl cyclase and through G_q to activate the PLC-IP_3-Ca^{2+} pathway. Activation of the G_i pathway can also activate K^+ channels, leading to hyperpolarization. Li^+ inhibits breakdown of inositol phosphates (IP) and thereby enhances IP accumulation and sequelae (Ca^{2+} mobilization, PKC activation, depletion of cellular I). Li^+ may also alter release of neurotransmitter by a variety of putative mechanisms (see text). D_2-like autoreceptors suppress synthesis of DA by diminishing phosphorylation of rate-limiting tyrosine hydroxylase (TH), and by limiting DA release. In contrast, presynaptic α_2 receptors activate the adenylyl cyclase–cAMP–PKA pathway and thence TH activity. All antipsychotic agents act at D_2 receptors and autoreceptors; some also block D_1 receptors (Table 19–2). Stimulant agents inhibit DA reuptake by DAT, thereby prolonging the dwell time of synaptic DA. Initially in antipsychotic treatment, DA neurons release more DA, but following repeated treatment, they enter a state of physiological depolarization inactivation, with diminished production and release of DA, in addition to continued receptor blockade. Symbols: ——|, inhibition or blockade; ⊕, elevation of activity; ⊖, reduction of activity. Abbreviations: cAMP, cyclic AMP; IP_3, inositol 1,4,5-trisphosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate.

dysfunction, although the impact on the last two symptom domains is modest at best with existing agents. Continuous antipsychotic treatment reduces 1-year relapse rates from 80% among unmedicated patients to 15%. Poor adherence to antipsychotic treatment increases relapse risk, and nonadherence is often related to adverse effects, cognitive dysfunction, substance use, and limited illness insight (Meyer and Stahl, 2021).

Regardless of the underlying pathology, the immediate goal of antipsychotic treatment is a decrease in acute symptoms that induce patient distress, particularly behavioral symptoms (e.g., hostility, agitation) that may present a danger to the patient or others. The dosing, route of administration, and choice of antipsychotic depend on the underlying disease state, clinical acuity, drug-drug interactions with concomitant medications, and patient sensitivity to short- or long-term adverse effects. With the exception of *pimavanserin* for PDP and *clozapine*'s superior efficacy for treatment-resistant schizophrenia or for schizophrenia patients with suicidality, psychogenic polydipsia, or persistent impulsive aggression (Meyer and Stahl, 2019), neither the clinical presentation nor biomarkers predict the likelihood of response to a specific antipsychotic class or agent. As a result, avoidance of adverse effects based on patient and drug characteristics and exploitation of certain medication properties (e.g., the availability of a long-acting injectable [LAI] preparation) are the principal determinants for choosing initial antipsychotic therapy.

Short-Term Antipsychotic Treatment

Delirium, Dementia, and Parkinson's Disease Psychosis

Psychotic symptoms of delirium or dementia are generally treated with low medication doses, although doses in delirium patients may have to be repeated at frequent intervals initially to achieve adequate behavioral control. Despite widespread clinical use, no antipsychotic has yet received approval for DRP. As of 2021, all antipsychotic drugs carry warnings on increased mortality in elderly dementia patients, although *pimavanserin* has a modified warning since the psychosis of PDP can exist with cognitive impairment. *Pimavanserin* for PDP has a $t_{1/2}$ of 57 h, and clinical effects are seen over 2 to 6 weeks. (See Use in Pediatric Populations and Use in Geriatric Populations, below.) Because central nervous system (CNS) anticholinergic effects may worsen delirium, high-potency FGAs (e.g., *haloperidol*) or SGAs with limited antimuscarinic properties (e.g., *risperidone*) are often the drugs of choice for agitated delirium (Meyer et al., 2016). There are no data to support antipsychotic use in hypoactive delirium. The only role for antipsychotics is short-term management of psychosis and related behavioral symptoms; antipsychotics do not address the underlying pathophysiology of delirium nor shorten recovery (Girard et al., 2018). DRP patients may require extended treatment, so avoidance of extrapyramidal symptoms (EPSs) is paramount due to the fall risk. For this reason, FGAs are avoided due to their potent D_2 receptor blockade in lieu of SGAs given in modest dosages, especially agents with limited anticholinergic properties. Antipsychotic doses for patients with dementia are one-fourth of adult schizophrenia doses (e.g., *risperidone* 0.5–1.5 mg/day), as EPSs, orthostasis, and sedation are particularly problematic in this patient population (see Chapter 21).

Mania

All SGA medications with the exception of *brexpiprazole*, *clozapine*, *iloperidone*, *lumateperone*, and *lurasidone* have indications for acute mania, and doses are titrated rapidly toward the maximum FDA-approved dose over the first 24 to 72 h of treatment. FGAs are also effective in acute mania but may be eschewed due to the risk for EPSs. Clinical response (decreased psychomotor agitation and irritability, increased sleep, and reduced or absent delusions and hallucinations) usually occurs within 7 days but may be apparent as early as day 2. Patients with mania may need to continue on antipsychotic treatment for many months after the resolution of psychotic and manic symptoms, typically in combination with a mood stabilizer such as *lithium* (Li^+) or *valproic acid* (VPA) preparations (e.g., *divalproex*) (Wingård et al., 2019). Oral *aripiprazole* and *olanzapine* have indications as monotherapy for bipolar disorder maintenance treatment, but the use of *olanzapine* has decreased dramatically due to concerns over adverse metabolic effects (e.g., weight gain, hyperlipidemia, hyperglycemia). LAI *risperidone* also has indications for maintenance monotherapy and adjunctively with Li^+ or VPA in patients with bipolar I disorder, but population-based cohort data indicate that patients on any antipsychotic monotherapy have higher relapse rates than those on Li^+ or VPA monotherapy or on antipsychotic/mood stabilizer combinations (Wingård et al., 2019). While combining an antipsychotic with a mood stabilizer improves control of mood symptoms and reduces relapse risk, weight gain from the additive effects of both agents is a significant clinical problem. Antipsychotic agents with greater weight-gain liabilities (e.g., *quetiapine*, *olanzapine*, *clozapine*) should be avoided unless patients are unresponsive to preferred treatments.

The recommended duration of treatment after resolution of bipolar mania varies considerably, but as symptoms permit, a gradual drug taper should be attempted after 6 months of treatment to lessen weight gain when combined with a mood stabilizer. This must be balanced by the accruing data on the benefits of combination therapy and the need for antipsychotics that specifically treat the depressive pole of the illness (e.g., *cariprazine*, *lumateperone*, *lurasidone*, *quetiapine*).

Major Depression

Management of major depressive disorder with psychotic features requires lower-than-average doses of antipsychotics, and these must be given in combination with an antidepressant to treat the underlying mood disorder. Extended antipsychotic treatment is not usually required, but certain SGAs provide adjunctive antidepressant benefit. Most antipsychotics show limited antidepressant benefit when used as monotherapy with the exception of *amisulpride*, *cariprazine*, *lumateperone*, *lurasidone*, and *quetiapine*. Some SGAs are effective as adjunct therapy in unipolar major depression (e.g., *aripiprazole*, *brexpiprazole*, *quetiapine*) and might be preferentially used for unipolar depression with psychotic features. The postulated antidepressant mechanisms include $5HT_7$ antagonism (*amisulpride*, *lurasidone*), $5HT_{2c}$ antagonism (*olanzapine*, and *quetiapine*'s metabolite, *norquetiapine*), DA D_3 partial agonism (*aripiprazole*, *brexpiprazole*, *cariprazine*), or a combination of $5HT_{2A}$ antagonism and D_1 activation (*lumateperone*). Potent D_3 antagonists are hypothesized to block autoreceptors on the cell body of mesocortical DA neurons, resulting in increased prefrontal D_1-mediated activity at glutamatergic pathways.

Schizophrenia

The immediate goals of acute antipsychotic treatment are the reduction of agitated, disorganized, or hostile behavior; decreased impact of hallucinations; improvement in the organization of thought; and the reduction of social withdrawal. Doses used acutely may be higher than those required for maintenance treatment of stable patients, but not always. In acute psychosis, significant antipsychotic benefits are usually seen within 60 to 120 min after drug administration. Delirious or demented patients may be reluctant or unable to swallow tablets, but oral dissolving tablet (ODT) preparations or liquid concentrate forms are available. Intramuscular administration of *ziprasidone* or *olanzapine* represents an option for treating agitated and minimally cooperative patients and presents less risk for drug-induced dystonia than does *haloperidol*. An inhaled form of *loxapine* 10 mg is available in the U.S. with a median t_{max} of less than 2 min. Following rapid distribution, levels drop 75% over the next 10 min and then follow typical kinetics with a $t_{1/2}$ of 7.6 h. Inhaled *loxapine* was approved for agitation associated with schizophrenia or bipolar I disorder, so use outside of those conditions is off-label. It can also only be administered in healthcare facilities that can provide advanced airway management in the rare event of acute bronchospasm.

Aside from *clozapine*, which is uniquely efficacious in treatment resistant schizophrenia, SGAs are not more effective than FGAs, but they do offer a more favorable neurological side effect profile (Huhn et al., 2019). Excessive D_2 blockade, as is often the case with use of high-potency FGAs (e.g., *haloperidol*), not only increases risk for short-term neurological adverse effects (e.g., muscular rigidity, bradykinesia, tremor, dystonia, akathisia) but also slows mentation (bradyphrenia) and interferes with central reward pathways, resulting in patient complaints of anhedonia (loss of capacity to experience pleasure) or even dysphoria with symptomatic worsening (Meyer and Stahl, 2021). Low-potency FGAs such as *chlorpromazine* are not commonly used due to the high affinities for H_1, M_1, and α_1 adrenergic receptors that result in undesirable effects (sedation, anticholinergic properties, orthostasis). Concerns regarding QT_c prolongation further limit their clinical usefulness. In agitated acutely psychotic patients, sedation may be desirable, but the use of a sedating antipsychotic drug may interfere with cognitive function and assessment.

Because schizophrenia requires long-term treatment, antipsychotics with greater metabolic liabilities, especially weight gain (discussed further in this chapter), should be avoided as first-line therapies. High-potency FGAs (e.g., *haloperidol*, *fluphenazine*, *perphenazine*) have low risk for metabolic adverse effects, and this is one reason why these agents are still used (Huhn et al., 2019). Among the SGAs, *ziprasidone*, *aripiprazole*, *iloperidone*, *brexpiprazole*, *cariprazine*, *lumateperone*, and *lurasidone* are the most metabolically benign. Patients with schizophrenia have a 2-fold higher prevalence of metabolic syndrome and type 2 diabetes mellitus and have 2-fold greater cardiovascular (CV)-related mortality rates than the general population. Consensus guidelines therefore recommend baseline determination of serum glucose, lipids, weight, blood pressure, and personal and family histories of metabolic and CV disease (American Psychiatric Association, 2021).

With the lower EPS risk experienced during therapy with SGAs, or even when FGAs are used in modest dosages, prophylactic use of antiparkinsonian medications (e.g., *benztropine*, *trihexyphenidyl*) must be avoided, especially given the cognitive impairment that results. Nonetheless, drug-induced parkinsonism can occur at higher dosages or among elderly patients exposed to antipsychotics with higher D_2 affinity; recommended doses in older patients are often 50% of those used in younger patients with schizophrenia. (See also Use in Pediatric Populations and Use in Geriatric Populations further in the chapter.) If an antiparkinsonian medication must be used, *amantadine* is preferable to anticholinergics (Silver and Geraisy, 1995).

Long-Term Treatment

The need for long-term treatment is the foundation for chronic psychotic illnesses such as schizophrenia and schizoaffective disorder. However, long-term antipsychotic treatment is sometimes used for bipolar I disorder patients, for ongoing psychosis in patients with DRP, for PDP, and for adjunctive use in treatment-resistant depression. Safety concerns combined with limited long-term efficacy data have dampened enthusiasm for extended antipsychotic drug use in patients with dementia (Maust et al., 2015). Justification for ongoing use, based on documentation of patient response to tapering of antipsychotic medication, is often mandated in long-term care settings.

Antipsychotic Agents

The choice of antipsychotic for long-term schizophrenia treatment is based primarily on avoidance of adverse effects, prior history of patient response, and the need for a LAI formulation due to oral nonadherence. While concerns over EPSs and tardive dyskinesia (TD) decreased with the introduction of SGAs, the estimated risk for TD with chronic SGA use is still 7.2% among those with no prior FGA exposure (Carbon et al., 2017). Since the advent of SGAs, there has been increased concern over metabolic effects of treatment: weight gain, dyslipidemia (particularly hypertriglyceridemia), and an adverse impact on glucose-insulin homeostasis (Huhn et al., 2019). *Clozapine*, *olanzapine*, and higher dose *quetiapine* have the highest metabolic risk and are not considered first-line agents. For treatment-resistant schizophrenia, response rates for most antipsychotics are less than 5%, but for *olanzapine*, the rate is 7% to 9% (Meyer and Stahl, 2019). While the *clozapine* response rate for treatment-resistant schizophrenia is 40% to 60%, *olanzapine* is sometimes used prior to *clozapine* after failure of more metabolically benign agents, since response to *olanzapine* lessens the monitoring burden and risk for certain unusual adverse effects associated with *clozapine* use.

Acutely psychotic patients usually exhibit some response within hours after drug administration, and extensive analyses of symptom response in clinical trials indicate that the majority of response to any antipsychotic dose in acute schizophrenia is seen by week 2. Failure of response after 2 weeks should prompt clinical reassessment, including determination of medication adherence and, ideally, extent of drug exposure using plasma antipsychotic levels, before a decision is made to increase the dose or consider switching to another agent (Meyer and Stahl, 2021). Patients with first-episode schizophrenia often respond to lower doses, and chronic patients may require doses that exceed recommended ranges, but the best proxy for CNS drug exposure is the peripheral plasma level. For many antipsychotics, there are sufficient data from fixed-dose trials and imaging studies to define plasma level response thresholds and an upper limit (the point of futility) beyond which response rates are less than 5% even if tolerated (Meyer and Stahl, 2021).

Usual dosages, plasma level response thresholds, and the point of futility are noted in Table 19–1. *While the dose in milligrams is what is ordered, issues with oral medication nonadherence, drug-drug interactions, and population variation in drug metabolism mean that plasma levels correlate significantly better with response than does the prescribed dose. Dosing should be adjusted based on clinically observable signs of antipsychotic benefit and adverse effects.* As noted above, less than minimal response after 2 weeks of a given dose portends a low likelihood of response at week 6. Obtaining a plasma level as a 12-h trough (for oral antipsychotics) or 1 to 72 h prior to the next LAI injection can be helpful in ruling out kinetic or adherence failures and determining whether the current level exceeds the response threshold (Meyer and Stahl, 2021). Having a level beyond the response threshold is no guarantee of response, as some patients may need higher levels, and certain patients may indeed be treatment resistant. Nonetheless, *antipsychotic titration in nonresponding schizophrenia patients should proceed until one of three hard endpoints is reached:*

1. The patient is markedly improved
2. The patient experiences dose-limiting adverse effects, or
3. The *point of futility* is reached

While intolerability is often a clinical endpoint signaling the end of an antipsychotic trial, a small proportion of patients may never exhibit dose-limiting adverse effects and will tolerate further antipsychotic titration, even with high-potency FGAs (see Table 19–1). Defining the upper limit of the response range as the *point of futility* encapsulates these important concepts: *intolerability may not limit the drug trial in some instances, but ongoing titration beyond a certain plasma level (the point of futility) is fruitless as less than 5% of patients will respond to these higher plasma levels* (Meyer and Stahl, 2021).

Certain antipsychotic adverse effects, including weight gain, sedation, orthostasis, and EPSs, can be predicted based on potencies at neurotransmitter receptors (Table 19–2). The detection of dyslipidemia or hyperglycemia is based on laboratory monitoring (see Table 19–1). Dose reduction often resolves EPSs, orthostasis, and sedation, but hyperprolactinemia and metabolic abnormalities may only improve with discontinuation of the offending agent and switching to a less offending medication. The decision to switch a medication for stable schizophrenia patients with either metabolic dysfunction or hyperprolactinemia must be individualized based on patient preferences, severity of the metabolic or endocrine disturbance, likelihood of improvement with antipsychotic switching, and history of response to prior agents. Patients with treatment-resistant schizophrenia on *clozapine* are not good candidates for switching because they are resistant to other medications (see the definition of treatment-resistant schizophrenia further in this section). Other strategies must be used to manage metabolic adverse effects as discussed below.

Psychotic Relapse

There are many reasons for psychotic relapse or inadequate response to antipsychotic treatment in patients with schizophrenia; these reasons include substance use, psychosocial stressors, and poor medication adherence. The common problem of medication nonadherence among patients with schizophrenia has led to the development of LAI antipsychotic medications, often referred to as depot antipsychotics (Meyer and Stahl, 2021). The kinetic properties and dosing of commonly used LAI formulations are summarized in Table 19–2. Patients receiving LAI antipsychotic medications show consistently lower relapse rates compared to patients receiving the identical oral forms and may have fewer adverse effects due to lower peak plasma levels (Kishimoto et al., 2021).

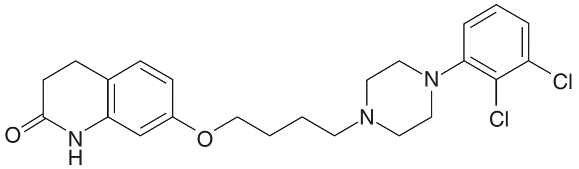
Treatment-Resistant Schizophrenia

Lack of response to adequate antipsychotic drug doses for adequate periods of time may indicate treatment-resistant illness. Use of antipsychotic plasma levels is critical to separate those who are nonadherent or are kinetic failures from those who are not responding to adequate medication exposure (Meyer and Stahl, 2021). In treatment-resistant schizophrenia, response rates are less than 5% for FGAs and less than 10% for *olanzapine*, but consistently 40% to 60% for *clozapine* (Meyer and Stahl, 2019). The therapeutic threshold for *clozapine* is 350 ng/mL, but patients may require levels as high as 1000 ng/mL for response (Meyer and Stahl, 2021). When ongoing titration finally achieves a therapeutic plasma concentration *for that patient*, response to *clozapine* occurs on average after 17 ± 14 days (Meyer and Stahl, 2019). *Clozapine* can have numerous adverse effects: risk of severe neutropenia (requires hematological monitoring), high metabolic burden, myocarditis, dose-dependent lowering of the seizure threshold, tachycardia, orthostasis, constipation and ileus, sedation, anticholinergic effects (especially constipation), and sialorrhea. Electroconvulsive therapy also has proven efficacy for treatment-resistant schizophrenia (Meyer and Stahl, 2019). Most clinicians recognize that treatment-resistant patients have virtually no viable option to *clozapine* and so become adept at monitoring for, and managing, these adverse effects.

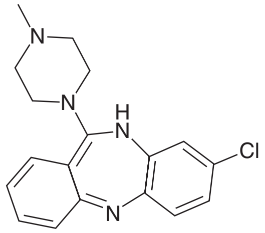
Pharmacology of Antipsychotic Agents

Chemistry

Antipsychotic agents include many different chemical structures with a range of activities at different neurotransmitter receptors. As a result, structure-function relationships that were relied on in the past have become less important, while receptor binding and functional assays are more clinically relevant. *Aripiprazole* represents a good example of how an examination of the structure provides little insight into the mechanism of partial agonism at D_2 DA receptors. Detailed knowledge of receptor affinities (see Table 19–2) and the functional effect at specific receptors (e.g., full, partial, or inverse agonism or antagonism) provides useful information about the therapeutic and adverse effects of antipsychotic agents. Nevertheless, there are limits. For example, it is not known which properties are responsible for *clozapine*'s unique effectiveness in resistant schizophrenia, although there are several hypotheses. Other notable antipsychotic properties not fully explained by receptor parameters include reduced seizure threshold, weight-independent effects on glucose and lipid metabolism, and increased risk for cerebrovascular events and mortality among dementia patients (see Adverse Effects and Drug Interactions, below).



ARIPIPRAZOLE



CLOZAPINE

Mechanism of Action

With the exception of *pimavanserin* for PDP, no antipsychotic approved through the end of 2021 is devoid of D_2-modulating activity

TABLE 19–1 ■ DRUGS FOR PSYCHOSIS AND SCHIZOPHRENIA: ORAL DOSING AND METABOLIC RISK PROFILE

	ORAL DOSAGE INFORMATION**		PLASMA LEVEL RANGE FOR SCHIZOPHRENIA (ng/mL)		METABOLIC SIDE EFFECTS		
GENERIC NAME *DOSAGE FORMS*	RANGE (mg/day)	CONCENTRATION DOSE RELATIONSHIP	THERAPEUTIC THRESHOLD	POINT OF FUTILITY[a]	WEIGHT GAIN	LIPIDS	GLUCOSE
Phenothiazines							
Chlorpromazine ***O, S, IM***	150–800	0.06	3–30	100	+++	+++	++
Perphenazine ***O*** decanoate ***Depot IM***	12–60	CYP2D6 EM 0.04 CYP2D6 PM 0.08	0.81	5.0	+/–	–	–
Trifluoperazine ***O***	5–40	???	1.0	2.3	+/–	–	–
Fluphenazine ***O, S, IM*** decanoate ***Depot IM***	2.5–20	Smoker 0.06 Nonsmoker 0.10	1.0	4.0	+/–	–	–
Selected other first-generation agents							
Loxapine ***O, Inhaled***	15–60	0.22	3.8	18.4	+	–	–
Thiothixene ***O***	5–40	Smoker 0.04 Nonsmoker 0.05	1.0	12.0	+/–	–	–
Haloperidol ***O, S, IM*** decanoate ***Depot IM***	2.5–30	0.78	2.0	18.0	+/–	–	–
Second-generation agents							
Aripiprazole ***O, S, ODT*** monohydrate/lauroxil ***Depot IM***	10–45	11.0	110	500	+/–	–	–
Amisulpride ***O***[b]	200–1200	Male 0.46 Female 0.60	100	550–700	+/–	–	–
Asenapine ***ODT, Transdermal***[c]	10–20	5 mg SL BID 0.15 10 mg SL BID 0.20	1.0	???	+/–	–	–
Brexpiprazole ***O***	2–4	CYP2D6 EM 18 CYP2D6 IntM 46	36	???	+/–	–	–
Cariprazine ***O***	3–6	1.91	5.6	???	+/–	–	–
Clozapine ***O, S, ODT, IM***	200–900	Female: Smoker 0.80 Nonsmoker 1.32 Male: Smoker 0.67 Nonsmoker 1.08	350	1000	++++	+++	+++
Iloperidone ***O***[d]	12–24	???	???	???	+	+/–	+/–
Lumateperone[e]	42	???	???	???	+/–	+/–	+/–
Lurasidone ***O***[f]	40–160	0.18	7.2	???	+	+/–	+/–
Olanzapine ***O, ODT, IM*** pamoate ***Depot IM***	7.5–40[g]	Smoker 1.43 Nonsmoker 2.00	23	150	++++	+++	+++
Paliperidone ***O*** palmitate ***Depot IM***	6–15	4.09	20	90	+	+/–	+/–
Pimavanserin ***O***[h]	34	???	???	???	–	–	–
Quetiapine ***O***	200–900	???	???	???	++	+	+/–
Risperidone ***O, S, ODT*** microspheres, subcutaneous gel ***Depot IM***	2–10	7.0 (active moiety: sum of risperidone + paliperidone)	15	112	+	+/–	+/–
Sertindole ***O***[b]	4–16	12–20	12–20	12–32	+/–	–	–
Ziprasidone ***O, IM***[i]	120–200	???	???	???	+/–	–	–

BID, twice a day; EM, extensive metabolizer; IM, acute intramuscular; IntM, intermediate metabolizer; O, tablet or capsule; ODT, orally dissolving tablet; PM, poor metabolizer; S, solution; SL, sublingual.

**Concentration dose relationship value, multiplied by the oral dose (mg), estimates the 12h trough level in ng/ml (e.g.: female nonsmoker on clozapine 300 mg qhs: 300 × 1.32 = 396 ng/ml).

[a] A small proportion of patients may never exhibit dose-limiting adverse effects even at supratherapeutic dosages; however, ongoing titration beyond the plasma level point of futility is fruitless as less than 5% of patients will respond at these higher plasma levels (Meyer, 2014; Meyer and Stahl, 2021).

[b] Not available in the U.S.

[c] Transdermal doses per 24 h are 3.8, 5.7, and 7.6 mg, equivalent to sublingual asenapine doses of 10, 15, and 20 mg/day, respectively.

[d] Due to orthostasis risk, dose titration of iloperidone is 1 mg twice daily on day 1, increasing to 2, 4, 6, 8, 10, and 12 mg twice daily on days 2 to 7 (as needed).

[e] Dose must be given with 350 kcal food to facilitate absorption. Administration with evening meal improves tolerability.

[f] Only one dose approved for schizophrenia (42 mg). A 21 mg dose and 10.5 mg dose are available for those on CYP 3A4 moderate or strong inhibitors, respectively.

[g] A combination tablet with olanzapine (doses of 5, 10, 15, or 20 mg) and 10 mg of samidorphan was approved in the U.S. in June 2021 for schizophrenia or bipolar I disorder with manic or mixed episodes as monotherapy, or for adjunctive use to lithium or valproate in bipolar I. Samidorphan is a μ-opioid receptor antagonist and modest κ-opioid receptor partial agonist that blunts the endorphin signal that reinforces eating behavior.

[h] Indicated for Parkinson's disease psychosis and dementia-related psychosis.

[i] Oral dose must be given with 500 kcal food to facilitate absorption.

TABLE 19–2 ■ MEAN HALF-LIFE AND KINETIC PROPERTIES OF COMMONLY USED LONG-ACTING INJECTABLE ANTIPSYCHOTICS

DRUG	VEHICLE	DOSAGE	t_{max}	$t_{1/2}$ MULTIPLE DOSING	ABLE TO BE LOADED
First-generation antipsychotics					
Fluphenazine decanoate	Sesame oil	12.5–75 mg/2 weeks **Max:** 75 mg/week	0.3–1.5 days	14 days	Yes
Haloperidol decanoate	Sesame oil	25–300 mg/4 weeks **Max:** 300 mg/2 weeks	3–9 days	21 days	Yes
Perphenazine decanoate[a]	Sesame oil	27–216 mg/3–4 weeks **Max:** 216 mg/3 weeks	7 days	27 days	Yes
Flupenthixol decanoate[a]	Coconut oil	20–40 mg/2–4 weeks **Max:** 100 mg/2 weeks	4–7 days	17 days	Yes
Zuclopenthixol decanoate[a]	Coconut oil	25–100 mg/2–4 weeks **Max:** 400 mg/2 weeks	3–7 days	19 days	Yes
Newer antipsychotics					
Risperidone subcutaneous (Perseris)	Water	90–120 mg/4 weeks **Max:** 120 mg/4 weeks	7–8 days	9–11 days	Not needed
Risperidone subcutaneous (Uzedy)	Water	50–125 mg/4 weeks 100–250 mg/8 weeks **Max:** 125 mg/4 weeks 250 mg/8 weeks	8–14 days	15–21 days	Not needed
Risperidone microspheres (Risperdal Consta)	Water	12.5–50 mg/2 weeks **Max:** 50 mg/2 weeks	21 days	See note[a]	**No** (21–28 days oral overlap)
Paliperidone palmitate (monthly) (Invega Sustenna)[b]	Water	39–234 mg/4 weeks (25–150 mg/4 weeks) **Max:** 234 mg/4 weeks (150 mg/4 weeks)	13 days	25–49 days	Yes
Paliperidone palmitate (3 month) (Invega Trinza)[c]	Water	273–819 mg/12 weeks (175–525 mg/12 weeks) **Max:** 819 mg/12 weeks (525 mg/12 weeks)	84–95 days (deltoid) 118–139 days (gluteal)	30–33 days	No
Paliperidone palmitate (6 month) (Invega Hafyera)[d]	Water	1092–1560 mg/26 weeks (700–1000 mg/26 weeks) **Max:** 1560 mg/26 weeks (1000 mg/26 weeks)	148–159 days (gluteal)	29–32 days	No
Olanzapine pamoate (Zyprexa Relprevv)	Water	150–300 mg/2 weeks 300–405 mg/4 weeks **Max:** 300 mg/2 weeks	7 days	30 days	Yes
Aripiprazole monohydrate (Abilify Maintena)	Water	300–400 mg/4 weeks **Max:** 400 mg/4 weeks	6.5–7.1 days	29.9–46.5 days	**No** (14 days oral overlap)
Aripiprazole lauroxil (Aristada)[e]	Water	441, 662, 882 mg/4 wks 882 mg/6 weeks 1064 mg/8 weeks **Max:** 882 mg/4 weeks	41 days (single dose) 24.4–35.2 days (repeated dosing)	53.9 – 57.2 days	No (start with AL_{NCD} 675 mg IM + 30 mg oral ***OR*** 21 days oral overlap)
Aripiprazole lauroxil nanocrystal (Aristada Initio)[f]	Water	675 mg once	27 days (range: 16–35 days)	15–18 days (single dose)	—

AL_{NCD}, nanocrystalline milled dispersion of aripiprazole lauroxil.

[a] Not available in the U.S.

[b] The dosages in parentheses reflect those used outside the U.S., expressed as paliperidone equivalent doses.

[c] Only for those on paliperidone palmitate monthly for 4 months. Cannot be converted from oral medication.

[d] Only for those on paliperidone palmitate monthly for 4 months or on paliperidone palmitate 3-month. Cannot be converted from oral medication.

[e] Requires 21 days of oral overlap unless starting with aripiprazole lauroxil nanocrystal plus a single 30-mg oral dose.

[f] AL_{NCD} is only used for initiation of treatment with aripiprazole lauroxil or for resumption of treatment. It is always administered together with the clinician-determined dose of aripiprazole lauroxil, although the latter can be given up to 10 days after the aripiprazole lauroxil nanocrystal injection.

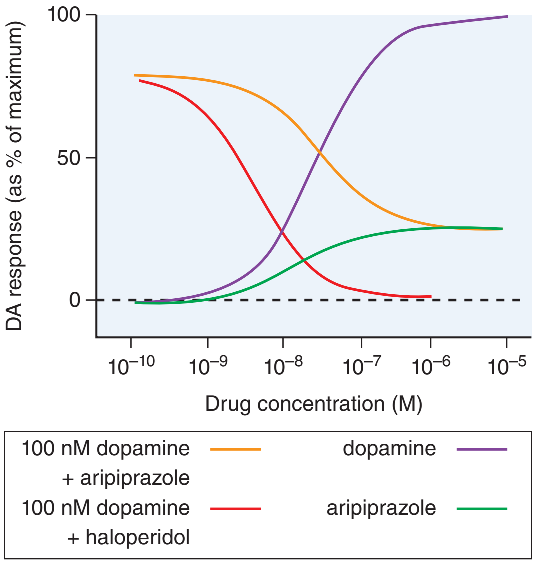


Figure 19–2 *Partial agonist activity of aripiprazole at D_2 receptors.* *Aripiprazole* is a partial D_2 agonist. In this stylized representation, *aripiprazole* inhibits the effects of DA and reduces stimulation at the D_2 receptor only to the extent of its own capacity as an agonist (orange tracing); in the absence of DA, its partial agonist effects are apparent (green line), becoming maximal at about 25% of the maximal effect of DA alone (purple line). *Haloperidol*, an antagonist without agonist activity, completely antagonizes D_2 receptor activation by 100 nM DA (red tracing). Here, receptor activation is measured as inhibition of forskolin-induced cAMP accumulation in cultured cells transfected with human D_{2l} DNA. (Data from Burris et al., 2002).

(Egerton et al., 2021). This direct reduction in dopaminergic neurotransmission is presently achieved through one of two mechanisms: D_2 antagonism or partial D_2 agonism (*aripiprazole*, *brexpiprazole*, and *cariprazine*). The mechanism of action for partial agonist antipsychotics relies on intrinsic activity at D_2 receptors that is a fraction of the efficacy of DA (i.e., 18%–25% of DA's activity), as depicted in Figure 19–2 for *aripiprazole*. (Recall that a partial agonist will also occupy the receptor and antagonize the binding of other antagonists and also full agonists; see Chapter 3.) Antipsychotics that primarily rely on D_2 antagonism need striatal D_2 occupancy (i.e., reduction in postsynaptic D_2 signal) of 60% to 80% for optimal efficacy. While higher levels of D_2 occupancy increase the risk for EPSs with DA antagonists, partial agonist antipsychotics require significantly higher D_2 occupancy levels (80%–100%). The presence of intrinsic DA agonism from the partial agonist generates a sufficient postsynaptic DA signal to remain below the EPS threshold, although reports of parkinsonism and dystonia do exist, primarily in antipsychotic-naïve, younger patients.

Clozapine was not suspected to possess antipsychotic activity until experimental human use in the mid-1960s revealed it to be an effective treatment of schizophrenia with virtually no EPS risk, particularly in patients who failed other antipsychotic medications. *Clozapine* possesses weaker D_2 antagonism than other antipsychotics, combined with potent $5HT_{2a}$ antagonism. *Clozapine* and its active metabolite *N*-desmethylclozapine also possess activity at numerous other receptors, including agonism at various muscarinic receptor subtypes, NMDA receptor activation via glycine site agonism (Meyer and Stahl, 2019), and activation of TAAR1 (Karmacharya et al., 2011).

Dopamine Receptor Occupancy and Behavioral Effects

Dopaminergic projections from the midbrain terminate on septal nuclei, the olfactory tubercle and basal forebrain, the amygdala, and other structures within the temporal and prefrontal cerebral lobes and the hippocampus. Excessive DA neurotransmission in the associative striatum and adjacent sensorimotor striatum is central to the positive symptoms of psychosis for schizophrenia patients (McCutcheon et al., 2019). The behavioral effects and the time course of antipsychotic response parallel the decrease in postsynaptic D_2 activity in this region. DA D_2 receptor occupancy predicts clinical efficacy, EPSs, and plasma level–clinical response relationships for the D_2 antagonist antipsychotics. Occupancy of greater than 78% of D_2 receptors in the basal ganglia is associated with greater risk of EPSs across all DA antagonist antipsychotic agents, while occupancies in the range of 60% to 75% are associated with antipsychotic efficacy and lower EPS risk (Figure 19–3). With the exception of the D_2 partial agonists, all SGAs at low doses have much greater occupancy of $5HT_{2a}$ receptors (e.g., 75%–99%) than FGAs (Table 19–3). Given the large variations in drug metabolism, plasma levels of antipsychotic agents (rather than doses) are the best predictors of D_2 occupancy (see Table 19–1).

The Role of Nondopamine Receptors for Atypical Antipsychotic Agents. The concept of atypicality was initially based on *clozapine*'s absence of EPSs combined with potent $5HT_2$ receptor antagonism. $5HT_{2a}$ antagonism exerts its greatest effect on prefrontal and basal ganglia DA release, thereby decreasing EPS risk in the context of nigrostriatal D_2 antagonism. That $5HT_{2A}$ antagonism conveys antipsychotic activity is exemplified by *pimavanserin* and *lumateperone*, antipsychotics that possess no or very low D_2 occupancy. As discussed with *clozapine*, muscarinic M_4 agonism is hypothesized to be an antipsychotic mechanism and is the basis for trials of the M_4 preferring agonist *xanomeline*. Most recently, there have been studies of TAAR1 agonists resulting from preclinical

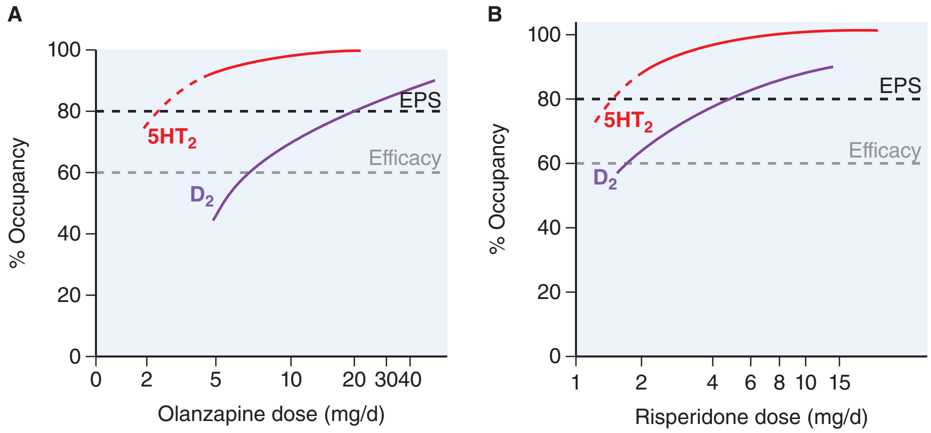


Figure 19–3 *Receptor occupancy and clinical response for D_2 receptor antagonist antipsychotics.* Antipsychotic effects are seen when D_2 receptor occupancy exceeds 60%, while occupancy greater than 80% increases risk of EPSs. Atypical agents combine weak D_2 receptor blockade with more potent $5HT_{2a}$ antagonism/inverse agonism. Inverse agonism at $5HT_2$ receptor subtypes may contribute to the reduced EPS risk of *olanzapine* (**A**) and *risperidone* (**B**) and efficacy at lower D_2 receptor occupancy (*olanzapine*, **A**). *Aripiprazole* is a partial D_2 agonist that can achieve only 75% functional blockade (see Figure 19–2).

TABLE 19–3 ■ POTENCIES OF ANTIPSYCHOTIC AGENTS AT NEUROTRANSMITTER RECEPTORS[a]

		DOPAMINE	SEROTONIN	MUSCARINIC	ADRENERGIC		HISTAMINE
		D_2	$5HT_{2A}$	M_1	α_{1A}	α_{1B}	H_1
First-generation agents (oral equivalent) (Leucht et al., 2020)							
Haloperidol	1 mg	1.2	57	>10,000	12	7.6	1700
Fluphenazine	1 mg	0.8	3.2	1100	6.5	13	14
Trifluoperazine	2.5 mg	1.1	74	>660	24	–	63
Thiothixene	3.75 mg	0.7	50	>10,000	12	35	8
Perphenazine	3.75 mg	0.8	5.6	1500	10	–	8.0
Molindone	6.25 mg	20	>5000	>10,000	2600	–	2130
Loxapine	12.5 mg	11	4.4	120	42	53	4.9
Chlorpromazine	37.5 mg	3.6	3.6	32	0.3	0.81	3.1
Second-generation agents							
Lurasidone		1.0	0.5	>1000	48	–	>1000
Aripiprazole[b]		1.6[b]	8.7	6800	26	34	28
Brexpiprazole[b]		0.35[b]	0.47	>1000	–	0.17	19
Cariprazine[b]		0.59[b]	19	>1000	132	>1000	23
Asenapine		1.4	0.1	>10,000	1.2	3.9	1.0
Ziprasidone		6.8	0.6	>10,000	18	9.0	63
Sertindole[c]		2.7	0.4	>5000	1.8	–	130
Zotepine[c]		8.0	2.7	330	6.0	5.0	3.2
Risperidone		3.2	0.2	>10,000	5.0	9.0	20
Paliperidone		4.2	0.7	>10,000	2.5	0.7	19
Iloperidone		6.3	5.6	4900	0.3	–	12
Amisulpride[c]		2.2	8304	>10,000	>10,000	>10,000	>10,000
Olanzapine		31	3.7	2.5	110	263	2.2
Lumateperone[d]		32	0.54	>100	73	–	>1000
Quetiapine		380	640	37	22	39.0	6.9
Clozapine		160	5.4	6.2	1.6	7.0	1.1
Pimavanserin[e]		>10,000	0.087	>10,000	>10,000	>10,000	>10,000

[a] Data are averaged K_i values (nM) from published sources determined by competitive binding at cloned human receptors. Data derived from receptor binding to human or rat brain tissue is used when cloned human receptor data are lacking. Unless noted otherwise, data are from PDSP Ki Database (https://pdsp.unc.edu/databases/pdsp.php; accessed June 27, 2022).

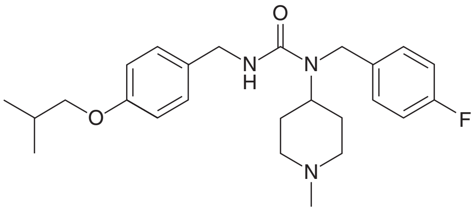
[b] Partial agonist at D_2 receptor.

[c] Not available in the U.S. for schizophrenia treatment.

[d] Snyder et al., 2015.

[e] Acadia Pharmaceuticals, Inc. Nuplazid Package Insert. San Diego, California 2020.

assays that predicted antipsychotic activity, with positive human data reported in 2020 in a phase II schizophrenia trial of *ulotaront* (Koblan et al., 2020).



PIMAVANSERIN

Tolerance and Physical Dependence

As defined in Chapter 28, antipsychotics are not addicting; however, tolerance to the α_1 adrenergic, antihistaminic, and anticholinergic effects usually develops over days or weeks. Loss of efficacy with prolonged treatment is not known to occur with antipsychotics; however, tolerance to antipsychotic drugs and cross-tolerance among agents are demonstrable in behavioral and biochemical experiments in animals. One correlate of tolerance in striatal dopaminergic systems is the pathological development of postsynaptic DA receptor supersensitivity due to the upregulation of supersensitive D_2 receptors, referred to as D_2^{High} receptors (Seeman et al., 2005). These changes in the nigrostriatal pathway underlie the pathophysiology of TD, while comparable changes in the associative striatum are associated with supersensitivity psychosis (Nakata et al., 2017).

ADME

Absorption for most antipsychotics following oral administration is quite high, with notable exceptions including *asenapine* (<2%), *fluphenazine* (3%), *lumateperone* (4%), and *lurasidone* (9%–19%). Most ODTs and liquid preparations provide similar pharmacokinetics because there is little mucosal absorption and absorption occurs in the gastrointestinal (GI) tract. *Asenapine* is the only exception: it was available initially only as an ODT preparation administered sublingually; absorption occurred via the oral mucosa with bioavailability of 35%. If *asenapine* is swallowed (i.e., the patient consumes water within 2 min after the ODT is placed in the mouth), the first-pass effect is greater than 98% and the drug is essentially not bioavailable. A transdermal form of *asenapine* is now available that avoids the kinetic issues of the ODT preparation.

Intramuscular forms for acute administration exist for many antipsychotics, and this route provides measurable concentrations in plasma within 15 to 30 min. There is also an inhaled form of *loxapine* that generates peak levels within 2 min.

The pharmacokinetic binding constants and metabolic pathways for many SGAs and FGAs are listed in Tables 19–3 and 19–4. Antipsychotics are highly lipophilic and accumulate in the brain, lung, and other tissues with a rich blood supply. Most antipsychotic agents are highly protein bound, primarily to α_1-glycoproteins, and do not significantly displace other medications bound to prealbumin or albumin. Antipsychotics also enter the fetal circulation and breast milk. Despite half-lives that may be short, the biological effects of single doses of most antipsychotic medications usually persist for at least 24 h, permitting once-daily dosing after the patient has adjusted to initial side effects. Due to accumulation in tissue stores, both parent compound and metabolites of LAI medications can be detected several months after discontinuation, a useful property for those who may miss injections (see Table 19–2).

Other Therapeutic Uses

Antipsychotics are also utilized in several nonpsychotic neurological disorders and as antiemetics.

Anxiety Disorders

Double-blind, placebo-controlled trials have shown the benefit of adjunctive treatment with several SGAs for obsessive-compulsive disorder, with a recent meta-analysis showing no significant efficacy differences between agents, although *quetiapine* and *paliperidone* were significantly less tolerable than placebo. Data from the largest double-blind, placebo-controlled study did not support routine use of adjunctive *risperidone* for posttraumatic stress disorder (Krystal et al., 2011). The use of SGAs for generalized anxiety disorder is not evidenced based. The last comprehensive meta-analysis was published in 2011 and concluded that SGAs are not superior to placebo as augmentation for refractory generalized anxiety disorder (LaLonde and Van Lieshout, 2011).

Tourette Disorder

The ability of antipsychotic drugs to suppress tics in patients with Tourette disorder relates to reduced D_2 neurotransmission at sites within the basal ganglia. *Aripiprazole* is the only antipsychotic FDA-approved for the treatment of Tourette disorder starting at doses of 2 mg/day and increasing (if needed) to a maximum of 10 mg/day for those weighing less than 50 kg or to 20 mg/day if weighing 50 kg or more (Yang et al., 2020). In prior decades, low-dose, high-potency FGAs (e.g., *haloperidol*) were treatments of choice, but nonpsychotic patients are extremely sensitive to the impact of DA blockade on cognitive processing speed and on reward centers. Moreover, safety concerns regarding *pimozide*'s QTc prolongation and increased risk for ventricular arrhythmias have largely ended its clinical use.

Huntington's Disease

Huntington's disease is another neuropsychiatric condition that, like tic disorders, is associated with basal ganglia pathology. DA blockade can suppress the severity of choreoathetotic movements but is not strongly endorsed due to the risks associated with excessive DA antagonism that outweigh the marginal benefit. Inhibition of the vesicular monoamine transporter 2 (VMAT2) with tetrabenazine compounds (e.g., *tetrabenazine*, *deutetrabenazine*) has replaced DA receptor blockade in the management of choreiform movements for these patients (see Chapter 21).

Autism

Autism is a disease whose neuropathology is incompletely understood but that can be associated with explosive behavioral outbursts and aggressive or self-injurious behaviors that may be stereotypical. *Risperidone* and *aripiprazole* are FDA-approved for irritability associated with autism in child and adolescent patients ages 5 to 16 and are used temporarily for disruptive behavior problems in autism and intellectual disability when behavioral programs are not effective. Long-term use is not supported by existing data, and children with intellectual disabilities may be very sensitive to weight gain and somnolence. Initial *risperidone* daily doses are 0.25 mg for patients weighing less than 20 kg and 0.5 mg for others, with a target dose of 0.5 mg/day in those weighing less than 20 kg and 1.0 mg/day for other patients, with a range of 0.5 to 3.0 mg/day. For *aripiprazole*, the starting dose is 2 mg/day, with a target range of 5 to 10 mg/day and maximum daily dose of 15 mg.

Antiemetic Use

The D_2 antagonist antipsychotics protect against the nausea- and emesis-inducing effects of DA agonists such as *apomorphine* that act at central DA receptors in the medullary chemoreceptor trigger zone (see Figure 54–6). Medications or other stimuli that cause emesis by an action on the nodose ganglion, or locally on the GI tract, are not antagonized by antipsychotics, but potent piperazines and butyrophenones are sometimes effective against nausea caused by vestibular stimulation. The commonly used antiemetic phenothiazines (e.g., *prochlorperazine*) and *metoclopramide* are weak DA antagonists without antipsychotic activity but can be associated with EPSs, akathisia, and TD if used chronically. Both *prochlorperazine* and *metoclopramide* have label warnings for TD. Emesis and antiemetic agents are discussed at length in Chapter 54.

Adverse Effects and Drug Interactions

Adverse Effects Predicted by Monoamine Receptor Affinities

Dopamine D_2 Receptors. With the exception of *pimavanserin* and the D_2 partial agonists (*aripiprazole*, *brexpiprazole*, *cariprazine*), all other antipsychotic agents possess D_2 antagonist properties, the strength of which determines the likelihood for short-term EPSs, akathisia, neuroleptic malignant syndrome (NMS), and hyperprolactinemia. As expected, FGAs also have 4-fold higher TD risk than SGAs (Carbon et al., 2017).

Extrapyramidal Symptoms. The manifestations of EPSs are described in Table 19–5, along with the usual treatment approach. Acute dystonic reactions occur in the early hours and days of treatment, with highest risk among younger patients (peak incidence ages 10–19), especially antipsychotic-naïve individuals, in response to abrupt decreases in nigrostriatal D_2 neurotransmission. Dystonia typically involves head and neck muscles and the tongue and, in its severest form, the oculogyric crisis, extraocular muscles, all of which are frightening to the patient.

Parkinsonism resembling its idiopathic form may occur; it will respond to dose reduction or switching to an antipsychotic with weaker D_2 antagonism. If this is neither possible nor desirable, antiparkinsonian medication may be employed but with the caveat that use of anticholinergic antiparkinsonian medication induces cognitive dysfunction. Elderly patients are at greatest risk for parkinsonism and are most sensitive to the cognitive and peripheral adverse effects of anticholinergic antiparkinsonian medications.

Muscarinic M_1 cholinergic receptors are present on nigrostriatal medium spiny neurons. Acetylcholine released from adjacent cholinergic interneurons stimulates these M_1 receptors and balances out the effects of DA agonism at D_2 receptors. When a D_2 antagonist causes disinhibition of medium spiny neurons, M_1 antagonists help mitigate this effect, resulting in reduced symptoms of parkinsonism and dystonia. Important issues in the use of anticholinergics include the negative impact on cognition and memory; peripheral antimuscarinic adverse effects (e.g., urinary retention, dry mouth, cycloplegia); exacerbation of TD; and risk of cholinergic rebound following abrupt anticholinergic withdrawal. For parenteral administration, intramuscular (IM) *diphenhydramine* (25–50 mg) and *benztropine* (1–2 mg IM) are most commonly used. *Diphenhydramine* possesses both antihistaminic and anticholinergic properties and is used preferentially in intramuscular combinations with *haloperidol* to provide dystonia prophylaxis and sedation. *Benztropine* combines a benzhydryl group with a tropane group to create a compound that is more anticholinergic than trihexyphenidyl but less antihistaminic than *diphenhydramine* and therefore less sedating in routine usage. The clinical effect of a single dose is sufficiently short that patients typically require two or three daily doses. *Benztropine* dosing usually starts at 0.5 to 1 mg twice daily,

TABLE 19–4 ■ DRUG DISPOSITION AND EFFECTS OF CYP INHIBITION AND INDUCTION ON ORAL ANTIPSYCHOTIC LEVELS

	t_{max}; ORAL BIOAVAILABILITY	METABOLISM	EFFECT OF CYP INHIBITION	EFFECT OF CYP INDUCTION
Commonly used atypical antipsychotics				
Aripiprazole	Bioavailability: 87% t_{max}: 3–5 h	CYPs 2D6 and 3A4 produce active metabolite, dehydroaripiprazole. $t_{1/2}$: aripiprazole, 75 h; dehydroaripiprazole, 94 h Metabolite = 40% of AUC at steady state	In 2D6 PM: ↑ AUC of aripiprazole up to 80%, 30% ↓ AUC of metabolite. $t_{1/2}$: 146 h in PMs. Strong CYP2D6 inhibitors (e.g., ketoconazole) can double AUC of parent drug. Ketoconazole (a strong 3A4 inhibitor) increased the AUCs of aripiprazole and its active metabolite by 63% and 77%, respectively.	3A4 induction ↓ max concentration and AUC of aripiprazole and metabolite by 70%.
Asenapine (transdermal)	Relative bioavailability: the 3.8 mg/24 h, 5.7 mg/24 h, and 7.6 mg/24 h doses are bioequivalent to the sublingual doses of 5 mg BID, 15 mg/day, and 10 mg BID, respectively t_{max}: 12–24 h	Primarily CYP1A2 and direct glucuronidation by the phase 2 enzyme UGT1A4. CYP3A4 and CYP2D6 are minor pathways. No active metabolites. $t_{1/2}$: 24 h	Fluvoxamine, (25 mg twice daily for 8 days) ↑ C_{max} by 13% and AUC 29%, but a greater effect from higher fluvoxamine doses is possible. The asenapine dose may need to be adjusted in those circumstances. There is no impact from the strong CYP2D6 inhibitor paroxetine or the UGT1A4 inhibitor valproate. Of note, asenapine increases paroxetine exposure 2-fold, so paroxetine doses should be decreased by 50%.	Smoking: no effect on clearance or other kinetic parameters. Carbamazepine, can ↓ C_{max} and AUC (16%).
Asenapine (sublingual)	Bioavailability: 32%–40% after 10 min of buccal residence time. If water is given before 10 min, absorption is decreased. Oral: <2% t_{max}: 1 h	Primarily CYP1A2 and direct glucuronidation by the phase 2 enzyme UG 1A4. CYP3A4 and CYP2D6 are minor pathways. No active metabolites. $t_{1/2}$: 30 h	Fluvoxamine (25 mg twice daily for 8 days) ↑ C_{max} by 13% and AUC 29%, but a greater effect from higher fluvoxamine doses is possible. The asenapine dose may need to be adjusted in those circumstances. There is no impact from the strong CYP2D6 inhibitor paroxetine or the UGT1A4 inhibitor valproate. Of note, asenapine increases paroxetine exposure 2-fold, so paroxetine doses should be decreased by 50%.	Smoking: no effect on clearance or other kinetic parameters. Carbamazepine, can ↓ C_{max} and AUC (16%).
Brexpiprazole	Bioavailability: 95% t_{max}: 4 h	CYPs 2D6 and 3A4 convert brexpiprazole to inactive metabolite (DM-3411). $t_{1/2}$: 91 h	Strong CYP2D6 or CYP3A4 inhibitors increase AUC_{0-24h} by 2-fold. Combined use of a strong CYP3A4 and CYP2D6 inhibitor (or with CYP2D6 PM) increased AUC_{0-24h} 4.8- to 5.1-fold. Reduce the dose by 50% with strong 2D6 or 3A4 inhibitors and by 75% with combined strong CYP2D6/CYP3A4 inhibitors.	Strong 3A4 inducers reduce exposure AUC_{0-24h} by ~70%. Double the dose with strong CYP3A4 inducers.

(*Continued*)

TABLE 19–4 ■ DRUG DISPOSITION AND EFFECTS OF CYP INHIBITION AND INDUCTION ON ORAL ANTIPSYCHOTIC LEVELS (*CONTINUED*)

	t_{max}; ORAL BIOAVAILABILITY	METABOLISM	EFFECT OF CYP INHIBITION	EFFECT OF CYP INDUCTION
Cariprazine	Bioavailability: 65% t_{max}: 3–6 h	CYP3A4 converts cariprazine to active metabolites DCAR and DDCAR. At steady state on 6 mg/day: cariprazine 28%, DCAR 9%, and DDCAR 63%. CYP2D6 is a minor pathway. Parent drug and DDCAR show good brain penetration; after oral cariprazine, brain-to-plasma ratios for both are ~9.8. $t_{1/2}$: 31.6–68.4 h; DCAR, 29.7–39.5 h; DDCAR, 314–446 h ≥50% of DDCAR present 1 week after discontinuation.	The strong CYP3A4 inhibitor ketoconazole at the dose of 400 mg/day increased cariprazine C_{max} and AUC_{0-24h} by 3.5- and 4.0-fold, respectively. Ketoconazole also increased DDCAR C_{max} and AUC_{0-24h} by 1.5-fold and decreased DCAR C_{max} and AUC_{0-24h} by about one-third. Reduce the dose by 50% with strong 3A4 inhibitors. There is no impact from 2D6 inhibitors.	Not studied. Impact unknown. Not recommended with 3A4 inducers.
Clozapine	Bioavailability: 60% t_{max}: 2.5 h	The mean contributions of CYPs 1A2, 2C19, 3A4, 2C9, and 2D6 are 30%, 24%, 22%, 12%, and 6%, respectively, but in some instances CYP1A2 is responsible for 40%–55% of clozapine biotransformation. CYP1A2 is the most important form at low concentrations, which is in agreement with clinical findings. Norclozapine is an active metabolite. In the largest data set available, the steady-state ratio of clozapine to norclozapine in nonsmokers is 1.32. Other studies report different values (e.g., 1.73). The important concept is that this is genetically determined and only modified by exposure to medications or other chemicals (e.g., cigarette smoke) that alter CYP activity or by erratic timing of the clozapine dose with respect to plasma level determinations. $t_{1/2}$: 12 h (4–66) with chronic dosing $t_{1/2}$: 22.5 h (norclozapine)	Fluvoxamine, a strong CYP1A2 inhibitor, increases plasma levels 5- to 10-fold. Strong CYP2D6 or CYP3A4 inhibitors increase trough plasma levels approximately 2-fold.	Carbamazepine, phenytoin, phenobarbital, rifampin, and omeprazole ↓ clozapine trough levels up to 50%. Smoking as few as 7–12 cigarettes/day is sufficient to fully induce CYP1A2, and ↑ its activity 1.66-fold. Upon smoking cessation, CYP1A2 activity declines with a half-life of 38.6 h (range, 27.4–54.4 h), and CYP1A2 activity will return to baseline after 5 half-lives, or 8 days on average. Loss of smoking-related 1A2 induction results in ≥50% increase in plasma levels.
Iloperidone	Bioavailability: well absorbed, but data limited t_{max}: 2–4 h	CYP2D6/3A4 produce active metabolites P88 and P95. Exposure to P88 and P95 can be significant. P88 $t_{1/2}$: EM, 26 h; PM, 37 h P95 $t_{1/2}$: EM, 23 h; PM, 31 h $t_{1/2}$: 18 h (CYP2D6 EMs), 33 h (CYP2D6 PMs)	Ketoconazole, fluoxetine, and paroxetine can ↑ AUC of iloperidone and metabolites by 50%–300%, with similar effects on C_{max} at steady state.	Impact of 3A4 inducers not documented.

Lumateperone	Bioavailability: 4.4% t_{max}: 1–2 h	CYP3A4 is the primary pathway. UGT1A4 is involved in phase 2 metabolism. Lumateperone is not a Pgp or BCRP substrate.	Itraconazole, a strong 3A4 inhibitor, increased the AUC 4-fold, while diltiazem, a moderate CYP3A4 inhibitor, increased AUC 2-fold. Due to the lack of multiple dosage forms, cannot be used with CYP3A4 inhibitors or UGT inhibitors (e.g., VPA).	Rifampin ↓ AUC by >95%. Cannot be taken with CYP3A4 inducers.
Lurasidone	Bioavailability: 9%–19% Mean C_{max} and AUC increased 3-fold and 2-fold, respectively, when administered with food. t_{max}: 1–3 h	CYP3A4 $t_{1/2}$: 18–36 h	The strong CYP3A4 inhibitor ketoconazole at a dose of 400 mg/day for 7 days in 10 healthy volunteers increased AUC 9.3-fold from baseline. Lurasidone cannot be used with strong CYP3A4 inhibitors. Exposure to the moderate CYP3A4 inhibitor diltiazem 240 mg/day for 7 days increased the single-dose AUC 2.2-fold for lurasidone and similarly for its metabolite ID-14283. The lurasidone dose should be decreased by 50% with moderate CYP3A4 inhibitors.	DO NOT USE with strong inducers of CYP3A4. Concurrent rifampin ↓ C_{max} to a seventh of prior levels and decreases AUC by 80%.
Olanzapine	Bioavailability: 60% t_{max}: 6 h	Direct glucuronidation via UGT1A4 or UGT2B10, or CYP1A2-mediated oxidation to *N*-desmethylolanzapine (inactive). $t_{1/2}$: 30 (21–54) h	The mean increases in olanzapine AUC following fluvoxamine exposure are 52% and 108%, respectively, in female nonsmokers and male smokers, respectively, but the effect is dependent on the fluvoxamine dose. Fluvoxamine 100 mg/day for 8 weeks resulted in an increase in trough olanzapine levels from 31 ± 15 ng/mL to 56 ± 31 ng/mL (81%), with a range of 12%–112%. A smaller fluvoxamine dose (25 mg/day) increased exposure only 26%.	Carbamazepine use ↑ clearance by 50%. The olanzapine concentration:dose ratio is at least 30% lower in smokers and may be 50% lower in some patients.
Paliperidone	Bioavailability: 28% t_{max}: 24 h	59% excreted unchanged, 32% appears as metabolites (primarily phase 2 via UGT1A4). 80% of elimination is renal, related to active excretion by the efflux transporter Pgp.	No significant impact of CYP inhibitors. At steady state (5 days), divalproex extended-release 1000 mg once daily increased the AUC of paliperidone 12 mg by 50%.	Carbamazepine use ↓ steady-state C_{max} and AUC by 37%.
Pimavanserin	Bioavailability: not determined t_{max}: 6 (4–24) h	Predominantly CYP3A4 to metabolite *N*-desmethylpimavanserin, which is active peripherally but has limited CNS penetration. $t_{1/2}$: 57 h $t_{1/2}$: 200 h (*N*-desmethylpimavanserin)	Ketoconazole increases pimavanserin AUC by 3-fold.	One week exposure to 600 mg rifampin ↓ pimavanserin AUC by 91%. A moderate CYP3A4 inducer (efavirenz) may ↓ AUC of pimavanserin by 70%.

(*Continued*)

TABLE 19–4 ■ DRUG DISPOSITION AND EFFECTS OF CYP INHIBITION AND INDUCTION ON ORAL ANTIPSYCHOTIC LEVELS (*CONTINUED*)

	t_{max}; ORAL BIOAVAILABILITY	METABOLISM	EFFECT OF CYP INHIBITION	EFFECT OF CYP INDUCTION
Quetiapine	Bioavailability: 9% t_{max}: 1–2 h t_{max}: 6 h (extended release)	CYP3A4 converts quetiapine to active metabolite norquetiapine. Steady-state mean AUC of norquetiapine is ~ half, of that for quetiapine. Unlikely to be a Pgp substrate at clinical concentrations. $t_{1/2}$: 6 h $t_{1/2}$: 9–12 h (norquetiapine)	Ketoconazole (200 mg once daily for 4 days) reduced oral clearance of quetiapine by 84%, resulting in a 335% increase in maximum plasma concentration.	Phenytoin increases clearance 5-fold.
Risperidone	Bioavailability: 70% t_{max}: 1 and 3 h for risperidone and 9-OH risperidone, respectively. In CYP2D6 PM, t_{max} for 9-OH risperidone is 17 h.	CYP2D6 converts risperidone to 9-OH risperidone (active). $t_{1/2}$: 3–4 h; 20–24 h (9-OH risperidone) In 2D6 PMs, half-lives are: risperidone, 20 h; 9-OH risperidone, 30 h.	Strong CYP2D6 inhibitors increase exposure to the active moiety (i.e., the AUC) by 1.5-fold (range, 1.3–1.8). Strong CYP3A4 inhibitors have no significant impact on active moiety levels.	Carbamazepine decreases exposure to the active moiety (i.e., the AUC) by 49%.
Ziprasidone	Bioavailability: 60% when given with food A 500-kcal meal (of any composition) ↑ AUC of a 20-mg, 40-mg, and 80-mg capsule by 48%, 87%, and 101%, respectively. t_{max}: 6–8 h	Aldehyde oxidase (66%), CYP3A4 (34%) $t_{1/2}$: 7.5 h	35%–40% increase in ziprasidone AUC by concomitantly administered ketoconazole.	35% decrease in ziprasidone AUC by carbamazepine.
Typical antipsychotics				
Haloperidol	Bioavailability: 60% t_{max}: 2–6 h	Multiple CYP pathways, particularly 2D6, 3A4. No active metabolites. $t_{1/2}$: 24 h (12–36 h)	Strong CYP2D6 inhibitors increase exposure 25%–50%. Individuals with only one functional CYP2D6 gene experience 2-fold greater trough serum levels. Those with no functioning alleles 3- to 4-fold higher. Strong CYP1A2 inhibitors increase exposure on average 62.5% (range, 48%–79%). Strong 3A4 inhibitors increase exposure 17%.	Carbamazepine, phenobarbital, or phenytoin ↓ C_p by 40%–72%. Rifampin ↓ C_p by 70%. Discontinuation of carbamazepine results in a 2- to 5-fold increase in C_p.
Chlorpromazine	Bioavailability is dose dependent: 25 mg: mean, 8.07%; max, 14.7% 100 mg: mean, 18.4%; max, 34.2% t_{max}: 1.4–2.0 h	CYP2D6 and to a lesser extent CYP1A2 convert chlorpromazine to an active metabolite, 7-OH chlorpromazine, which is present at levels ~34% of the parent compound. $t_{1/2}$: 11.05–15 h (range, 8–33 h with chronic dosing)	Strong CYP1A2 and CYP2D6 inhibitors will increase exposure as much as 38% and 70%, respectively.	Carbamazepine lowers C_p by 61% (range, 28%–84%). Phenobarbital lowers C_p by 36%–60%. Cigarette or cannabis smoking ↑ clearance 38%–50%.

AUC, area under the curve; BCRP, breast cancer resistance protein; BID, twice a day; EM, extensive metabolizer; PM, poor metabolizer.

TABLE 19–5 ■ NEUROLOGICAL SIDE EFFECTS OF ANTIPSYCHOTIC DRUGS

REACTION	FEATURES	TIME OF ONSET AND RISK INFO	PROPOSED MECHANISM	TREATMENT
Acute dystonia	Spasm of muscles of tongue, face, neck, back	Time: 1–5 days. Young, antipsychotic-naïve patients at highest risk	Acute DA antagonism	Antiparkinsonian agents are diagnostic and curative[a]
Akathisia	Subjective and objective restlessness; *not* anxiety or “agitation”	Time: 5–60 days	Unknown, but relates in part to DA antagonism. Can also be seen with DA partial agonists.	Reduce dose or change drug; low-dose mirtazapine (7.5–15 mg), clonazepam, and propranolol more effective than antiparkinsonians. Long-term use of benzodiazepines associated with higher mortality in schizophrenia patients, especially suicide mortality.[b]
Parkinsonism	Bradykinesia, rigidity, variable tremor, mask facies, shuffling gait	Time: 5–30 days. Elderly at greatest risk	DA antagonism	Dose reduction; change medication; antiparkinsonian agents. If possible, use amantadine and avoid anticholinergic medications due to adverse cognitive effects.[c]
Neuroleptic malignant syndrome	Extreme rigidity, fever, unstable blood pressure, myoglobinemia; can be fatal	Time: weeks–months. Can persist for days after stopping antipsychotic	DA antagonism	Stop antipsychotic immediately; supportive care; dantrolene and bromocriptine.[d]
Perioral tremor (“rabbit syndrome”)	Perioral tremor (may be a late variant of parkinsonism)	Time: months or years of treatment	DA antagonism	Antiparkinsonian agents often help.[c] If possible, use amantadine and avoid anticholinergic medications due to adverse cognitive effects.
Tardive dyskinesia	Orofacial dyskinesia, choreiform movements, tic-like movements, stereotypy, and possibly dystonia. Approximately one-third do not have head/neck involvement.	Time: months or years of treatment. Elderly at 5-fold greater risk. Risk proportional to potency of D_2 blockade	Upregulation of supersensitive postsynaptic dopamine D_2 receptors	Typically irreversible but may be reversible with early recognition and drug discontinuation. VMAT2 inhibitors valbenazine and deutetrabenazine are FDA-approved for tardive dyskinesia and very effective with effect sizes as high as 0.90. Anticholinergics exacerbate tardive dyskinesia but may be used for residual tardive dystonia not responsive to VMAT2 inhibition.

[a] Treatment: diphenhydramine 25–50 mg IM or benztropine 1–2 mg IM. Due to long antipsychotic $t_{1/2}$, may need to repeat or follow with oral medication.

[b] Propranolol often effective in relatively low doses (20–80 mg/day in divided doses). β_1-Selective adrenergic receptor antagonists are less effective. Nonlipophilic β adrenergic antagonists (e.g., atenolol) have limited CNS penetration and are of no benefit.

[c] Use of amantadine avoids anticholinergic effects of benztropine or diphenhydramine.

[d] Despite the response to dantrolene, there is no evidence of abnormal Ca^{2+} transport in skeletal muscle; with persistent antipsychotic effects (e.g., long-acting injectable agents), prolonged bromocriptine may be necessary in large doses (10–40 mg/day). Antiparkinsonian agents are not effective.

with a daily maximum of 6 mg, although slightly higher doses are used in rare circumstances. The piperidine compound *trihexyphenidyl* was one of the first synthetic anticholinergics. It also inhibits the presynaptic DA reuptake transporter, and this property creates a higher risk of abuse than *diphenhydramine* or *benztropine*. *Trihexyphenidyl* has good GI absorption, achieving peak plasma levels in 1 to 2 h, with a serum $t_{1/2}$ of about 10 to 12 h generally necessitating multiple daily dosing to achieve satisfactory clinical results. The total daily dosage range is 5 to 15 mg, given two or three times a day as divided doses. *Biperiden* is another drug in this class.

Amantadine, originally marketed as an antiviral agent for influenza A, is an alternative medication for antipsychotic-induced parkinsonism and avoids the adverse CNS and peripheral effects of anticholinergic medications (Silver and Geraisy, 1995). Its mechanisms of action involve presynaptic DA reuptake blockade, facilitation of DA release, postsynaptic DA agonism, and/or DA receptor modulation. *Amantadine* is well absorbed after oral administration with peak levels achieved 1 to 4 h after ingestion; clearance is renal with more than 90% recovered unmetabolized in the urine. The plasma $t_{1/2}$ is 12 to 18 h in healthy young adults but is longer with renal impairment. For those with creatinine clearance of 30 to 50 mL/min, the recommended dose is 200 mg given once followed by 100 mg every 24 h. The starting dosage is 100 mg orally once daily in healthy adults, which should be increased to 100 mg twice daily. Maximal doses for management of antipsychotic-related parkinsonism are 300 to 400 mg/day. A dose of 100 mg twice daily yields peak plasma levels of 500 to 800 ng/mL and trough levels of 300 ng/mL. Toxicity is seen at serum levels between 1000 and 5000 ng/mL.

Tardive Dyskinesia. Tardive dyskinesia results from increased postsynaptic nigrostriatal dopaminergic activity due to upregulation of postsynaptic supersensitive D_2 receptors from chronic D_2 blockade (and possible direct toxic effects of high-potency DA antagonists). TD occurs five times more frequently in elderly patients, and the risk may be somewhat greater in patients with mood disorders than in those with schizophrenia. The prevalence of TD averages 25% in adults treated with FGAs for more than a year but is estimated at 7.2% in those exposed only to SGAs (Carbon et al., 2017).

Tardive dyskinesia is characterized by stereotyped, repetitive, involuntary, quick choreiform (tic-like) movements of the face, eyelids (blinks or spasm), mouth (grimaces), tongue, extremities, or trunk, with varying degrees of slower athetosis (twisting movements). As part of the syndrome, there may also be tardive dystonia, tardive akathisia, and, rarely, tardive pain. TD movements disappear during sleep, vary in intensity over time, and are dependent on the level of arousal or emotional distress, sometimes reappearing during acute psychiatric illnesses following prolonged disappearance. TD movements can be suppressed partially by using a potent DA antagonist to block supersensitive postsynaptic D_2 receptors, but such interventions may worsen the severity over time as this was part of the initial pharmacological insult. Switching patients from potent D_2 antagonists to weaker antagonists is not effective and should not be employed to manage TD (Bhidayasiri et al., 2018). The sole exception is switching to *clozapine* for patients with moderate/severe TD, but this should be performed only in patients who have other indications for *clozapine* (e.g., treatment-resistant schizophrenia), since the newly approved VMAT2 inhibitors are well tolerated and effective when given with existing antipsychotic therapy (Mentzel et al., 2018). In patients who do not require continuous antipsychotic therapy (e.g., adjunctive use for mood disorders), drug discontinuation may be beneficial when TD is recognized early but is effective in only 12% of cases with more established symptoms (Bhidayasiri et al., 2018).

The VMAT2 inhibitors *valbenazine* and *deuterated-tetrabenazine* (*deutetrabenazine*) are FDA-approved for TD. Both are derivatives of *tetrabenazine* but with more favorable kinetics that also improve on the tolerability profile of *tetrabenazine* (Bhidayasiri et al., 2018). *Valbenazine* is a prodrug metabolized to a *tetrabenazine* metabolite (α-dihydrotetrabenazine) that is a potent VMAT2 inhibitor. *Valbenazine* is dosed once daily and is titrated after 1 week from 40 mg to 80 mg unless tolerability or kinetic issues limit use of the highest dosage. The clearance of *valbenazine* and its active metabolite involve CYPs 2D6 and 3A4. Thus, exposure to the parent drug and its active metabolite will be increased in CYP2D6-poor metabolizers, in the presence of strong inhibitors of CYP2D6 (e.g., *paroxetine*) or CYP3A4 (e.g., *ketoconazole*), or in patients with moderate-to-severe hepatic impairment. Use of *valbenazine* in the presence of strong inducers of CYP3A4 (e.g., *rifampin*) is not recommended; concomitant use of monoamine oxidase inhibitors should be avoided. *Valbenazine* also inhibits the P-glycoprotein (Pgp) efflux transporter and will increase *digoxin* exposure. *Deutetrabenazine* is approved for TD and for managing the chorea of Huntington's disease. (See Chapter 21 for discussion of *deutetrabenazine* and *tetrabenazine* use for Huntington's chorea.) *Deutetrabenazine* is given twice daily and has a titration of 6 mg/week from the starting dose of 6 mg twice daily to the effective dosage range of 24 to 48 mg/day (Bhidayasiri et al., 2018). Both *valbenazine* and *deutetrabenazine* were well tolerated in clinical trials, with high completion rates (80%–90%) that were comparable to placebo. Efficacy is sustained during long-term use, but given the irreversible nature of TD in most patients, symptoms typically return to baseline when a VMAT2 inhibitor is discontinued.

Akathisia. Unlike antipsychotic-induced parkinsonism and acute dystonia, the phenomenology and treatment of akathisia suggest involvement of structures outside the nigrostriatal pathway. Despite the association with D_2 blockade, akathisia has minimal response to anticholinergic antiparkinsonian drugs, so other treatment strategies are employed acutely, including high-potency benzodiazepines (e.g., *clonazepam*), nonselective β adrenergic blockers with excellent CNS penetration (e.g., *propranolol*), and the $5HT_{2A}$ antagonist antidepressant *mirtazapine* at doses of 7.5 to 15 mg at bedtime (American Psychiatric Association, 2021). Over time, one should pursue dose reduction or switching to another antipsychotic. That *clonazepam* and *propranolol* have significant cortical activity and are ineffective for other forms of EPSs points to an extrastriatal origin for akathisia symptoms.

Neuroleptic Malignant Syndrome. NMS resembles a severe form of parkinsonism, but with additional symptoms including autonomic instability (hyperthermia and labile pulse, blood pressure, and respiration rate), stupor, elevation of serum creatine kinase, and resultant myoglobinuria with potential nephrotoxicity. At its most severe, this syndrome may persist for more than a week after the offending agent is discontinued and can be associated with mortality. NMS has been associated with myriad antipsychotic agents, but risk is greatest with relatively high doses of potent D_2 antagonists. Aside from cessation of antipsychotic treatment and provision of supportive care (e.g., aggressive cooling measures), specific pharmacological treatment with a DA agonist such as *bromocriptine* is helpful, with conflicting data on the value of *dantrolene*. Electroconvulsive therapy (ECT) can also be considered in patients with inadequate response to other measures. There are fewer reports of NMS with SGAs compared to those from FGA exposure, so this syndrome is less commonly seen in its full presentation (Gurrera et al., 2017). Consensus criteria to assist in NMS diagnosis were published in 2011, with a subsequent validation study released in 2017 using the proposed scoring scheme from the 2011 paper (Gurrera et al., 2017).

Hyperprolactinemia. Hyperprolactinemia results from blockade of tuberoinfundibular D_2 receptors. Dopaminergic neurons project from the arcuate nucleus of the hypothalamus to the median eminence where they deliver DA to the anterior pituitary via the hypophyseoportal vessels. D_2 receptors on lactotrophs in the anterior pituitary mediate the tonic prolactin-inhibiting action of DA. Correlations between the D_2 potency of antipsychotic drugs and prolactin elevations are excellent. With the exception of *amisulpride*, *risperidone*, and *paliperidone*, SGAs show limited effects (*asenapine*, *iloperidone*, *olanzapine*, *quetiapine*, *ziprasidone*) to almost no effects (*clozapine*, *aripiprazole*, *brexpiprazole*, *cariprazine*, *lumateperone*) on prolactin secretion. *Amisulpride*, *risperidone*, and *paliperidone* have the highest risk for prolactin elevation, even more so than high-potency FGAs, and this is related to their high affinity for the Pgp efflux transporter (Huhn et al., 2019). High Pgp affinity results in reflux at the blood-brain barrier, thereby exposing pituitary cells to locally high concentrations of the antipsychotic. The impact on prolactin secretion is thus disproportionate to the D_2 affinity.

Hyperprolactinemia can directly induce breast engorgement and galactorrhea and can cause amenorrhea in women and sexual dysfunction or infertility in women and men. Dose reduction can be tried to decrease serum prolactin levels, but caution must be exercised to keep treatment within the antipsychotic therapeutic range. Switching from offending antipsychotic agents (especially from *amisulpride*, *risperidone*, and *paliperidone*) is the preferred strategy, but when not feasible, *bromocriptine* can be employed. The hyperprolactinemia from antipsychotic drugs is rapidly reversed when the drugs are discontinued. Use of the partial agonist *aripiprazole* is generally not advisable as there are case reports of exacerbation due to the displacement of D_2 antagonist antipsychotics (e.g., *haloperidol*, *risperidone*, *paliperidone*) by *aripiprazole* (Takeuchi and Remington, 2013). Like all partial agonist antipsychotics, *aripiprazole* has extremely high D_2 affinity, even higher than that of FGAs.

Histamine H_1 Receptors.

Antagonism of CNS of H_1 receptors is associated with two major adverse effects: sedation and weight gain via appetite stimulation. Certain antipsychotic agents cause these adverse effects by virtue of their high H_1 binding (see Table 19–3).

Sedation. Examples of sedating antipsychotic drugs include low-potency FGAs such as *chlorpromazine* and the SGAs *clozapine* and *quetiapine*. The sedating effect is predicted by their high H_1 receptor affinities (see Table 19–3). Some tolerance to the sedative properties will develop, a helpful fact to remember when considering switching a patient to a nonsedating agent. Rapid discontinuation of sedating antihistaminic antipsychotics is inevitably followed by significant complaints of rebound insomnia and sleep disturbance. If discontinuation of sedating antipsychotic treatment is deemed necessary, the sedating antipsychotic should be tapered slowly over 4 to 12 weeks, and the clinician should be prepared to utilize a sedative at the end of the taper. *Hydroxyzine* (an antihistamine) or *diphenhydramine* (an anticholinergic antihistamine) are reasonable options. Following emergency cessation of *clozapine* for severe neutropenia or myocarditis, high doses of anticholinergics must be used to prevent cholinergic rebound. Sedation may be useful during acute psychosis, but excessive sedation can interfere with patient evaluation, may prolong emergency room and psychiatric hospital stays unnecessarily, and is poorly tolerated among elderly patients with dementia and delirium; thus, the prescriber must exercise caution with the choice of agent and the dose.

Weight Gain. Weight gain is a significant problem during long-term use of antipsychotic drugs and represents a major barrier to medication adherence as well as a significant threat to the physical and emotional health of the patient. Weight gain has effectively replaced concerns over EPS as the adverse effect causing the most consternation among patients and clinicians alike. Appetite stimulation is the primary mechanism involved, with little evidence to suggest that decreased activity (due to sedation) is a main contributor to antipsychotic-related weight gain. Laboratory studies indicated that medications with significant H_1 antagonism induce appetite stimulation through effects at hypothalamic sites. Antagonism at $5HT_{2c}$ receptors may play an additive role in promoting weight gain for medications that possess high H_1 affinities (e.g., *clozapine*, *olanzapine*) but appears to have no effect in the absence of significant H_1 blockade, as evidenced by *ziprasidone*, an antipsychotic with low weight gain risk, extremely high $5HT_{2c}$ affinity, and poor affinity for H_1 histamine receptors. *Chlorpromazine* and the SGAs *olanzapine*, *quetiapine*, and *clozapine* are the agents of highest risk, but some weight gain occurs with nearly all antipsychotic drugs (Huhn et al., 2019).

Younger and antipsychotic-naïve patients are more sensitive to the weight gain from all antipsychotics, including agents that appear more weight neutral in adults. Switching to more weight-neutral medications can achieve significant results; however, when changing medications is not feasible or unsuccessful, behavioral strategies must be employed and should be considered. *Metformin* is also used to moderate the antipsychotic-induced weight gain from *olanzapine* and *clozapine* and should be introduced when starting the antipsychotic. Glucagon-like peptide-1 agonists such as *exenatide* and *liraglutide* can also be helpful (Siskind et al., 2019). In June 2021, a combination tablet of *olanzapine* and *samidorphan* was approved in the U.S. for schizophrenia and bipolar I disorder. *Samidorphan* is a μ-opioid receptor antagonist and modest κ-opioid receptor partial agonist whose role is to limit *olanzapine*-related weight gain in patients newly started on *olanzapine* by blunting the endorphin signal that reinforces eating behavior.

Muscarinic M_1 Receptors. Muscarinic antagonism is responsible for the central and peripheral anticholinergic effects of medications. The clinically relevant anticholinergic burden of most SGAs are limited, whereas *clozapine* and low-potency phenothiazines have significant anticholinergic adverse effects (see Table 19–3). *Quetiapine* has modest muscarinic affinity; its active metabolite norquetiapine is likely responsible for the anticholinergic effects. *Clozapine* is particularly associated with significant constipation and ileus due to its anticholinergic properties and possibly effects at $5HT_3$ receptors. Routine use of multiple stool softeners and secretagogues is necessary to manage the problem. Psyllium should be avoided since the colonic transit time for *clozapine*-treated patients may exceed 100 h, thereby resulting in inspissation of psyllium into a gel that itself can cause intestinal obstruction (Meyer and Stahl, 2019). Anticholinergic medications should be avoided in elderly patients, especially those with dementia or delirium, due to the adverse effects on cognition, with *clozapine* being the notable exception due to lack of other options for treatment-resistant schizophrenia.

Adrenergic α_1 Receptors. α_1 Adrenergic antagonism is associated with orthostatic hypotension, and this can be particularly problematic for elderly patients who have poor vasomotor tone. The extent to which an antipsychotic causes this effect in clinical practice is dependent on the doses employed and the rapidity of titration. Compared to high-potency FGAs, low-potency FGAs pose greater risk for orthostasis. Among SGAs, *iloperidone* carries a warning regarding minimization of orthostasis risk through slower titration. *Clozapine* can be associated with significant orthostasis, even when titrated slowly. Because *clozapine*-treated patients have few other antipsychotic options, the potent mineralocorticoid *fludrocortisone* starting at 0.1 mg/day (maximum dose 0.5 mg/day) is used as a volume expander (Meyer and Stahl, 2019).

Adverse Effects Not Predicted by Monoamine Receptor Affinities

Adverse Metabolic Effects. Metabolic effects are an area of great concern during long-term antipsychotic treatment, paralleling the overall concern for the high prevalence of prediabetic conditions, type 2 diabetes, and 2-fold greater CV mortality among patients with schizophrenia.

Aside from weight gain, the two predominant metabolic adverse effects seen with antipsychotic drugs are dyslipidemia, primarily elevated serum triglycerides, and impairments in glycemic control.

Low-potency phenothiazines elevate serum triglyceride levels, an effect not seen with high-potency phenothiazines. Among SGAs, significant increases in fasting triglyceride levels are noted during *clozapine*, *olanzapine*, and high-dose *quetiapine* (>400 mg/day) exposure. Effects on total cholesterol and cholesterol fractions are significantly less but show expected associations related to agents of highest risk: *clozapine*, *olanzapine*, and *quetiapine* (Huhn et al., 2019). Weight gain can induce deleterious lipid changes; however, evidence indicates that antipsychotic-induced hypertriglyceridemia is a weight-independent adverse event that occurs within weeks of starting an offending medication and improves within 6 weeks after medication discontinuation. In individuals not exposed to antipsychotics, elevated fasting triglycerides are a direct consequence of insulin resistance, as insulin-dependent lipases in fat cells are normally inhibited by insulin. As insulin resistance worsens, inappropriately high levels of lipolysis lead to the release of excess amounts of free fatty acids, which are transformed into triglyceride particles. Elevated fasting triglyceride levels thus become a sensitive marker of insulin resistance, leading to the hypothesis that the triglyceride increases seen during antipsychotic treatment are the result of derangements in glucose-insulin homeostasis.

The ability of antipsychotics to induce hyperglycemia was first noted during low-potency phenothiazine treatment; indeed, *chlorpromazine* was occasionally exploited for this specific property as adjunctive presurgical treatment of insulinoma. As SGAs gained widespread use, numerous papers documented the association of new-onset diabetes and diabetic ketoacidosis with SGA exposure, especially during *clozapine*, *olanzapine*, and *quetiapine* therapy. The mechanism by which antipsychotic drugs disrupt glucose-insulin homeostasis is not known, but *in vivo* animal experiments document immediate dose-dependent effects of *clozapine* and *olanzapine* on whole-body and hepatic insulin sensitivity.

There may also be inherent disease-related mechanisms that increase risk for metabolic disorders among schizophrenia patients, but medication choice is a primary modifiable risk factor, and all SGAs in the U.S. include a warning about hyperglycemia on the drug label. Use of metabolically more benign agents is recommended for the initial treatment of all patients for whom long-term treatment is expected. Clinicians should obtain baseline metabolic data, including a fasting glucose or hemoglobin A_{1c}, a fasting lipid panel, and weight, and then establish a plan for ongoing monitoring of metabolic parameters. As with weight gain, changes in fasting glucose and lipids should prompt reevaluation of ongoing treatment, institution of measures to improve metabolic health (diet, exercise, nutritional counseling), and consideration of switching antipsychotic agents.

Adverse Cardiac Effects. Multiple ion channels are involved in the depolarization and repolarization of cardiac ventricular cells (see Chapters 33 and 34). Some antipsychotics can interfere with the functioning of these channels, elevating the risk of ventricular arrhythmias and sudden cardiac death (SCD). While most FGAs (e.g., *thioridazine*) significantly inhibited inwardly rectifying K^+ channels (I_{kr}) in cardiac myocytes, this effect is much less pronounced for SGAs (Xiong et al., 2020). Antagonism of voltage-gated Na^+ channels causes QRS widening and an increase in the PR interval, with increased risk for ventricular arrhythmia. Myocyte repolarization is mediated in part by K^+ current through two channels: the rapid I_{kr} and the slow I_{ks} channels. The α subunit of the I_{kr} channel, $K_{v11.1}$, is encoded by *hERG*, the human-ether-à-go-go related gene. $K_{v11.1}$ is thus part of the K^+ channel that mediates the repolarizing I_{kr} current of the cardiac action potential. Polymorphisms of *hERG* are involved in the congenital long QT syndrome associated with syncope and SCD. Antagonism of I_{kr} channels is responsible for most cases of drug-induced QT prolongation and is the suspected mechanism for the majority of antipsychotic-induced SCDs (Xiong et al., 2020).

Aside from individual agents, for which anecdotal and pharmacosurveillance data indicate risk for torsades de pointes (e.g., *thioridazine*, *pimozide*), most of the commonly used newer antipsychotic agents are not associated with a known increased risk for ventricular arrhythmias. Prior associations between *clozapine* use and QT prolongation are now recognized as spurious artifacts related to use of the outdated and inaccurate Bazett QT correction formula in a group of patients who are often tachycardic (Meyer and Stahl, 2019). One current exception is *sertindole*, an agent not available in the U.S. that was withdrawn in 1998 based on anecdotal reports of torsades de pointes, but reintroduced in Europe in 2006 with strict electrocardiogram (ECG) monitoring guidelines. Although *in vitro* data revealed *sertindole*'s affinity for I_{kr}, several epidemiological studies published over the past decade were unable to confirm an increased risk of sudden death due to *sertindole* exposure, thereby providing justification for its reintroduction.

Currently, no data suggest a benefit of routine ECG monitoring for prevention of SCD among patients using antipsychotic drugs (Xiong et al., 2020). Nonetheless, all antipsychotic medications marketed in the U.S. (with the exception of *lurasidone* and *cariprazine*) carry a class label warning regarding QTc prolongation. A specific black-box warning exists for *thioridazine*, *pimozide*, intramuscular *droperidol*, and *haloperidol* (intravenous formulation but not oral or intramuscular) concerning torsades de pointes and subsequent fatal ventricular arrhythmias (discussed next and in Chapter 34). Overdose with FGAs is of particular concern with certain agents (e.g., *chlorpromazine*, *thioridazine*, *pimozide*) due to the risk of torsades de pointes and, for the lower potency antipsychotics, a likelihood of sedation, anticholinergic effects, and orthostasis. Patients who overdose on high-potency FGAs (e.g., *haloperidol*) and the substituted benzamides are at greater risk for EPSs (due to the high D_2 affinity) and for ECG changes. Overdose experience with SGAs indicates a much lower risk for torsades de pointes and other ventricular arrhythmias compared to older antipsychotic medications; however, combinations of antipsychotic agents with other medications can lead to fatality, primarily through respiratory depression.

Effects of *clozapine* on the heart include tachycardia that occurs independently of orthostasis, myocarditis, and dilated cardiomyopathy. Myocarditis risk is 1% and occurs only during the first 6 weeks of treatment. It is suspected by clinical signs (e.g., chest pain, malaise, fever) with diagnosis confirmed by troponin I or T levels greater than or equal to 2 times the upper limit of normal or a C-reactive protein greater than or equal to 100 mg/L (Meyer and Stahl, 2019).

Other Adverse Effects. In the U.S., there is a class label warning for seizure risk (<1%) on all antipsychotics except *pimavanserin*. Among commonly used SGAs, only *clozapine* has a dose-dependent seizure risk, with a prevalence of 3% to 5%. The structurally related *olanzapine* had an incidence of 0.9% in premarketing studies. Patients with seizure disorder who commence antipsychotic treatment must receive adequate prophylaxis, with consideration given to avoiding *carbamazepine* and *phenytoin* due to their potent induction of CYPs and Pgp. *Carbamazepine* is also contraindicated during *clozapine* treatment due to its rare risk for bone marrow effects. VPA derivatives (e.g., *divalproex*) are used for *clozapine*-associated seizures as they best cover the spectrum of generalized and myoclonic or atonic seizures. *Divalproex* should not be used prophylactically for two reasons: seizure rates even at *clozapine* doses greater than 600 mg/day are less than 2%, and VPA itself is associated with neutropenia risk, which may complicate *clozapine* treatment (Meyer and Stahl, 2019).

Clozapine is associated with several adverse effects, the most concerning of which is severe neutropenia, with an incidence of slightly under 1%; the highest risk occurs during the initial 6 months of treatment, peaking at months 2 to 3 and diminishing rapidly thereafter (Meyer and Stahl, 2019). The mechanism is immune mediated, and patients who have severe neutropenia are usually not rechallenged, with rare exceptions (Meyer and Stahl, 2019). An extensive algorithm guiding clinical response to neutropenia is available from manufacturer websites and must be followed, along with mandated absolute neutrophil count monitoring. An important update to the U.S. prescribing guidelines lowered the starting neutropenia threshold for those with benign ethnic neutropenia to 1000/mm^3.

Other uncommon antipsychotic-related adverse effects include pigmentary retinopathy (*thioridazine* at daily doses ≥800 mg/day), photosensitivity, and elevations of alkaline phosphatase and, rarely, hepatic transaminases (low-potency phenothiazines).

Increased Mortality in Patients With Dementia. Perhaps the least-understood adverse effect is the increased risk for cerebrovascular events and all-cause mortality among elderly patients with dementia exposed to antipsychotics (~1.7-fold increased mortality risk for drug vs. placebo) (Maust et al., 2015). Mortality is due to heart failure, sudden death, or pneumonia. The underlying etiology for antipsychotic-related cerebrovascular and mortality risk is unknown, but the finding of virtually equivalent mortality risk for FGAs and SGAs (including *aripiprazole*) suggests an impact of reduced D_2 signaling regardless of individual antipsychotic mechanisms.

Drug-Drug Interactions

Antipsychotics are not significant inhibitors of CYPs, with a few notable exceptions: *chlorpromazine*, *perphenazine*, and *thioridazine* inhibit CYP2D6, and *asenapine* increases *paroxetine* levels 2-fold, presumably by CYP inhibition. The plasma half-lives of most antipsychotics are influenced by induction or inhibition of hepatic CYPs and by genetic polymorphisms that alter specific CYP activities (see Table 19–4). While antipsychotics are highly protein bound, the binding sites are to α_1-glycoproteins. There is no evidence of significant displacement of other protein-bound medications that bind to albumin or prealbumin, so dosage adjustment is not required for anticonvulsants, *warfarin*, or other agents with narrow therapeutic indices. Antipsychotics are not removed by renal dialysis (Meyer and Stahl, 2021).

It is also important to consider the kinetic effects of environmental exposures (smoking, nutraceuticals, grapefruit juice) on drug metabolism and the impact of changes in these behaviors. Aromatic hydrocarbons in tobacco smoke induce CYP1A2, so changes in smoking status can be especially problematic for *clozapine*-treated patients (Meyer and Stahl, 2019). Placing a smoker in a smoke-free environment will result in decreased CYP1A2 activity and an elevation of *clozapine* plasma levels, with potentially toxic results. Conversely, a patient discharged from a nonsmoking facility who resumes smoking will experience an increase in CYP1A2 activity and a 50% decrease in plasma *clozapine* levels. Monitoring of plasma *clozapine* concentrations, anticipation of changes in smoking habits, and dosage adjustment can minimize development of subtherapeutic or supratherapeutic levels. Serious bacterial or viral infections (e.g., COVID-19), and occasionally the COVID-19 vaccination itself, cause marked cytokine release, and this significantly downregulates CYP1A2 expression. This decreased CYP1A2 availability can induce *clozapine* toxicity (Meyer and Stahl, 2019; Tio et al., 2021).

Use in Pediatric Populations

Aripiprazole, *olanzapine*, *quetiapine*, *risperidone*, *lurasidone*, and *paliperidone* have indications for adolescent schizophrenia (ages 13–17). *Aripiprazole*, *quetiapine*, and *risperidone* are approved in child and adolescent acute mania (ages 10–17), and *lurasidone* is approved for bipolar I depression in this same age group; *risperidone* and *aripiprazole* are also FDA-approved for irritability associated with autism in child and adolescent patients (ages 5–16). As discussed in the sections on adverse effects, antipsychotic drug-naïve patients and younger patients are more susceptible than other patients to EPSs and weight gain (Correll et al., 2020). Use of the minimum effective dose can minimize EPS risk, and use of agents with lower weight gain liability is critical. The greater impact of *risperidone* and *paliperidone* on serum prolactin must be monitored. Delayed sexual maturation was not seen in adolescents in clinical trials with *risperidone*; nonetheless, the prescriber must be alert for such changes and for issues such as amenorrhea in girls and gynecomastia in boys and girls. There are compelling data for the use of *metformin* to mitigate the higher weight gain liability of SGAs in child/adolescent patients (Correll et al., 2020).

Use in Geriatric Populations

The increased sensitivity to EPSs, orthostasis, sedation, and anticholinergic effects are important for the geriatric population and often dictate the choice of antipsychotic medication. Avoidance of drug-drug interactions is also important, as older patients are on numerous concomitant medications. Dose adjustment can offset known drug-drug interactions, but clinicians must be attentive to changes in concurrent medications and the potential pharmacokinetic and pharmacodynamic consequences. Vigilance must also be maintained for the additive pharmacodynamic effects of α_1 adrenergic, antihistaminic, and anticholinergic properties of other agents.

Elderly patients have an increased risk for TD and parkinsonism, with TD rates about 5-fold higher than those seen with younger patients. With FGAs, the reported annual TD incidence among elderly patients is 20% to 25%, compared to 4% to 5% for younger patients. With SGAs, the annual TD rate in elderly patients is much lower (2%–3%). Increased risk for cerebrovascular events and all-cause mortality is also seen in elderly patients with dementia (see Increased Mortality in Patients With Dementia). Compared to younger patients, antipsychotic-induced weight gain is lower in elderly patients.

Use During Pregnancy and Lactation

Results from a recent large database study comprising 9991 women with first-trimester exposure did not show increased rates of major congenital malformations (MCMs) after first-trimester antipsychotic exposure (Huybrechts et al., 2016). While concerns over MCM risk have lessened, antipsychotic drugs are designed to cross the blood-brain barrier and also have high rates of placental passage. Placental passage ratios are estimated to be highest for *olanzapine* (72%), followed by *haloperidol* (42%), *risperidone* (49%), and *quetiapine* (24%). This has implications for neonatal care at time of delivery, including concerns over EPSs, sedation, or withdrawal syndromes in the newborn. Neonates exposed to *olanzapine*, the atypical agent with highest placental passage ratio, exhibit a trend toward greater neonatal intensive care unit admission, and exposure to sedating medications such as *clozapine* can be associated with decreased arousal and hypotonia ("floppy baby" syndrome). Schizophrenia spectrum patients should continue medication throughout pregnancy, but tapering antipsychotics used for other indications can be considered for 1 to 2 weeks prior to the expected delivery date to lessen neonatal exposure. Use in nursing mothers raises a separate set of concerns due to the reduced capacity of the newborn to metabolize xenobiotics, thus presenting a significant risk for antipsychotic toxicity. Available data do not provide adequate guidance on choice of agent, and only *clozapine* is absolutely contraindicated in breastfeeding mothers due to risk for neutropenia, sedation, and seizures in the infant (Meyer and Stahl, 2019).

Major Drugs Available in the Class

Atypical antipsychotics have largely replaced older agents, primarily due to their more favorable EPS profile. However, high-potency FGAs are widely used when a higher level of D_2 antagonism is required. Table 19–1 describes the acute and maintenance doses for adult schizophrenia treatment based on consensus recommendations. There are numerous LAI FGA formulations (see Table 19–2), but in the U.S., the only available LAI FGAs are *fluphenazine* and *haloperidol* (as decanoate esters). There are now multiple LAI SGAs approved, including a 2-month form of *aripiprazole* (*aripiprazole lauroxil*), and both 3- and 6-month forms of LAI *paliperidone*. *Pimavanserin* is the only medication indicated for PDP; it does not worsen motor symptoms due to the lack of DA antagonism (Cummings et al., 2014).

Treatment of Mania

Mania is a period of elevated, expansive, or irritable mood with coexisting symptoms of increased energy and goal-directed activity and decreased need for sleep. Mania represents one pole of bipolar I disorder. As with psychosis, mania may be induced by prescribed medications (e.g., DA agonists, antidepressants, stimulants) or substances of abuse, primarily cocaine and amphetamines, although periods of substance-induced mania should not be relied on solely to make a diagnosis of bipolar disorder. However, a full manic episode that emerges during antidepressant treatment (for example) *but persists at a fully syndromal level beyond the physiological effect of that treatment* is sufficient evidence for a manic episode and, therefore, a bipolar I diagnosis.

Mania is distinguished from its less-severe form, hypomania, by the fact that hypomania, by definition, does not result in functional impairment or hospitalization. Patients who experience periods of hypomania and major depression have bipolar II disorder; those with mania at any time, bipolar I; and those with hypomania but less-severe forms of depression, cyclothymia. The prevalence of bipolar I disorder is roughly 1% of the population, and the prevalence of all forms of bipolar disorder is 3% to 5%.

Genetic studies of bipolar disorder have yielded several loci of interest associated with disease risk and predictors of treatment response, but the data are not yet at the phase of clinical application. Unlike schizophrenia, for which the biological understanding of monoamine neurotransmission has permitted synthesis of numerous effective compounds, no medication has yet been designed to treat the full spectrum of bipolar disorder based on biological hypotheses of the illness. Lithium carbonate was introduced fortuitously in 1949 for the treatment of mania and approved for this purpose in the U.S. in 1970. While many classes of agents demonstrate efficacy in acute mania, including Li^+, antipsychotic drugs, and certain anticonvulsants, no medication has surpassed Li^+'s efficacy for prophylaxis of future manic and depressive phases of bipolar disorder, and no other medication has demonstrated Li^+'s reduction in suicidality among bipolar patients (Baldessarini et al., 2019). There is also compelling data supporting Li^+'s neuroprotective effects based on decreased dementia risk in older bipolar patients maintained on Li^+ (Velosa et al., 2020).

Pharmacological Properties of Agents for Mania

Antipsychotic Agents

The chemistry and pharmacology of antipsychotics are addressed earlier in this chapter. When used for acute mania, the dosages are often at the high end of approved maximum dosing, and the antimanic effect relates to D_2 blockade, not sedation from H_1 antagonism. *Clozapine* can be beneficial in patients with treatment-resistant mania as adjunctive therapy and as monotherapy (Meyer and Stahl, 2019). Certain antipsychotics have efficacy for adjunctive use (*olanzapine/fluoxetine* combination, *lumateperone*, *lurasidone*, *quetiapine*, *lumateperone*) or as monotherapy (*quetiapine*, *lurasidone*, *cariprazine*, *lumateperone*) for bipolar depression, typically at lower dosages than those used for schizophrenia or acute mania.

Anticonvulsants

The pharmacology and chemistry of the anticonvulsants used in treating acute mania (VPA compounds, *carbamazepine*) and for bipolar maintenance (*lamotrigine*) are covered extensively in Chapter 20. The therapeutic serum levels for the commonly used mood-stabilizing anticonvulsants and for Li^+ are listed in Table 19–6.

Lithium

Lithium is the lightest of the alkali metals (group Ia). Salts of Li^+ share some characteristics with those of Na^+ and K^+. Li^+ is readily assayed in biological fluids and can be detected in brain tissue by magnetic resonance spectroscopy. Traces of the ion occur normally in animal tissues, but it has no known physiological role. Li^+ carbonate and Li^+ citrate are used therapeutically in the U.S.

Therapeutic concentrations of Li^+ have almost no discernible psychotropic effects in individuals without psychiatric symptoms. There are numerous molecular and cellular actions of Li^+, some of which overlap with identified properties of other mood-stabilizing agents (particularly VPA) and are discussed next. An important characteristic of Li^+ is that, unlike Na^+ and K^+, Li^+ develops a relatively small gradient across

TABLE 19–6 ■ COMPARATIVE EFFICACY AND TARGET SERUM LEVELS FOR MOOD STABILIZERS

	ACUTE MANIA	PROPHYLAXIS	BIPOLAR DEPRESSION
Lithium	+++ 1.0–1.5 mEq/L[a]	+++ 0.6–1.0 mEq/L[b]	+ 0.6–1.0 mEq/L
Valproate	++++ 100–120 μg/mL[c]	+++ 60–100 μg/mL	—
Carbamazepine	+ 6–12 μg/mL	++ 6–12 μg/mL	+ 6–12 μg/mL
Lamotrigine	—	++	++ 2.5–20 μg/mL[d]

[a] Lithium can be loaded with three 10 mg/kg doses of an extended-release preparation administered at 4 PM, 6 PM, and 8 PM (Kook et al., 1985). Treatment should continue on day 2 with lithium carbonate given once nightly to minimize the risk of polyuria and renal insufficiency.

[b] Outpatient levels should never exceed 1.2 mEq/L, as this increases the risk of long-term renal insufficiency by 74% (Castro et al., 2016).

[c] Divalproex can be loaded at 30 mg/kg over 24 h, administered as a single dose or separated into two doses.

[d] These levels are based on anticonvulsant use and do not necessarily correlate with efficacy for mood disorders.

biological membranes. Although it can replace Na^+ in supporting a single action potential in a nerve cell, it is not a substrate for the Na^+ pump and therefore cannot maintain membrane potentials. It is uncertain whether therapeutic concentrations of Li^+ (0.5–1.0 mEq/L) affect the transport of other monovalent or divalent cations by nerve cells.

Hypotheses for the Mechanism of Action of Lithium and Relationship to Anticonvulsants

Plausible hypotheses for the mechanism of action focus on Li^+'s impact on monoamines implicated in the pathophysiology of mood disorders and on second-messenger and other intracellular molecular mechanisms involved in signal transduction, gene regulation, and cell survival. Li^+ has limited effects on catecholamine-sensitive adenylyl cyclase activity or on the binding of ligands to monoamine receptors in brain tissue, although it can influence response of 5HT autoreceptors to agonists. Presynaptic 5HT release is regulated by $5HT_{1a}$ autoreceptors located on the cell body and $5HT_{1b}$ receptors on the nerve terminal. *In vitro* electrophysiological studies suggest that Li^+ facilitates 5HT release. Li^+ augments effects of antidepressants, and in animal models of depression, Li^+'s activity appears to be mediated through desensitizing actions at $5HT_{1b}$ sites; Li^+ also antagonizes mouse behaviors induced by administration of selective $5HT_{1b}$ agonists.

Li^+ inhibits inositol monophosphatase and interferes with the cycling of the phosphatidylinositol (PI) pathway (see Figure 19–1). One result is an enhancement of IP_3 accumulation when the G_q-PLC-IP_3-Ca^{2+} pathway is activated. As a result, IP_3 signaling and consequent mobilization of Ca^{2+} from intracellular stores may also be enhanced acutely, along with the sequelae of those effects: Ca^{2+} mobilization and PKC activation; another result is a decrease in available inositol for resynthesis/reincorporation into membrane PI phosphates. The uncompetitive inhibition of inositol phosphatase by Li^+ occurs within the range of therapeutic Li^+ concentrations. A genome-wide association study implicated diacylglycerol kinase in the etiology of bipolar disorder, strengthening the association between Li^+ actions and PI metabolism. Further support for the role of inositol signaling in mania rests on the finding that VPA and its derivatives decrease intracellular inositol concentrations. Unlike Li^+, VPA decreases inositol through inhibition of myo-inositol-1-phosphate synthase. In cultured cell systems, *carbamazepine* also appears to act via inositol depletion and may contribute to VPA's and *carbamazepine*'s mood-stabilizing properties.

Treatment with Li^+ ultimately leads to decreased activity of several protein kinases in brain tissue, including PKC, particularly isoforms α and β (Valvassori et al., 2020). Among other proposed antimanic or mood-stabilizing agents, this effect is also shared with VPA (particularly for PKC) but not with *carbamazepine* (Einat et al., 2007). Long-term treatment of rats with Li^+ carbonate or VPA decreases cytoplasm-to-membrane translocation of PKC and reduces PKC stimulation–induced release of 5HT from cerebral cortical and hippocampal tissue. Excessive PKC activation can disrupt prefrontal cortical regulation of behavior, but pretreatment of monkeys and rats with Li^+ carbonate or VPA blocks the impairment in working memory induced by activation of PKC in a manner also seen with the PKC inhibitor *chelerythrine*. A major substrate for cerebral PKC is the MARCKS protein, which is implicated in synaptic and neuronal plasticity. The expression of MARCKS protein is reduced by treatment with both Li^+ and VPA but not by *carbamazepine*, antipsychotics, or antidepressants. This proposed mechanism of PKC inhibition has been the basis for therapeutic trials of *tamoxifen*, a selective estrogen receptor modulator that is also a potent centrally active PKC inhibitor. In acutely manic bipolar I patients, *tamoxifen* has shown evidence of efficacy as adjunctive treatment (Valvassori et al., 2020). The impact of Li^+ or VPA on PKC activity may secondarily alter the activity of tyrosine hydroxylase.

Both Li^+ and VPA treatment also inhibit the activity of GSK-3β. GSK-3 inhibition increases hippocampal levels of β-catenin, a function implicated in mood stabilization. In animal models, Li^+ induces molecular and behavioral effects comparable to those seen when one GSK-3β gene locus is inactivated (Alda, 2015). These Li^+-sensitive behaviors are related to the impact of Li^+ on the stability of the β-arrestin-2/PKB/PP2A signaling complex. Li^+ disrupts β-arrestin-2/PKB/PP2A complex formation and thus prevents PP2A from dephosphorylating (and thereby inactivating) the serine-threonine kinase Akt. While Akt remains active, it continues to phosphorylate the ser^9 residue on GSK-3β, directly inhibiting GSK-3β activity.

Another proposed common mechanism for the actions of Li^+ and VPA relates to reduction in arachidonic acid turnover in brain membrane phospholipids. Rats fed Li^+ in amounts that achieve therapeutic CNS drug levels have 83% reduced turnover of PI and 73% reduced turnover of phosphatidylcholine; chronic intraperitoneal VPA achieves reductions of 34% and 36%, respectively. Li^+ also decreases gene expression of phospholipase A_2 and levels of cyclooxygenase 2 (COX-2) and its products (Alda, 2015).

ADME

Li^+ is almost completely absorbed from the GI tract. Peak plasma concentrations occur 2 to 4 h after an oral dose, although peak CNS levels occur on average 3 hours later (Malhi and Tanious, 2011). As noted below, Li^+ is always dosed once daily at night for two reasons: once-daily dosing minimizes renal adverse effects, and nighttime dosing allows one to obtain 12-h trough levels in the morning (Castro et al., 2016). Slow-release preparations of Li^+ carbonate have lower maximal levels and a delayed peak, which may help mitigate GI adverse effects (Malhi and Tanious, 2011). Li^+ initially distributes to the extracellular fluid, does not bind appreciably to plasma proteins, and gradually accumulates in tissues, with a volume of distribution of 0.7 to 0.9 L/kg. The concentration gradient across plasma membranes is much smaller than those for Na^+ and K^+. Passage through the blood-brain barrier is slow, and when a steady state is achieved, the concentration of Li^+ in the cerebrospinal fluid and in brain tissue is about 40% to 50% of the concentration in plasma. The kinetics of Li^+ can be monitored in human brain with magnetic resonance spectroscopy.

Approximately 95% of a single dose of Li^+ is eliminated in the urine, with a $t_{1/2}$ of about 24 h (varies with age and can be ~12 h in the young and ~36 h in the elderly [secondary to reduced glomerular filtration rate (GFR)]). That the CNS $t_{1/2}$ is approximately 28 h also supports once-daily dosing. As noted above, once-nightly dosing not only improves adherence but decreases long-term risk for renal insufficiency by at least 20% compared to multiple daily dosing (Castro et al., 2016). With repeated administration, Li^+ levels and excretion increase until a steady state is achieved (after 4–5 half-lives). When Li^+ is stopped, there is a rapid phase of renal excretion followed by a slow 10- to 14-day phase. Although the pharmacokinetics of Li^+ vary considerably among subjects, the volume of distribution and clearance are relatively stable in an individual patient.

Less than 1% of ingested Li^+ leaves the human body in the feces; 4% to 5% is secreted in sweat. Li^+ is secreted in saliva in concentrations about twice those in plasma, while its concentration in tears is about equal to that in plasma. Li^+ is secreted in human milk, but serum levels in breastfed infants are only 20% that of maternal levels and are not associated with notable behavioral effects, although impact on thyroid function was noted in one case (Imaz et al., 2019).

Li^+ competes with Na^+ for proximal tubular reabsorption, and Li^+ retention can be increased by Na^+ loss related to diuretic use, diarrhea, and other GI illness. Heavy sweating leads to a preferential secretion of Li^+ over Na^+ in sweat and may lower Li^+ levels slightly; however, the repletion of excessive sweating using free water without electrolytes can cause hyponatremia and promote Li^+ retention.

Serum-Level Monitoring and Dose

Because of Li^+'s narrow therapeutic index, regular determination of serum concentrations is crucial. Concentrations considered to be effective and acceptably safe are between 0.6 and 1.5 mEq/L. The range of 1.0 to 1.5 mEq/L is favored *only* for treatment of acutely manic patients. Lower values (0.6–1.0 mEq/L) are safer for long-term prophylaxis, with long-term risk for renal insufficiency associated with any outpatient levels that exceed 1.2 mEq/L (Castro et al., 2016). Serum concentrations of Li^+ have been found to follow a dose-effect relationship between 0.4 and 1.0 mEq/L, but with a corresponding dose-dependent rise in polyuria and tremor as indices of adverse effects (Gitlin, 2016). Nonetheless, patients who maintain trough levels of 0.8 to 1.0 mEq/L experience decreased mania relapse risk compared to those maintained at lower serum concentrations. There are patients who may do well with serum levels of 0.5 to 0.8 mEq/L, but there are no current clinical or biological predictors to permit *a priori* identification of these individuals. Individualization of serum levels is often necessary to obtain a favorable risk-benefit relationship.

By convention, the serum Li^+ concentration is measured from samples obtained 10 to 12 h after the evening dose. Single daily doses at night generate relatively large oscillations of plasma Li^+ concentration but lower mean trough levels than with multiple daily dosing; moreover, single nightly dosing means that peak serum levels occur during sleep, so complaints of CNS adverse effects are minimized (Gitlin, 2016). While relatively uncommon, GI complaints are a compelling reason for using delayed-release Li^+ preparations, also given once daily at night.

Therapeutic Uses

Drug Treatment of Bipolar Disorder. Treatment with Li^+ ideally is initiated in patients with acceptable renal function, typically defined as estimated glomerular filtration rate (eGFR) greater than 70 mL/min. Occasionally, patients with severe systemic illnesses are treated with Li^+ provided that the indications are compelling, but the need for medications that pose potential kinetic problems often precludes Li^+ use in those with multiple medical problems. Treatment of acute mania and the prevention of recurrences of bipolar illness in adults or adolescents are uses approved by the FDA. Li^+ is the mood stabilizer with the most robust data on suicide reduction in bipolar patients (Baldessarini et al., 2019); Li^+ is also efficacious for augmentation in unipolar depressive patients who respond inadequately to antidepressant therapy and is also associated with a 5-fold reduction in suicidality in that population compared to nonlithium therapies.

Pharmacotherapy of Mania. The modern treatment of the manic, depressive, and mixed-mood phases of bipolar disorder was revolutionized by the introduction of Li^+ in 1949, initially for acute mania only and later for prevention of mania recurrence. While Li^+, VPA, and *carbamazepine* have efficacy in acute mania, in clinical practice, these are usually combined with atypical antipsychotic drugs, even in manic patients without psychotic features, due to their complementary modes of action. Li^+, *carbamazepine*, and VPA preparations are effective only with daily dosing that maintains adequate serum levels, and thus require monitoring of serum levels. Patients with mania are often irritable and poorly cooperative with medication administration and phlebotomy; thus, atypical antipsychotic drugs may be the sole initial therapy. While antipsychotics have proven efficacy as monotherapy for acute mania, population-based cohort studies indicate that relapse rates are high when used long term as monotherapy (Wingård et al., 2019). Acute intramuscular forms of *olanzapine* and *ziprasidone* can be used to achieve rapid control of psychosis and agitation. Benzodiazepines are often used adjunctively for agitation and sleep induction, but use outside of acute hospital settings is discouraged due to concerns about tolerance and dependence.

Li^+ is effective in acute mania and can be loaded in those with normal renal function using three individual 10-mg/kg doses of a sustained-release preparation administered at 2-h intervals in the evening (Kook et al., 1985). Since Li^+ has a 28-h $t_{1/2}$ in the CNS there is no plausible reason for split daily dosing or routine use of sustained-release preparations. The sustained-release form is only used to minimize GI adverse effects (e.g., nausea, diarrhea) due to the lower C_{max} and delayed peripheral t_{max}. Acutely manic patients may require higher dosages to achieve therapeutic serum levels, and downward adjustment may be necessary once the patient is euthymic. Efficacy following loading can be achieved within 5 days. When adherence with oral capsules or tablets is an issue, the liquid Li^+ citrate can be used. A 300-mg dose of Li^+ carbonate provides 56 mg of elemental Li^+, which is equivalent to 8 mEq. Li^+ citrate can be ordered in either milligram or milliequivalent doses, with most forms of the syrup containing 300 mg (or 8 mEq) per 5 mL.

The anticonvulsant VPA also provides antimanic effects, with therapeutic benefit seen within 3 to 5 days when loaded. The most common form of VPA is *divalproex* due to its lower incidence of GI and other adverse effects. *Divalproex* is initiated at 30 mg/kg given as single or divided doses in the first 24 h, and then titrated to effect based on the desired 12-h trough serum level. Trough serum concentrations of 90 to 120 μg/dL show the best response in clinical studies. With immediate-release forms of VPA and *divalproex*, 12-h troughs are used to guide treatment. With the extended-release *divalproex* preparation, the trough occurs 24 h after dosing. However, obtaining serum levels at night may be difficult in outpatient settings, so 12-h troughs are commonly used, bearing in mind that 12-h trough levels for sustained-release *divalproex* are 18% to 25% higher than the 24-h trough.

Divalproex lacks Li^+'s risk for renal and thyroid adverse effects, but there is concern about its use in women of reproductive age related to risks of polycystic ovary syndrome and also the known mutagenic potential, which demands routine use of contraception (Anmella et al., 2019). *Divalproex* is also associated with hyperammonemia due to effects on the carnitine shuttle and can induce thrombocytopenia and neutropenia. Given Li^+'s superior efficacy for depressive episodes and for reduction in suicidal behavior, Li^+ should be preferentially used for bipolar I prophylaxis unless there is a clear medical contraindication to initiation (e.g., baseline eGFR <60 mL/min). *Carbamazepine* is effective for acute mania, but *carbamazepine* cannot be loaded or rapidly titrated over 24 h due to the development of adverse effects such as dizziness or ataxia, even within the therapeutic range (6–12 μg/dL). An extended-release form of *carbamazepine* is effective as monotherapy with once-daily dosing.

Carbamazepine response rates are lower than those for VPA compounds or for Li^+, with mean rates of 45% to 60% (Post et al., 2007). Nevertheless, certain individuals respond to *carbamazepine* after failing Li^+ and VPA. Initial doses are 400 mg/day in two divided doses. Titration proceeds by 200-mg increments every 24 to 48 h based on clinical response and serum trough levels, not to exceed 1600 mg/day.

Serious and potentially fatal skin reactions (e.g., Stevens-Johnson syndrome and toxic epidermal necrolysis) may occur with administration of *carbamazepine* in patients positive for the HLA-B*1502 allele. The FDA recommends genetic screening in patients of Asian ancestry (among whom the prevalence of this allele is >15%) before initiation of *carbamazepine* therapy and recommends using alternative therapies in patients positive for the allele. See Chapter 20 for more information on *carbamazepine*.

Lamotrigine has no role in acute mania due to the slow, extended titration necessary to minimize risk of Stevens-Johnson syndrome. It is used for bipolar maintenance and for treatment of depressive phases, although the antidepressant effect is delayed considerably due to the slow titration to effective doses (200–400 mg/day).

Prophylactic Treatment of Bipolar Disorder. The medication choice for prophylaxis is determined by the need for continued antipsychotic drug use to mitigate manic or depressive relapse and for use of a mood-stabilizing agent. Both *aripiprazole* and *olanzapine* are effective as monotherapy for mania prophylaxis, but *olanzapine* use is eschewed out of concern for metabolic effects, and *aripiprazole* offers no benefit for prevention of depressive relapse. LAI *risperidone* is also approved for bipolar maintenance treatment as monotherapy or adjunctively with Li^+ or VPA. Despite these indications, population-based cohort data indicate that bipolar I patients on antipsychotic monotherapy after a manic episode fare poorly, with higher relapse rates than patients on Li^+ monotherapy (Wingård et al., 2019). Relapse rates are lowest for bipolar I patients maintained on combination therapy with a primary mood stabilizer (e.g., Li^+ or VPA) and an antipsychotic, although many patients do well on mood stabilizer monotherapy and thus avoid adverse effects. In patients exhibiting sustained remission following a manic episode, the optimal duration of antipsychotic treatment is unclear.

Overriding concerns during bipolar treatment are the high recurrence rate and the high risk of suicide. Individuals who experience mania have an 80% to 90% lifetime risk of subsequent manic episodes. As with schizophrenia, lack of insight, poor psychosocial support, and substance abuse all interfere with treatment adherence. While the anticonvulsants *lamotrigine*, *carbamazepine*, and *divalproex* have data supporting their use in bipolar prophylaxis, only Li^+ has consistently been shown to reduce the risk of suicide compared to other treatments. *Lamotrigine* is effective for bipolar patients whose most recent mood episode was manic or depressed, with greater effect on depressive relapse (Baldessarini et al., 2019). The ability to provide prophylaxis for future depressive episodes combined with data in acute bipolar depression have made *lamotrigine* a useful choice for bipolar treatment, given that patients with bipolar I and bipolar II disorders spend 32% and 50% of the time, respectively, in a depressive phase. While *lamotrigine* requires no serum level monitoring and lacks many of the adverse effects seen with Li^+ and VPA (e.g., weight gain, tremor), it causes class IB antiarrhythmic activity at therapeutically relevant concentrations. Based on this finding, the U.S. FDA issued a warning in March 2021 that *lamotrigine* could slow ventricular conduction and induce arrhythmias, including sudden death, in those with cardiac conduction disorders (second- or third-degree heart block), ventricular arrhythmias, myocardial ischemia, heart failure, structural heart disease, Brugada syndrome, or other Na^+ channelopathies. Concomitant use of other Na^+ channel blockers may increase the risk of arrhythmia.

Bipolar disorder is a lifetime illness with high recurrence rates. Individuals who experience an episode of mania should be educated about the probable need for ongoing treatment. Stopping mood stabilizer therapy can be considered in patients who have experienced only one lifetime manic episode, particularly when there may have been a pharmacological precipitant (e.g., substance or antidepressant use), and who have been euthymic for extended periods. For patients with bipolar II, the impact of hypomania is relatively limited, so the decision to recommend prolonged maintenance treatment with a mood stabilizer is based on clinical response and the risk:benefit ratio. Discontinuation of maintenance Li^+ treatment in patients with bipolar I carries a high risk of early recurrence and of suicidal behavior over a period of several months, even if the treatment had been successful for several years. Recurrence is much more rapid than is predicted by the natural history of untreated bipolar disorder, in which cycle lengths average about 1 year. This risk may be moderated by slow, gradual removal of Li^+; rapid discontinuation should be avoided unless dictated by medical emergencies.

Other Uses of Lithium. Li^+ is effective as adjunct therapy in treatment-resistant major depression. Meta-analyses indicated that Li^+'s benefit on suicide reduction extends to patients with unipolar mood disorder (Tondo et al., 2001). While maintenance Li^+ levels in the range of 0.6 to 1.0 mEq/L are used for bipolar prophylaxis, a lower range (0.4–0.6 mEq/L) is recommended for antidepressant augmentation.

When older patients maintain sufficient eGFR to continue Li^+ (e.g., ≥50 mL/min), it is well-tolerated without evidence of disproportionate safety concerns, especially when compared to *divalproex* (Fotso Soh et al., 2019). The most compelling reasons to continue Li^+ in older bipolar I individuals with acceptable eGFR are the neuroprotective properties. Long-term use of Li^+ is associated with a decreased dementia risk of 50% (Velosa et al., 2020), and this effect emerges with as little as 301 to 365 days of treatment. Based on animal and human data documenting neuroprotective properties, Li^+ treatment has been suggested for conditions associated with excitotoxic and apoptotic cell death, such as stroke and spinal cord injury, and in neurodegenerative disorders, including dementia of the Alzheimer type, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, progressive supranuclear palsy, and spinocerebellar ataxia type I (Morris and Berk, 2016).

Drug Interactions. Li^+ levels are increased with medical conditions and with use of medications that promote hyponatremia due to the decreased competition with Na^+ for proximal resorption. Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) increase Li^+ levels, and this effect can be managed by obtaining an Li^+ level after 1 week of combination therapy and downward adjustment of the Li^+ dose to prevent toxicity (Meyer et al., 2005). The unique exception is the ACEI *lisinopril* due to the fact that *lisinopril* is 100% renally cleared. *Lisinopril* accumulates in those with subnormal renal function, and the steadily increasing *lisinopril* levels lead to higher Li^+ levels, decreased renal function, and even higher *lisinopril* levels in a positive feedback loop (Meyer et al., 2005). *Lisinopril should never be used with* Li^+; any other ACEI can be used with dosing adjustment based on levels. Distal collecting tubule diuretics cause Na^+ wasting and thereby increase reabsorption of Li^+ proximally. Among the commonly used agents in this class, the thiazides present the greatest risk and must be avoided. *Amiloride* causes less Na^+ wasting and can be used to manage Li^+-related polyuria as discussed below. Loop diuretics such as *furosemide* decrease Li^+ clearance only approximately 20% in healthy younger individuals and are the diuretics of choice for most patients on Li^+. However, older patients, particularly those with some degree of renal dysfunction, may become sufficiently volume contracted and Na^+ depleted during *furosemide* therapy that virtually all filtered Li^+ is reabsorbed in the proximal tubule leading to risk of toxicity (Juurlink et al., 2004). Through alteration of renal perfusion, some nonsteroidal anti-inflammatory agents can facilitate renal proximal tubular resorption of Li^+ and thereby increase serum concentrations. This interaction appears to be particularly prominent with *indomethacin*, but also may occur with *ibuprofen*, *naproxen*, *meloxicam*, *ketorolac*, and COX-2 inhibitors, and less so with *sulindac* and *aspirin*.

Adverse Effects of Lithium

CNS Effects. The most common effect of Li^+ in the therapeutic dose range is fine postural hand tremor, indistinguishable from essential tremor. Severity and risk for tremor are dose and serum level dependent, with incidence ranging from 15% to 70%. In addition to the avoidance of caffeine and other agents that increase tremor amplitude, therapeutic options include dose reduction (bearing in mind the increased relapse risk with lower serum Li^+ levels) and the β-adrenergic antagonist *propranolol* (also effective for VPA-induced tremor). At peak serum (and CNS) levels of Li^+, patients may complain of incoordination, ataxia, or slurred speech, all of which can be avoided by dosing Li^+ at bedtime.

Seizures have been rarely reported in nonepileptic patients with therapeutic plasma concentrations of Li^+. Li^+ treatment has also been associated with increased risk of post-ECT confusion and is generally tapered off prior to a course of ECT. In some instances, addition of Li^+ to existing antipsychotics may increase the sensitivity to D_2 blockade resulting in EPSs.

Li^+ treatment results in weight gain, a problem magnified by concurrent use of antipsychotic drugs. There is one case report of *metformin* use to mitigate Li^+-related weight gain (Praharaj, 2016), and this should be considered especially in those on concurrent SGA therapy.

Renal Effects. The kidney's ability to concentrate urine decreases during Li^+ therapy, and about 60% of individuals exposed to Li^+ experience some form of polyuria and compensatory polydipsia. The mechanism of polyuria is related to the fact that Li^+ has 1.5- to 2.0-fold greater affinity than Na^+ for the epithelial Na^+ channel (ENaC) present on the apical (i.e., luminal) surfaces of distal tubular cells (Grünfeld and Rossier, 2009). Intracellular Li^+ is a poor substrate for the Na^+/K^+-ATPase present on the basal membrane, leading to accumulation of Li^+ in these distal tubular cells; high intracellular Li^+ concentrations inhibit GSK-3β, causing vasopressin insensitivity, downregulation of aquaporin-2 channels, and nephrogenic diabetes insipidus (NDI). Multiple daily dosing increases this risk at least 20%. Renal function should be monitored with semiannual serum blood urea nitrogen and creatinine levels for calculation of eGFR, and urine osmolality to monitor development of NDI (Morriss and Benjamin, 2008). Patient complaints of polyuria should be solicited throughout the course of Li^+ treatment so that the extent of the problem can be quantified using a spot urine osmolality. Li^+ discontinuation or a switch to single daily dosing may reverse the impact on renal concentrating ability in patients with less than 5 years of Li^+ exposure, but the preferred option is use of *amiloride,* which blocks entry of Li^+ into renal distal tubule ENaCs and has been used to safely manage NDI associated with Li^+ therapy (Bedford et al., 2008). This use of *amiloride* requires electrolyte monitoring to track Na^+ depletion that may necessitate Li^+ dosage adjustments (Bedford et al., 2008). Reassessment of Li^+ treatment should be considered when the eGFR is less than 60 mL/min on several periodic measurements, when daily urinary volume exceeds 4 L, or when serum creatinine continues to rise on three separate occasions; discontinuation is recommended when eGFR is less than 50 mL/min to prevent toxicity and further declines in renal function (Schoot et al., 2020). With modern monitoring principles, no patient should develop stage 3b chronic kidney disease (eGFR 30–44 mL/min), and patients should never have renal dysfunction to the extent of requiring renal dialysis.

Thyroid and Endocrine Effects. A small number of patients on Li^+ develop a benign, diffuse, nontender thyroid enlargement suggestive of compromised thyroid function; many of these patients will have normal thyroid function. Measurable effects include overt hypothyroidism (7%–10%) and subclinical thyroid disease (23%), with women at 3 to 9 times greater risk than men (Gitlin, 2016). Ongoing monitoring of thyrotropin (TSH) and free thyroxine is recommended throughout the course of Li^+ treatment. The development of hypothyroidism is easily treated through exogenous replacement and is not a reason to discontinue Li^+ therapy. Rare reports of hyperthyroidism during Li^+ treatment also exist. Hypercalcemia related to hyperparathyroidism has been reported in about 10% of Li^+-treated patients. Routine monitoring of serum Ca^{2+} should be included with measurements of electrolytes, thyroid indices, renal function, and serum Li^+ levels.

ECG Effects. Li^+ induces ECG changes related to dose-dependent inhibition of myocyte voltage-gated Na^+ channels and decreases in intracellular K^+ levels (Mehta and Vannozzi, 2017). T-wave depression is the most widely reported ECG change (incidence, 16%–33%). Those with more substantial T-wave changes may have U waves as seen with hypokalemia. T-wave changes depend on the duration of treatment rather than the Li^+ level and are usually reversible. Sinus node dysfunction and bradycardia are the second most common finding. Li^+ slows atrial conduction time, with complete right bundle branch block seen in 6.5% and incomplete intraventricular conduction delay in 6.5%. Li^+ may unmask rare asymptomatic Brugada syndrome patients (up to 0.5%). Li^+ can cause electrical instability in both the atria and ventricles; significant cardiac toxicity with symptomatic bradycardia, ST depression or elevation, and conduction problems is usually only seen with Li^+ levels greater than 1.5 mEq/L (Mehta and Vannozzi, 2017). Routine ECG monitoring may be considered in patients greater than 60 years old, those with a history of arrhythmia or coronary heart disease, and patients with a concerning family history of cardiac issues (e.g., sudden death, Brugada syndrome) (Mehta and Vannozzi, 2017).

Skin Effects. Allergic reactions such as dermatitis, folliculitis, and vasculitis can occur with Li^+ administration. Worsening of acne vulgaris, psoriasis, and other dermatological conditions is a common problem that is usually treatable by topical measures, but a small number of patients may improve only on discontinuation of Li^+ (Gitlin, 2016). Ten percent to 12% of patients on Li^+ (and VPA) may experience alopecia. Li^+ and VPA attenuate the dihydrotestosterone-induced downregulation of intracellular β-catenin levels by inhibiting GSK-3β. Daily multivitamins with at least 100 μg of selenium and 15 mg of zinc may help, but consider early use of topical 5% *minoxidil* as soon as the complaint surfaces.

Pregnancy and Lactation. The use of Li^+ in early pregnancy may be associated with Ebstein anomaly and related right ventricular outflow tract malformations; however, the risk of Ebstein anomaly (about 1 per 20,000 live births in controls) is now known to be only modestly increased with first-trimester Li^+ exposure (Patorno et al., 2017). In balancing the risk versus benefit of using Li^+ during the first trimester of pregnancy, it is important to evaluate the risk of inadequate prophylaxis for a bipolar I patient and subsequent risk that mania poses for the patient and fetus. If the history of decompensation upon stopping Li^+ or inadequate mood control on antipsychotic monotherapy presents a compelling need for Li^+, screening ultrasonography for CV anomalies is recommended. In patients who choose to forgo Li^+ exposure during the first trimester, potentially safer treatments for acute mania include antipsychotics or ECT.

In pregnancy, maternal polyuria may be exacerbated by Li^+. Concomitant use of Li^+ with medications that waste Na^+ or a low-Na^+ diet during pregnancy can contribute to maternal and neonatal Li^+ intoxication. Li^+ freely crosses the placenta, and fetal or neonatal Li^+ toxicity may develop when maternal blood levels are within the therapeutic range. Fetal Li^+ exposure is associated with neonatal goiter, CNS depression, hypotonia ("floppy baby" syndrome), and cardiac murmur. Most experts recommend withholding Li^+ therapy for 24 to 48 h before delivery if possible, and this is considered standard practice to avoid potentially toxic increases in maternal and fetal serum Li^+ levels associated with postpartum diuresis. The physical and CNS sequelae of late-term neonatal Li^+ exposure are reversible once Li^+ exposure has ceased and no long-term neurobehavioral consequences are observed (Diav-Citrin et al., 2014).

Other Effects. A benign, sustained increase in circulating polymorphonuclear leukocytes commonly occurs (at times up to 12,000–15,000 cells/mm^3), related to Li^+-induced increases in levels of granulocyte colony-stimulating factor and augmented production of granulocyte colony-stimulating factor by peripheral blood mononuclear cells (Focosi et al., 2009). Li^+ also directly stimulates the proliferation of pluripotent stem cells. Some patients may complain of a metallic taste, making food less palatable.

Acute Toxicity and Overdose. The occurrence of toxicity is related to the serum concentration of Li^+ and its rate of rise following administration. Acute intoxication is characterized by vomiting, profuse diarrhea, coarse tremor, ataxia, coma, and convulsions. Symptoms of milder toxicity are most likely to occur at the absorptive peak of Li^+ and include nausea, vomiting, abdominal pain, diarrhea, sedation, and fine tremor. The more serious effects involve the CNS and include mental confusion, hyperreflexia, gross tremor, dysarthria, seizures, and cranial nerve and focal neurological signs, progressing to coma and death. Sometimes both cognitive and motor neurological damage may be irreversible, with persistent cerebellar tremor the most common (Kores and Lader, 1997). Other toxic effects are cardiac arrhythmias, hypotension, and albuminuria.

Treatment of Lithium Intoxication. There is no specific antidote for Li^+ intoxication, and treatment is supportive, including intubation if indicated and continuous cardiac monitoring. Levels greater than 1.5 mEq/L are considered toxic, but inpatient medical admission is usually not indicated (in the absence of symptoms) until levels exceed 2 mEq/L. Care must be taken to ensure that the patient is not Na^+ and water depleted. There are several issues involved in the assessment, including whether the ingestion occurred in an Li^+-naïve patient or one on chronic therapy, and whether the intoxication represents an acute event or a chronic problem (e.g., high level for an extended period of time). In acute ingestion, levels should be checked every 2 to 4 h until they peak, especially as the peak may be delayed with controlled-release Li^+. Importantly, serum levels do not always correlate well with clinical toxicity since the danger lies in the Li^+ level in the CNS, which reaches a maximal level more slowly than plasma. Dialysis is the most effective means of removing Li^+ from its intracellular and CNS locations and is indicated in the following situation: Li^+ levels greater than or equal to 5.2 mEq/L and/or serum creatinine greater than or equal to 2.26 mg/dL (Vodovar et al., 2020). Complete recovery occurs with an average maximal level of 2.5 mEq/L; permanent neurological symptoms result from mean levels of 3.2 mEq/L; death occurs with mean maximal levels of 4.2 mEq/L (Kores and Lader, 1997).

Use in Pediatric Populations. Li^+ is FDA-approved for child/adolescent bipolar disorder for ages 12 years or older (Duffy and Grof, 2018). *Aripiprazole*, *quetiapine*, and *risperidone* are FDA-approved for acute mania in children and adolescents aged 10 to 17 years. Children and adolescents have higher volumes of body water and higher eGFR than adults. The resulting shorter $t_{1/2}$ of Li^+ demands dosing increases on a milligram/kilogram basis, and multiple daily dosing may be required. In children ages 6 to 12 years, a dose of 30 mg/kg per day given in three divided doses will produce an Li^+ concentration of 0.6 to 1.2 mEq/L in 5 days, although dosing is always guided by serum levels and clinical response (Duffy and Grof, 2018). Use in children under 12 represents an off-label use for Li^+, and caregivers should be alert to signs of toxicity. As with adults, ongoing monitoring of renal and thyroid function is important, along with clinical inquiry into extent of polyuria.

A limited number of controlled studies suggested that VPA has efficacy comparable to that of Li^+ for mania in children or adolescents. As with Li^+, weight gain and tremor can be problematic; moreover, there are reports of hyperammonemia in children with urea cycle disorders. Ongoing monitoring of platelets and liver function tests, in addition to serum drug levels, is recommended.

Use in Geriatric Populations. The majority of older patients on Li^+ therapy are those maintained for years on the medication, but any person with adequate renal function (eGFR >70 mL/min) is a Li^+ candidate, *especially given its neuroprotective properties*. Elderly patients frequently take medications for other illnesses, and the potential for drug-drug interactions is substantial. An increased risk of Li^+ toxicity is seen within a month of initiating treatment with a loop diuretic or an ACEI, but not with thiazide diuretics or nonsteroidal anti-inflammatory drugs (Juurlink et al., 2004).

Age-related reductions in total body water and creatinine clearance reduce the safety margin for Li^+ treatment in older patients. Targeting lower maintenance serum levels (0.6–0.8 mEq/L) may reduce the risk of toxicity. As eGFR drops below 50 mL/min, strong consideration must be given to use of alternative agents, despite Li^+'s therapeutic advantages (Morriss and Benjamin, 2008).

Elderly patients who are drug naïve may be more sensitive to the CNS adverse effects of all types of medications used for acute mania, especially parkinsonism and TD from D_2 antagonism, confusion from antipsychotic medications with antimuscarinic properties, and ataxia or sedation from Li^+ or anticonvulsants.

Drug Facts for Your Personal Formulary: *Antipsychotic and Mood-Stabilizing Agents*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
First-Generation Antipsychotics • Low-potency D_2 antagonists		
Chlorpromazine	• Schizophrenia • Acute mania	• High M_1, H_1, and α_1 adrenergic affinities increase rates of anticholinergic side effects, sedation and weight gain, and hypotension, respectively; high risk of adverse metabolic events • Less QTc risk than thioridazine; photosensitivity
First-Generation Antipsychotics • Medium- and high-potency D_2 antagonists		
Haloperidol	• Schizophrenia • Acute mania	• Higher rates of EPSs, akathisia, hyperprolactinemia • Limited anticholinergic side effects, sedation, weight gain, and hypotension • Avoid IV use (QTc prolongation); chlorpromazine 75 mg po equivalence: 2 mg
Fluphenazine	• Schizophrenia • Acute mania	• Higher rates of EPSs, akathisia, hyperprolactinemia • Limited anticholinergic side effects, sedation, weight gain, and hypotension • Chlorpromazine 75 mg oral equivalence: 2 mg
Trifluoperazine	• Schizophrenia • Acute mania	• Higher rates of EPSs, akathisia, hyperprolactinemia • Limited anticholinergic side effects, sedation, weight gain, and hypotension • Chlorpromazine 75 mg oral equivalence: 5 mg
Thiothixene	• Schizophrenia • Acute mania	• Higher rates of EPSs, akathisia, hyperprolactinemia • Limited anticholinergic side effects, sedation, weight gain, and hypotension • Chlorpromazine 75 mg oral equivalence: 7.5 mg
Perphenazine	• Schizophrenia • Acute mania	• Modest rates of EPSs, akathisia • Limited anticholinergic side effects, sedation, weight gain, and hypotension • Chlorpromazine 75 mg oral equivalence: 7.5 mg
Loxapine	• Schizophrenia • Acute mania	• Modest rates of EPS, akathisia; chlorpromazine 75 mg oral equivalence: 25 mg • Limited anticholinergic side effects, sedation, weight gain, and hypotension

Drug Facts for Your Personal Formulary: *Antipsychotic and Mood-Stabilizing Agents (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Second-Generation Antipsychotics • 5HT$_{2a}$ and D$_2$ antagonists		
Asenapine	• Schizophrenia • Acute mania	• Available in ODT formulation due to 98% first-pass effect if swallowed. The ODT tablet is administered sublingually: avoid water for 10 min to achieve maximum oral-buccal absorption (avoiding water for 2 min achieves 80% of maximum absorption) • Available in transdermal formulation: 3.8 mg/24 h = 10 mg/day ODT; 5.7 mg/24 h = 15 mg/day ODT; 7.6 mg/24 h = 20 mg/day ODT • Low risk of metabolic adverse effects • Limited drug-drug kinetic interactions
Clozapine	• Resistant schizophrenia • Schizophrenia with suicidality, polydipsia, or persistent impulsive aggression • Resistant mania	• Must register patient and prescriber due to mandatory hematological monitoring • High M$_1$, H$_1$, and α$_1$ adrenergic affinity increases rates of anticholinergic side effects, sedation and weight gain, and hypotension, respectively • High risk of metabolic adverse effects • Significant constipation; avoid other anticholinergic agents, iron, and psyllium. Manage aggressively with docusate, polyethylene glycol 3350, bisacodyl, and if needed, linaclotide. • Sialorrhea; manage with locally administered agents (sublingual atropine 1% drops or ipratropium 0.06% spray)
Iloperidone	• Schizophrenia	• High α$_1$ adrenergic affinity; titrate to minimize orthostasis • Low risk of metabolic adverse effects
Lumateperone	• Schizophrenia • Bipolar depression (monotherapy and adjunct)	• Very low risk for anticholinergic side effects, sedation and weight gain, and hypotension, respectively • Very low risk of EPSs and metabolic and endocrine adverse effects
Lurasidone	• Schizophrenia • Bipolar depression (monotherapy and adjunct)	• Low risk for anticholinergic side effects, sedation and weight gain, and hypotension, respectively • Low risk of metabolic adverse effects • Absorption increased 100% by administration with 350 kcal food
Olanzapine	• Schizophrenia • Acute mania • Bipolar depression (in combination with fluoxetine)	• High risk of metabolic adverse effects • Anticholinergic effects at high dosages
Paliperidone	• Schizophrenia	• Moderate risk of metabolic adverse effects • High rates of hyperprolactinemia
Quetiapine	• Schizophrenia • Acute mania • Bipolar depression (monotherapy) • Unipolar depression (adjunct)	• High risk of metabolic adverse effects at full therapeutic dosages for schizophrenia • High H$_1$ and α$_1$ adrenergic affinities increase rates of sedation and hypotension, respectively • Low rates of EPSs, akathisia, and hyperprolactinemia
Risperidone	• Schizophrenia • Acute mania	• Moderate risk of metabolic adverse effects • High rates of hyperprolactinemia
Sertindole	• Schizophrenia	• Not available in the U.S. • Restricted use in Europe, with extensive monitoring for QTc prolongation • Low risk of metabolic adverse effects
Ziprasidone	• Schizophrenia • Acute mania	• Low risk of metabolic adverse effects • Absorption increased 100% by administration with 500 kcal food • Improved tolerability at starting doses >80 mg/day with food
Second-Generation Antipsychotics • D$_2$ partial agonists		
Aripiprazole	• Schizophrenia • Acute mania • Unipolar depression (adjunct)	• Low risk of metabolic adverse effects • Lowers serum prolactin • Akathisia noted in depression trials—can be lessened with starting dose of 2.0–2.5 mg at bedtime
Brexpiprazole	• Schizophrenia • Unipolar depression (adjunct)	• Low risk of metabolic adverse effects • Lowers serum prolactin
Cariprazine	• Schizophrenia • Acute mania/bipolar mixed • Bipolar depression (monotherapy)	• Low risk of metabolic adverse effects • Lowers serum prolactin • Akathisia
Second-Generation Antipsychotics • D$_2$ and D$_3$ antagonists		
Amisulpride	• Schizophrenia • Unipolar depression (adjunct, at low dosages)	• Higher rates of EPSs • Higher rates of hyperprolactinemia • Low risk of metabolic adverse effects

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Drug Facts for Your Personal Formulary: *Antipsychotic and Mood-Stabilizing Agents* (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
5HT$_{2a}$ Inverse Agonist Without D$_2$ Binding		
Pimavanserin	• Parkinson's disease psychosis (PDP)	• Potent 5HT$_{2a}$ inverse agonist with no D$_2$ affinity • Monotherapy efficacy data for psychosis available only for PDP • Only one dose available: 34 mg once daily, with or without food • Decrease dose by 50% with concurrent strong 3A4 inhibitors; may lose efficacy with strong 3A4 inducers • Clinical effects may not be seen for 2–6 weeks
Mood Stabilizers • Acute mania and/or bipolar maintenance		
Lithium	• Acute mania • Bipolar maintenance • Unipolar depression (adjunct)	• Reduces suicidality more than other treatments • Evidence for neuroprotective properties in older bipolar patients • Renally cleared • Risk for weight gain • Monitor TSH, renal function tests, serum calcium, lithium levels • May cause tremor, hair loss • Therapeutic serum level: acute mania 1.0–1.5 mEq/L • Therapeutic serum level: maintenance 0.6–1.0 mEq/L
VPA (divalproex)	• Acute mania • Bipolar maintenance	• Can be loaded in acute mania: 30 mg/kg over 24 h • Highly protein bound • Risk for weight gain • Risk for polycystic ovary syndrome—avoid in women of childbearing age if possible • High risk for neural tube defects—women of childbearing age must be using contraception • May cause thrombocytopenia, neutropenia, hyperammonemia, tremor, hair loss • Monitor complete blood count, liver function tests, levels • Therapeutic serum level: acute mania 100–120 μg/dL • Therapeutic serum level: maintenance 60–100 μg/dL
Carbamazepine	• Acute mania • Bipolar maintenance	• Less effective than lithium and valproic acid • Highly protein bound • Human leukocyte antigen testing for those from East Asia to identify high risk of Stevens-Johnson syndrome • May cause hyponatremia, leukopenia • Strong inducer of CYP3A4 and Pgp • Cannot be loaded. Avoid rapid titration to minimize risk of sedation, ataxia • Therapeutic serum level 6–12 μg/dL
Lamotrigine	• Bipolar maintenance	• Prolonged titration to minimize risk of Stevens-Johnson syndrome • 50% dosage reduction required if patient on valproic acid or divalproex • Warning by U.S. FDA in March 2021 regarding arrhythmia risk in those with preexisting conditions. Should be avoided in those with cardiac conduction disorders (e.g., second- or third-degree heart block), ventricular arrhythmias, myocardial ischemia, heart failure, structural heart disease, Brugada syndrome, or other sodium channelopathies.

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Chapter 20

Pharmacotherapy of the Epilepsies

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TERMINOLOGY AND EPILEPTIC SEIZURE CLASSIFICATION

NATURE AND MECHANISMS OF SEIZURES AND ANTISEIZURE DRUGS
- Focal Epilepsies
- Generalized-Onset Epilepsies: Absence Seizures
- Genetic Approaches to the Epilepsies

ANTISEIZURE DRUGS: GENERAL CONSIDERATIONS
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- Therapeutic Aspects

HYDANTOINS
- Phenytoin

BENZODIAZEPINES
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- Mechanism of Action
- Pharmacokinetic Properties
- Toxicity
- Plasma Drug Concentrations
- Therapeutic Uses

ANTISEIZURE BARBITURATES
- Phenobarbital
- Primidone

IMINOSTILBENES
- Carbamazepine
- Oxcarbazepine
- Eslicarbazepine Acetate

SUCCINIMIDES
- Ethosuximide

OTHER ASDs
- Acetazolamide
- Cannabidiol
- Cenobamate
- Ezogabine
- Felbamate
- Gabapentin and Pregabalin
- Lacosamide
- Lamotrigine
- Levetiracetam and Brivaracetam
- Perampanel
- Rufinamide
- Stiripentol
- Tiagabine
- Topiramate
- Valproic Acid
- Vigabatrin
- Zonisamide

GENERAL PRINCIPLES AND CHOICE OF DRUGS FOR THE THERAPY OF THE EPILEPSIES
- Duration of Therapy
- Focal and Focal to Bilateral Tonic-Clonic Seizures
- Generalized Absence Seizures
- Myoclonic Seizures
- Febrile Convulsions
- Seizures in Infants and Young Children
- Status Epilepticus and Other Convulsive Emergencies
- Antiseizure Therapy and Pregnancy
- Development of Novel Treatments for Epilepsy

The epilepsies are common and frequently devastating disorders, affecting approximately 2.5 million people in the U.S. alone. More than 40 distinct forms of epilepsy have been identified. Epileptic seizures often cause transient impairment of awareness, leaving the individual at risk of bodily harm and often interfering with education and employment. Current therapy is symptomatic, as available antiseizure drugs (ASDs) inhibit seizures, but neither effective prophylaxis nor cure is available. Adherence to prescribed treatment regimens is a major problem because of the need for long-term therapy together with unwanted effects of many drugs.

The mechanisms of action of ASDs fall into the following major categories (Porter et al., 2012):

1. Modulation of sodium, potassium, or calcium ion channels. This can include prolongation of the inactivated state of voltage-gated Na^+ channels, positive modulation of K^+ channels, and inhibition of Ca^{2+} channels.
2. Enhancement of γ-aminobutyric acid (GABA) neurotransmission through actions on $GABA_A$ receptors, modulation of GABA metabolism, or inhibition of GABA reuptake into the synaptic terminal.
3. Modulation of synaptic release through actions on the synaptic vesicle protein SV2A or Ca^{2+} channels containing the $\alpha 2\delta$ subunit.
4. Diminishing synaptic excitation mediated by ionotropic glutamate receptors (e.g., α-amino-3-hydroxy 5-methyl-4-isoxazolepropionic acid [AMPA] receptors).

Despite these broad classifications, it is noteworthy that many ASDs act through mechanisms distinct from the primary known mode of action. Furthermore, ASDs with similar mechanistic categories may have disparate clinical uses.

Although many treatments are available, much effort is being devoted to elucidating the genetic causes and the cellular and molecular mechanisms by which a normal brain becomes epileptic, insights that promise to provide molecular targets for both symptomatic and preventive therapies.

Terminology and Epileptic Seizure Classification

The term *seizure* (from the Latin *sacire* meaning "to take possession of") refers to a transient alteration of behavior due to the disordered, synchronous, and rhythmic firing of populations of brain neurons. The term *epilepsy* refers to a disorder of brain function characterized by the risk of periodic and unpredictable occurrence of seizures. Seizures can be

Abbreviations

AMPA: α-amino-3-hydroxy 5-methyl-4-isoxazolepropionic acid
ASD: antiseizure drugs
CBD: cannabidiol
CSF: cerebrospinal fluid
CYP: cytochrome P450
EEG: electroencephalogram
ENT-1B: equilibrative nucleoside transporter 1
GABA: γ-aminobutyric acid
GI: gastrointestinal
MRI: magnetic resonance imaging
NMDA: *N*-methyl-D-aspartate receptor
PEMA: phenylethylmalonamide
SV2A: synaptic vesicle protein 2A
THC: tetrahydrocannabinol
UGT: uridine diphosphate-glucuronosyltransferase

"nonepileptic," when evoked in a normal brain by treatments such as electroshock or chemical convulsants, or "epileptic," when occurring without evident provocation. While agents in current clinical use inhibit seizures, whether any of these prevent the development of epilepsy (epileptogenesis) is uncertain.

Seizures are generally thought to arise from the cerebral cortex and not from other central nervous system (CNS) structures such as the thalamus, brainstem, or cerebellum. Recently, the classification for epileptic seizures has been revised, and this new nomenclature will be used in this chapter. Thus, those epileptic seizures previously classified as *partial* seizures will be referred to as *focal* seizures, whereas *generalized* seizures, those that involve both hemispheres widely from the outset, will still be referred to as generalized seizures (Commission on Classification and Terminology, 2016). In addition, the International League Against Epilepsy has added a classification for seizures with *unknown onset*, which include such seizure types as tonic-clonic, atonic, and epileptic spasms. The behavioral manifestations of a seizure are determined by the functions normally served by the cortical site at which the seizure arises. For example, a seizure involving motor cortex is associated with clonic jerking of the body in the area controlled by this region of cortex. This type of *focal* seizure is associated with preservation of awareness. *Focal* seizures may also be associated with impairments of awareness. The majority of such focal seizures originate from the temporal lobe. Generalized seizures are now distinguished by the involvement of the motor system or those that lack motor involvement (e.g., typical and atypical absence and eyelid myoclonic). The type of epileptic seizure is one determinant of the drug selected for therapy. Detailed information pertaining to seizure classifications is presented in Figure 20–1 (modified from Ayala et al., 1973). More detailed information is presented in Table 20–1.

Apart from this epileptic seizure classification, an additional classification specifies epileptic syndromes, which refer to a cluster of symptoms frequently occurring together and include seizure types, etiology, age of onset, and other factors (Commission on Classification and Terminology, 1989). More than 50 distinct epileptic syndromes have been identified and categorized into focal versus generalized epilepsies. The focal epilepsies may consist of any of the focal seizure types (see Table 20–1) and

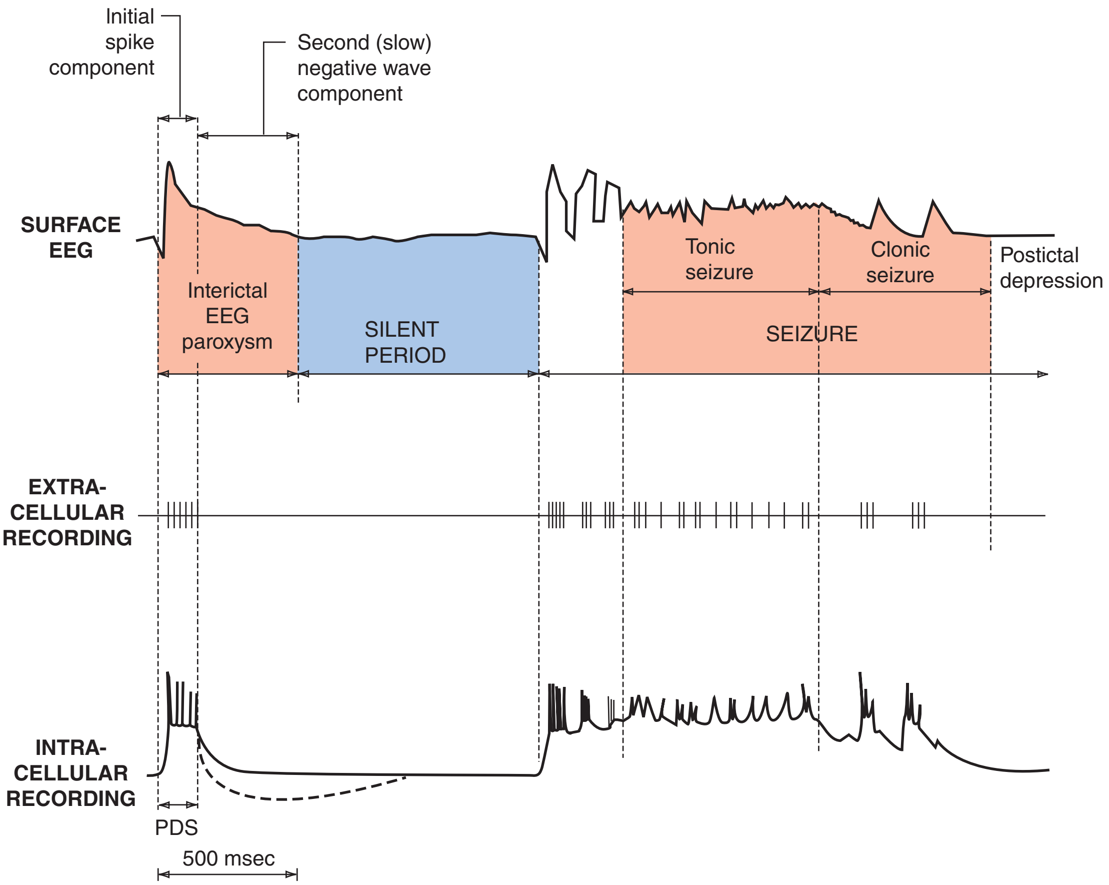


Figure 20–1 *Relationship of cortical EEG, extracellular, and intracellular recordings in a seizure focus induced by local application of a convulsant agent to mammalian cortex.* The extracellular recording was made through a high-pass filter. Note the high-frequency firing of the neuron evident in both the extracellular and intracellular recording during the paroxysmal depolarization shift (PDS). (Modified with permission from Ayala GF, et al. Genesis of epileptic interictal spikes. New knowledge of cortical feedback systems suggests a neurophysiological explanation of brief paroxysms. *Brain Res*, **1973**, *52*:1–17. Copyright © Elsevier.)

TABLE 20–1 ■ CLASSIFICATION OF EPILEPTIC SEIZURES

SEIZURE TYPE	FEATURES	CONVENTIONAL ANTISEIZURE DRUGS	RECENTLY DEVELOPED ANTISEIZURE DRUGS
Focal Seizures			
Focal Aware	Diverse manifestations determined by the region of cortex activated by the seizure (e.g., if motor cortex representing left thumb, clonic jerking of left thumb results; if somatosensory cortex representing left thumb, paresthesia of left thumb results), lasting approximately 20–60 sec. *Key feature is preservation of awareness.*	Carbamazepine, phenytoin, valproate	Brivaracetam, cenobamate, eslicarbazepine, ezogabine, gabapentin, lacosamide, lamotrigine, levetiracetam, perampanel, rufinamide, tiagabine, topiramate, zonisamide
Focal With Impaired Awareness	Impaired awareness lasting 30 sec to 2 min, often associated with purposeless movements such as lip smacking or hand wringing.		
Focal to Bilateral Tonic-Clonic	Simple or complex focal seizure evolves into a tonic-clonic seizure with loss of awareness and sustained contractions (tonic) of muscles throughout the body followed by periods of muscle contraction alternating with periods of relaxation (clonic), typically lasting 1–2 min.	Carbamazepine, phenobarbital, phenytoin, primidone, valproate	
Generalized Seizures			
Generalized Absence	Abrupt onset of impaired awareness associated with staring and cessation of ongoing activities typically lasting less than 30 sec.	Ethosuximide, valproate, clonazepam	Lamotrigine
Generalized Myoclonic	A brief (perhaps a second), shocklike contraction of muscles that may be restricted to part of one extremity or may be generalized.	Valproate, clonazepam	Levetiracetam
Generalized Tonic-Clonic	As described earlier in table for partial with secondarily generalized tonic-clonic seizures except that it is not preceded by a partial seizure.	Carbamazepine, phenobarbital, phenytoin, primidone, valproate	Lamotrigine, levetiracetam, topiramate
Syndrome-Specific Uses	Seizures arising from - Dravet syndrome - Lennox-Gastaut syndrome - Tuberous sclerosis complex		Cannabidiol, fenfluramine (Dravet syndrome)

account for roughly 60% of all epilepsies. The etiology commonly consists of a lesion in some part of the cortex, such as a tumor, developmental malformation, or damage due to trauma or stroke. Such lesions often are evident on brain magnetic resonance imaging (MRI). Alternatively, the etiology may be genetic. The generalized epilepsies are characterized most commonly by one or more of the generalized seizure types listed in Table 20–1 and account for approximately 40% of all epilepsies. The etiology is usually genetic. The most common generalized epilepsy is referred to as juvenile myoclonic epilepsy, accounting for approximately 10% of all epileptic syndromes. The age of onset is in the early teens, and the condition is characterized by myoclonic, tonic-clonic, and often absence seizures. Like most of the generalized-onset epilepsies, juvenile myoclonic epilepsy is a complex genetic disorder that is probably due to inheritance of multiple susceptibility genes; there is a familial clustering of cases, but the pattern of inheritance is not Mendelian. The classification of epileptic syndromes guides clinical assessment and management and, in some instances, selection of ASDs.

Nature and Mechanisms of Seizures and Antiseizure Drugs

Focal Epilepsies

More than a century ago, John Hughlings Jackson, the father of modern concepts of epilepsy, proposed that seizures were caused by "occasional, sudden, excessive, rapid and local discharges of gray matter," and that a generalized convulsion resulted when normal brain tissue was invaded by the seizure activity initiated in the abnormal focus. This insightful proposal provided a valuable framework for thinking about mechanisms of focal epilepsy. The advent of the electroencephalogram (EEG) in the 1930s permitted the recording of electrical activity from the scalp of humans with epilepsy and demonstrated that the epilepsies are disorders of neuronal excitability.

The pivotal role of synapses in mediating communication among neurons in the mammalian brain suggested that defective synaptic function might lead to a seizure. That is, a reduction of inhibitory synaptic activity or enhancement of excitatory synaptic activity might be expected to trigger a seizure; pharmacological studies of seizures support this notion. The neurotransmitters mediating the bulk of synaptic transmission in the mammalian brain are amino acids, with GABA and glutamate being the principal inhibitory and excitatory neurotransmitters, respectively. Pharmacological studies disclosed that *antagonists* of the $GABA_A$ receptor or *agonists* of different glutamate receptor subtypes (*N*-methyl-D-aspartate receptor [NMDA], AMPA, or kainic acid) (see Chapter 16) trigger seizures in experimental animals *in vivo*. Conversely, pharmacological agents that enhance GABA-mediated synaptic inhibition suppress seizures in diverse models. Glutamate receptor antagonists also inhibit seizures in diverse models, including seizures evoked by electroshock and chemical convulsants, such as *pentylenetetrazol*.

These findings suggest pharmacological regulation of synaptic function can regulate the propensity for seizures and provide a framework for electrophysiological analyses aimed at elucidating the role of both synaptic and nonsynaptic mechanisms in seizures and epilepsy. Progress in techniques has fostered the progressive refinement of the analysis of seizure mechanisms from the EEG to populations of neurons (field potentials) to individual neurons to individual synapses and individual ion channels on individual neurons. Beginning in the mid-1960s, cellular electrophysiological studies of epilepsy focused on elucidating the mechanisms underlying the depolarization shift, the intracellular correlate of the "interictal spike" (see Figure 20–1). The interictal (or between-seizures) spike is a sharp waveform recorded in the EEG of patients with epilepsy; it is asymptomatic, as it is not accompanied by overt change in the patient's behavior. However, the location of the interictal spike helps localize the brain region from which seizure activity originates in a given patient. The depolarization shift consists of a large depolarization of the neuronal membrane associated with a burst of action potentials. In most cortical neurons, the depolarization shift is generated by a large excitatory synaptic current that can be enhanced by activation of voltage-gated intrinsic membrane currents. Although the mechanisms generating the depolarization shift and whether the interictal spike triggers a seizure, inhibits a seizure, or is an epiphenomenon remain unclear, the study of the mechanisms underlying depolarization shift generation set the stage for inquiry into the cellular mechanisms of a seizure.

During the 1980s, various *in vitro* models of seizures were developed in isolated brain slice preparations in which many synaptic connections are preserved. Electrographic events with features similar to those recorded during seizures *in vivo* have been produced in hippocampal slices by multiple methods, including altering ionic constituents of media bathing the brain slices (McNamara, 1994) such as low Ca^{2+}, zero Mg^{2+}, or elevated K^+. The accessibility and experimental control provided by these *in vitro* preparations have permitted mechanistic investigations into the induction of seizures. Analyses of multiple *in vitro* models confirmed the importance of synaptic function for initiating a seizure, demonstrating that subtle reductions (e.g., 20%) of inhibitory synaptic function could lead to epileptiform activity and that activation of excitatory synapses could be pivotal in seizure initiation. Other important factors were identified, including the volume of the extracellular space as well as intrinsic properties of a neuron, such as voltage-gated ion channels (e.g., K^+, Na^+, and Ca^{2+} channels) (Traynelis and Dingledine, 1988). Identification of these diverse synaptic and nonsynaptic factors controlling seizures *in vitro* provides potential pharmacological targets for regulating seizure susceptibility *in vivo*.

Additional studies have centered on understanding the mechanisms by which a normal brain is transformed into an epileptic brain. Some common forms of focal epilepsy arise months to years after cortical injury sustained as a consequence of stroke, trauma, infection, or other factors. Effective prophylaxis administered to patients at high risk would be highly desirable in the clinical setting. However, no effective antiepileptogenic agent has yet been identified. The drugs described in this chapter provide symptomatic therapy; that is, the drugs inhibit seizures in patients with epilepsy.

Understanding the mechanisms of epileptogenesis in cellular and molecular terms should provide a framework for development of novel therapeutic approaches. The availability of animal models provides an opportunity to investigate the underlying mechanisms and have also enabled the discovery of numerous ASDs that have proven safe and efficacious in humans.

One model, termed *kindling*, is induced by periodic administration of brief, low-intensity electrical stimulation of the amygdala or other limbic structures that evokes a brief electrical seizure recorded on the EEG without behavioral change. Repeated (e.g., 10–20) stimulations result in progressive intensification of seizures, culminating in tonic-clonic seizures that, once established, persist for the life of the animal. Additional models are produced by induction of continuous seizures that last for hours (*status epilepticus*). The inciting agent used in these models is typically either a chemoconvulsant, such as *kainic acid* or *pilocarpine*, or sustained electrical stimulation. The fleeting episode of status epilepticus is followed weeks later by the onset of spontaneous seizures, an intriguing parallel to the scenario of complicated febrile seizures in young children preceding the emergence of spontaneous seizures years later. In contrast to the limited or absent neuronal loss characteristic of the kindling model, overt destruction of hippocampal neurons occurs in the *status epilepticus* models, reflecting aspects of hippocampal sclerosis observed in humans with severe limbic seizures. Indeed, the discovery that complicated febrile seizures precede and presumably are the cause of hippocampal sclerosis in young children (VanLandingham et al., 1998) establishes yet another commonality between these preclinical models and the human condition.

Several questions arise with respect to these models. What transpires during the latent period between status epilepticus and emergence of spontaneous seizures that causes the epilepsy? Might an antiepileptogenic agent that was effective in one of these models demonstrate disease-modifying effects in other models?

Important insights into the mechanisms of action of drugs that are effective against focal seizures have emerged in the past two decades (Rogawski and Löscher, 2004). These insights largely have come from electrophysiological studies of relatively simple *in vitro* models, such as neurons isolated from the mammalian CNS and maintained in primary culture. The experimental control and accessibility provided by these models—together with careful attention to clinically relevant concentrations of the drugs—led to clarification of their mechanisms. Although it is difficult to prove unequivocally that a given drug effect observed *in vitro* is both necessary and sufficient to inhibit a seizure in an animal or humans *in vivo*, there is an excellent likelihood that the putative mechanisms identified do in fact underlie the clinically relevant antiseizure effects. Table 20–2 summarizes putative mechanisms of action of ASDs.

Electrophysiological analyses of individual neurons during a focal seizure demonstrate that the neurons undergo depolarization and fire action potentials at high frequencies (see Figure 20–1). This pattern of neuronal firing is characteristic of a seizure and is uncommon during physiological neuronal activity. Thus, selective inhibition of this pattern of firing would be expected to reduce seizures with minimal adverse effects. *Carbamazepine*, *lamotrigine*, *phenytoin*, *lacosamide*, and *valproic acid* inhibit high-frequency firing at concentrations known to be effective at limiting seizures in humans (Rogawski and Löscher, 2004). Inhibition of the high-frequency firing is thought to be mediated by reducing the ability of Na^+ channels to recover from inactivation (Figure 20–2). That is, depolarization-triggered opening of the Na^+ channels in the axonal membrane of a neuron is required for an action potential; after opening, the channels spontaneously close, a process termed *inactivation*. This inactivation is thought to cause the refractory period, a short time after an action potential during which it is not possible to evoke another action potential. Upon recovery from inactivation, the Na^+ channels are again poised to participate in another action potential. Because firing at a slow rate permits sufficient time for Na^+ channels to recover from inactivation, inactivation has little or no effect on low-frequency firing. However, reducing the rate of recovery of Na^+ channels from inactivation would limit the ability of a neuron to fire at high frequencies, an effect that likely underlies the effects of *carbamazepine*, *lamotrigine*, *lacosamide*, *phenytoin*, *topiramate*, *valproic acid*, and *zonisamide* against focal seizures.

Insights into mechanisms of seizures suggest that enhancing GABA-mediated synaptic inhibition would reduce neuronal excitability and raise the seizure threshold. Several drugs are thought to inhibit seizures by regulating GABA-mediated synaptic inhibition through an action at distinct sites of the synapse (Rogawski and Löscher, 2004). The principal postsynaptic receptor of synaptically released GABA is termed the $GABA_A$ receptor (see Chapter 16). Activation of the $GABA_A$ receptor inhibits the postsynaptic cell by increasing the inflow of Cl^- ions into the cell, which tends to hyperpolarize the neuron. Clinically relevant concentrations of both benzodiazepines and barbiturates enhance $GABA_A$ receptor–mediated inhibition through distinct actions on the $GABA_A$ receptor (Figure 20–3), and this enhanced inhibition probably underlies the effectiveness of these compounds against focal and tonic-clonic seizures in humans. At higher concentrations, such as might be used for status epilepticus, these drugs also can inhibit high-frequency firing of action potentials. A second mechanism of enhancing GABA-mediated

TABLE 20–2 ■ PROPOSED MECHANISMS OF ACTION OF ANTISEIZURE DRUGS

MOLECULAR TARGET AND ACTIVITY	DRUG	CONSEQUENCES OF ACTION
Na$^+$ channel modulators that:		
Enhance fast inactivation	PHT, CBZ, LTG, FBM, OxCBZ, TPM, VPA, ESL	• Block action potential propagation • Stabilize neuronal membranes • ↓ Neurotransmitter release, focal firing, and seizure spread
Enhance slow inactivation	LCM	• ↑ Spike frequency adaptation • ↓ AP bursts, focal firing, and seizure spread • Stabilize neuronal membrane
Ca^{2+} channel blockers	ESM, VPA, LTG	• ↓ Neurotransmitter release (N- and P-types) • ↓ Slow depolarization (T-type) and spike-wave discharges
α2δ ligands	GBP, PGB	• Modulate neurotransmitter release
GABA$_A$ receptor allosteric modulators	BZDs, PB, FBM, TPM, CBZ, OxCBZ, STP, CLB	• ↑ Membrane hyperpolarization and seizure threshold • ↓ Focal firing BZDs—attenuate spike-wave discharges PB, CBZ, OxCBZ—aggravate spike-wave discharges
GABA uptake inhibitors/GABA-transaminase inhibitors	TGB, VGB	• ↑ Extrasynaptic GABA levels and membrane hyperpolarization • ↓ Focal firing • Aggravate spike-wave discharges
NMDA receptor antagonists	FBM	• ↓ Slow excitatory neurotransmission • ↓ Excitatory amino acid neurotoxicity • Delay epileptogenesis
AMPA/kainate receptor antagonists	PB, TPM	• ↓ Fast excitatory neurotransmission and focal firing
Enhancers of HCN channel activity	LTG	• Buffers large hyperpolarizing and depolarizing inputs • Suppresses action potential initiation by dendritic inputs
Positive allosteric modulator of KCNQ2-5	EZG	• Suppresses bursts of action potentials • Hyperpolarizes membrane potentials
SV2A protein ligand	LEV, BRV	• Unknown; may decrease transmitter release
Inhibitors of brain carbonic anhydrase	ACZ, TPM, ZNS	• ↑ HCN-mediated currents • ↓ NMDA-mediated currents • ↑ GABA-mediated inhibition
Mixed/unknown	CNB	Mixed, not fully understood mechanism • Inhibit voltage-gated sodium channels (persistent current) • Positive modulation of GABA$_A$
Unknown	CBD	Unknown; potential mechanisms include • Activation of TRPV1 • Antagonism of GPR55 and GPR55-mediated elevations of presynaptic Ca^{2+} • Activation of 5HT$_{1A}$ receptors • Inhibition of ENT-1 (modulation of adenosine tone)
Mixed	FEN	• Inhibition of serotonin transporter-mediated uptake • Activation of 5HT receptors: 5HT$_{1D}$, 5HT$_{2C}$ • Sigma-1–positive allosteric modulator

ACZ, acetazolamide; AP, action potential; BRV, brivaracetam; BZDs, benzodiazepines; CBD, cannabidiol; CBZ, carbamazepine; CLB, clobazam; CNB, cenobamate; ESL, eslicarbazepine; EZG, ezogabine; FEN, fenfluramine; FBM, felbamate; GBP, gabapentin; HCN, hyperpolarization-activated cyclic nucleotide–gated; 5HT, serotonin; LEV, levetiracetam; LCM, lacosamide; LTG, Lamotrigine; OxCBZ, oxcarbazepine; PER, perampanel; PB, phenobarbital; PGB, pregabalin; PHT, phenytoin; STP, stiripentol; TGB, tiagabine; TPM, topiramate; VGB, vigabatrin; VPA, valproic acid; ZNA, zonisamide.

Source: Modified with permission from Leppik IE, Kelly KM, deToledo-Morrell L et al. Basic research in epilepsy and aging. *Epilepsy Res*, **2006**, *68*(suppl 1):21. Copyright © Elsevier B.V.

synaptic inhibition is thought to underlie the antiseizure mechanism of *tiagabine*; *tiagabine* inhibits the GABA transporter, GAT-1, and reduces neuronal and glial uptake of GABA (Rogawski and Löscher, 2004). Finally, ASDs may either increase the production (i.e., *gabapentin*) or decrease the transaminase metabolism (i.e., *valproic acid*, *vigabatrin*) of GABA, resulting in increased GABA concentrations (Ben-Menachem, 2011; Cai et al., 2012; Larsson et al., 1986).

Generalized-Onset Epilepsies: Absence Seizures

In contrast to focal seizures, which arise from localized regions of the cerebral cortex, generalized-onset seizures arise from the reciprocal firing of the thalamus and cerebral cortex (Huguenard and McCormick, 2007). Among the diverse forms of generalized seizures, absence seizures have been studied most intensively. The striking synchrony in appearance of

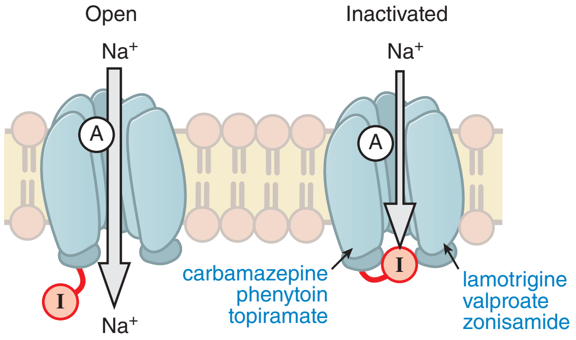


Figure 20–2 *ASD-enhanced Na$^+$ channel inactivation.* Some ASDs (shown in blue text) prolong the inactivation of the Na$^+$ channels, thereby reducing the ability of neurons to fire at high frequencies. Note that the inactivated channel appears to remain open but is blocked by the inactivation gate **I**. A, activation gate.

generalized seizure discharges in widespread areas of neocortex led to the idea that a structure in the thalamus and/or brainstem (the "centrencephalon") synchronized these seizure discharges. Focus on the thalamus in particular emerged from the demonstration that low-frequency stimulation of midline thalamic structures triggered EEG rhythms in the cortex similar to spike-and-wave discharges characteristic of absence seizures. Intracerebral electrode recordings from humans subsequently demonstrated the presence of thalamic and neocortical involvement in the spike-and-wave discharge of absence seizures.

Many of the structural and functional properties of the thalamus and neocortex that lead to the generalized spike-and-wave discharges have been elucidated (Huguenard and McCormick, 2007).

The EEG hallmark of an absence seizure is generalized spike-and-wave discharges at a frequency of 3 per second (3 Hz). These bilaterally synchronous spike-and-wave discharges, recorded locally from electrodes in both the thalamus and the neocortex, represent oscillations between the thalamus and neocortex. A comparison of EEG and intracellular recordings reveals that the EEG spikes are associated with the firing of action potentials and the following slow wave with prolonged inhibition. These reverberatory, low-frequency rhythms are made possible by a combination of factors, including reciprocal excitatory synaptic connections between the neocortex and thalamus as well as intrinsic properties of neurons in the thalamus (Huguenard and McCormick, 2007). One intrinsic property of thalamic neurons that is pivotally involved in the generation of the 3-Hz spike-and-wave discharges is a particular type of Ca^{2+} current, the low threshold ("T-type") current. T-type Ca^{2+} channels are activated at a much more negative membrane potential (hence, "low threshold") than most other voltage-gated Ca^{2+} channels expressed in the brain. T-type currents are much larger in many thalamic neurons compared to neurons outside the thalamus. Indeed, bursts of action potentials in thalamic neurons are mediated by activation of the T-type currents. T-type currents amplify thalamic membrane potential oscillations, with one oscillation being the 3-Hz spike-and-wave discharge of the absence seizure. Importantly, the principal mechanism by which anti–absence seizure drugs (*ethosuximide, valproic acid*) are thought to act is by inhibition of the T-type Ca^{2+} channels (Figure 20–4) (Rogawski and Löscher, 2004). Thus, inhibiting voltage-gated ion channels is a common mechanism of action among ASDs, with anti–focal seizure drugs inhibiting voltage-activated Na$^+$ channels and anti–absence seizure drugs inhibiting voltage-activated Ca^{2+} channels.

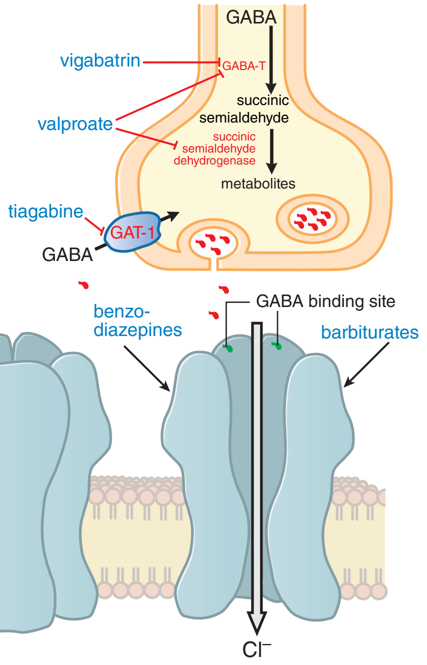


Figure 20–3 *Enhanced GABA synaptic transmission.* In the presence of GABA, the GABA$_A$ receptor (structure on left) is opened, allowing an influx of C1^-, which in turn increases membrane polarization (see Figure 16–12). Some antiseizure drugs (shown in larger blue text) act by reducing the metabolism of GABA. Others act at the GABA$_A$ receptor, enhancing C1^- influx in response to GABA. As outlined in the text, gabapentin acts presynaptically to promote GABA release; its molecular target is currently under investigation. (●), GABA molecules; GABA-T, GABA transaminase; GAT-1, GABA transporter.

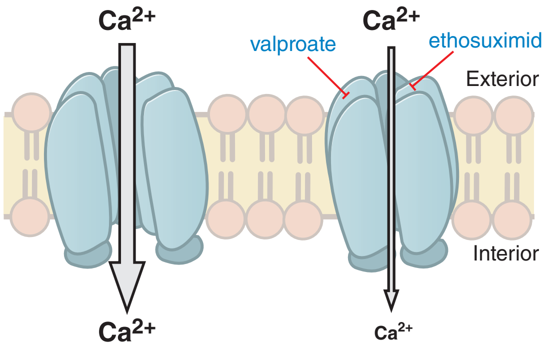


Figure 20–4 *ASD-induced reduction of current through T-type Ca^{2+} channels.* Some ASDs (shown in blue text) reduce the flow of Ca^{2+} through T-type Ca^{2+} channels thus reducing the pacemaker current that underlies the thalamic rhythm in spikes and waves seen in generalized absence seizures.

Genetic Approaches to the Epilepsies

Genetic causes contribute to a wide diversity of human epilepsies. Genetic causes are solely responsible for rare forms inherited in an autosomal dominant or autosomal recessive manner. Genetic causes also are mainly responsible for more common forms such as Dravet syndrome, juvenile myoclonic epilepsy, or childhood absence epilepsy, the majority of which are likely due to inheritance of two or more susceptibility genes. Genetic determinants also may contribute some degree of risk to epilepsies caused by injury of the cerebral cortex.

Enormous progress has been made in understanding the genetics of human epilepsy, with more than 70 genes whose mutations are now known to contribute to epilepsy. Gene mutations have been identified for a number of symptomatic epilepsies, in which the epilepsy is a manifestation of the underlying neurodegenerative disease. Because most patients with epilepsy are neurologically normal, elucidating the genes underlying familial epilepsy in otherwise normal individuals is of particular interest. Perhaps not surprisingly, many of the identified epilepsy-conferring mutations are in genes that encode voltage- or ligand-gated ion channels (Reid et al., 2009). However, mutations have also been identified in signaling pathways, transporters, and even synaptic vesicle proteins (EpiPM Consortium, 2015). Furthermore, many of the mutations arise *de novo*, thus complicating efforts in diagnoses. The genotype-phenotype correlations of these genetic syndromes are complex; the same mutation in one channel can be associated with divergent clinical syndromes ranging from simple febrile seizures to intractable seizures with intellectual decline. Conversely, clinically indistinguishable epilepsy syndromes have been associated with mutation of distinct genes. The implication of genes encoding ion channels in familial epilepsy is particularly interesting because episodic disorders involving other organs also result from mutations of these genes. For example, episodic disorders of the heart (cardiac arrhythmias), skeletal muscle (periodic paralyses), cerebellum (episodic ataxia), vasculature (familial hemiplegic migraine), and other organs all have been linked to mutations in genes encoding components of voltage-gated ion channels (Ptacek and Fu, 2001).

The cellular electrophysiological consequences of these mutations can inform on the mechanisms of seizures, inform as to the actions of ASDs, and allow for the determination of precision therapies for patients with specific mutations. For example, generalized epilepsy with febrile seizures is caused, in some cases, by a point mutation in the β subunit of a voltage-gated Na^+ channel (*SCN1B*). As described previously, several ASDs act on Na^+ channels to promote their inactivation; the phenotype of the mutated Na^+ channel appears to involve defective inactivation (Wallace et al., 1998).

Spontaneous mutations in *SCN1A* (encoding the α subunit of the major voltage-gated Na^+ channel in neurons, Nav1.1) that result in truncations and presumed loss of Na^+ channel function have been identified in a subset of infants with a catastrophic severe myoclonic epilepsy of infancy or Dravet syndrome. The fact that these loss-of-function mutations in Na^+ channels result in seizures is somewhat surprising. However, seizures may arise as a consequence of the cell types that express these channels within neural circuits that underlie seizure initiation. Interestingly, patients with these mutations are generally found to be refractory to ASDs that block Na^+ channels.

Advances in the Genetics of Epilepsy

Some deletions or additions to segments of DNA, referred to as *copy number variants*, can contribute to the pathology of epilepsy. These variants have been identified by chromosome microarray studies and whole-genome sequencing (Hirabayashi et al., 2019; Monlong et al., 2018; Myers and Mefford, 2015). Genome-wide association studies have been used to identify risk factors and variants that contribute to epilepsy (International League Against Epilepsy Consortium on Complex Epilepsies, 2018; Myers and Mefford, 2015). Identification of critical genetic variants that contribute to risk may, in turn, lead to novel therapeutic approaches (e.g., antisense oligonucleotides, novel drug targets, and antibody-based therapies).

Antiseizure Drugs: General Considerations

History

The first antiepileptic drug was *bromide*, which was used in the late 19th century. *Phenobarbital* was the first synthetic organic agent recognized as having antiseizure activity. Its usefulness, however, was limited to generalized tonic-clonic seizures and, to a lesser degree, focal seizures. It had no effect on absence seizures. Merritt and Putnam developed the electroshock seizure test in experimental animals to screen chemical agents for antiseizure effectiveness; in the course of screening a variety of drugs, they discovered that *diphenylhydantoin* (later renamed *phenytoin*) suppressed seizures in the absence of sedative effects. The maximal electroshock seizure test is extremely valuable because drugs that are effective against the tonic hind limb extension induced by corneal electroshock generally have proven to be effective against focal and generalized tonic-clonic seizures in humans. In contrast, seizures induced by the chemoconvulsant *pentylenetetrazol* are most useful in the identification of ASDs that are effective against myoclonic seizures in humans. These screening tests are still used in addition to many other phenotypically and/or etiologically relevant acute and chronic animal models.

The chemical structures of most of the drugs introduced before 1965 were closely related to *phenobarbital*. These included the hydantoins and the succinimides. Between 1965 and 1990, the chemically distinct structures of the benzodiazepines, an iminostilbene (*carbamazepine*), and a branched-chain carboxylic acid (*valproic acid*) were introduced, followed in the 1990s by a phenyltriazine (*lamotrigine*), a cyclic analogue of GABA (*gabapentin*), a sulfamate-substituted monosaccharide (*topiramate*), a nipecotic acid derivative (*tiagabine*), and a pyrrolidine derivative (*levetiracetam*). The Epilepsy Therapy Screening Project, formerly known as the Anticonvulsant Screening Project, was established by the National Institutes of Health with a contract to the University of Utah in the 1970s as a way to incentivize the continued development of ASDs, with academic scientists working in partnership with private industry and government. Using a wide array of animal models of seizures and epilepsy, the program has contributed to the preclinical identification and development of many of the clinically available ASDs.

Therapeutic Aspects

The ideal ASD would suppress all seizures without causing any unwanted effects. Unfortunately, drugs used currently not only fail to control seizure activity in approximately one-third of patients but also frequently cause unwanted adverse effects that range in severity from minimal impairment of the CNS to death from aplastic anemia or hepatic failure. In 2009, all manufacturers of ASDs were required by the FDA to update their product labeling to include a warning about an increased risk of suicidal thoughts or actions and to develop information targeted at helping patients understand this risk (FDA, 2008). The risk applies to all ASDs used for any indication. A recent meta-analysis of medications approved since 2008 (*eslicarbazepine*, *perampanel*, *brivaracetam*, *cannabidiol*, and *cenobamate*) suggests that there is no risk of suicidality associated with these newer agents (Klein et al., 2021).

The clinician who treats patients with epilepsy is faced with the task of selecting the appropriate drug or combination of drugs that best controls seizures in an individual patient at an acceptable level of untoward effects. As a general rule, complete control of seizures can be achieved in up to 50% of patients, while another 25% can be improved significantly. The degree of success varies as a function of seizure type, cause, and other factors.

To minimize adverse effects, treatment with a single drug is preferred. If seizures are not controlled with the initial agent at adequate plasma concentrations, substitution of a second drug is preferred to the concurrent administration of another agent. However, multiple-drug therapy may be required, especially when two or more types of seizure occur in the same patient.

Measurement of drug concentrations in plasma facilitates optimizing antiseizure medication, especially when therapy is initiated, after dosage adjustments, in the event of therapeutic failure, when toxic effects appear, or when multiple-drug therapy is instituted. However, clinical effects of some drugs do not correlate well with their concentrations in plasma, and recommended concentrations are only guidelines for therapy. The ultimate therapeutic regimen must be determined by clinical assessment of effect and toxicity.

The individual agents are introduced in the next sections, followed by a discussion of some general principles of the drug therapy of the epilepsies.

Hydantoins

Phenytoin

Phenytoin is effective against all types of focal and tonic-clonic seizures but not absence seizures. Properties of other hydantoins, such as *ethotoin*, are described in previous editions of this book.

Pharmacological Effects

Central Nervous System. Phenytoin exerts antiseizure activity without causing general depression of the CNS. In toxic doses, it may produce excitatory signs, and at lethal levels, it may produce a type of decerebrate rigidity.

Mechanism of Action

Phenytoin limits the repetitive firing of action potentials evoked by a sustained depolarization of mouse spinal cord neurons maintained *in vitro*

(McLean and Macdonald, 1986a). This effect is mediated by a slowing of the rate of recovery of voltage-activated Na^+ channels from inactivation, an action that is both voltage dependent (greater effect if membrane is depolarized) and use dependent. At therapeutic concentrations, the effects on Na^+ channels are selective, and no changes of spontaneous activity or responses to iontophoretically applied GABA or glutamate are detected. At concentrations 5- to 10-fold higher, multiple effects of *phenytoin* are evident, including reduction of spontaneous activity and enhancement of responses to GABA; these effects may underlie some of the unwanted toxicity associated with high levels of *phenytoin*.

Pharmacokinetic Properties

Phenytoin is available in two types of oral formulations that differ in their pharmacokinetics: rapid-release and extended-release forms. Once-daily dosing is possible only with the extended-release formulations, and due to differences in dissolution and other formulation-dependent factors, the plasma *phenytoin* level may change when converting from one formulation to another. Confusion also can arise because different formulations can include either *phenytoin* or *phenytoin sodium*. Therefore, comparable doses can be approximated by considering "*phenytoin* equivalents," but serum level monitoring is also necessary to assure therapeutic safety.

The pharmacokinetic characteristics of *phenytoin* are influenced markedly by its binding to serum proteins, by the nonlinearity of its elimination kinetics, and by its metabolism by hepatic cytochromes P450 (CYPs) (Table 20–3). *Phenytoin* is extensively bound (~90%) to serum proteins, mainly albumin. Small variations in the percentage of *phenytoin* that is bound dramatically affect the absolute amount of free (active) drug. Some agents can compete with *phenytoin* for binding sites on plasma proteins and increase free *phenytoin* at the time the new drug is added to the regimen. However, the effect on free *phenytoin* is only short-lived and usually does not cause clinical complications unless inhibition of *phenytoin* metabolism also occurs. For example, *valproate* competes for protein binding sites *and* inhibits *phenytoin* metabolism, resulting in marked and sustained increases in free *phenytoin*. Measurement of free rather than total *phenytoin* permits direct assessment of this potential problem in patient management.

TABLE 20–3 ■ INTERACTIONS OF ANTISEIZURE DRUGS WITH HEPATIC MICROSOMAL ENZYMES

	INDUCES		INHIBITS		METABOLIZED BY	
DRUG	**CYP**	**UGT**	**CYP**	**UGT**	**CYP**	**UGT**
Brivaracetam	No	No	No	No	2C19/2C9	No
Cannabidiol	?	?	2C9/3A4/2C19/2D6/1A1	1A9/2B7	3A4/2C19	?
Carbamazepine	1A2/2C9/3A4	Yes	No	No	1A2/2C8/3A4	No
Cenobamate	Yes	?	Yes	?	2E1/2A6/2B6	Yes
Clobazam	No	No	No	No	3A4	No
Clonazepam	No	No	No	No	3A4	No
Eslicarbazepine	3A4	No	No	No	No	Yes
Ethosuximide	No	No	No	No	3A4	No
Ezogabine	No	No	No	No	No	Yes
Felbamate	3A4	No	2C19	No	3A4/2E1	?
Fenfluramine	No	?	No	?	Yes	?
Gabapentin	No	No	No	No	No	No
Lacosamide	No	No	No	No	2C19	?
Lamotrigine	No	No	No	No	No	Yes (UGT1A4)
Levetiracetam	No	No	No	No	No	No
Oxcarbazepine	3A4/5	Yes (UGT1A4)	2C19	Weak	No	Yes
Perampanel	No	No	Weak	Weak	3A4/3A5	Yes
Phenobarbital	2C9/3A4/1A2	Yes	No	No	2C9/19/2E1	Yes
Phenytoin	2C9/3A4/1A2	Yes	2C9	No	2C9/19	No
Pregabalin	No	No	No	No	No	No
Primidone	2C/3A	Yes	Yes	No	2C9/19	No
Rufinamide	3A4	2C9/19	No	No	No	Yes
Stiripentol	No	No	1A2/3A4/2C19/2D6	No	No	No
Tiagabine	No	No	No	No	3A4	No
Topiramate	3A4 (>200 mg/day)	No	2C19	No	Yes	No
Valproate	No	No	2C9/3A4?	Yes	2C9/2C19/2A6/2B6	Yes (UGT1A3/2B7)
Vigabatrin	No	No	No	No	No	No
Zonisamide	No	No	No	No	3A4	No

CYP, cytochrome P450; UGT, uridine diphosphate-glucuronosyltransferase.

Source: Data modified from Johannessen and Landmark, 2010; Wheles JW, Vasquez B. **2010**; and Cawello W. **2015**. CBD data from Alsherbiny and Li, 2018.

Phenytoin is one of the few drugs for which the rate of elimination varies as a function of its concentration (i.e., the rate is nonlinear). The plasma $t_{1/2}$ of *phenytoin* ranges between 6 and 24 h at plasma concentrations below 10 μg/mL. At low blood levels, metabolism follows first-order kinetics. However, as blood levels rise, the maximal limit of the liver to metabolize *phenytoin* is approached; as a result, plasma drug concentration increases disproportionately as dosage is increased, even with small adjustments for levels near the therapeutic range.

The majority (95%) of *phenytoin* is metabolized in the hepatic endoplasmic reticulum by CYP2C9/10 and to a lesser extent CYP2C19 (see Table 20–3). The principal metabolite, a parahydroxyphenyl derivative, is inactive. Because its metabolism is saturable, other drugs that are metabolized by these CYPs can inhibit the metabolism of *phenytoin* and increase its plasma concentration. Conversely, the degradation rate of other drugs that serve as substrates for these enzymes can be inhibited by *phenytoin*; one such drug is *warfarin*, and addition of *phenytoin* to a patient receiving *warfarin* can lead to bleeding disorders (see Chapter 36). An alternative mechanism of drug interactions arises from *phenytoin*'s ability to induce various CYPs (see Chapter 5). Of particular note in this regard are oral contraceptives, which are metabolized by CYP3A4; treatment with *phenytoin* can enhance the metabolism of oral contraceptives and lead to unplanned pregnancy. The potential teratogenic effects of *phenytoin* underscore the importance of attention to this interaction. *Carbamazepine*, *oxcarbazepine*, *phenobarbital*, and *primidone* also induce CYP3A4 and likewise might increase degradation of oral contraceptives.

The low water solubility of *phenytoin* hindered its intravenous use and led to production of *fosphenytoin*, a water-soluble prodrug. *Fosphenytoin* is converted into *phenytoin* by phosphatases in liver and red blood cells with a $t_{1/2}$ of 8 to 15 min. *Fosphenytoin* is extensively bound (95%–99%) to human plasma proteins, primarily albumin. This binding is saturable, and *fosphenytoin* displaces *phenytoin* from protein-binding sites. *Fosphenytoin* is useful for adults with focal or generalized seizures when either the intravenous or intramuscular route of administration is indicated.

Toxicity

The toxic effects of *phenytoin* depend on the route of administration, the duration of exposure, and the dosage.

When *fosphenytoin*, the water-soluble prodrug, is administered intravenously at an excessive rate in the emergency treatment of status epilepticus, the most notable toxic signs are cardiac arrhythmias with or without hypotension and/or CNS depression. Although cardiac toxicity occurs more frequently in older patients and in those with known cardiac disease, it also can develop in young, healthy patients. These complications can be minimized by administering *fosphenytoin* at a rate of less than 150 mg of *phenytoin sodium* equivalents per minute. Acute oral overdose results primarily in cerebellar and vestibular symptoms; high doses have been associated with marked cerebellar atrophy. Toxic effects associated with chronic treatment also are primarily dose-related cerebellar-vestibular effects but also include other CNS effects, behavioral changes, increased frequency of seizures, gastrointestinal (GI) symptoms, gingival hyperplasia, osteomalacia, and megaloblastic anemia. Hirsutism is a particularly problematic untoward effect in females. Usually, these phenomena can be diminished by proper adjustment of dosage. Serious adverse effects, including those on the skin, bone marrow, and liver, probably are manifestations of drug allergy. Although rare, they necessitate withdrawal of the drug. Moderate transient elevation of the plasma concentrations of hepatic transaminases sometimes can also occur.

Gingival hyperplasia occurs in approximately 20% of all patients during chronic treatment and can be minimized by good oral hygiene. Related to this, *phenytoin* can also produce coarsening of facial features. Inhibition of release of antidiuretic hormone has been observed. Hyperglycemia and glycosuria appear to be due to inhibition of insulin secretion. Osteomalacia, with hypocalcemia and elevated alkaline phosphatase activity, has been attributed to both altered metabolism of vitamin D and the attendant inhibition of intestinal absorption of Ca^{2+}. *Phenytoin* also increases the metabolism of vitamin K and reduces the concentration of vitamin K–dependent proteins that are important for normal Ca^{2+} metabolism in bone. This may explain why the osteomalacia is not always ameliorated by the administration of vitamin D.

Hypersensitivity reactions include morbilliform rash in 2% to 5% of patients and occasionally more serious skin reactions, including Stevens-Johnson syndrome and toxic epidermal necrolysis. Drug-induced systemic lupus erythematosus, hepatic necrosis, hematological reactions including neutropenia and leukopenia, red cell aplasia, agranulocytosis, and mild thrombocytopenia also have been reported. Hypoprothrombinemia and hemorrhage have occurred in the newborns of mothers who received *phenytoin* during pregnancy; vitamin K is effective treatment or prophylaxis.

Plasma Drug Concentrations

A good correlation usually is observed between the total concentration of *phenytoin* in plasma and its clinical effect. Thus, control of seizures generally is obtained with total concentrations above 10 μg/mL, while toxic effects such as nystagmus develop at total concentrations around 20 μg/mL. Control of seizures generally is obtained with free *phenytoin* concentrations of 0.75 to 1.25 μg/mL.

Drug Interactions

Concurrent administration of any drug metabolized by CYP2C9 or CYP2C10 can increase the plasma concentration of *phenytoin* by decreasing its rate of metabolism (see Table 20–3). Conversely, the degradation rate of other drugs that are substrates for these enzymes can be inhibited by *phenytoin*. *Carbamazepine*, which may enhance the metabolism of *phenytoin*, causes a well-documented decrease in *phenytoin* concentration. *Phenytoin* can also induce expression of a number of different CYPs, leading to increased degradation of coadministered drugs such as oral contraceptives. Conversely, *phenytoin* reduces the concentration of *carbamazepine*.

Therapeutic Uses

Epilepsy. *Phenytoin* is one of the more widely used ASDs, and it is effective against focal and generalized tonic-clonic, focal to bilateral tonic-clonic, and tonic-clonic of unknown onset (tonic-clonic) seizures, but not generalized absence (absence) seizures. The use of *phenytoin* and other agents in the therapy of epilepsies is discussed further at the end of this chapter. *Phenytoin* preparations differ significantly in bioavailability and rate of absorption. In general, patients should consistently be treated with the same drug from a single manufacturer. However, if it becomes necessary to temporarily switch between products, care should be taken to select a therapeutically equivalent product and patients should be monitored for loss of seizure control or onset of new toxicities.

Other Uses. Trigeminal and related neuralgias occasionally respond to *phenytoin*, but *carbamazepine* may be preferable. The use of *phenytoin* in the treatment of cardiac arrhythmias is discussed in Chapter 34.

Benzodiazepines

The benzodiazepines are used primarily as sedative-antianxiety drugs; their pharmacology is described in Chapters 16 and 22. Discussion here is limited to their use in the therapy of the epilepsies. A large number of benzodiazepines have broad antiseizure properties, but only *clonazepam* and *clorazepate* have been approved in the U.S. for the long-term treatment of certain types of seizures. *Midazolam* was designated an orphan drug in 2006 for intermittent treatment of bouts of increased seizure activity in refractory patients with epilepsy who are on stable regimens of ASDs. *Diazepam* and *lorazepam* have well-defined roles in the management of status epilepticus. Figure 22–1 shows the basic structure of the benzodiazepines. Unlike other marketed 1,4-benzodiazepines, *clobazam* is a 1,5-benzodiazepine that is less lipophilic, less acidic, and may be better tolerated than traditional 1,4-benzodiazepines. *Clobazam* is used in a variety of seizure phenotypes and is approved in the U.S. for the treatment of Lennox-Gastaut syndrome in patients aged 2 years or older

Antiseizure Properties

In animal models, inhibition of *pentylenetetrazol*-induced seizures by the benzodiazepines is much more prominent than is their modification of the maximal electroshock seizure pattern. *Clonazepam* is unusually potent in antagonizing the effects of *pentylenetetrazol*, but it is almost without action on seizures induced by maximal electroshock. Benzodiazepines, including *clonazepam*, suppress the spread of kindled seizures and generalized convulsions produced by stimulation of the amygdala but do not abolish the abnormal discharge at the site of stimulation.

Mechanism of Action

The antiseizure actions of the benzodiazepines, as well as other effects that occur at nonsedating doses, result in large part from their ability to enhance GABA-mediated synaptic inhibition. Molecular cloning and study of recombinant receptors have demonstrated that the benzodiazepine receptor is an integral part of the $GABA_A$ receptor. At therapeutically relevant concentrations, benzodiazepines act at subsets of $GABA_A$ receptors and increase the frequency, but not duration, of openings at GABA-activated Cl^- channels (Twyman et al., 1989). At higher concentrations, *diazepam* and many other benzodiazepines can reduce sustained high-frequency firing of neurons, similar to the effects of *phenytoin*, *carbamazepine*, and *valproate*. Although these concentrations correspond to concentrations achieved in patients during treatment of status epilepticus with *diazepam*, they are considerably higher than those associated with antiseizure or anxiolytic effects in ambulatory patients. *Clobazam* potentiates GABA-mediated neurotransmission in the same fashion as other benzodiazepines at $GABA_A$ receptors.

Pharmacokinetic Properties

Benzodiazepines are well absorbed after oral administration, and concentrations in plasma are usually maximal within 1 to 4 h. After intravenous administration, they are redistributed in a manner typical of that for highly lipid-soluble agents. Central effects develop promptly but wane rapidly as the drugs move to other tissues. *Diazepam* is redistributed especially rapidly, with a $t_{1/2}$ of redistribution of approximately 1 h. The extent of binding of benzodiazepines to plasma proteins correlates with lipid solubility, ranging from approximately 99% for *diazepam* to approximately 85% for *clonazepam*.

The major metabolite of *diazepam*, *N*-desmethyl-diazepam, is somewhat less active than the parent drug and may behave as a partial agonist. This metabolite also is produced by the rapid decarboxylation of *clorazepate* following its ingestion. Both *diazepam* and *N*-desmethyl-diazepam are slowly hydroxylated to other active metabolites, such as *oxazepam*. The $t_{1/2}$ of *diazepam* in plasma is between 1 and 2 days, while that of *N*-desmethyl-diazepam is approximately 60 h. *Clonazepam* is metabolized principally by reduction of the nitro group to produce inactive 7-amino derivatives. Less than 1% of the drug is recovered unchanged in the urine. The $t_{1/2}$ of *clonazepam* in plasma is approximately 23 h. *Lorazepam* is metabolized chiefly by conjugation with glucuronic acid; its $t_{1/2}$ in plasma is approximately 14 h. *Clobazam* has a $t_{1/2}$ of 18 h and is effective at doses between 0.5 and 1 mg/kg daily, with limited development of tolerance. The active metabolite of *clobazam* is norclobazam.

Toxicity

The principal side effects of long-term oral therapy with *clonazepam* are drowsiness and lethargy. These occur in approximately 50% of patients initially, but tolerance often develops with continued administration. Muscular incoordination and ataxia are less frequent. Although these symptoms usually can be kept to tolerable levels by reducing the dosage or the rate at which it is increased, they sometimes force drug discontinuation.

Other side effects include hypotonia, dysarthria, and dizziness. Behavioral disturbances, especially in children, can be very troublesome; these include aggression, hyperactivity, irritability, and difficulty in concentration. Both anorexia and hyperphagia have been reported. Increased salivary and bronchial secretions may cause difficulties in children. Seizures are sometimes exacerbated, and status epilepticus may be precipitated if the drug is discontinued abruptly. Other aspects of the toxicity of the benzodiazepines are discussed in Chapter 22. Cardiovascular and respiratory depression may occur after the intravenous administration of *diazepam*, *clonazepam*, or *lorazepam*, particularly if other ASDs or central depressants have been administered previously.

Plasma Drug Concentrations

Because tolerance affects the relationship between drug concentration and drug antiseizure effect, plasma concentrations of benzodiazepines are of limited value.

Therapeutic Uses

Clonazepam is useful in the therapy of absence seizures as well as myoclonic seizures in children. However, tolerance to its antiseizure effects usually develops after 1 to 6 months of administration, after which some patients will no longer respond to *clonazepam* at any dosage. The initial dose of *clonazepam* for adults should not exceed 1.5 mg/day and for children 0.01 to 0.03 mg/kg per day. The dose-dependent side effects are reduced if two or three divided doses are given each day. The dose may be increased every 3 days in amounts of 0.25 to 0.5 mg/day in children and 0.5 to 1 mg/day in adults. The maximal recommended dose is 20 mg/day for adults and 0.2 mg/kg per day for children. *Clonazepam* intranasal spray is designated as an orphan drug for recurrent acute repetitive seizures.

While *diazepam* is an effective agent for treatment of status epilepticus, its short duration of action is a disadvantage, leading to the more frequent use of *lorazepam*. Although *diazepam* is not useful as an oral agent for the treatment of seizure disorders, *clorazepate* is effective in combination with certain other drugs in the treatment of focal seizures. The maximal initial dose of *clorazepate* is 22.5 mg/day in three portions for adults and children older than age 12 years and 15 mg/day in two divided doses in children 9 to 12 years of age. *Clorazepate* is not recommended for children under the age of 9. *Clobazam* is used in a variety of seizure phenotypes and is approved in the U.S. for the treatment of Lennox-Gastaut syndrome in patients aged 2 years or older. In patients weighing greater than 30 kg, *clobazam* is initiated orally at 5 mg every 12 h and then titrated up to a maximum of 40 mg/day, if tolerated. Dose escalation must be done gradually, not exceeding more than once per week.

Antiseizure Barbiturates

The pharmacology of the barbiturates as a class is described in Chapter 22; discussion in this chapter is limited to *phenobarbital* and *primidone*.

Phenobarbital

Phenobarbital was the first effective organic antiseizure agent. It has relatively low toxicity, is inexpensive, and is still one of the more effective and widely used drugs for this purpose. While most barbiturates have antiseizure properties, only some barbiturates, such as *phenobarbital*, exert maximal antiseizure effects at doses below those that cause hypnosis. This therapeutic index determines a barbiturate's clinical utility as an antiseizure therapeutic drug.

Mechanism of Action

The mechanism by which phenobarbital inhibits seizures likely involves potentiation of synaptic inhibition through an action on the $GABA_A$ receptor (see Figure 20–3). Intracellular recordings of mouse cortical or spinal cord neurons demonstrated that *phenobarbital* enhances responses to iontophoretically applied GABA. These effects have been observed at therapeutically relevant concentrations of *phenobarbital*. Analyses of single channels in outside-out patches isolated from mouse spinal cord neurons demonstrated that *phenobarbital* increased the $GABA_A$ receptor–mediated current by increasing the duration of bursts of $GABA_A$ receptor–mediated currents without changing the frequency of bursts

(Twyman et al., 1989). At levels exceeding therapeutic concentrations, *phenobarbital* also limits sustained repetitive firing; this may underlie some of the antiseizure effects of higher concentrations of *phenobarbital* achieved during therapy of status epilepticus.

Pharmacokinetic Properties

Oral absorption of *phenobarbital* is complete but somewhat slow; peak concentrations in plasma occur several hours after a single dose. It is 40% to 60% bound to plasma proteins and bound to a similar extent in tissues, including brain. Up to 25% of a dose is eliminated by pH-dependent renal excretion of the unchanged drug; the remainder is inactivated by hepatic microsomal enzymes, principally CYP2C9, with minor metabolism by CYP2C19 and CYP2E1. *Phenobarbital* induces uridine diphosphate-glucuronosyltransferases (UGTs) as well as the CYP2C and CYP3A subfamilies. Drugs metabolized by these enzymes can be more rapidly degraded when coadministered with *phenobarbital*; importantly, oral contraceptives are metabolized by CYP3A4. The terminal $t_{1/2}$ of *phenobarbital* varies with age, with values ranging in adults from 5 to 140 h and in children less than 5 years of age from 40 to 70 h. *Phenobarbital*'s duration of effect usually exceeds 6 to 12 h in nontolerant patients.

Toxicity

Sedation, the most frequent undesired effect of *phenobarbital*, is apparent to some extent in all patients upon initiation of therapy, but tolerance develops during chronic medication. Nystagmus and ataxia occur at excessive dosage. *Phenobarbital* can produce irritability and hyperactivity in children and agitation and confusion in the elderly. Scarlatiniform or morbilliform rash, possibly with other manifestations of drug allergy, occurs in 1% to 2% of patients. Exfoliative dermatitis is rare. Hypoprothrombinemia with hemorrhage has been observed in the newborns of mothers who have received *phenobarbital* during pregnancy; vitamin K is effective for treatment or prophylaxis. As with *phenytoin*, megaloblastic anemia that responds to folate and osteomalacia that responds to high doses of vitamin D occur during chronic *phenobarbital* therapy of epilepsy. Other adverse effects of *phenobarbital* are discussed in Chapter 22.

Plasma Drug Concentrations

During long-term therapy in adults, the plasma concentration of *phenobarbital* averages 10 μg/mL per daily dose of 1 mg/kg; in children, the value is 5 to 7 μg/mL per 1 mg/kg. Although a precise relationship between therapeutic results and concentration of drug in plasma does not exist, plasma concentrations of 10 to 35 μg/mL are usually recommended for control of seizures.

The relationship between plasma concentration of *phenobarbital* and adverse effects varies with the development of tolerance. Sedation, nystagmus, and ataxia usually are absent at concentrations below 30 μg/mL during long-term therapy, but adverse effects may be apparent for several days at lower concentrations when therapy is initiated or whenever the dosage is increased. Concentrations greater than 60 μg/mL may be associated with marked intoxication in the nontolerant individual. Since significant behavioral toxicity may be present despite the absence of overt signs of toxicity, the tendency to maintain patients, particularly children, on excessively high doses of *phenobarbital* should be resisted. The plasma *phenobarbital* concentration should be increased above 30 to 40 μg/mL only if the increment is adequately tolerated and only if it contributes significantly to control of seizures.

Drug Interactions

Interactions between *phenobarbital* and other drugs usually involve induction of the hepatic CYPs by *phenobarbital* (see Table 20–3 and Chapters 5 and 22). The interaction between *phenytoin* and *phenobarbital* is variable. Concentrations of *phenobarbital* in plasma may be elevated by as much as 40% during concurrent administration of *valproic acid*.

Therapeutic Uses

Phenobarbital is an effective agent for generalized tonic-clonic, focal to bilateral tonic-clonic, tonic-clonic of unknown onset (generalized tonic-clonic), and focal seizures. Its efficacy, low toxicity, and low cost make it an important agent for these types of epilepsy. However, its sedative effects and its tendency to disturb behavior in children have reduced its use as a primary agent. It is not effective for absence seizures.

Primidone

Although primidone is indicated in the U.S. for patients with focal and/or generalized epilepsy, it has largely been replaced by *carbamazepine* and other newer ASDs that possess a lower incidence of sedation.

Mechanism of Action

Primidone, also known as 2-desoxyphenobarbitol, is metabolized to two active metabolites: *phenobarbital* and *phenylethylmalonamide* (PEMA). Each of these three compounds has antiseizure effects on focal and generalized tonic-clonic seizures. The exact mechanism of *primidone*'s antiseizure effect is not fully understood.

Pharmacokinetics

Primidone is completely absorbed and generally reaches peak plasma concentration within approximately 3 h of oral administration. *Primidone* is not highly protein bound in plasma (30%) and is rapidly metabolized to both *phenobarbital* and PEMA. Both *primidone* and *phenobarbital* undergo extensive conjugation prior to excretion. *Primidone*'s $t_{1/2}$ is approximately 6 to 8 h. In contrast, the terminal $t_{1/2}$ of *phenobarbital* varies with age, with values ranging in adults from 5 to 140 h and in children less than 5 years of age from 40 to 70 h. Because of its slow clearance, *phenobarbital* reaches therapeutic concentrations approximately two to three times higher than that of *primidone*. Care should be taken and *phenobarbital* plasma levels closely monitored during titration of *primidone* doses since *primidone* may reach steady-state levels rapidly (1–2 days), whereas the metabolites, *phenobarbital* and PEMA, each attain steady state more slowly (2–20 days and 3–4 days, respectively).

Toxicity

The dose-dependent adverse effects of *primidone* are similar to those of *phenobarbital*, except that pronounced drowsiness is observed early after *primidone* administration. Common adverse effects include ataxia and vertigo, both of which diminish and may disappear with continued therapy. *Primidone* is contraindicated in patients with either porphyria or with hypersensitivity to *phenobarbital*.

Therapeutic Uses

Doses of 10 to 20 mg/kg per day reach clinically relevant steady-state plasma concentrations (8–12 μg/mL), although interpatient variation is common. In addition to its early use in patients with focal-onset and/or generalized epilepsy, *primidone* is still considered to be a first-line therapy for essential tremor with the β adrenergic antagonist *propranolol*.

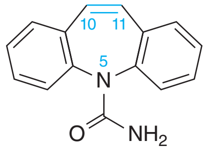
IMINOSTILBENES

Carbamazepine

Carbamazepine has been employed since the 1960s for the treatment of trigeminal neuralgia. It was initially approved in the U.S. for use as an antiseizure agent in 1974 and is now considered to be a primary drug for the treatment of generalized tonic-clonic, focal to bilateral tonic-clonic, tonic-clonic of unknown onset (generalized tonic-clonic), and focal seizures.

Chemistry

Carbamazepine is related chemically to the tricyclic antidepressants. It is a derivative of iminostilbene with a carbamyl group at the 5 position; this moiety is essential for potent antiseizure activity.



CARBAMAZEPINE

Mechanism of Action

Like *phenytoin*, *carbamazepine* limits the repetitive firing of action potentials evoked by a sustained depolarization of mouse spinal cord or cortical neurons maintained *in vitro* (McLean and Macdonald, 1986a). This appears to be mediated by a slowing of the rate of recovery of voltage-activated Na^+ channels from inactivation. These effects of *carbamazepine* are evident at concentrations in the range of therapeutic drug levels in cerebrospinal fluid (CSF) in humans. At those concentrations, the effects of *carbamazepine* are selective, in that there are no effects on spontaneous activity or on responses to iontophoretically applied GABA or glutamate. The *carbamazepine* metabolite, 10,11-epoxycarbamazepine, also limits sustained repetitive firing at therapeutically relevant concentrations, suggesting that this metabolite may contribute to the antiseizure efficacy of *carbamazepine*.

Pharmacokinetic Properties

The pharmacokinetics of *carbamazepine* are complex. They are influenced by its limited aqueous solubility and by the ability of many ASDs, including *carbamazepine* itself, to increase their conversion to active metabolites by hepatic oxidative enzymes (see Table 20–3). *Carbamazepine* is absorbed slowly and erratically after oral administration. Peak concentrations in plasma usually are observed 4 to 8 h after oral ingestion but may be delayed by as much as 24 h, especially following the administration of a large dose. The drug distributes rapidly into all tissues. Approximately 75% of *carbamazepine* binds to plasma proteins, and concentrations in the CSF appear to correspond to the concentration of free drug in plasma. The predominant metabolic pathway in humans involves conversion to the 10,11-epoxide, a metabolite as active as the parent compound in various animals; its concentrations in plasma and brain may reach 50% of those of *carbamazepine*, especially during the concurrent administration of *phenytoin* or *phenobarbital*. The 10,11-epoxide is metabolized further to inactive compounds, which are excreted in the urine, principally as glucuronides. *Carbamazepine* also is inactivated by conjugation and hydroxylation. Hepatic CYP3A4 is primarily responsible for biotransformation of *carbamazepine*. *Carbamazepine* induces CYP2C, CYP3A, and UGT, thus enhancing the metabolism of drugs degraded by these enzymes. Of particular importance in this regard are oral contraceptives, which are also metabolized by CYP3A4.

Toxicity

Acute intoxication with *carbamazepine* can result in stupor or coma, hyperirritability, convulsions, and respiratory depression. During long-term therapy, the more frequent untoward effects of the drug include drowsiness, vertigo, ataxia, diplopia, and blurred vision. The frequency of seizures may increase, especially with overdose. Other adverse effects include nausea, vomiting, serious hematological toxicity (aplastic anemia, agranulocytosis), and hypersensitivity reactions (dangerous skin reactions, eosinophilia, lymphadenopathy, splenomegaly). A late complication of therapy with *carbamazepine* is retention of water, with decreased osmolality and concentration of Na^+ in plasma, especially in elderly patients with cardiac disease.

Some tolerance develops to the neurotoxic effects of *carbamazepine*, and they can be minimized by gradual increase in dosage or adjustment of maintenance dosage. Various hepatic or pancreatic abnormalities have been reported during therapy with *carbamazepine*, most commonly a transient elevation of hepatic transaminases in plasma in 5% to 10% of patients. A transient, mild leukopenia occurs in approximately 10% of patients during initiation of therapy and usually resolves within the first 4 months of continued treatment; transient thrombocytopenia also has been noted. In approximately 2% of patients, a persistent leukopenia may develop that requires withdrawal of the drug. The initial concern that aplastic anemia might be a frequent complication of long-term therapy with *carbamazepine* has not materialized. In most cases, the administration of multiple drugs or the presence of another underlying disease has made it difficult to establish a causal relationship. The prevalence of aplastic anemia is approximately 1 in 200,000 patients who are treated with the drug. It is not clear whether monitoring of hematological function can avert the development of irreversible aplastic anemia. Although *carbamazepine* is carcinogenic in rats, it is not known to be a human carcinogen. Possible teratogenic effects are discussed later in the chapter.

Plasma Drug Concentrations

There is no simple relationship between the dose of *carbamazepine* and concentrations of the drug in plasma. Therapeutic concentrations are reported to be 6 to 12 μg/mL, but with considerable variation. Side effects referable to the CNS are frequent at concentrations above 9 μg/mL.

Drug Interactions

Phenobarbital, *phenytoin*, and *valproate* may increase the metabolism of *carbamazepine* by inducing CYP3A4; *carbamazepine* may enhance the biotransformation of *phenytoin*. Concurrent administration of *carbamazepine* may lower concentrations of *valproate*, *lamotrigine*, *tiagabine*, and *topiramate*. *Carbamazepine* reduces both the plasma concentration and therapeutic effect of *haloperidol*. The metabolism of *carbamazepine* may be inhibited by *propoxyphene*, *erythromycin*, *cimetidine*, *fluoxetine*, and *isoniazid*.

Therapeutic Uses

Carbamazepine is useful in patients with generalized tonic-clonic and both focal aware and focal with impaired awareness seizures (see Table 20–1). When it is used, renal and hepatic function and hematological parameters should be monitored. The therapeutic use of *carbamazepine* is discussed further at the end of this chapter.

Carbamazepine can produce therapeutic responses in patients with bipolar disorder, including some in whom *lithium carbonate* is ineffective. Further, *carbamazepine* has antidiuretic effects that are sometimes associated with increased concentrations of antidiuretic hormone in plasma via poorly understood mechanisms.

Carbamazepine is the primary agent for treatment of trigeminal and glossopharyngeal neuralgias. It is also effective for lightning-type ("tabetic") pain associated with bodily wasting. *Carbamazepine* is also used in the treatment of bipolar affective disorders, as discussed further in Chapter 19.

Oxcarbazepine

Oxcarbazepine (10,11-dihydro-10-oxocarbamazepine) is a keto analogue of *carbamazepine*. *Oxcarbazepine* has been approved for monotherapy or adjunct therapy for focal seizures in adults, as monotherapy for focal seizures in children ages 4 to 16, and as adjunctive therapy in children 2 years of age and older with epilepsy. *Oxcarbazepine* is a prodrug that is almost immediately converted to its metabolite, *eslicarbazepine*. *Eslicarbazepine* is then extensively converted to the active S(+) enantiomer, *S-licarbazepine*. *Oxcarbazepine* is inactivated by glucuronide conjugation, is eliminated by renal excretion, and has a $t_{1/2}$ of only 1 to 2 h.

Oxcarbazepine has a mechanism of action similar to that of *carbamazepine* but is a less potent enzyme inducer than *carbamazepine*. Substitution of *oxcarbazepine* for *carbamazepine* is associated with increased levels of *phenytoin* and *valproic acid*, presumably because of reduced induction of hepatic enzymes. *Oxcarbazepine* does not induce the hepatic enzymes involved in its own degradation. Although *oxcarbazepine* does not appear to reduce the anticoagulant effect of *warfarin*, it does induce CYP3A and thus reduces plasma levels of steroid oral contraceptives. Fewer hypersensitivity reactions have been associated with *oxcarbazepine*, and cross-reactivity with *carbamazepine* does not always occur. Although most adverse effects are similar to those with *carbamazepine*, hyponatremia may occur more commonly with *oxcarbazepine* than with *carbamazepine*.

Eslicarbazepine Acetate

Eslicarbazepine acetate is a prodrug approved in the U.S. as a monotherapy and adjunctive treatment of focal-onset seizures. As described previously, *eslicarbazepine* is converted to its active metabolite, *S-licarbazepine*, faster than its prodrug, *oxcarbazepine*; ultimately, *eslicarbazepine* has a similar mechanism of action as *oxcarbazepine*, since both serve as prodrugs of same active metabolite, *S-licarbazepine*. *Eslicarbazepine* competitively inhibits fast voltage-gated sodium channels, stabilizing the inactivated state and the sodium-dependent release of neurotransmitters. *Eslicarbazepine* has a $t_{1/2}$ similar to that of *carbamazepine* (8–12 h); it is excreted as a glucuronide. *Eslicarbazepine acetate* in adults may be initiated at 400 to 1200 mg/day. Higher doses require careful titration based on patient response. Reduction in dosing is necessary in patients with renal impairment.

Succinimides

Ethosuximide

Ethosuximide is a primary agent for the treatment of absence seizures.

Structure-Activity Relationship

The structure-activity relationship of the succinimides is in accord with that for other antiseizure classes. *Ethosuximide*, with alkyl substituents, is the most active of the succinimides against seizures induced by pentylenetetrazol and is the most selective for absence seizures. A related compound, *methsuximide*, has phenyl substituents and is more active against maximal electroshock seizures. It is no longer in common use. Discussion of its properties can be found in previous editions of this book.

Pharmacological Effects

The most prominent characteristic of *ethosuximide* at nontoxic doses is protection against clonic motor seizures induced by *pentylenetetrazol*. By contrast, at nontoxic doses, *ethosuximide* does not inhibit tonic hind limb extension of electroshock seizures or kindled seizures. This profile correlates with efficacy against absence seizures in humans.

Mechanism of Action

Ethosuximide reduces low-threshold Ca^{2+} currents (T-type currents) in thalamic neurons (Coulter et al., 1989). The thalamus plays an important role in generation of 3-Hz spike-and-wave rhythms typical of absence seizures (Huguenard and McCormick, 2007). Neurons in the thalamus exhibit large-amplitude T-type currents that underlie bursts of action potentials and likely play an important role in thalamic oscillatory activity such as 3-Hz spike-and-wave activity. At clinically relevant concentrations, *ethosuximide* inhibits the T-type current, as is evident in voltage-clamp recordings of acutely isolated, ventrobasal thalamic neurons from rats and guinea pigs. *Ethosuximide* reduces this current without modifying the voltage dependence of steady-state inactivation or the time course of recovery from inactivation. By contrast, *succinimide* derivatives with convulsant properties do not inhibit this current. *Ethosuximide* does not inhibit sustained repetitive firing or enhance GABA responses at clinically relevant concentrations. Inhibition of T-type currents likely is the mechanism by which *ethosuximide* inhibits absence seizures.

Pharmacokinetic Properties

Absorption of *ethosuximide* appears to be complete, with peak concentrations in plasma within approximately 3 h after a single oral dose. *Ethosuximide* is not significantly bound to plasma proteins; during long-term therapy, its concentration in the CSF is similar to that in plasma. The apparent volume of distribution averages 0.7 L/kg.

Approximately 25% of the drug is excreted unchanged in the urine. The remainder is metabolized by hepatic microsomal enzymes, but whether CYPs are responsible is unknown. The major metabolite, the hydroxyethyl derivative, accounts for approximately 40% of *ethosuximide* metabolism, is inactive, and is excreted in the urine, as such and as the glucuronide. The plasma $t_{1/2}$ of *ethosuximide* averages between 40 and 50 h in adults and approximately 30 h in children.

Toxicity

The most common dose-related side effects are GI complaints (nausea, vomiting, and anorexia) and CNS effects (drowsiness, lethargy, euphoria, dizziness, headache, and hiccough). Some tolerance to these effects develops. Parkinson-like symptoms and photophobia also have been reported. Restlessness, agitation, anxiety, aggressiveness, inability to concentrate, and other behavioral effects have occurred primarily in patients with a prior history of psychiatric disturbance.

Urticaria and other skin reactions, including Stevens-Johnson syndrome, as well as systemic lupus erythematosus, eosinophilia, leukopenia, thrombocytopenia, pancytopenia, and aplastic anemia, also have been attributed to the drug. The leukopenia may be transient despite continuation of the drug, but several deaths have resulted from bone marrow depression. Renal and hepatic toxicity have not been reported.

Plasma Drug Concentrations

During long-term therapy, the plasma concentration of *ethosuximide* averages approximately 2 μg/mL per daily dose of 1 mg/kg. A plasma concentration of 40 to 100 μg/mL usually is required for satisfactory control of absence seizures.

Therapeutic Uses

Ethosuximide is effective against absence seizures but not tonic-clonic seizures. An initial daily dose of 250 mg in children (3–6 years old) and 500 mg in older children and adults is increased by 250-mg increments at weekly intervals until seizures are adequately controlled or toxicity intervenes. Divided dosage is required occasionally to prevent nausea or drowsiness associated with once-daily dosing. The usual maintenance dose is 20 mg/kg per day. Increased caution is required if the daily dose exceeds 1500 mg in adults or 750 to 1000 mg in children. The therapeutic use of *ethosuximide* is discussed further at the end of the chapter.

Other ASDs

Acetazolamide

Acetazolamide, the prototype for carbonic anhydrase inhibitors, is discussed in Chapter 29. Its antiseizure actions have been discussed in previous editions of this textbook. Although it is sometimes effective against absence seizures, its usefulness is limited by the rapid development of tolerance. Adverse effects are minimal when it is used in moderate dosage for limited periods.

Cannabidiol

Cannabidiol (CBD) is a nonintoxicating phytocannabinoid derived from *Cannabis sativa*. CBD has been demonstrated to have significant analgesic, anti-inflammatory, anticonvulsant, and anxiolytic activities without the psychoactive effect of tetrahydrocannabinol (THC) (Costa et al., 2007; Devinsky et al., 2014). CBD is supplied as a highly purified solution. This phytocannabinoid was approved by the U.S. FDA in 2018 and by the European Commission. Chapter 26 describes the biosynthesis and pharmacological effects of CBD and other cannabinoids.

Mechanism of Action

While the mechanism(s) of action contributing to CBD's reduction of seizures is unknown, CBD has demonstrated affinity for a diverse array of targets that functionally modulate neuronal excitability. CBD has little binding affinity for the endogenous cannabinoid receptors, CB_1 or CB_2 (Thomas et al., 2007). CBD acts as a noncompetitive negative allosteric modulator of CB_1 receptor when in the presence of agonists, such as THC (Laprairie et al., 2015), as an inverse agonist of the CB_2 receptor (Dos Santos et al., 2021), and as an inhibitor of the reuptake and hydrolysis of the endocannabinoid *anandamide* (Leweke et al., 2012).

CBD's antiseizure effects likely involve one or more of its actions on a diverse array of non–cannabinoid receptor–mediated targets. These targets include:

1. Activation of the vanilloid receptor, TRPV1 (Bisogno et al., 2001)
2. Antagonism of the orphan GPR55 and GPR55-mediated elevation of presynaptic Ca^{2+} (Sylantyev et al., 2013)
3. Activation of $5HT_{1A}$ receptors (Resstel et al., 2009)
4. Competitive inhibition of the equilibrative nucleoside transporter 1 (ENT-1) to regulate adenosine tone (Devinsky et al., 2014; Gray and Whalley, 2020)

Pharmacokinetics

CBD is a highly lipophilic phytocannabinoid with low water solubility and is administered orally (Rosenberg et al., 2015). According to Perucca and Bialer (2020), the oral bioavailability of CBD under fasting conditions is approximately 6% and increases 4-fold when the medication is coadministered with a high-fat meal. CBD is primarily protein bound in the blood and preferentially deposits in brain and adipose tissue (Rodriguez de Fonseca et al., 1994). The release of CBD from adipose tissue is responsible for its prolonged terminal elimination half-life of 18 to 32 h (Devinsky et al., 2014). CBD is metabolized in the liver and intestines by CYPs. Despite undergoing first-pass hepatic metabolism, a large proportion of the CBD dose is excreted unchanged in the feces (Wall et al., 1976).

Plasma Concentrations

Taylor et al. (2018) reported pharmacokinetic data obtained from the phase I, randomized, double-blind, placebo-controlled clinical trial of CBD conducted in healthy human subjects. After single oral dosing, CBD appeared rapidly in plasma with a time to maximum plasma concentration (t_{max}) of 4 to 5 h. The major circulating metabolites were 7-carboxy-CBD > parent CBD > 7-hydroxy-CBD (active metabolite) > 6-hydroxy-CBD (a relatively minor metabolite). The maximum plasma concentration (C_{Pmax}) and area under the plasma concentration-time curve from time zero to time t (AUC) increased in a less than dose-proportional manner. CBD reached steady state after approximately 2 days with moderate accumulation. CBD elimination was multiphasic. The terminal elimination $t_{1/2}$ was approximately 60 h following twice-daily dosing of either 750 or 1500 mg of CBD, and the half-life of effect was estimated to be 10 to 17 h. Similarly, after twice-daily dosing with 1500 mg of CBD, the C_{Pmax} was 541 ng/mL and AUCτ was 3236 ng·h/mL. Intake of a high-fat meal increased CBD plasma exposure by approximately 4.5-fold with no effect of food on either the t_{max} or the terminal half-life.

Drug Interactions

CBD is metabolized by hepatic CYPs. CBD inhibits CYP2C isozymes at low concentrations and CYP3A4 isozymes at higher concentrations (Bornheim et al., 1993; Jiang et al., 2011; Stout and Cimino, 2014). CBD also inhibits other CYPs, including 2D6 (Yamaori et al., 2011) and 1A1 (Yamaori et al., 2010). Since CYP2C and CYP3A4 are induced by ASDs, such as *carbamazepine*, *topiramate*, and *phenytoin*, and inhibited by other ASDs, such as *valproate*, there is a potential for bidirectional drug-drug interactions with CBD (Patsalos et al., 2020). For example, during open-label CBD clinical trials, patients treated with CBD and the benzodiazepine *clobazam* had elevated levels of the nordesmethyl metabolite of *clobazam*, which may contribute to both the sedation and the efficacy of CBD in these studies (Devinski et al., 2014; Friedman and Devinski, 2015; Pauli et al., 2020).

Toxicity

Human CBD studies have reported CBD-induced drug-drug interactions and dose-dependent hepatic abnormalities. The most common adverse events included diarrhea, fatigue, vomiting, and somnolence (Huestis et al., 2019).

Therapeutic Uses

In the U.S., CBD is indicated for the treatment of seizures associated with Lennox-Gastaut syndrome, Dravet syndrome, and tuberous sclerosis complex in patients who are 1 year of age and older. In the European Union, CBD has received orphan drug designation from the European Medicines Agency and is approved as adjunctive therapy with *clobazam* to treat seizures associated with Lennox-Gastaut syndrome and Dravet syndrome and for adjunctive use to treat seizures associated with tuberous sclerosis complex.

Cenobamate

Cenobamate is approved for use in partial-onset seizures in adults both as a monotherapy and as an add-on agent.

Pharmacological Effects and Mechanism of Action

Cenobamate reduces seizure burden, increases the responder rate, and increases the rate of seizure freedom in patients with partial-onset seizures (Buckley et al., 2021). The mechanism of action of *cenobamate* is not fully understood but may involve enhancement of slow and fast sodium channel inactivation as well as inhibition of the persistent sodium current (Buckley et al., 2021). It also acts as a positive allosteric modulator of the $GABA_A$ channel (Sharma et al., 2020).

Pharmacokinetics

Cenobamate is orally bioavailable and at therapeutic doses has a $t_{1/2}$ of 30 to 76 h (Buckley et al., 2021). Further, *cenobamate* may possess nonlinear pharmacokinetics, particularly at higher doses at which greater concentrations can prolong $t_{1/2}$. *Cenobamate* is metabolized primarily in the liver by CYPs (2E1, 2A6, and 2B6, and to a lesser extent 2C19 and 3A4) and UGT2B7; it is excreted mainly as a glucuronides in the urine and feces. *Cenobamate* is an inducer of CYP3A4, which may increase the potential for drug-drug interactions with concomitantly administered antiseizure drugs as well as a variety of other medications including oral contraceptives.

Therapeutic Use

Multicenter, double-blind, randomized, placebo-controlled trials have demonstrated that *cenobamate* reduces focal seizures. Adjunctive use of *cenobamate* reduced partial-onset seizures in patients with pharmacoresistant epilepsy in a dose-dependent manner (Krauss et al., 2020). *Cenobamate* is initiated slowly (initial doses 12.5–25 mg) and increased gradually over several weeks (escalating to 200–400 mg daily doses).

Toxicity

Common adverse effects include somnolence, dizziness, fatigue, diplopia, and headache. Major adverse effects include risk for allergic reactions and potential to shorten QT interval (contraindicated patients with history of shortened QT). Drug reaction with eosinophilia and systemic symptoms (DRESS; also known as multiorgan hypersensitivity) can occur in some patients, particularly when dose escalations are rapid.

Ezogabine

Pharmacological Effects and Mechanisms of Action

Ezogabine, known as *retigabine* in the European Union, is a first-in-class potassium channel opener. *Ezogabine* enhances transmembrane potassium currents mediated by the KCNQ family of ion channels (i.e., Kv7.2–Kv7.5). Through its activation of the KCNQ channels, *ezogabine* may stabilize the resting membrane potential and reduce brain excitability. *In vitro* studies suggest that *ezogabine* may also enhance GABA-mediated currents.

Therapeutic Use

Ezogabine was approved in the U.S. as adjunctive treatment of focal-onset seizures in patients aged 18 years and older who have inadequately responded to alternative ASDs and for whom the benefits outweigh the risk of retinal abnormalities and visual acuity deficits. Due to addition by the FDA of a black-box warning in 2017, the drug was removed from distribution.

Pharmacokinetics

Dosing in adults is typically initiated at 300 mg/day and gradually titrated to 600 to 1200 mg/day over several weeks. *Ezogabine* is rapidly absorbed after oral administration; absorption is unaffected by food. *Ezogabine* is approximately 80% protein bound in plasma. *Ezogabine* is metabolized by glucuronidation and acetylation, with a $t_{1/2}$ of 7 to 11 h. The parent drug and its metabolites are excreted in the urine. Thus, *ezogabine* generally requires three times daily dosing. Concomitant administration of *phenytoin* or *carbamazepine* may reduce plasma concentrations of *ezogabine*; thus, an increase in *ezogabine* dosage should be considered when adding *phenytoin* or *carbamazepine*.

Toxicity

The most common adverse effects associated with *ezogabine* include dizziness, somnolence, fatigue, confusion, and blurred vision. Vertigo, diplopia, memory impairment, gait disturbance, aphasia, dysarthria, and balance problems also may occur. Serious side effects, such as skin discoloration, QT prolongation, and neuropsychiatric symptoms, including suicidal thoughts and behavior, psychosis, and hallucinations, have been reported. Due to the presence of Kv7.2–Kv7.5 in the bladder uroepithelium, *ezogabine* is also associated with urinary retention. Blue pigmentation of skin and lips occurs in as many as one-third of patients maintained on long-term *ezogabine* therapy. Chronic treatment with *ezogabine* may also cause retinal abnormalities, independent of changes in skin coloration. The FDA has changed the labeling of *ezogabine* to warn about the risks of serious adverse effects, all of which may be permanent. *Ezogabine* should thus be discontinued if clinical benefit is not achieved after careful titration. However, the discontinuation of *ezogabine* should be done gradually, while under the care of a physician.

Felbamate

Felbamate is not indicated as a first-line therapy for any type of seizure activity. Rather, *felbamate* was FDA-approved for focal seizures in patients who have inadequately responded to alternative ASDs and in patients for whom the severity of their epilepsy outweighs the substantial risk of aplastic anemia and hepatitis. The potential for such serious and life-threatening adverse effects has limited the clinical utility of *felbamate*.

Pharmacological Effects and Mechanisms of Action

Clinically relevant concentrations of *felbamate* inhibit NMDA-evoked responses and potentiate GABA-evoked responses in whole-cell, voltage-clamp recordings of cultured rat hippocampal neurons (Rho et al., 1994). This dual action on excitatory and inhibitory transmitter responses may contribute to the wide spectrum of action of the drug in seizure models.

Therapeutic Use

Despite the potential serious adverse effects, *felbamate* is used at doses ranging from 2 to 4 g/day. Randomized, double-blind, appropriately controlled clinical studies have demonstrated the efficacy of *felbamate* in patients with poorly controlled focal and secondarily generalized seizures (Sachdeo et al., 1992). *Felbamate* also reduced seizures in patients with Lennox-Gastaut syndrome (Felbamate Study Group in Lennox-Gastaut Syndrome, 1993). The clinical efficacy of this unique compound, which inhibits responses to NMDA while potentiating GABAergic neurotransmission, underscores the potential therapeutic value of identifying additional ASDs with novel mechanisms of action.

Fenfluramine

Fenfluramine was originally approved for treating obesity but was removed from the market by the FDA in 1997 due to the potential of serious adverse cardiac events due to finding of valvulopathies and primary pulmonary hypertension in significant numbers of patients. However, during this time, *fenfluramine* was also being examined for the potential use as an ASD when used at lower doses than those used for appetite suppression. Subsequently, it proved to successfully reduce seizures in children with Dravet syndrome, a severe epileptic encephalopathy often resulting from loss of function mutations in the SCN1A sodium channel subunit (Lagae et al., 2019; Nabbout et al., 2020). Low-dose *fenfluramine* was then approved by the FDA for the treatment of refractory seizures in Dravet syndrome in 2020.

Pharmacological Effects and Mechanism of Action

Fenfluramine, an amphetamine derivative, can increase extracellular levels of serotonin (5HT) by preventing uptake by the serotonin transporter SERT. In addition, fenfluramine exhibits agonist activity at the $5HT_2$, $5HT_{1D}$, and $5HT_{2C}$ receptors. *Fenfluramine* is also a positive modulator of the sigma-1 receptor (Martin et al., 2020). Such additional mechanisms may contribute to its superiority in seizure suppression compared to other 5HT uptake inhibitors.

Pharmacokinetics

Fenfluramine is orally bioavailable with a half-life of nearly 20 h. It is metabolized to norfenfluramine primarily by CYP1A2, CYP2B6, and CYP2D6. Additionally, CYP2C9, CYP2C19, and CYP3A4/5 are involved in *fenfluramine* metabolism, albeit to a lesser extent. Norfenfluramine is then deaminated and oxidized to form inactive metabolites that are excreted in the urine. *Fenfluramine* does not induce or inhibit CYPs. However, given the role of these enzymes in metabolizing *fenfluramine*, care should be taken when administering this drug with other drugs that either induce or inhibit these enzymes. This is of primary importance in Dravet syndrome, given that the standard of care can include the use of *stiripentol*, *clobazam*, and/or *valproic acid*, all drugs that increase risk for drug-drug interactions.

Therapeutic Use

Fenfluramine was approved for the use as an ASD in Dravet syndrome in patients 2 years of age and older in 2020. The initial starting and maintenance dosage is 0.1 mg/kg twice daily, although this can be increased weekly based on both efficacy and tolerability. The maximum daily maintenance dose of *fenfluramine* is 0.35 mg/kg twice daily. If patients are also taking *stiripentol* plus *clobazam*, then the maximum maintenance dosage is 0.2 mg/kg twice daily.

Toxicity

As noted above, the greatest risks for serious adverse events are for valvulopathies and primary pulmonary hypertension, risks that prompted a boxed warning from the FDA on the package insert. It is also suggested that cardiac assessments of patients be routinely performed. Of note, because the doses used for seizure control are less than those prescribed for weight loss, the risk of these adverse events appears to be reduced (Schoonjans et al., 2017). While *fenfluramine* is generally well tolerated, other adverse effects include decreased appetite, fatigue, pyrexia, diarrhea, nasopharyngitis, and decreased blood glucose (Lagae et al., 2019; Nabbout et al., 2020).

Gabapentin and Pregabalin

Gabapentin and *pregabalin* are ASDs that consist of a GABA molecule covalently bound to either a lipophilic cyclohexane ring or isobutane. *Gabapentin* was designed to be a centrally active GABA agonist, with its high lipid solubility aimed at facilitating its transfer across the blood-brain barrier.

Pharmacological Effects and Mechanisms of Action

Gabapentin inhibits tonic hind limb extension in the electroshock seizure model. Interestingly, *gabapentin* also inhibits clonic seizures induced by *pentylenetetrazol*. Its efficacy in both these tests parallels that of *valproic acid* and distinguishes it from *phenytoin* and *carbamazepine*. Despite their design as GABA agonists, neither *gabapentin* nor *pregabalin* mimics GABA when iontophoretically applied to neurons in primary culture. These compounds interact with multiple targets in neurons. They bind with high affinity to a protein in cortical membranes with an amino acid sequence identical to that of the α_2-δ1 subunit of Ca_V, the voltage-gated Ca^{2+} channel (Gee et al., 1996). *Gabapentin* also binds to the voltage-gated K^+ channels KCNQ2/3 (the molecular correlate of neuronal M-currents) and homomeric KCNQ3 and KCNQ5 (Manville and Abbott, 2018). The proposed responses to *gabapentin*'s binding at these sites are the reduction of neuronal Ca^{2+} currents (via interaction with Ca_V) and activation of KCNQ2/3 and homomeric KCNQ3 and KCNQ5 channels, leading to hyperpolarization of susceptible neurons, with concomitant reduction in excitability. Whether the anticonvulsant effects of *gabapentin* are mediated by these interactions is not yet clear. *Pregabalin* binding is reduced but not eliminated in mice carrying a mutation in the α_2δ-1 protein (Field et al., 2006), and analgesic efficacy of *pregabalin* is eliminated; whether the anticonvulsant effects of *pregabalin* are also eliminated was not reported.

Pharmacokinetics

Gabapentin and *pregabalin* are absorbed after oral administration and are not metabolized in humans. These compounds are not bound to plasma proteins and are excreted unchanged, mainly in the urine. Their half-lives, when used as monotherapy, approximate 6 h. These compounds have no known interactions with other ASDs.

Therapeutic Uses

Gabapentin and *pregabalin* are effective for focal seizures, with and without progression to bilateral tonic-clonic seizures, when used in addition to other ASDs

Double-blind placebo-controlled trials of adults with refractory focal seizures demonstrated that addition of *gabapentin* or *pregabalin* to other ASDs is superior to placebo (French et al., 2003; Sivenius et al., 1991). A double-blind study of *gabapentin* monotherapy (900 or 1800 mg/day) disclosed that *gabapentin* was equivalent to *carbamazepine* (600 mg/day) for newly diagnosed focal or generalized epilepsy (Chadwick et al., 1998). *Gabapentin* also is being used for the treatment of migraine, chronic pain, and bipolar disorder.

Gabapentin usually is effective in doses of 900 to 1800 mg daily in three doses, although 3600 mg may be required in some patients to achieve reasonable seizure control. Therapy usually is begun with a low dose (300 mg once on the first day), which is increased in daily increments of 300 mg until an effective dose is reached.

Toxicity

Overall, *gabapentin* is well tolerated with the most common adverse effects of somnolence, dizziness, ataxia, and fatigue. These effects usually are mild to moderate in severity but resolve within 2 weeks of onset during continued treatment. With respect to use during pregnancy, *gabapentin* and *pregabalin* can cause fetal adverse effects in animal studies, but there are no adequate human studies; potential benefits may warrant use despite potential risks.

Lacosamide

Lacosamide is a stereo-selective enantiomer of the amino acid, L-serine. This functionalized amino acid was approved by the FDA in 2008 as adjunctive therapy for focal-onset seizures in patients 17 years of age or older. The FDA assigned *lacosamide* a Controlled Substance Act Schedule V designation, meaning it has a low potential for abuse. However, abuse of *lacosamide* may lead to limited physical or psychological dependence compared to the substances designated as Controlled Substance Act Schedule IV.

Pharmacological Effects and Mechanism of Action

Lacosamide is the first ASD to enhance the slow inactivation of voltage-gated Na^+ channels and to limit sustained repetitive firing, the neuronal firing pattern characteristic of focal seizures. *Lacosamide* also binds collapsin response mediator protein 2 (crmp-2), a phosphoprotein involved in neuronal differentiation and axon outgrowth, but the contribution of crmp-2 to *lacosamide*'s antiseizure efficacy remains unclear. Rather, *lacosamide*'s antiseizure mechanism is likely mediated by its enhancement of slow inactivation of Na^+ channels. *Lacosamide* was extensively evaluated by the Epilepsy Therapy Screening Project and found to be highly effective in numerous preclinical animal models of seizures and epilepsy, including maximal electroshock, hippocampal kindling, and the Frings and 6-Hz models, giving *lacosamide* a unique preclinical profile compared to other Na^+ channel blockers.

Pharmacokinetics

An injectable formulation of *lacosamide* is available for short-term use when oral administration is not possible. *Lacosamide* neither induces nor inhibits CYPs and thus has a low potential to induce harmful drug-drug interactions. As a monotherapy for the treatment of focal seizures, the initial recommended dose is 50 to 100 mg twice daily and, depending on patient response, may be increased at weekly intervals by 50 mg twice daily to a recommended maintenance dose of 100 to 200 mg twice daily, or 200 to 400 mg/day. Peak *lacosamide* plasma concentrations occur approximately 1 to 4 h after oral administration, and food consumption does not affect the absorption. *Lacosamide* has a $t_{1//2}$ of approximately 12 to 16 h and is 95% excreted in urine, 40% as the parent drug. The major metabolite, *O*-desmethyl-lacosmide, is inactive.

Therapeutic Use

Lacosamide has been approved for both monotherapy and add-on therapy for focal-onset seizures in patients aged 17 years and older. The pharmacological profile is advantageous for hospitalized patients, since it is available in an intravenous formulation, has minimal hepatic metabolism, and has no adverse respiratory effects. In addition, double-blind, placebo-controlled studies of adults with refractory focal seizures suggest that addition of *lacosamide* to other ASDs is superior to the addition of placebo.

Toxicity

Lacosamide is generally well tolerated. Although it has been associated with a brief (6 msec) prolongation of the PR interval, recent studies in healthy patients find that *lacosamide* does not appear to prolong the QT interval. No major adverse effects have been reported, although minor adverse effects include headache, dizziness, double vision, nausea, vomiting, fatigue, tremor, loss of balance, and somnolence. In addition, like most currently available ASDs, *lacosamide* may contribute to suicidal ideations and suicide. As a consequence, the FDA has mandated a boxed warning for this concern.

Lamotrigine

Lamotrigine is a phenyltriazine derivative initially developed as an antifolate agent based on the incorrect idea that reducing folate would effectively combat seizures. Structure-activity studies have since indicated that its effectiveness as an ASD is unrelated to its antifolate properties (Macdonald and Greenfield, 1997).

Pharmacological Effects and Mechanisms of Action

Lamotrigine suppresses tonic hind limb extension in the maximal electroshock model and focal and secondarily generalized seizures in the kindling model but does not inhibit clonic motor seizures induced by *pentylenetetrazol*. *Lamotrigine* blocks sustained repetitive firing of mouse spinal cord neurons and delays the recovery from inactivation of recombinant Na^+ channels, mechanisms similar to those of *phenytoin* and *carbamazepine* (Xie et al., 1995). This may explain *lamotrigine*'s actions on focal and secondarily generalized seizures. However, as mentioned below (see Therapeutic Use section), *lamotrigine* is effective against a broader spectrum of seizures than *phenytoin* and *carbamazepine*, suggesting that *lamotrigine* may have actions in addition to regulating recovery from inactivation of Na^+ channels. The mechanisms underlying its broad spectrum of actions are incompletely understood. One possibility is suggested by *lamotrigine*'s inhibition of glutamate release in rat cortical slices treated with *veratridine*, a Na^+ channel activator, raising the possibility that *lamotrigine* inhibits synaptic release of glutamate by acting at Na^+ channels themselves.

Pharmacokinetics

Lamotrigine is completely absorbed from the GI tract and is metabolized primarily by glucuronidation. The plasma $t_{1/2}$ of a single dose is 24 to 30 h. Administration of *phenytoin*, *carbamazepine*, or *phenobarbital* reduces the $t_{1/2}$ and plasma concentrations of *lamotrigine*. Conversely, addition of *valproate* markedly increases plasma concentrations of *lamotrigine*, likely by inhibiting glucuronidation. Addition of *lamotrigine* to *valproic acid* produces a reduction of *valproate* concentrations by approximately 25% over a few weeks. Concurrent use of *lamotrigine* and *carbamazepine* is associated with increases of the 10,11-epoxide of *carbamazepine* and clinical toxicity in some patients.

Therapeutic Use

Lamotrigine is useful for monotherapy and add-on therapy of focal and secondarily generalized tonic-clonic seizures in adults and Lennox-Gastaut syndrome in both children and adults. Lennox-Gastaut syndrome is a disorder of childhood characterized by multiple seizure types, intellectual disability, and refractoriness to antiseizure medication.

Lamotrigine monotherapy in newly diagnosed focal or generalized tonic-clonic seizures is equivalent to monotherapy with *carbamazepine* or *phenytoin* (Brodie et al., 1995; Steiner et al., 1999). A double-blind, placebo-controlled trial of addition of *lamotrigine* to existing ASDs further demonstrated effectiveness of *lamotrigine* against tonic-clonic seizures and drop attacks in children with the Lennox-Gastaut syndrome (Motte et al., 1997). *Lamotrigine* was also superior to placebo in a double-blind study of children with newly diagnosed absence epilepsy (Frank et al., 1999).

Patients who are already taking a hepatic enzyme–inducing ASD (e.g., *carbamazepine*, *phenytoin*, *phenobarbital*, or *primidone*, but not *valproate*) should be given *lamotrigine* initially at 50 mg/day for 2 weeks. The dose is increased to 50 mg twice per day for 2 weeks and then increased in increments of 100 mg/day each week up to a maintenance dose of 300 to 500 mg/day divided into two doses. For patients taking *valproate* in addition to an enzyme-inducing ASD, the initial dose should be 25 mg every other day for 2 weeks, followed by an increase to 25 mg/day for 2 weeks; the dose then can be increased by 25 to 50 mg/day every 1 to 2 weeks up to a maintenance dose of 100 to 150 mg/day divided into two doses.

Toxicity

The most common adverse effects are dizziness, ataxia, blurred or double vision, nausea, vomiting, and rash when *lamotrigine* is added to another ASD. A few cases of Stevens-Johnson syndrome and disseminated intravascular coagulation have been reported. The incidence of serious rash in pediatric patients (~0.8%) is higher than in the adult population (0.3%).

Levetiracetam and Brivaracetam

Levetiracetam is a pyrrolidine, the racemically pure S-enantiomer of α-ethyl-2-oxo-1-pyrrolidineacetamide, and FDA-approved for adjunctive therapy for myoclonic, focal-onset, and primary generalized tonic-clonic seizures in adults and children as young as 4 years old. *Brivaracetam*, an analogue of *levetiracetam*, was FDA-approved in 2016 as an adjunctive therapy for focal-onset seizures in patients with epilepsy aged 16 years and older.

Pharmacological Effects and Mechanism of Action

Levetiracetam exhibits a novel pharmacological profile: It inhibits focal and secondarily generalized tonic-clonic seizures in the kindling model yet is ineffective against maximum electroshock- and *pentylenetetrazol*-induced seizures, findings consistent with clinical effectiveness against focal and secondarily generalized tonic-clonic seizures. The mechanism by which *levetiracetam* exerts these antiseizure effects is not fully understood. However, the correlation between binding affinity of *levetiracetam* and its analogues and their potency toward audiogenic seizures suggests that the synaptic vesicle protein, SV2A, mediates the anticonvulsant effects of *levetiracetam* (Rogawski and Bazil, 2008). Hypothetically, binding of *levetiracetam* to SV2A might affect cellular function or neuronal excitability by modifying the release of glutamate and GABA through an action on vesicular function. In addition, *levetiracetam* inhibits calcium fluxes from intracellular stores and via N-type calcium channels.

Brivaracetam is a novel, high-affinity (selective) ligand for SVA2 that also demonstrates inhibitory effects on neuronal voltage-gated sodium channels (Kenda et al., 2004; Zona et al., 2010). Preclinical studies suggest a broad spectrum of anticonvulsant protection.

Pharmacokinetics

Levetiracetam is rapidly and almost completely absorbed after oral administration and is not bound to plasma proteins. The plasma half-life is 6 to 8 h but may be longer in elderly patients. Ninety-five percent of the drug and its inactive metabolite are excreted in the urine, 65% of which is unchanged drug; 24% of the drug is metabolized by hydrolysis of the acetamide group. It neither induces nor is a high-affinity substrate for CYPs or glucuronidases and thus is devoid of known interactions with other ASDs, oral contraceptives, or anticoagulants. Oral, extended-release, and intravenous formulations are all available. In comparison, *brivaracetam* is rapidly absorbed and well tolerated with an elimination $t_{1/2}$ of approximately 7 to 8 h.

Therapeutic Use

Levetiracetam is marketed for the adjunctive treatment of focal seizures in adults and children, for primary generalized tonic-clonic seizures, and for myoclonic seizures of juvenile myoclonic epilepsy. It is available in tablet (10, 25, 50, 75, or 100 mg), oral solution (10 mg/mL), or injectable form (50 mg/5 mL). Adult dosing is initiated at 500 to 1000 mg/day and increased every 2 to 4 weeks by 1000 mg to a maximum dose of 3000 mg/day. The drug is administered twice daily. Double-blind, placebo-controlled trials of adults with either refractory focal seizures or uncontrolled generalized tonic-clonic seizures associated with idiopathic generalized epilepsy revealed that addition of *levetiracetam* to other antiseizure medications was superior to placebo. *Levetiracetam* also has efficacy as adjunctive therapy for refractory generalized myoclonic seizures (Andermann et al., 2005). There is insufficient evidence on its use as monotherapy for focal or generalized epilepsy. The recommended starting dose for *brivaracetam* is 50 mg twice daily, which based on patient response and tolerability may be adjusted to either 25 mg twice daily or 100 mg twice daily, as needed.

Toxicity

Both *levetiracetam* and *brivaracetam* are well tolerated. The most frequently reported adverse effects associated with *levetiracetam* are somnolence, asthenia, ataxia, and dizziness. Behavioral and mood changes are serious but less common. For *brivaracetam*, the most common adverse effects are similarly mild and include somnolence, sedation, dizziness, and GI upset. In patients with hepatic insufficiency, dose adjustment may be required with *brivaracetam* to 25 mg twice daily and a maximal dosage of 75 mg twice daily. Hypersensitivity reactions may occur.

Perampanel

Pharmacological Effects and Mechanisms of Action

Perampanel is a first-in-class selective, noncompetitive antagonist of the AMPA-type ionotropic glutamate receptor (see Table 16–2; Bialer and White, 2010; Stephen and Brodie, 2011). Unlike NMDA antagonists, which shorten the duration of repetitive discharges, AMPA receptor antagonists prevent repetitive neuronal firing. Preclinical studies show a broad spectrum of activity in both acute and chronic seizure models, indicating that *perampanel* reduces fast excitatory signaling critical to the seizure generation (Tortorella et al., 1997) and spread (Namba et al., 1994; Rogawski and Donevan, 1999). However, data suggest that *perampanel* contributes a greater inhibitory effect on seizure propagation than on their initiation, since afterdischarge firing persists despite complete inhibition of behavioral seizures (Hanada et al., 2011).

Therapeutic Use

Perampanel is FDA-approved as an adjunctive therapy for the treatment of focal-onset seizures in patients 12 years and older with or without secondarily generalized seizures. The recommended oral starting dose is 2 mg once daily, titrated to a maximal dose of 4 to 12 mg/day at bedtime.

Pharmacokinetics

Perampanel is absorbed well after oral administration, with a plasma $t_{1/2}$ of approximately 105 h, allowing it to be administered once daily. It is 95% bound to plasma protein, mainly albumin, and is metabolized by hepatic oxidation and glucuronidation. A linear relationship between *perampanel* dose and plasma concentration has been reported over the dose range of 2 to 12 mg/day.

Drug Interactions

Primary metabolism is mediated by hepatic CYP3A, so specific drug interactions and dose adjustments may need to be considered. For example, *perampanel* may decrease the effectiveness of progesterone-containing contraceptives, *carbamazepine*, *clobazam*, *lamotrigine*, and *valproic acid*, but it may increase exposure to *oxcarbazepine*. Furthermore, serum *perampanel* may be decreased when taken with *carbamazepine*, *oxcarbazepine*, and *topiramate*.

Toxicity

Common adverse effects include somnolence, anxiety, confusion, imbalance, double vision, dizziness, GI distress or nausea, imbalance, and weight gain. Rare but serious adverse behavioral reactions, including hostility, aggression, and suicidal thoughts and/or behaviors, independent of clinical history of psychiatric disorder, have also been reported.

Rufinamide

Rufinamide is novel triazole derivative that is structurally unrelated to other marketed ASDs. It is FDA-approved for adjunctive treatment of seizures related to Lennox-Gastaut syndrome in children aged 4 years and older and adults.

Pharmacological Effects and Mechanism of Action

Rufinamide enhances slow inactivation of voltage-gated Na^+ channels and limits sustained repetitive firing, the firing pattern characteristic of focal seizures. The complete mechanism of action of *rufinamide* remains unclear.

Pharmacokinetics

Rufinamide is well absorbed orally, minimally protein bound in plasma, and reaches peak plasma concentrations approximately 4 to 6 h after oral administration. The $t_{1/2}$ is approximately 6 to 10 h. *Rufinamide* is metabolized by carboxylesterases, and the products are excreted in the urine.

Therapeutic Use

Rufinamide is effective against all seizure phenotypes in Lennox-Gastaut syndrome. In adults, 400 to 800 mg/day *rufinamide* is initially administered in two equal doses. Doses are then adjusted every other day by 10 mg/kg to whichever is less between maximal doses of 45 mg/kg per day or 3200 mg/day. Children are initiated at 10 mg/kg per day divided into two equal daily doses that are increased to the lesser of 45 mg/kg per day or 3200 mg/day.

Toxicity

Common adverse effects include headache, dizziness, somnolence, fatigue, and nausea.

Stiripentol

Therapeutic Use

Stiripentol is an aromatic alcohol that is structurally unrelated to any other ASDs. Although *stiripentol* was granted orphan drug status for the treatment of Dravet syndrome in 2008, it did not receive FDA approval until 2018. *Stiripentol* is used clinically in conjunction with *clobazam* and *valproate* as an adjunctive therapy for refractory generalized tonic-clonic seizures in patients with severe myoclonic epilepsy in infancy (Dravet syndrome) whose seizures are not adequately controlled with *clobazam* and *valproate* (Aneja and Sharma, 2013; Plsker, 2012). A randomized, double-blind, placebo-controlled clinical trial demonstrated that adjunctive *stiripentol* in children with Dravet syndrome who failed to respond to *valproate* and *clobazam* produced a 71% response rate (Chiron et al., 2000; Nabbout and Chiron, 2012). *Stiripentol* will also reduce the frequency and severity of tonic-clonic seizures as well as status epilepticus in infants and children with a variety of epilepsy syndromes (Inoue et al., 2009; Perez et al., 1999; Rey et al., 1999).

Pharmacological Effects and Mechanisms of Action

Although the exact nature of its antiseizure mechanism is not clear, *stiripentol* may increase central levels of GABA, the major inhibitory neurotransmitter in mammalian brain, by inhibition of synaptosomal uptake of GABA and/or by inhibition of GABA transaminase. *Stiripentol* has also been shown to enhance $GABA_A$ receptor–mediated neurotransmission and increase the mean open duration of $GABA_A$ receptor chloride channels in a barbiturate-like fashion in immature rat hippocampus (Fisher, 2011; Quilichini et al., 2006).

Drug Interactions

Stiripentol has diverse pharmacokinetic and pharmacodynamic interactions with other concomitantly administered drugs. It is a potent inhibitor of CYPs 3A4, 1A2, and 2C19. Thus, adjunctively administered ASDs, such as *carbamazepine*, *sodium valproate*, *phenytoin*, *phenobarbital*, and other benzodiazepines, may require dose adjustments due to the potent inhibition of their hepatic metabolism (Tran et al., 1997). *Stiripentol* can increase *clobazam* and *valproate* concentrations by 2- to 3-fold when given concomitantly, and reduction of their doses may be necessary to avoid toxicity. The initiation of adjunctive therapy with *stiripentol* is complex and should be undertaken gradually with frequent plasma monitoring for both the parent ASDs as well as their active metabolites. Plasma monitoring is important to inform reductions in concomitant ASDs as needed, based on patient response.

Pharmacokinetics

Stiripentol is quickly absorbed, with a time to peak plasma concentration of approximately 1.5 h, and is highly protein bound in plasma. *Stiripentol*'s kinetics are nonlinear. Plasma clearance decreases markedly at high doses and after repeated administration, possibly due to inhibition of the CYPs responsible for phase 1 of its metabolism. The elimination $t_{1/2}$ ranges from 4 to 13 h, increasing in a dose-dependent manner. A large fraction of the drug (73%) is ultimately metabolized to glucuronides and eliminated in the urine; most of the remainder is excreted unchanged in the feces.

Toxicity

The most commonly reported adverse effects in patients on *stiripentol* include anorexia, weight loss, insomnia, drowsiness, ataxia, hypotonia, and dystonia.

Tiagabine

Tiagabine is a derivative of nipecotic acid and approved by the FDA as adjunct therapy for focal seizures in adults.

Pharmacological Effects and Mechanism of Action

Tiagabine inhibits the GABA transporter, GAT-1, and thereby reduces GABA uptake into neurons and glia. In CA1 neurons of the hippocampus, *tiagabine* increases the duration of inhibitory synaptic currents, findings consistent with prolonging the effect of GABA at inhibitory synapses through reducing its reuptake by GAT-1. *Tiagabine* inhibits maximum electroshock seizures and both limbic and secondarily generalized tonic-clonic seizures in the kindling model, results suggestive of clinical efficacy against focal and tonic-clonic seizures. Paradoxically, *tiagabine* has been associated with the occurrence of seizures in patients without epilepsy, and off-label use of the drug is discouraged.

Pharmacokinetics

Tiagabine is rapidly absorbed after oral administration, extensively bound to serum or plasma proteins, and metabolized mainly in the liver, predominantly by CYP3A. Its $t_{1/2}$ of approximately 8 h is shortened by 2 to 3 h when coadministered with hepatic enzyme–inducing drugs such as *phenobarbital*, *phenytoin*, or *carbamazepine*.

Therapeutic Use

Double-blind, placebo-controlled trials have established *tiagabine*'s efficacy as add-on therapy of refractory focal seizures with or without secondary generalization. Its efficacy as monotherapy for newly diagnosed or refractory focal and generalized epilepsy has not been established.

Toxicity

The principal adverse effects include dizziness, somnolence, and tremor; they seem mild to moderate in severity and appear shortly after initiation of therapy. *Tiagabine* and other drugs that enhance effects of synaptically released GABA can facilitate spike-and-wave discharges in animal models of absence seizures. Case reports suggest that *tiagabine* treatment of patients with a history of spike-and-wave discharges causes exacerbations of their EEG abnormalities. Thus, *tiagabine* may be contraindicated in patients with generalized absence epilepsy.

Topiramate

Topiramate is a sulfamate-substituted monosaccharide that is FDA-approved as initial monotherapy (in patients at least 10 years old) and as adjunctive therapy (for patients as young as 2 years of age) for focal-onset or primary generalized tonic-clonic seizures, for Lennox-Gastaut syndrome in patients 2 years of age and older, and for migraine headache prophylaxis in adults.

Pharmacological Effects and Mechanisms of Action

Topiramate reduces voltage-gated Na^+ currents in cerebellar granule cells and may act on the inactivated state of the channel similar to *phenytoin*.

In addition, *topiramate* activates a hyperpolarizing K$^+$ current, enhances postsynaptic GABA$_A$-receptor currents, and limits activation of the AMPA-kainate subtype(s) of glutamate receptor (GluA and GluK receptors; see Table 16–2). *Topiramate* is a weak carbonic anhydrase inhibitor. *Topiramate* inhibits maximal electroshock- and *pentylenetetrazol*-induced seizures as well as focal and secondarily generalized tonic-clonic seizures in the kindling model, findings predictive of a broad spectrum of antiseizure actions clinically.

Pharmacokinetics

Topiramate is rapidly absorbed after oral administration, exhibits little (10%–20%) binding to plasma proteins, and is mainly excreted unchanged in the urine. The remainder undergoes metabolism by hydroxylation, hydrolysis, and glucuronidation with no single metabolite accounting for greater than 5% of an oral dose. Its $t_{1/2}$ is approximately 1 day. Reduced estradiol plasma concentrations occur with concurrent *topiramate*, suggesting the need for higher doses of oral contraceptives when coadministered with *topiramate*.

Therapeutic Use

Topiramate is equivalent to *valproate* and *carbamazepine* in children and adults with newly diagnosed focal and primary generalized epilepsy (Privitera et al., 2003). *Topiramate* is also effective as monotherapy for refractory focal epilepsy (Sachdeo et al., 1997) and refractory generalized tonic-clonic seizures (Biton et al., 1999) and is significantly more effective than placebo against both drop attacks and tonic-clonic seizures in patients with Lennox-Gastaut syndrome (Sachdeo et al., 1999).

Toxicity

Topiramate is well tolerated. The most common adverse effects are somnolence, fatigue, weight loss, and nervousness. It may precipitate renal calculi (kidney stones), likely due to inhibition of carbonic anhydrase. *Topiramate* has been associated with cognitive impairment, and patients may complain about a change in the taste of carbonated beverages.

Valproic Acid

The antiseizure properties of *valproic acid* were discovered serendipitously when it was employed as a vehicle for other compounds that were being screened for antiseizure activity.

Chemistry

Valproic acid (*n*-dipropylacetic acid) is a simple branched-chain carboxylic acid. Certain other branched-chain carboxylic acids have potencies similar to that of *valproic acid* in antagonizing *pentylenetetrazol*-induced convulsions. However, increasing the number of carbon atoms to nine introduces marked sedative properties. Straight-chain carboxylic acids have little or no activity.

Pharmacological Effects

Valproic acid is strikingly different from *phenytoin* and *ethosuximide* in that it is effective in inhibiting seizures in a variety of models. Like *phenytoin* and *carbamazepine*, *valproate* inhibits tonic hind limb extension in maximal electroshock seizures and kindled seizures at nontoxic doses. Like *ethosuximide*, *valproic acid* at subtoxic doses inhibits clonic motor seizures induced by *pentylenetetrazol*. Its efficacy in diverse models parallels its efficacy against absence as well as focal and generalized tonic-clonic seizures in humans.

Mechanism of Action

Valproic acid produces effects on isolated neurons similar to those of *phenytoin* and *ethosuximide* (see Table 20–2). At therapeutically relevant concentrations, *valproate* inhibits sustained repetitive firing induced by depolarization of mouse cortical or spinal cord neurons (McLean and Macdonald, 1986b). The action is similar to that of both *phenytoin* and *carbamazepine* and appears to be mediated by a prolonged recovery of voltage-activated Na$^+$ channels from inactivation. *Valproic acid* does not modify neuronal responses to iontophoretically applied GABA. In neurons isolated from the nodose ganglion, *valproate* also produces small reductions of T-type Ca^{2+} currents (Kelly et al., 1990) at clinically relevant but slightly higher concentrations than those that limit sustained repetitive firing; this effect on T-type currents is similar to that of *ethosuximide* in thalamic neurons (Coulter et al., 1989). Together, these actions of limiting sustained repetitive firing and reducing T-type currents may contribute to the effectiveness of *valproic acid* against focal and tonic-clonic seizures and absence seizures, respectively.

Another potential mechanism that may contribute to *valproate*'s antiseizure actions involves metabolism of GABA. Although *valproate* has no effect on responses to GABA, it does increase the amount of GABA that can be recovered from the brain after the drug is administered to animals. *In vitro*, *valproate* can stimulate the activity of the GABA synthetic enzyme, glutamic acid decarboxylase, and inhibit GABA degradative enzymes, GABA transaminase, and succinic semialdehyde dehydrogenase. Thus far, it has been difficult to relate the increased GABA levels to the antiseizure activity of *valproate*.

Finally, it is known that *valproic acid* is a potent inhibitor of histone deacetylase. Thus, some of its antiseizure activity may be due to its ability to modulate gene expression through this mechanism.

Pharmacokinetic Properties

Valproic acid is absorbed rapidly and completely after oral administration. Peak plasma concentration occurs in 1 to 4 h, although this can be delayed for several hours if the drug is administered in enteric-coated tablets or is ingested with meals.

The apparent volume of distribution for *valproate* is approximately 0.2 L/kg. Its extent of binding to plasma proteins is usually approximately 90%, but the fraction bound is reduced as the total concentration of *valproate* is increased through the therapeutic range. Although concentrations of *valproate* in CSF suggest equilibration with free drug in the blood, there is evidence for carrier-mediated transport of *valproate* both into and out of the CSF.

The majority of *valproate* (95%) undergoes hepatic metabolism, with less than 5% excreted unchanged in urine. Its hepatic metabolism occurs mainly by UGT enzymes and β-oxidation. *Valproate* is a substrate for CYP2C9 and CYP2C19, but metabolism by these enzymes accounts for a relatively minor portion of its elimination. Some of the drug's metabolites, notably 2-propyl-2-pentenoic acid and 2-propyl-4-pentenoic acid, demonstrate near equipotent antiseizure activity as the parent compound; however, only the 2-en-valproate accumulates in plasma and brain to a potentially significant extent. The $t_{1/2}$ of *valproate* is approximately 15 h but is reduced in patients taking other antiepileptic drugs.

Toxicity

The most common side effects are transient GI symptoms, including anorexia, nausea, and vomiting (~16%). Effects on the CNS include sedation, ataxia, and tremor; these symptoms occur infrequently and usually respond to a decrease in dosage. Rash, alopecia, and stimulation of appetite have been observed occasionally, and weight gain has been seen with chronic *valproic acid* treatment in some patients. *Valproic acid* has several effects on hepatic function; elevation of hepatic transaminases in plasma is observed in up to 40% of patients and often occurs asymptomatically during the first several months of therapy.

A rare complication is a fulminant hepatitis that is frequently fatal (Dreifuss et al., 1989). Pathological examination reveals a microvesicular steatosis without evidence of inflammation or hypersensitivity reaction. Children below 2 years of age with other medical conditions who were given multiple ASDs were especially likely to suffer fatal hepatic injury. At the other extreme, there were no deaths reported for patients over the age of 10 years who received only *valproate*. Acute pancreatitis and hyperammonemia have been frequently associated with the use of *valproic acid*. *Valproic acid* can also produce teratogenic effects such as neural tube defects.

Plasma Drug Concentrations

Valproate plasma concentrations associated with therapeutic effects are approximately 30 to 100 μg/mL. However, there is a poor correlation between the plasma concentration and efficacy. There appears to be a threshold at approximately 30 to 50 μg/mL; this is the concentration at which binding sites on plasma albumin begin to become saturated.

Drug Interactions

Valproate inhibits the metabolism of drugs that are substrates for CYP2C9, including *phenytoin* and *phenobarbital*. *Valproate* also inhibits UGTs and thus inhibits the metabolism of *lamotrigine* and *lorazepam*. A high proportion of *valproate* is bound to albumin, and the high molar concentrations of *valproate* in the clinical setting result in *valproate* displacing *phenytoin* and other drugs from albumin. With respect to *phenytoin* in particular, *valproate*'s inhibition of the drug's metabolism is exacerbated by displacement of *phenytoin* from albumin. The concurrent administration of *valproate* and *clonazepam* has been associated with the development of absence status epilepticus; however, this complication appears to be rare.

Therapeutic Uses

Valproate is a broad-spectrum ASD that is effective in the treatment of absence, myoclonic, focal, and tonic-clonic seizures. The initial daily dose usually is 15 mg/kg, increased at weekly intervals by 5 to 10 mg/kg per day to a maximum daily dose of 60 mg/kg. Divided doses should be given when the total daily dose exceeds 250 mg. The therapeutic uses of *valproate* in epilepsy are discussed further at the end of this chapter.

Vigabatrin

Vigabatrin is FDA-approved as adjunctive therapy of refractory focal seizures with impaired awareness in adults. In addition, *vigabatrin* is designated as an orphan drug for treatment of infantile spasms (see below).

Pharmacological Effects and Mechanism of Action

Vigabatrin is a structural analogue of GABA that irreversibly inhibits the major degradative enzyme for GABA, GABA transaminase, thereby leading to increased concentrations of GABA in the brain. *Vigabatrin*'s mechanism of action is thought to involve effects of increased extracellular GABA concentrations in the brain and enhancement of GABA-mediated inhibition.

Pharmacokinetics

Although *vigabatrin* has a $t_{1/2}$ of only 6 to 8 h, the pharmacodynamics effects are prolonged and do not correlate well with plasma $t_{1/2}$. Adult dosing is generally initiated orally at 500 mg twice daily and then increased in 500-mg increments weekly to 1.5 g twice daily.

Therapeutic Use

A 2-week, randomized, single-masked clinical trial of *vigabatrin* for infantile spasms in children less than 2 years old revealed time- and dose-dependent increases in responders, evident as freedom from spasms for 7 consecutive days. The subset of children in whom infantile spasms were caused by tuberous sclerosis was particularly responsive to *vigabatrin*.

Toxicity

Due to progressive and permanent bilateral vision loss, *vigabatrin* must be reserved for patients who have failed several alternative therapies, and its availability is limited. The most common side effects (>10% patients) include weight gain, concentric visual field constriction, fatigue, somnolence, dizziness, hyperactivity, and seizures.

Zonisamide

Zonisamide has a small ringed structure related to sulfonamide antibiotics. It is FDA-approved as adjunctive therapy of focal seizures in children and adults aged 12 years or older.

Pharmacological Effects and Mechanism of Action

Zonisamide inhibits the sustained, repetitive firing of spinal cord neurons, presumably by prolonging the inactivated state of voltage-gated Na^+ channels in a manner similar to actions of *phenytoin* and *carbamazepine* and preventing neurotransmitter release. In addition, *zonisamide* also inhibits T-type Ca^{2+} currents and prevents influx of calcium. *Zonisamide* has been shown to scavenge free radicals, which may contribute to its neuroprotective effects.

Pharmacokinetics

Zonisamide is almost completely absorbed after oral administration, has a long $t_{1/2}$ (~60 h), is approximately 40% bound to plasma protein, and has linear kinetics at doses ranging from 100 to 400 mg. *Zonisamide* is metabolized by hepatic CYPs and glucuronidases. Approximately 85% of an oral dose is excreted in the urine, principally as unmetabolized *zonisamide* and a glucuronide of sulfamoylacetyl phenol, which is a product of metabolism by CYP3A4. As such, *phenobarbital*, *phenytoin*, and *carbamazepine* decrease the plasma concentration/dose ratio of *zonisamide*, whereas *lamotrigine* increases this ratio. Conversely, *zonisamide* has little effect on the plasma concentrations of other ASDs.

Therapeutic Use

Double-blind, placebo-controlled studies of patients with refractory focal seizures demonstrated that addition of *zonisamide* to other drugs was superior to placebo. There is insufficient evidence for its efficacy as monotherapy for newly diagnosed or refractory epilepsy.

Toxicity

Overall, *zonisamide* is well tolerated. The most common adverse effects include somnolence, dizziness, cognitive impairment, ataxia, anorexia, nervousness, and fatigue. Potentially serious skin rashes are rare but may occur. Approximately 1% of individuals develop renal calculi during treatment with *zonisamide*, which may relate to its ability to inhibit carbonic anhydrase. Patients with predisposing conditions (e.g., renal disease, severe respiratory disorders, diarrhea, surgery, ketogenic diet) may be at greater risk for metabolic acidosis while taking *zonisamide*. The risk of *zonisamide*-induced metabolic acidosis also appears to be more frequent and severe in younger patients. Measurement of serum bicarbonate prior to initiating therapy and periodically thereafter, even in the absence of symptoms, is recommended. Lastly, spontaneous abortions and congenital abnormalities have been reported in female patients of childbearing age receiving polytherapy including *zonisamide* at twice the rate (7%) of the healthy control population (2%–3%).

General Principles and Choice of Drugs for the Therapy of the Epilepsies

Early diagnosis and treatment of seizure disorders with a single appropriate agent offers the best prospect of achieving prolonged seizure-free periods with the lowest risk of toxicity. An attempt should be made to determine the cause of the epilepsy with the hope of discovering a correctable lesion, either structural or metabolic. The drugs commonly used for distinct seizure types are listed in Table 20–1. The cost/benefit ratio of the efficacy and the unwanted effects of a given drug are considered to determine which particular drug is optimal for a given patient.

The first decision to make is whether and when to initiate treatment (French and Pedley, 2008). For example, it may not be necessary to initiate therapy after an isolated tonic-clonic seizure in a healthy young adult who lacks a family history of epilepsy and who has a normal neurological exam, a normal EEG, and a normal brain MRI scan. The odds of seizure recurrence in the next year (15%) are similar to the risk of a drug reaction sufficiently severe to warrant discontinuation of medication (Bazil and Pedley, 1998). On the other hand, a similar seizure occurring in an individual with a positive family history of epilepsy, an abnormal neurological exam, an abnormal EEG, and an abnormal MRI carries a risk of recurrence approximating 60%, odds that favor initiation of therapy.

Unless extenuating circumstances such as status epilepticus exist, only monotherapy should be initiated. Initial dosing should target steady-state plasma drug concentrations within at least the lower portion of the range associated with clinical efficacy. At the same time, the initial dose should be as low as possible to minimize dose-related adverse effects. Dosage is increased at appropriate intervals as required for control of seizures or as limited by toxicity. Such adjustment should be assisted by monitoring of drug concentrations in plasma. Compliance with a properly selected, single drug in maximal tolerated dosage results in complete control of seizures in approximately 50% of patients. If a seizure occurs despite optimal drug levels, the physician should assess the presence of potential precipitating factors such as sleep deprivation, a concurrent febrile illness, or drugs (e.g., large amounts of caffeine or over-the-counter medications that can lower the seizure threshold).

If compliance has been confirmed yet seizures persist, another drug should be substituted. Unless serious adverse effects of the drug dictate otherwise, dosage always should be reduced gradually to minimize risk of seizure recurrence. In the case of focal seizures in adults, the diversity of available drugs permits selection of a second drug that acts by a different mechanism (see Table 20–2). Among previously untreated patients, 47% became seizure free with the first drug and an additional 14% became seizure free with a second or third drug (Kwan and Brodie, 2000).

If therapy with a second single drug also is inadequate, combination therapy is warranted. This decision should not be taken lightly, because most patients obtain optimal seizure control with the fewest unwanted effects when taking a single drug. Nonetheless, some patients will not be controlled adequately without the simultaneous use of two or more ASDs. No properly controlled studies have systematically compared one particular drug combination with another. The chances of complete control with this approach are not high; Kwan and Brodie (2000) found that epilepsy was controlled by treatment with two drugs in only 3% of patients. Moreover, a rational approach may be to select two drugs that act by distinct mechanisms (e.g., one that promotes Na^+ channel inactivation and another that enhances GABA-mediated synaptic inhibition). Side effects of each drug and the potential drug interactions also should be considered. As specified in Table 20–3, many of these drugs induce expression of CYPs and thereby impact the metabolism of themselves and/or other drugs (see Table 20–3).

Essential to optimal management of epilepsy is the filling out of a seizure chart by the patient or a relative. Frequent visits to the physician may be necessary early in the period of treatment, since hematological and other possible side effects may require a change in medication. Long-term follow-up with neurological examinations and possibly EEG and neuroimaging studies is appropriate. Most crucial for successful management is patient adherence to the drug regimen; noncompliance is the most frequent cause for failure of therapy with ASDs. Pharmacotherapy has also benefitted from recent advances in genetic testing. For example, Na^+ channel blockers can be detrimental to patients with mutations in SCN1A (Perucca and Perucca, 2019); therefore, these medications can be avoided in favor of more beneficial therapies (Cross et al., 2019).

Measurement of plasma drug concentration at appropriate intervals greatly facilitates the initial adjustment of dosage to minimize dose-related adverse effects without sacrificing seizure control. Periodic monitoring during maintenance therapy can also detect noncompliance. Knowledge of plasma drug concentrations can be especially helpful during multiple-drug therapy. If toxicity occurs, monitoring helps to identify the drug(s) responsible and can guide adjustment of dosage.

Finally, there have been several improvements in recent years that have added to the treatment of women with epilepsy. A greater understanding of sex-based differences in disease pathophysiology and therapy response (Reddy, 2017) contributes to better patient outcomes. Similarly, catamenial epilepsy (i.e., linked to the menstrual cycle) may contribute to cyclical changes both in epilepsy symptoms and drug metabolism (Navis and Harden, 2016; Reddy, 2016). Pregnancy and breastfeeding also present unique challenges to epilepsy therapy over concerns of exposure of the developing child to antiseizure medications. Ongoing research is aimed at the identification of safe and effective pharmacotherapy for women during and following pregnancy.

Duration of Therapy

Once initiated, ASDs are typically continued for at least 2 years. Tapering and discontinuing therapy should be considered if the patient is seizure free after 2 years.

Factors associated with high risk for recurrent seizures following discontinuation of therapy include EEG abnormalities, known structural lesions, abnormalities on neurological exam, and history of frequent seizures or medically refractory seizures prior to control. Conversely, factors associated with low risk for recurrent seizures include idiopathic epilepsy, normal EEG, onset in childhood, and seizures easily controlled with a single drug. The risk of recurrent seizures ranges from 12% to 66% (French and Pedley, 2008). Typically, 80% of recurrences will occur within 4 months of discontinuing therapy. The clinician and patient must weigh the risk of recurrent seizure and the associated potential deleterious consequences (e.g., loss of driving privileges) against the various implications of continuing medication including cost, unwanted effects, implications of diagnosis of epilepsy, and so on. Medications should be tapered slowly over a period of several months.

Focal and Focal to Bilateral Tonic-Clonic Seizures

The efficacy and toxicity of *carbamazepine*, *phenobarbital*, and *phenytoin* for treatment of focal and secondarily generalized tonic-clonic seizures in adults have been examined in a double-blind prospective study (Mattson et al., 1985). *Carbamazepine* and *phenytoin* were the most effective agents. The choice between *carbamazepine* and *phenytoin* required assessment of toxic effects of each drug. All three drugs decreased libido and increased impotence (*carbamazepine* 13%, *phenobarbital* 16%, and *phenytoin* 11%). In direct comparison with *valproate*, *carbamazepine* provided superior control of complex focal seizures (Mattson et al., 1992). With respect to adverse effects, *carbamazepine* was more commonly associated with skin rash, but *valproate* was more commonly associated with tremor and weight gain. Overall, the data demonstrated that *carbamazepine* and *phenytoin* are preferable for treatment of focal seizures, but *phenobarbital* and *valproic acid* are also efficacious.

Control of secondarily generalized tonic-clonic seizures did not differ significantly with *carbamazepine*, *phenobarbital*, or *phenytoin* (Mattson et al., 1985). *Valproate* was as effective as *carbamazepine* for control of secondarily generalized tonic-clonic seizures (Mattson et al., 1992). Since secondarily generalized tonic-clonic seizures usually coexist with focal seizures, these data indicate that among drugs introduced before 1990, *carbamazepine* and *phenytoin* are the first-line drugs for these conditions.

One key issue confronting the treating physician is choosing the optimal drug for initiating treatment in new-onset epilepsy. At first glance, this issue may appear unimportant because approximately 50% of newly diagnosed patients become seizure free with the first drug, whether old or new drugs are used (Kwan and Brodie, 2000). However, responsive patients typically receive the initial drug for several years, underscoring the importance of proper drug selection. Among the drugs available before 1990, *phenytoin*, *carbamazepine*, and *phenobarbital* induce hepatic CYPs, thereby complicating use of multiple ASDs as well as impacting metabolism of oral contraceptives, *warfarin*, and many other drugs. These drugs also enhance metabolism of endogenous compounds including gonadal steroids and vitamin D, potentially impacting reproductive function and bone density. Factors arguing against use of recently introduced drugs include higher costs and less clinical experience with the compounds.

Ideally, a prospective study would systematically compare newly introduced ASDs with drugs available before 1990 in a study design adjusting dose as needed and observing responses for extended periods of time (e.g., 2 years or more), in much the same manner as that used when comparing the older ASDs with one another, as described earlier (Mattson et al., 1985).

Unfortunately, such a study has not been performed. Many of the studies referenced in description of newer drugs compared a new ASD with an older ASD, but study design did not permit declaring a clearly superior drug; moreover, differences in study design and patient populations preclude comparing a new drug with multiple older drugs or with other new drugs. The use of recently introduced ASDs for newly diagnosed epilepsy was analyzed by subcommittees of the American Academy of Neurology and the American Epilepsy Society (French et al., 2004a, 2004b). The authors concluded that available evidence supported the use of *gabapentin*, *lamotrigine*, and *topiramate* for newly diagnosed focal or mixed seizure disorders. None of these drugs, however, has been FDA-approved for either of these indications. Insufficient evidence was available on the remaining newly introduced drugs to permit meaningful assessment of their effectiveness for this indication.

Generalized Absence Seizures

Ethosuximide and *valproate* are considered equally effective in the treatment of generalized absence seizures (Mikati and Browne, 1988).

Between 50% and 75% of newly diagnosed patients are free of seizures following therapy with either drug. If tonic-clonic seizures are present or emerge during therapy, *valproate* is the agent of first choice. Available evidence also indicates that *lamotrigine* is effective for newly diagnosed absence epilepsy despite the fact that *lamotrigine* is not approved for this indication by the FDA (Ben-Menachem, 2011).

Myoclonic Seizures

Valproic acid is the drug of choice for myoclonic seizures in the syndrome of juvenile myoclonic epilepsy, in which myoclonic seizures often coexist with tonic-clonic and absence seizures. *Levetiracetam* also has demonstrated efficacy as adjunctive therapy for refractory generalized myoclonic seizures.

Febrile Convulsions

Two to four percent of children experience a convulsion associated with a febrile illness. From 25% to 33% of these children will have another febrile convulsion. Only 2% to 3% become epileptic in later years, a 6-fold increase in risk compared with the general population. Several factors are associated with an increased risk of developing epilepsy: preexisting neurological disorder or developmental delay, a family history of epilepsy, or a complicated febrile seizure (i.e., the febrile seizure lasted >15 min, was one-sided, or was followed by a second seizure in the same day). If all of these risk factors are present, the risk of developing epilepsy is approximately 10%.

The increased risk of developing epilepsy or other neurological sequelae led many physicians to prescribe ASDs prophylactically after a febrile seizure. Uncertainties regarding the efficacy of prophylaxis for reducing epilepsy combined with substantial side effects of *phenobarbital* prophylaxis (Farwell et al., 1990) argue against the use of chronic therapy for prophylactic purposes (Freeman, 1992). For children at high risk of developing recurrent febrile seizures and epilepsy, rectally administered *diazepam* at the time of fever may prevent recurrent seizures and avoid side effects of chronic therapy.

Seizures in Infants and Young Children

Infantile spasms with *hypsarrhythmia* (abnormal interictal high-amplitude slow waves and multifocal asynchronous spikes on EEG) are refractory to the usual ASD. *Corticotropin* or the glucocorticoids are commonly used, and repository *corticotropin* was designated as an orphan drug for this purpose in 2003. A randomized study found *vigabatrin* (γ-vinyl GABA) to be efficacious in comparison to placebo (Appleton et al., 1999). Constriction of visual fields has been reported in a high percentage of patients treated with *vigabatrin* (Miller et al., 1999). The potential for progressive and permanent vision loss has resulted in *vigabatrin* being labeled with a black-box warning and marketed under a restrictive distribution program. The drug received orphan drug status for the treatment of infantile spasms in the U.S. in 2000 (and was FDA-approved in 2009 as adjunctive therapy for adults with refractory focal seizures with impaired awareness). *Ganaxolone* also has been designated as an orphan drug since 1994 for the treatment of infantile spasms and completed a phase II clinical trial for uncontrolled focal-onset seizures in adults in 2009. In 2020, the FDA granted Rare Pediatric Disease designation to *ganaxolone* for the treatment of patients with CDKL5 deficiency disorder, a rare refractory form of pediatric epilepsy.

Lennox-Gastaut syndrome is a severe form of epilepsy that usually begins in childhood and is characterized by cognitive impairments and multiple types of seizures including tonic-clonic, tonic, atonic, myoclonic, and atypical absence seizures. Addition of *lamotrigine* to other ASDs resulted in improved seizure control in comparison to placebo in a double-blind trial (Motte et al., 1997), demonstrating *lamotrigine* to be an effective and well-tolerated drug for this treatment-resistant form of epilepsy. *Felbamate* is also effective for seizures in this syndrome, but the occasional occurrence of aplastic anemia and hepatic failure limit its use (French et al., 1999). *Topiramate* is effective for Lennox-Gastaut syndrome (Sachdeo et al., 1999), and *clobazam* is approved for the adjunctive treatment in Lennox-Gastaut syndrome.

Dravet syndrome, previously referred to as severe myoclonic epilepsy of infancy, clinically presents in infancy (before 15 months of age) usually as focal or generalized convulsive seizures and a variety of behavioral, cognitive, and physiological comorbidities. A majority of individuals with this disorder have a mutation in the Na^+ channel gene *SCN1A* (Bender et al., 2012). Early-life seizures in Dravet syndrome can persist and develop into a variety of seizure types later in life. Patients with this disorder are also at an increased risk of sudden unexplained death in epilepsy (Shmuely et al., 2016). One or more medications are often required to achieve full control of seizures, and it is often the case that patients do not experience full seizure control (Cross et al., 2019). Initial treatment options may include *clobazam*, *stiripentol*, *valproic acid*, *topiramate*, and other medications (Cross et al., 2019). However, Na^+ channel blockers are contraindicated, as they may worsen seizures.

Status Epilepticus and Other Convulsive Emergencies

Status epilepticus is a neurological emergency. Mortality for adults approximates 20% (Lowenstein and Alldredge, 1998). The goal of treatment is rapid termination of behavioral and electrical seizure activity; the longer the episode of status epilepticus is untreated, the more difficult it is to control and the greater the risk of permanent brain damage. Critical to the management is a clear plan, prompt treatment with effective drugs in adequate doses, and attention to hypoventilation and hypotension. Since hypoventilation may result from high doses of drugs used for treatment, it may be necessary to assist respiration temporarily. To assess the optimal initial drug regimen, a double-blind, multicenter trial compared four intravenous treatments: *diazepam* followed by *phenytoin*; *lorazepam*; *phenobarbital*; and *phenytoin* alone (Treiman et al., 1998). The treatments had similar efficacies, with success rates ranging from 44% to 65%. *Lorazepam* alone was significantly better than *phenytoin* alone. No significant differences were found with respect to recurrences or adverse reactions. A more recent randomized, double-blind clinical trial (RAMPART) indicated that *midazolam* administered via the IM route was equally effective as *lorazepam* administered via the IV route and was not associated with either respiratory distress or seizure recurrence. Thus, emergency treatment with *midazolam* (IM) may prove to be the preferred treatment prior to arrival to the hospital.

Antiseizure Therapy and Pregnancy

Use of ASDs has diverse implications of great importance for the health of women. Issues include interactions with oral contraceptives, potential teratogenic effects, and effects on vitamin K metabolism in pregnant women (Pack, 2006). Guidelines for the care of women with epilepsy have been published by the American Academy of Neurology (Morrell, 1998). The FDA's Pregnancy and Lactation Labeling rule phased out the assignment of letter categories (A, B, C, D, and X) for prescription drug products. The FDA now requires labeling to include a summary of a prescription drug's known risk, a discussion of the data supporting that summary, and relevant information regarding its use during pregnancy and lactation (Epilepsy Foundation, 2013).

The effectiveness of oral contraceptives appears to be reduced by concomitant use of ASDs. The failure rate of oral contraceptives is 3.1/100 years in women receiving ASDs compared to a rate of 0.7/100 years in nonepileptic women. One attractive explanation of the increased failure rate is the increased rate of oral contraceptive metabolism caused by ASDs that induce hepatic enzymes (see Table 20–2), especially CYP3A4.

Teratogenicity

Epidemiological evidence suggests that ASDs have teratogenic effects (Pack, 2006). These teratogenic effects add to the deleterious consequences of oral contraceptive failure. Infants of epileptic mothers are at 2-fold greater risk of major congenital malformations than offspring of nonepileptic mothers (4%–8% compared to 2%–4%). These malformations include congenital heart defects, neural tube defects, cleft lip, cleft palate, and others. Inferring causality from the associations found in large epidemiological studies with many uncontrolled variables can be hazardous, but a causal role for ASDs is suggested by association of congenital defects with higher concentrations of a drug or with polytherapy compared to monotherapy. *Phenytoin*, *carbamazepine*, *valproate*, *lamotrigine*, and *phenobarbital* all have been associated with teratogenic effects. Newer ASDs have teratogenic effects in animals, but whether such effects occur in humans is uncertain (Güveli et al., 2017). One consideration for a woman with epilepsy who wishes to become pregnant is a trial free of ASD; monotherapy with careful attention to drug levels is another alternative. Polytherapy with toxic levels should be avoided. Folate supplementation (0.4 mg/day) has been recommended by the U.S. Public Health Service for all women of childbearing age to reduce the likelihood of neural tube defects, and this is appropriate for epileptic women as well.

ASDs that induce CYPs have been associated with vitamin K deficiency in the newborn, which can result in a coagulopathy and intracerebral hemorrhage. Treatment with vitamin K_1 (phylloquinone), 10 mg/day during the last month of gestation, has been recommended for prophylaxis. Collard and turnip greens, raw spinach and kale, broccoli, and soybeans are vegetables rich in phylloquinone (National Institutes of Health, 2021).

Development of Novel Treatments for Epilepsy

Nearly one-third of all patients with epilepsy do not experience full control of seizures, and numerous antiseizure drugs have dose-limiting side effects. Furthermore, there are no known treatments that halt or stop disease progression in epilepsy. Therefore, there is an ongoing need for the development of novel treatments for epilepsy. Many therapies have emerged that target specific epilepsy subpopulations (e.g., cannabidiol for Dravet syndrome and Lennox-Gastaut syndrome). In addition to refinement and optimization of current drug targets, numerous additional targets have been identified that include various signaling, inflammatory, transcriptional, and metabolic pathways (see Raut and Bhatt, 2020, for review). For example, glial cells continue to emerge as critical regulators of epilepsy pathophysiology and represent a significant therapeutic target. Further, the widespread use of the ketogenic diet has furthered understanding of signaling pathways and therapeutic targets contributing to the antiseizure properties of ketone bodies. Researchers continue to elucidate additional therapeutic targets and to develop novel therapies. We can be hopeful.

Drug Facts for Your Personal Formulary: *Antiseizure Agents*

Drugs	Therapeutic Uses (Seizure Types)	Clinical Pharmacology and Tips
Sodium Channel Modulators • Enhance fast inactivation		
Phenytoin	*Focal* • Aware • With impaired awareness *Generalized* • Tonic-clonic	• Once-daily dosing only available with extended-release formulation • Intravenous use with fosphenytoin • Nonlinear pharmacokinetics • May interfere with drugs metabolized by CYP2C9/10/19 • Induces CYP enzymes (e.g., CYP3A4) • *Side effects*: gingival hyperplasia, facial coarsening; hypersensitivity (rare)
Carbamazepine	*Focal* • Aware • With impaired awareness • Focal to bilateral tonic-clonic *Generalized* • Tonic-clonic	• Induces CYPs (e.g., 2C, 3A4) and UGTs • Active metabolite, the 10,11-epoxide • *Side effects*: drowsiness, vertigo, ataxia, blurred vision, increased seizure frequency
Eslicarbazepine	*Focal* • Aware • With impaired awareness	
Lamotrigine	*Focal* • Aware • With impaired awareness *Generalized* • Absence • Tonic-clonic	• Reduced $t_{1/2}$ in presence of phenytoin, carbamazepine, or phenobarbital • ↑ concentration in presence of valproic acid • Also used in Lennox-Gastaut syndrome
Oxcarbazepine	*Focal* • Aware • With impaired awareness	• Prodrug, metabolized to eslicarbazepine • Short $t_{1/2}$ • ↓ enzyme induction vs. carbamazepine • *Side effects*: ↓ incidence of hypersensitivity reactions (vs. carbamazepine)
Rufinamide	*Focal* • Aware • With impaired awareness	• Can be used in Lennox-Gastaut syndrome
Sodium Channel Modulators • Enhance slow inactivation		
Lacosamide	*Focal* • Aware • With impaired awareness	
Calcium Channel Blockers • Block T-type calcium channels		
Ethosuximide	*Generalized* • Absence	• *Side effects*: GI complaints, drowsiness, lethargy, dizziness, headache, hypersensitivity/skin reactions • Titration can reduce side effect occurrence

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Drug Facts for Your Personal Formulary: *Antiseizure Agents* (*continued*)

Drugs	Therapeutic Uses (Seizure Types)	Clinical Pharmacology and Tips
Zonisamide	*Focal* • Aware • With impaired awareness	• *Side effects*: somnolence, ataxia, anorexia, fatigue
Calcium Channel Modulators • $\alpha_2\delta$ ligands		
Gabapentin	*Focal* • Aware • With impaired awareness	• *Side effects*: somnolence, dizziness, ataxia, fatigue
Pregabalin	*Focal* • Aware • With impaired awareness	• *Side effects*: dizziness, somnolence • Linear pharmacokinetics • Low potential for drug-drug interactions
GABA-Enhancing Drugs • GABA$_A$ receptor allosteric modulators (benzodiazepines, barbiturates)		
Clonazepam	*Generalized* • Absence • Myoclonic	• *Side effects*: drowsiness, lethargy, behavioral disturbances • Abrupt withdrawal can facilitate seizures • Tolerance to antiseizure effects
Clobazam	*Lennox Gastaut syndrome* *Generalized* • Atonic • Tonic • Myoclonic	• *N*-desmethylclobazam, active metabolite, ↑ in patients with poor CYP2C19 metabolism • *Side effects*: somnolence, sedation • Tapered withdrawal recommended
Diazepam	*Status epilepticus*	• Short duration of action • *Side effects*: drowsiness, lethargy, behavioral disturbances • Abrupt withdrawal can facilitate seizures • Tolerance to antiseizure effects
Phenobarbital	*Focal* • Focal to bilateral tonic-clonic *Generalized* • Tonic-clonic	• Induces CYPs and UGTs • *Side effects*: sedation, nystagmus, ataxia; irritability and hyperactivity (children); agitation and confusion (elderly); allergy, hypersensitivity (rare)
Primidone	*Focal* • Focal to bilateral tonic-clonic *Generalized* • Tonic-clonic	• Induces CYPs • Not commonly used
GABA-Enhancing Drugs • GABA uptake/GABA transaminase inhibitors		
Tiagabine	*Focal* • Aware • With impaired awareness	• Metabolized by CYP3A • *Side effects*: dizziness, somnolence, tremor
Stiripentol	*Generalized* • Tonic-clonic (Dravet syndrome)	• Used in Dravet syndrome • Inhibits CYPs 3A4 and 2C19
Vigabatrin	*Focal* • With impaired awareness	• Used in infantile spasms, especially when caused by tuberous sclerosis • *Side effects*: can cause progressive and bilateral vision loss
Glutamate Receptor Antagonists • AMPA receptor antagonists		
Perampanel	*Focal* • Aware • With impaired awareness	• Metabolized by CYP3A • *Side effects*: anxiety, confusion, imbalance, visual disturbance, aggressive behavior, suicidal thoughts
Potassium Channel Modulators • KCNQ2-5–positive allosteric modulator		
Ezogabine	*Focal* • Aware • With impaired awareness	• *Side effects*: blue pigmentation of skin and lips, dizziness, somnolence, fatigue, vertigo, tremor, attention disruption, memory impairment, retinal abnormalities, QT prolongation (rare)
Synaptic Vesicle (SV2A) Modulators		
Levetiracetam	*Focal* • Aware • With impaired awareness *Generalized* • Myoclonic • Tonic-clonic	• *Side effects*: somnolence, asthenia, ataxia, dizziness, mood changes
Brivaracetam	*Focal* • Aware • With impaired awareness	

Drug Facts for Your Personal Formulary: *Antiseizure Agents* (*continued*)

Drugs	Therapeutic Uses (Seizure Types)	Clinical Pharmacology and Tips
Mixed or Unknown Mechanisms of Action		
Topiramate	*Focal* • Aware • With impaired awareness *Generalized* • Tonic-clonic	• Used in Lennox-Gastaut syndrome • *Side effects*: somnolence, fatigue, cognitive impairment
Valproic acid	*Focal* • Aware • With impaired awareness • Focal to bilateral tonic-clonic *Generalized* • Absence • Myoclonic • Tonic-clonic	• *Side effects*: transient GI symptoms, sedation, ataxia, tremor, hepatitis (rare) • Inhibits CYP2C9, UGT
Cenobamate	*Focal* • Aware • With impaired awareness	• *Side effects*: somnolence, dizziness, fatigue, diplopia, headache • DRESS[*] more likely with rapid dose escalations
Cannabidiol	*Generalized* • Lennox-Gastaut syndrome • Dravet syndrome • Tuberous sclerosis complex	• *Side effects*: dry mouth, low blood pressure, light headedness, drowsiness. Signs of liver injury (elevated liver enzymes) have also been reported in some patients using higher doses of Epidiolex[**].
Fenfluramine	*Generalized* • Dravet syndrome	*Black-box warning*: valvulopathy and pulmonary hypertension risk

[*] DRESS, Drug reaction with eosinophilia and systemic symptoms.
[**] The FDA-approved prescription form of cannabidiol (CBD).

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Chapter 21

Treatment of Central Nervous System Degenerative Disorders

Erik D. Roberson and Talene A. Yacoubian

INTRODUCTION TO NEURODEGENERATIVE DISORDERS

COMMON FEATURES OF NEURODEGENERATIVE DISORDERS
- Proteinopathies
- Selective Vulnerability
- Genetics and Environment
- Approaches to Therapy

PARKINSON'S DISEASE
- Clinical Overview
- Pathophysiology
- Treatment

ALZHEIMER'S DISEASE
- Clinical Overview
- Genetics
- Pathophysiology
- Treatment

HUNTINGTON'S DISEASE
- Clinical Overview
- Genetics
- Pathophysiology
- Treatment

AMYOTROPHIC LATERAL SCLEROSIS
- Clinical Overview
- Genetics and Pathophysiology
- Treatment

Introduction to Neurodegenerative Disorders

Neurodegenerative disorders are characterized by progressive and irreversible loss of neurons from specific regions of the brain. Prototypical neurodegenerative disorders include PD and HD, where the loss of neurons from structures of the basal ganglia results in abnormalities in the control of movement; AD, where the loss of hippocampal and cortical neurons leads to impairment of memory and cognitive ability; and ALS, where degeneration of spinal, bulbar, and cortical motor neurons results in motor weakness. For the most part, currently available therapies for neurodegenerative disorders alleviate the disease symptoms without altering the underlying neurodegenerative process, but a new era of disease-modifying treatments targeting the molecules implicated in pathogenesis is beginning with the approval of the anti-Aβ antibody, *aducanumab*, for AD.

Common Features of Neurodegenerative Disorders

Proteinopathies

Each of the major neurodegenerative disorders is characterized by accumulation of particular proteins in cellular aggregates: *α-synuclein* in PD; *amyloid β* (Aβ) and the *microtubule-associated protein tau* in AD; *TDP-43* in most cases of ALS; and *huntingtin* in HD (Prusiner, 2013). The reason for accumulation of these proteins is unknown, and it is also unclear in most cases whether it is the large cellular aggregates or smaller soluble species of the proteins that most strongly drive pathogenesis.

Selective Vulnerability

A striking feature of neurodegenerative disorders is the selectivity of the disease processes for particular types of neurons in different brain regions. For example, in PD, there is extensive destruction of the dopaminergic neurons of the substantia nigra, whereas neurons in the cortex and many other areas of the brain are less affected. In contrast, neural injury in AD is most severe in the hippocampus and neocortex, and even within the cortex, the loss of neurons is not uniform but varies dramatically in different brain networks. In HD, the mutant gene responsible for the disorder is expressed throughout the brain and in many other organs, yet the pathological changes are most prominent in the neostriatum and cortex. In ALS, there is a loss of spinal motor neurons and the cortical neurons that provide their descending input. The diversity of these patterns of neural degeneration suggests that the process of neural injury results from the interaction of intrinsic properties of different neural circuits, genetics, and environmental influences. The intrinsic factors may include susceptibility to excitotoxic injury, regional variation in capacity for oxidative metabolism, and the production of toxic free radicals as by-products of cellular metabolism.

Genetics and Environment

Each of the major neurodegenerative disorders can be inherited due to genetic mutations, but how commonly this occurs varies widely among the diseases. *HD is exclusively genetic*; it is transmitted by autosomal dominant inheritance of an expanded repeat in the *huntingtin* gene. Nevertheless, environmental factors importantly influence the age of onset and rate of progression of HD symptoms. PD, AD, and ALS are usually sporadic, but for each, there are well-recognized genetic forms. For example, there are both dominant (*α-synuclein, LRRK2*) and recessive (*parkin, DJ-1, PINK1*) gene mutations that may give rise to PD (Kumar et al., 2012; Singleton et al., 2013). In AD, mutations in the genes coding for *APP* and the *presenilins* (involved in APP processing) lead to inherited forms of the disease. About 10% of ALS cases are familial, most commonly due to mutations in the *C9ORF72* gene (Renton et al., 2014).

There are also genetic risk factors that influence the probability of disease onset and modify the phenotype. For example, the *APOE* genotype constitutes an important risk factor for AD. Three common alleles of this gene encode different isoforms of apoE protein, and individuals with even one copy of the high-risk allele, ε4, have a several-fold higher risk of developing AD than those with the most common allele, ε3. In PD, risk factor genes include *α-synuclein, LRRK2, tau,* and *GBA*, among others.

Environmental factors, including infectious agents, environmental toxins, and acquired brain injury, have been proposed in the etiology of neurodegenerative disorders. Traumatic brain injury has been suggested

Abbreviations

AADC: aromatic L-amino acid decarboxylase
Aβ: amyloid β
ACh: acetylcholine
AChE: acetylcholinesterase
AD: Alzheimer's disease
ALDH: aldehyde dehydrogenase
ALS: amyotrophic lateral sclerosis
apoE: apolipoprotein E
APP: amyloid precursor protein
ARIA: amyloid-related imaging abnormality
ASO: antisense oligonucleotides
BPSD: behavioral and psychiatric symptoms in dementia
BuChE: butyrylcholinesterase
CNS: central nervous system
COMT: catechol-*O*-methyltransferase
CSF: cerebrospinal fluid
DA: dopamine
DAT: DA transporter
DβH: dopamine-β-hydroxylase
DOPAC: 3,4-dihydroxyphenylacetic acid
FALS: familial ALS
GABA: γ-aminobutyric acid
GBA: β-glucocerebrosidase
GI: gastrointestinal
Glu: glutamatergic
GPCR: G protein-coupled receptor
GPe: globus pallidus extern
GPi: globus pallidus interna
HD: Huntington's disease
5HT: serotonin
HTT: huntingtin
HVA: homovanillic acid
ICD: impulse control disorder
LRRK2: leucine-rich repeat kinase 2
MAO: monoamine oxidase
MCI: mild cognitive impairment
MPTP: *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI: magnetic resonance imaging
3MT: 3-methoxyltyramine
NIA-AA: National Institute on Aging and Alzheimer's Association
NE: norepinephrine
NET: NE transporter
NMDA: *N*-methyl-D-aspartate
3-OMD: 3-*O*-methyl dopa
PD: Parkinson's disease
PET: positron emission tomography
PH: phenylalanine hydroxylase
REM: rapid eye movement
SNpc: substantia nigra pars compacta
SNpr: substantia nigra pars reticulate
SOD: superoxide dismutase
SSRI: selective serotonin reuptake inhibitor
STN: subthalamic nucleus
TAR: transactivation response element
TDP-43: TAR DNA-binding protein 43
TH: tyrosine hydrolase
VA/VL: ventroanterior and ventrolateral
VMAT2: vesicular monoamine transporter 2

as a trigger for neurodegenerative disorders. At least one toxin, MPTP, can induce a condition closely resembling PD. More recently, evidence has linked pesticide exposure with PD. Exposure of soldiers to neurotoxic chemicals has been implicated in ALS (as part of "Gulf War syndrome").

Approaches to Therapy

Certain themes are apparent in the pharmacological approaches described in this chapter. Many of the existing therapies are *neurochemical*, aiming to replace or compensate for damage to specific neurotransmitter systems that are selectively impaired. For example, dopaminergic therapy is a mainstay of PD therapy, and the primary agents used in AD aim to boost acetylcholinergic transmission. The goal of much current research is to identify therapies that are *neuroprotective* and can modify the underlying neurodegenerative process.

One target of neuroprotective therapies is *excitotoxicity, neural injury* that results from the presence of excess glutamate in the brain. *Glutamate* is used as a neurotransmitter to mediate most excitatory synaptic transmission in the mammalian brain. The presence of excessive amounts of glutamate can lead to excitotoxic cell death (see Chapter 16). The destructive effects of glutamate are mediated by glutamate receptors, particularly those of the NMDA type (see Table 16–2). Excitotoxic injury contributes to the neuronal death that occurs in acute processes such as stroke and head trauma. The role of excitotoxicity is less certain in the chronic neurodegenerative disorders; nevertheless, *glutamate antagonists* have been developed as neuroprotective therapies for neurodegeneration, with two such agents (*memantine* and *riluzole*, described later in the chapter) currently in clinical use.

Aging is the most important risk factor for all neurodegenerative diseases; a likely contributor to the effect of age is the progressive impairment in the capacity of neurons for oxidative metabolism with consequent production of reactive compounds such as hydrogen peroxide and oxygen radicals. These reactive species can lead to DNA damage, peroxidation of membrane lipids, and neuronal death. This has led to pursuit of drugs that can enhance cellular metabolism (such as the mitochondrial cofactor coenzyme Q_{10}) and antioxidant strategies as treatments to prevent or retard degenerative diseases.

The discovery of specific proteins that accumulate and aggregate in each of the neurodegenerative disorders has opened the door to new therapeutic approaches. *Aducanumab*, a monoclonal antibody targeting Aβ, is the first of these to be approved. There is intensive ongoing research to bring disease-modifying treatments, including antibodies and antisense oligonucleotides, that target α-synuclein, tau, TDP-43, and huntingtin into clinical care.

The immune system has been increasingly recognized to play a role in the neurodegenerative process in several neurodegenerative diseases, and much research is focused on identifying which parts of the immune system are active in AD, PD, ALS, and HD. While no therapies that target the immune system are approved currently for neurodegenerative diseases, the lessons learned from therapies targeting multiple sclerosis (see Chapter 39) will aid in the development of new immune-modulating therapies as disease-modifying treatments for neurodegenerative disorders.

Parkinson's Disease

Clinical Overview

Parkinsonism is a clinical syndrome with four cardinal features:

- Bradykinesia (slowness and poverty of movement)
- Muscular rigidity
- Resting tremor (which usually abates during voluntary movement)
- Impairment of postural balance, leading to disturbances of gait and to falling

The most common form of parkinsonism is idiopathic PD, first described by James Parkinson in 1817 as *paralysis agitans,* or the "shaking palsy." *The pathological hallmark of PD is the loss of the pigmented, dopaminergic neurons of the substantia nigra pars compacta, with the* *appearance of intracellular inclusions known as Lewy bodies*. The principal component of the Lewy bodies is aggregated α-synuclein (Goedert et al., 2013). A loss of 70% to 80% of the DA-containing neurons accompanies symptomatic PD.

Without treatment, PD progresses over 5 to 10 years to a rigid, akinetic state in which patients are incapable of caring for themselves (Suchowersky et al., 2006). Death frequently results from complications of immobility, including aspiration pneumonia or pulmonary embolism. The availability of effective pharmacological treatment has radically altered the prognosis of PD; in most cases, good functional mobility can be maintained for many years. The life expectancy of adequately treated patients is increased substantially, but overall mortality remains higher than that of the general population.

While DA neuron loss is the most well-recognized feature of the disease, the disorder affects a wide range of other brain structures, including the brainstem, hippocampus, and cerebral cortex (Langston, 2006). There is increasing awareness of the "nonmotor" features of PD, which likely arise from pathology outside the DA system (Zesiewicz et al., 2010). Some nonmotor features may present before the characteristic motor symptoms: anosmia, or loss of the sense of smell; REM behavior disorder, a disorder of sleep with marked agitation and motion during periods of REM sleep; and disturbances of autonomic nervous system function, particularly constipation. Other nonmotor features are seen later in the disease and include depression, anxiety, and dementia. Features of cognitive impairment in PD include impaired attention, hallucinations, delusions, and executive and visuospatial dysfunction.

Several disorders other than idiopathic PD also may produce parkinsonism, including some relatively rare neurodegenerative disorders, stroke, and intoxication with DA receptor antagonists. Drugs that may cause parkinsonism include antipsychotics such as *haloperidol* and *chlorpromazine* (see Chapter 19) and antiemetics such as *prochlorperazine* and *metoclopramide* (see Chapter 54). The distinction between idiopathic PD and other causes of parkinsonism is important because parkinsonism arising from other causes usually is refractory to treatment.

Pathophysiology

The dopaminergic deficit in PD arises from a loss of the neurons in the substantia nigra pars compacta that provide innervation to the striatum (caudate and putamen). The current understanding of the pathophysiology of PD is based on the finding that the striatal DA content is reduced in excess of 80%, with a parallel loss of neurons from the substantia nigra, suggesting that replacement of DA could restore function. The direct and indirect pathway model of the function of the basal ganglia, described below, while incomplete, is still useful.

Dopamine Synthesis, Metabolism, and Receptors

Dopamine, a catecholamine, is synthesized in the terminals of dopaminergic neurons from tyrosine and stored, released, reaccumulated, and metabolized by processes described in Chapter 15 and summarized in Figure 21–1. The actions of DA in the brain are mediated by the DA receptor, of which there are two broad classes, D1 and D2, with five distinct subtypes, D_1–D_5. All the DA receptors are GPCRs. Receptors of the **D1 group** (D_1 and D_5 subtypes) couple to G_s and thence activate the cyclic AMP pathway. The **D2 group** (D_2, D_3, and D_4 receptors) couples to G_i to reduce the adenylyl cyclase activity and voltage-gated Ca^{2+} currents while activating K^+ currents. Each of the five DA receptors has a distinct anatomical pattern of expression in the brain. D_1 and D_2 proteins are abundant in the striatum and are the most important receptor sites with regard to the causes and treatment of PD. The D_4 and D_5 proteins are largely extrastriatal, whereas D_3 expression is low in the caudate and putamen but more abundant in the nucleus accumbens and olfactory tubercle.

Neural Mechanism of Parkinsonism: A Model of Basal Ganglia Function

Considerable effort has been devoted to understanding how the loss of dopaminergic input to the neurons of the neostriatum gives rise to the clinical features of PD (Hornykiewicz, 1973). The basal ganglia can be viewed as a modulatory side loop that regulates the flow of information from the cerebral cortex to the motor neurons of the spinal cord (Albin et al., 1989) (Figure 21–2).

The neostriatum is the principal input structure of the basal ganglia and receives excitatory glutamatergic input from many areas of the cortex. Most neurons within the striatum are projection neurons that innervate other basal ganglia structures. A small but important subgroup of striatal neurons consists of interneurons that connect neurons within the striatum but do not project beyond its borders. ACh and neuropeptides are used as transmitters by these striatal interneurons.

The outflow of the striatum proceeds along two distinct routes, termed the *direct* and *indirect pathways* (Calabresi et al., 2014). The direct pathway is formed by neurons in the striatum that project directly to the output structures of the basal ganglia, the SNpr and the GPi; these, in turn, relay to the VA and VL thalamus, which provides excitatory input to the cortex. The neurotransmitter in both links of the direct pathway is GABA, which is inhibitory, so that *the net effect of stimulation of the direct pathway at the level of the striatum is to increase the excitatory outflow from the thalamus to the cortex.*

The indirect pathway is composed of striatal neurons that project to the GPe. This structure, in turn, innervates the STN, which provides outflow to the SNpr and GPi output structures. The first two links—the projections from striatum to GPe and GPe to STN—use the inhibitory transmitter GABA; however, the final link—the projection from STN to SNpr and GPi—is an excitatory glutamatergic pathway. Thus, *the net effect of stimulating the indirect pathway at the level of the striatum is to reduce the excitatory outflow from the thalamus to the cerebral cortex*. The balance of activity in the direct and indirect pathways is thought critical to the modulation of movement. The differential effect of DA on the direct and indirect pathways is the key feature of this model of basal ganglia function, which accounts for the symptoms observed in PD as a result of loss of dopaminergic neurons (Figure 21–3).

The dopaminergic neurons of the SNpc innervate all parts of the striatum; however, the target striatal neurons express distinct types of DA receptors. The striatal neurons giving rise to the direct pathway express primarily the *excitatory* D_1 DA receptor protein, whereas the striatal neurons forming the indirect pathway express primarily the *inhibitory* D_2 type. *Thus, DA released in the striatum tends to increase the activity of the direct pathway and reduce the activity of the indirect pathway, whereas the depletion that occurs in PD has the opposite effect. The net effect of the reduced dopaminergic input in PD is to increase markedly the inhibitory outflow from the SNpr and GPi to the thalamus and reduce excitation of the motor cortex.* This model explains some of the key clinical features in PD, such as bradykinesia. However, there are several limitations of this model of basal ganglia function. For example, this model fails to explain the resting tremor or levodopa-induced dyskinesia observed in PD. The anatomical connections in the basal ganglia are considerably more complex, and many of the pathways involved use several neurotransmitters. Limitations notwithstanding, the model is useful and has important implications for the rational design and use of pharmacological agents in PD.

Treatment

Current management of PD is primarily focused on the replacement of DA signaling that is depleted in the disorder. These therapies are effective at reducing the motor symptoms of PD, yet they do not slow the disease progression.

Levodopa

Levodopa (also called L-DOPA or L-3,4-dihydroxyphenylalanine), the metabolic precursor of DA, is the single most effective agent in the treatment of PD (Cotzias et al., 1969; Fahn et al., 2004). DA itself is not used as a therapeutic agent in PD due to its inability to cross the blood-brain barrier. *Levodopa*, however, crosses across the blood-brain barrier by a membrane transported for aromatic amino acids. The effects of *levodopa* result from its decarboxylation to DA in the CNS. When administered orally, *levodopa* is absorbed rapidly from the small bowel by the transport system for aromatic amino acids. Concentrations of the drug in plasma

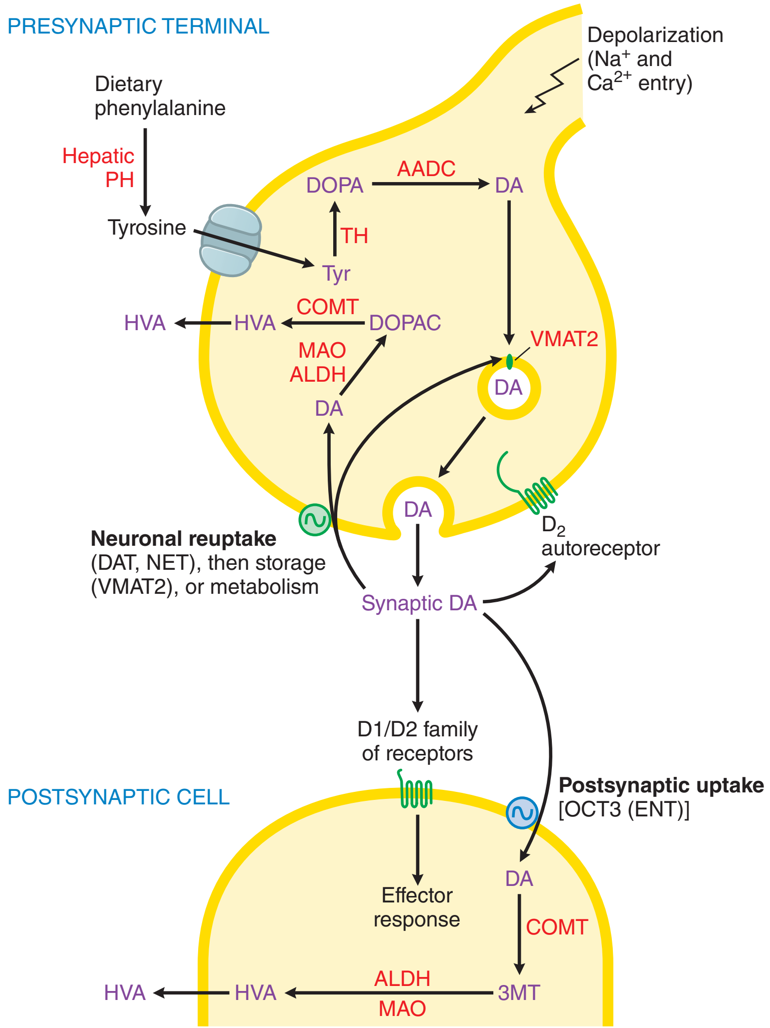


Figure 21–1 *Dopaminergic nerve terminal.* DA is synthesized from tyrosine in the nerve terminal by the sequential actions of TH and AADC. DA is sequestered by VMAT2 in storage granules and released by exocytosis. Synaptic DA activates presynaptic autoreceptors and postsynaptic D1 and D2 receptors. Synaptic DA may be taken up into the neuron via the DA and NE transporters (DAT, NET) or removed by postsynaptic uptake via the organic cation transporter, OCT3 (see Chapter 10). Cytosolic DA is subject to degradation by MAO and ALDH in the neuron and by COMT and MAO/ALDH in nonneuronal cells; the final metabolic product is HVA. See structures of neurotransmitters and metabolites in Figure 21–4.

usually peak between 0.5 and 2 h after an oral dose. The $t_{1/2}$ in plasma is short (1–3 h). The rate and extent of absorption of *levodopa* depend on the rate of gastric emptying, the pH of gastric juice, and the length of time the drug is exposed to the degradative enzymes of the gastric and intestinal mucosa. Administration of *levodopa* with high-protein meals delays absorption and reduces peak plasma concentrations. After entry across the blood-brain barrier by a membrane transporter for aromatic amino acids, *levodopa* is converted to DA by decarboxylation, primarily within the presynaptic terminals of dopaminergic neurons in the striatum. The DA produced is responsible for the therapeutic effectiveness of the drug in PD; after release, it is either transported back into dopaminergic terminals by the presynaptic uptake mechanism or metabolized by the actions of MAO and COMT (Figure 21–4).

In clinical practice, *levodopa* is almost always administered in combination with a peripherally acting inhibitor of AADC, such as *carbidopa* (used in the U.S.) or *benserazide* (available outside the U.S.), drugs that do not penetrate well into the CNS. If *levodopa* is administered alone, the drug is largely decarboxylated by enzymes in the intestinal mucosa and other peripheral sites so that relatively little unchanged drug reaches the cerebral circulation, and probably less than 1% penetrates the CNS. In addition, DA released into the circulation by peripheral conversion of *levodopa* produces undesirable effects, particularly nausea. Inhibition of peripheral decarboxylase markedly increases the fraction of administered *levodopa* that remains unmetabolized and available to cross the blood-brain barrier (Figure 21–5) and reduces the incidence of GI side effects and drug-induced orthostatic hypotension.

A daily dose of 75 mg *carbidopa* is generally sufficient to prevent the development of nausea. For this reason, the most commonly prescribed form of *carbidopa/levodopa* is the 25/100 form, containing 25 mg *carbidopa* and 100 mg *levodopa*. With this formulation, dosage schedules of three or more tablets daily provide acceptable inhibition of decarboxylase in most individuals.

Levodopa therapy can have a dramatic effect on motor signs and symptoms of PD. Early in the course of the disease, the degree of improvement in tremor, rigidity, and bradykinesia produced by *carbidopa/levodopa* may be nearly complete. Despite the short plasma $t_{1/2}$, *levodopa* dosing three to four times a day is sufficient to maintain a stable symptomatic benefit due to the storage of *levodopa* in surviving synaptic terminals. With long-term *levodopa* therapy, the “buffering” capacity is lost, and the patient’s motor state may fluctuate dramatically with each dose of *levodopa*, producing the *motor complications* of *levodopa* (Pahwa et al., 2006).

A common problem is the development of the “wearing-off” phenomenon: Each dose of *levodopa* effectively improves mobility for a period of time, perhaps 1 to 2 h, but rigidity and akinesia return rapidly at the end of the dosing interval. Increasing the dose and frequency of administration can improve this situation, but this often is limited by the development of

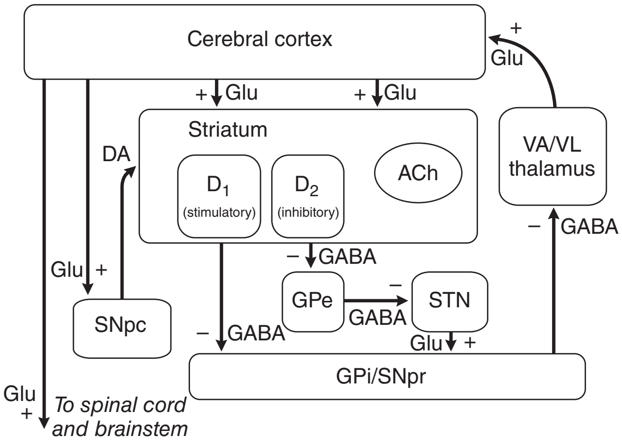

Cerebral cortex
+ Glu
+ Glu
+ Glu
Striatum
DA
D1 (stimulatory)
D2 (inhibitory)
ACh
VA/VL thalamus
− GABA
− GABA
Glu +
SNpc
GPe
− GABA
STN
− GABA
Glu +
GPi/SNpr
Glu +
To spinal cord and brainstem

Figure 21–2 *Schematic wiring diagram of the basal ganglia.* The striatum is the principal input structure of the basal ganglia and receives excitatory glutamatergic input from many areas of cerebral cortex. The striatum contains projection neurons expressing predominantly D_1 or D_2 DA receptors, as well as interneurons that use ACh as a neurotransmitter. Outflow from the striatum proceeds along two routes. The direct pathway, from the striatum to the SNpr and GPi, uses the inhibitory transmitter GABA. The indirect pathway, from the striatum through the GPe and the STN to the SNpr and GPi, consists of two inhibitory GABA-ergic links and one excitatory Glu projection. The SNpc provides dopaminergic innervation to the striatal neurons, giving rise to both the direct and the indirect pathways, and it regulates the relative activity of these two paths. The SNpr and GPi are the output structures of the basal ganglia and provide feedback to the cerebral cortex through the VA/VL nuclei of the thalamus.

dyskinesias, excessive and abnormal involuntary movements. In the later stages of PD, patients may fluctuate rapidly between being "off," having no beneficial effects from their medications, and being "on" but with disabling dyskinesias (the *on/off phenomenon*). A sustained-release formulation consisting of *carbidopa/levodopa* in an erodable wax matrix is helpful in some cases, but absorption of this older sustained-release formulation is not entirely predictable.

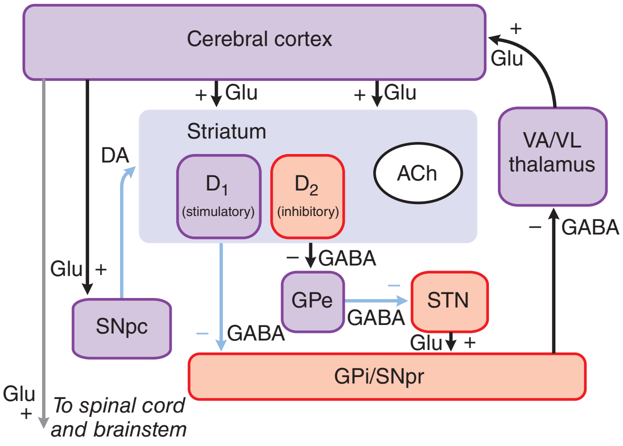

Cerebral cortex
+ Glu
+ Glu
+ Glu
Striatum
DA
D1 (stimulatory)
D2 (inhibitory)
ACh
VA/VL thalamus
− GABA
− GABA
Glu +
SNpc
GPe
− GABA
STN
− GABA
Glu +
GPi/SNpr
Glu +
To spinal cord and brainstem

Figure 21–3 *The basal ganglia in PD.* The primary defect is destruction of the dopaminergic neurons of the SNpc. The striatal neurons that form the direct pathway from the striatum to the SNpr and GPi express primarily the *excitatory* D_1 DA receptor, whereas the striatal neurons that project to the GPe and form the indirect pathway express the *inhibitory* D_2 DA receptor. Thus, loss of the dopaminergic input to the striatum has a differential effect on the two outflow pathways; the direct pathway to the SNpr and GPi is less active (structures in purple), whereas the activity in the indirect pathway is increased (structures in red). The net effect is that neurons in the SNpr and GPi become more active. This leads to increased inhibition of the VA/VL thalamus and reduced excitatory input to the cortex. Light blue lines indicate primary pathways with reduced activity. (See Abbreviations list for definitions of anatomical abbreviations.)

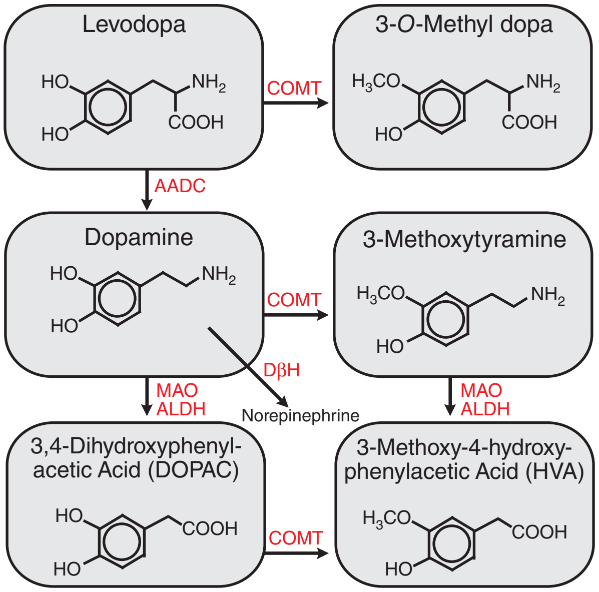

Levodopa
HO
HO
NH2
COOH
COMT
3-O-Methyl dopa
H3CO
HO
NH2
COOH
AADC
Dopamine
HO
HO
NH2
COMT
3-Methoxytyramine
H3CO
HO
NH2
DβH
MAO
ALDH
Norepinephrine
MAO
ALDH
3,4-Dihydroxyphenyl-acetic Acid (DOPAC)
HO
HO
COOH
COMT
3-Methoxy-4-hydroxy-phenylacetic Acid (HVA)
H3CO
HO
COOH

Figure 21–4 *Metabolism of levodopa (L-DOPA).*

Recently, several new formulations of *levodopa* intended to address the wearing off have been approved. A *carbidopa-levodopa* extended-release capsule contains both immediate- and extended-release beads that provide reduced off time in patients with motor fluctuations (Hauser et al., 2013). A *carbidopa-levodopa* intestinal gel is administered through a gastrostomy tube into the jejunum using a pump and can have a dramatic effect on reducing off time (Olanow et al., 2014). A *levodopa* inhalational powder is administered through an inhaler and is also effective for the treatment of off periods (LeWitt et al., 2019). Other *levodopa* formulations are under development, including subcutaneous delivery methods.

Does *levodopa* alter the course of the underlying disease or merely modify the symptoms? While the answer to this question is not entirely certain, the PD MED trial revealed that patients treated with *levodopa* early compared to DA agonists or MAO-B inhibitors had a slight but persistent benefit in mobility and activities of daily living at 7 years after initiation of therapy, although dyskinesia rates were higher among the levodopa group (PD MED Collaborative Group, 2014). Most practitioners have adopted a pragmatic approach, using *levodopa* only when the symptoms of PD cause functional impairment and other treatments are inadequate or not well tolerated.

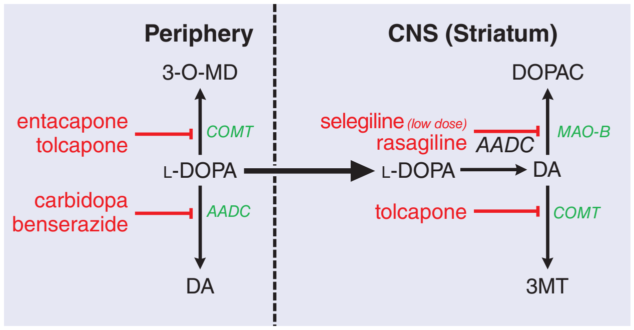

Periphery
CNS (Striatum)
3-O-MD
entacapone
tolcapone
COMT
L-DOPA
carbidopa
benserazide
AADC
DA
DOPAC
selegiline (low dose)
rasagiline
MAO-B
AADC
L-DOPA
DA
tolcapone
COMT
3MT

Figure 21–5 *Pharmacological preservation of levodopa (L-dopa) and striatal DA.* The principal site of action of inhibitors of COMT (e.g., *tolcapone, entacapone*, and *opicapone*) is in the peripheral circulation, where they block the *O*-methylation of L-dopa and increase the fraction of the drug available for delivery to the brain. Likewise, *carbidopa* and *benserazide* inhibit decarboxylation of L-dopa in the periphery, preserving it for delivery to the CNS. *Tolcapone* also has effects in the CNS. Inhibitors of MAO-B, such as low-dose *selegiline* and *rasagiline*, will act within the CNS to reduce oxidative deamination of DA, thereby enhancing vesicular stores.

A frequent and troubling adverse effect is the induction of hallucinations and confusion, especially in elderly patients or in patients with preexisting cognitive dysfunction. Conventional antipsychotic agents, such as the phenothiazines, are effective against *levodopa*-induced psychosis but may cause marked worsening of parkinsonism, probably through actions at the D_2 DA receptor, and should not be used in PD. An alternative approach has been to use "atypical" antipsychotic agents (see Chapter 19). The two atypical antipsychotics that are most effective and best tolerated in patients with advanced PD are *clozapine* and *quetiapine*. *Pimavanserin*, an inverse agonist at the serotonin $5HT2_A$ receptor, is FDA approved for the management of hallucinations and psychosis observed in PD. Monitoring of QT interval is important when using *pimavanserin* or atypical antipsychotics due to the risk of QT interval prolongation and resultant cardiac arrhythmias. Additionally, these drugs are associated with an increased rate of death when used in the elderly, a risk that must be weighed carefully against the risks created by hallucinations and psychosis. *Levodopa* (and the DA agonists, described in the next section) may also lead to the development of an impulse control disorder (ICD) (Weintraub et al., 2015). These include compulsive behaviors, gambling, and hypersexuality and can be destructive socially. PD also appears to be associated with an increased risk of suicidality, but whether this is associated with the disease or specific treatment is uncertain. Vigilance for signs of depression and suicidality should be practiced in all patients with PD.

Administration of *levodopa* with nonspecific inhibitors of MAO accentuates the actions of *levodopa* and may precipitate life-threatening hypertensive crisis and hyperpyrexia; nonspecific MAO inhibitors always should be discontinued at least 14 days before *levodopa* is administered (note that this prohibition does not include the MAO-B subtype-specific inhibitors *selegiline*, *rasagiline*, and *safinamide*). Abrupt withdrawal of *levodopa* or other dopaminergic medications may precipitate the *neuroleptic malignant syndrome* of confusion, rigidity, and hyperthermia, a potentially lethal adverse effect.

Dopamine Receptor Agonists

The DA receptor agonists in clinical use have durations of action substantially longer than that of *levodopa*; they are often used in the management of dose-related fluctuations in motor state and may be helpful in preventing motor complications (Parkinson Study Group, 2000). DA receptor agonists have been proposed to have the potential to modify the course of PD by reducing the endogenous release of DA as well as the need for exogenous *levodopa*, thereby reducing free radical formation. However, several clinical trials comparing DA receptor agonists to *levodopa* showed mixed results with regard to a protective advantage of DA receptor agonists compared to *levodopa* (Parkinson Study Group, 2002; PD MED Collaborative Group, 2014; Whone et al., 2003).

Two orally administered DA receptor agonists are commonly used for the treatment of PD: *ropinirole* and *pramipexole*. Both are well absorbed orally and have similar therapeutic actions. There is also a transdermal formulation of the DA agonist *rotigotine* available. *Ropinirole* and *pramipexole* have selective activity at D2 class sites (specifically at the D_2 and D_3 receptors). *Rotigotine* acts at D2 sites and also has activity at D1 sites. Like *levodopa*, these DA agonists can relieve the clinical symptoms of PD. The duration of action of the DA agonists (8–24 h) often is longer than that of *levodopa* (6–8 h), and they are particularly effective in the treatment of patients who have developed on/off phenomena. Both *ropinirole* and *pramipexole* are also available in once-daily sustained-release formulations, which are more convenient and may reduce adverse effects related to intermittent dosing. The transdermal delivery of *rotigotine* produces stable plasma drug levels over 24 h.

Pramipexole, *ropinirole*, and *rotigotine* may produce hallucinosis or confusion, similar to that observed with *levodopa*, and may cause nausea and orthostatic hypotension. These agents should be initiated at low dose and titrated slowly to minimize these effects. The DA agonists, as well as *levodopa* itself, are also associated with fatigue and somnolence. Patients should be warned about the potential for sleepiness, especially while driving. DA agonists cause a higher rate of ICD compared to levodopa, and screening for ICD is critical in patients treated with these drugs. Many practitioners prefer a DA agonist as initial therapy in younger patients to reduce the occurrence of motor complications. In older patients or those with substantial comorbidity, *levodopa/carbidopa* is generally better tolerated.

Apomorphine. *Apomorphine* is a dopaminergic agonist that can be administered by subcutaneous injection or as a sublingual formulation. It has a high affinity for D_4 receptors; moderate affinity for D_2, D_3, D_5, and adrenergic α_{1D}, α_{2B}, and α_{2C} receptors; and low affinity for D_1 receptors. *Apomorphine* is FDA approved as a "rescue therapy" for the acute intermittent treatment of off episodes in patients with a fluctuating response to dopaminergic therapy.

Apomorphine has the same side effects as the oral DA agonists. *Apomorphine* is highly emetogenic and requires pre- and posttreatment antiemetic therapy. Oral *trimethobenzamide*, at a dose of 300 mg, three times daily, should be started 3 days prior to the initial dose of *apomorphine* and continued at least during the first 2 months of therapy. Profound hypotension and loss of consciousness have occurred when *apomorphine* was administered with *ondansetron*; hence, the concomitant use of *apomorphine* with antiemetic drugs of the $5HT_3$ antagonist class is contraindicated. Other potentially serious side effects of *apomorphine* include QT prolongation, injection site reactions, and the development of a pattern of abuse characterized by increasingly frequent dosing leading to hallucinations, dyskinesia, and abnormal behavior. The sublingual formulation can cause oral sores and/or pain.

Because of these potential adverse effects, the use of *apomorphine* is appropriate only when other measures, such as oral DA agonists or COMT inhibitors, have failed to control the off episodes. *Apomorphine* injection should be initiated with a 2-mg test dose in a setting where the patient can be monitored carefully. If tolerated, it can be titrated slowly up to a maximum dosage of 6 mg. For effective control of symptoms, patients may require three or more injections daily. The starting dose for the sublingual formulation is 10 mg, and the dose can be titrated by 5-mg increments to a maximum of 30 mg/dose up to five doses total per day.

Catechol-O-Methyltransferase Inhibitors

Orally administered *levodopa* is largely converted by AADC to DA (see Figure 21–5), which causes nausea and hypotension. Addition of an AADC inhibitor such as *carbidopa* reduces the formation of DA but increases the fraction of *levodopa* that is methylated by COMT. COMT inhibitors block this peripheral conversion of *levodopa* to 3-*O*-methyl DOPA, increasing both the plasma $t_{1/2}$ of *levodopa* and the fraction of each dose that reaches the CNS. Additionally, centrally acting COMT inhibitors can block the metabolism of DA.

The COMT inhibitors *tolcapone* and *entacapone* reduce significantly "wearing-off" symptoms in patients treated with *levodopa/carbidopa* (Parkinson Study Group, 1997). The two drugs differ in their pharmacokinetic properties and adverse effects: *Tolcapone* has a relatively long duration of action and appears to act by inhibition of both central and peripheral COMT. *Entacapone* has a short duration of action (2 h) and principally inhibits peripheral COMT. Common adverse effects of both agents include nausea, orthostatic hypotension, vivid dreams, confusion, and hallucinations. An important adverse effect associated with *tolcapone* is hepatotoxicity, which has greatly limited its clinical use. At least three fatal cases of fulminant hepatic failure in patients taking *tolcapone* have been observed, leading to addition of a black-box warning to the label. *Tolcapone* should be used only in patients who have not responded to other therapies and with appropriate monitoring for hepatic injury. *Entacapone* has not been associated with hepatotoxicity. Both COMT inhibitors are given multiple times a day with each *levodopa* dose. *Entacapone* also is available in fixed-dose combinations with *levodopa/carbidopa*.

Opicapone is a new FDA-approved COMT inhibitor. Despite a short plasma $t_{1/2}$, its slow dissociation rate allows its use once daily. Its use with *levodopa* reduces off time comparable to *entacapone* (Ferreira et al., 2016).

Selective MAO-B Inhibitors

Two isoenzymes of MAO oxidize catecholamines: MAO-A and MAO-B. MAO-B is the predominant form in the striatum and is responsible for most of the oxidative metabolism of DA in the brain. Selective MAO-B inhibitors are used for the treatment of PD: *selegiline*, *rasagiline*, and *safinamide*. *Selegiline* and *rasagiline* selectively and irreversibly inactivate MAO-B; *safinamide* is a reversible inhibitor of MAO-B. These agents exert modest beneficial effects on the symptoms of PD. The basis of this efficacy is, presumably, inhibition of the breakdown of DA in the striatum.

Selective MAO-B inhibitors do not substantially inhibit the peripheral metabolism of catecholamines and can be taken safely with *levodopa*. These agents also do not exhibit the "cheese effect," the potentially lethal potentiation of catecholamine action observed when patients on nonspecific MAO inhibitors ingest indirectly acting sympathomimetic amines such as the tyramine found in certain cheeses and wine.

Selegiline is generally well tolerated in younger patients for symptomatic treatment of early or mild PD. In patients with more advanced PD or underlying cognitive impairment, *selegiline* may accentuate the adverse motor and cognitive effects of *levodopa* therapy. Metabolites of *selegiline* include amphetamine and methamphetamine, which may cause anxiety, insomnia, and other adverse symptoms. *Selegiline* is available in an orally disintegrating tablet as well as a transdermal patch. Both delivery routes are intended to reduce hepatic first-pass metabolism and limit the formation of amphetamine metabolites.

Unlike *selegiline*, *rasagiline* does not give rise to undesirable amphetamine metabolites. *Rasagiline* monotherapy is effective in early PD. Adjunctive therapy with *rasagiline* significantly reduces *levodopa*-related wearing-off symptoms in advanced PD (Olanow et al., 2008). A clinical trial comparing early versus late start of *rasagiline* suggested that *rasagiline* may have disease-modifying effects (Olanow et al., 2009), although this benefit was not clearly maintained in a later follow-up study (Rascol et al., 2016). Selective MAO-B inhibitors are generally well tolerated, but their drug interactions can be troublesome. Similar to the nonspecific MAO inhibitors, *selegiline* can lead to the development of stupor, rigidity, agitation, and hyperthermia when administered with the analgesic *meperidine*. Although the mechanics of this interaction are uncertain, the guidance is clear: *Selegiline* or *rasagiline* should not be given in combination with *meperidine*. *Tramadol*, *methadone*, *propoxyphene dextromethorphan*, St. John's wort, and *cyclobenzaprine* are also contraindicated with MAO-B inhibitors. Although the development of the *serotonin syndrome* has been reported with coadministration of MAO-B inhibitors and antidepressants (tricyclic or serotonin reuptake inhibitors), this appears to be rare, and many patients are treated with this combination without difficulty. If concurrent treatment with MAO-B inhibitors and antidepressants is undertaken, close monitoring and use of low doses of the antidepressant are advisable (Panisset et al., 2014).

Safinamide is a novel MAO-B inhibitor that is FDA approved for adjunctive therapy to *levodopa* for off periods in PD. Unlike *selegiline* or *rasagiline*, *safinamide* does not irreversibly inhibit MAO-B, and it also reduces glutamate release (Muller, 2018). Dose adjustment is required in moderate hepatic dysfunction, and *safinamide* should be avoided in severe hepatic disease. Side effect profile and drug interactions are similar to those of other MAO-B inhibitors.

Muscarinic Receptor Antagonists

Antimuscarinic drugs currently used in the treatment of PD include *trihexyphenidyl* and *benztropine mesylate*, as well as the antihistaminic *diphenhydramine hydrochloride*, which also interacts at central muscarinic receptors. The biological basis for the therapeutic actions of muscarinic antagonists is not completely understood. They may act within the neostriatum through the receptors that normally mediate the response to intrinsic cholinergic innervation of this structure, which arises primarily from cholinergic striatal interneurons.

These drugs have relatively modest antiparkinsonian activity and are used only in the treatment of early PD or as an adjunct to dopamimetic therapy. Adverse effects result from their anticholinergic properties. Most troublesome are sedation and mental confusion. All anticholinergic drugs must be used with caution in patients with narrow-angle glaucoma (see Chapter 74), and in general, anticholinergics are not well tolerated in the elderly. The pharmacology and signaling mechanisms of muscarinic receptors are thoroughly covered in Chapter 11.

Amantadine

Amantadine, an antiviral agent used for the prophylaxis and treatment of influenza A (see Chapter 62), has antiparkinsonian activity. *Amantadine* appears to alter DA release in the striatum, has anticholinergic properties, and blocks NMDA glutamate receptors. It is used as initial therapy for mild PD. It also may be helpful as an adjunct in patients on *levodopa* with dose-related fluctuations and dyskinesias. *Amantadine* is usually administered at a dose of 100 mg, twice per day, and is well tolerated. Once-a-day formulations are now available. Side effects, including dizziness, lethargy, anticholinergic effects, and sleep disturbance, as well as nausea and vomiting, are mild and reversible.

Istradefylline

Istradefylline is an adenosine A_{2A} receptor antagonist that is FDA approved for adjunctive treatment for off periods in PD. A_{2A} receptors are highly colocalized with D_2 receptors in the striatum, and activation of A_{2A} receptor inhibits D_2 receptor signaling, possibly via heterodimerization of A_{2A} and D_2 receptors. Therefore, in the presence of adenosine tone, antagonism of A_{2A} receptors could promote motor function by boosting DA signaling in the striatum. *Istradefylline* is dosed once daily and generally well tolerated. The most common side effects include hallucinations, nausea, and dizziness. A few small, open-label trials suggest that this agent may also help nonmotor symptoms, such as urinary dysfunction, daytime sleepiness, and cognitive impairment (Torti et al., 2018).

Future Therapies

The ultimate goal in PD therapeutics is disease-modifying treatments that slow disease progression. Advances in the understanding of the mechanisms underlying neurodegeneration have led to novel therapies that are undergoing preclinical and clinical investigation. α-Synuclein, the key protein that becomes misfolded and aggregates in PD, is a key target under investigation, and monoclonal antibodies against α-synuclein are in phase II clinical studies. The excess kinase activity of LRRK2 is implicated in patients with LRRK2 mutations and possibly in sporadic PD. Several compounds that inhibit LRRK2 kinase activity are under clinical investigation. Compounds to promote the activity of β-glucocerebrosidase, the protein encoded by the risk gene *GBA*, or to reduce its substrate are also under investigation in clinical trials.

Clinical Summary

Pharmacological treatment of PD should be tailored to the individual patient (Connolly and Lang, 2014). Drug therapy is not obligatory in early PD; many patients can be managed for a time with exercise and lifestyle interventions. For patients with mild symptoms, MAO-B inhibitors, *amantadine*, or (in younger patients) anticholinergics are reasonable choices. In most patients, treatment with a dopaminergic drug, either *levodopa* or a DA agonist, is eventually required. Many practitioners prefer a DA agonist as initial therapy in younger patients to reduce the occurrence of motor complications, although the evidence supporting this practice is inconclusive. In older patients or those with substantial comorbidity, *levodopa/carbidopa* is generally better tolerated.

Alzheimer's Disease

Clinical Overview

The brain region most vulnerable to neuronal dysfunction and cell loss in AD is the medial temporal lobe, including the entorhinal cortex and hippocampus. The proteins that accumulate in AD are Aβ in amyloid plaques and tau in neurofibrillar tangles. AD has three major stages:

1. An asymptomatic "preclinical" stage during which accumulation of Aβ and tau begins
2. An MCI stage with episodic memory loss (repeated questions, misplaced items, etc.) that is not severe enough to impair daily function
3. A dementia stage with progressive loss of functional abilities

Death usually ensues within 6 to 12 years of onset, most often from a complication of immobility such as pneumonia or pulmonary embolism.

Alzheimer's disease has traditionally been a clinical diagnosis based on the presence of memory impairment and other cognitive impairments that are insidious, progressive, and not well explained by another disorder. In recent years, there has been steady progress toward the inclusion of biomarkers in the diagnostic criteria. This includes both *fluid biomarkers*, such as changes in Aβ and tau in the cerebrospinal fluid or plasma, and *imaging biomarkers*, such as hippocampal atrophy on structural magnetic resonance imaging, cortical hypometabolism on fluorodeoxyglucose PET scans, and amyloid or tau deposition on PET scans. Three agents, *florbetapir, flutemetamol*, and *florbetaben,* are FDA approved for determining whether individuals with cognitive impairment have Aβ deposition, which would suggest AD as a possible etiology. *Flortaucipir* is approved for tau PET imaging. Under the 2018 NIA-AA research framework, amyloid (A), tau (T), and neurodegeneration (N) biomarkers are used to classify individuals' "ATN" status, with amyloid biomarkers required for a diagnosis of AD (Jack et al., 2018).

Genetics

Mutations in three genes have been identified as causes of autosomal dominant, early-onset AD: *APP*, which encodes Aβ precursor protein, and *PSEN1* and *PSEN2,* encoding presenilin 1 and 2, respectively. All three genes are involved in the production of Aβ peptides. Aβ is generated by sequential proteolytic cleavage of APP by two enzymes, β-secretase and γ-secretase; the presenilins form the catalytic core of γ-secretase. The genetic evidence, combined with the fact that Aβ accumulates in the brain in the form of soluble oligomers and amyloid plaques and is toxic when applied to neurons, forms the basis for the amyloid hypothesis of AD pathogenesis. Many genes have been identified as having alleles that increase AD risk. By far the most important of these is *APOE*, which encodes the lipid carrier protein apoE. Individuals inheriting the ε4 allele of *APOE* have a 3-fold or more higher risk of developing AD. While these individuals make up less than one-fourth of the population, they account for more than half of all AD cases.

Pathophysiology

The pathological hallmarks of AD are amyloid plaques, which are extracellular accumulations of Aβ, and intracellular neurofibrillary tangles composed of the microtubule-associated protein tau. The deposition of amyloid plaques occurs first, and tangle burden accrues over time in a manner that correlates more closely with the development of cognitive impairment. In autosomal dominant AD, Aβ accumulates due to mutations that cause its overproduction; in sporadic late-onset AD, the reasons for amyloid accumulation are less clear. While amyloid plaques consist of highly ordered fibrils of Aβ, it appears that soluble Aβ oligomers, perhaps as small as dimers, are more highly pathogenic. Tau also aggregates, forming the paired helical filaments that make up neurofibrillary tangles. Posttranslational modifications of tau, including phosphorylation, proteolysis, and other changes, increase tau's propensity to aggregate. Mechanisms by which Aβ and tau induce neuronal dysfunction and death may include direct impairment of synaptic transmission and plasticity, excitotoxicity, oxidative stress, and neuroinflammation.

Neurochemistry

The most striking neurochemical disturbance in AD is a *deficiency of ACh*. The anatomical basis of the cholinergic deficit is atrophy and degeneration of subcortical cholinergic neurons. The selective deficiency of ACh in AD and the observation that central cholinergic antagonists (e.g., atropine) can induce a confusional state resembling the dementia of AD have given rise to the "cholinergic hypothesis" that a deficiency of ACh is critical in the genesis of the symptoms of AD. AD, however, is complex and also involves multiple neurotransmitter systems, including glutamate, 5HT, and neuropeptides, and there is destruction not only of cholinergic neurons but also of cortical and hippocampal targets that receive cholinergic input.

Treatment

Three classes of drugs are FDA approved for the treatment of AD: orally administered cholinesterase inhibitors, *memantine*, and intravenously delivered anti-Aβ antibodies to clear amyloid plaques. These treatments are often used in combination (Cummings et al., 2019).

Cholinesterase Inhibitors

Augmentation of cholinergic transmission has been the first-line AD treatment for decades. Three drugs, *donepezil, rivastigmine*, and *galantamine*, are widely used for this purpose (Table 21–1). All three are reversible antagonists of cholinesterases (see Chapter 12) and target the cholinergic deficit in AD. Cholinesterase inhibitors are indicated for symptomatic treatment of mild or moderate dementia due to AD. They are also widely used to treat other neurodegenerative diseases with cholinergic deficits, including dementia with Lewy bodies and vascular dementia. Their effect is generally modest, usually producing a 6- to 12-month delay in progression without a dramatic improvement in symptoms, after which clinical decline resumes. The drugs are generally well tolerated, with the most common side effects being GI distress, muscle cramping, and abnormal dreams. They should be used with caution in patients with bradycardia or syncope.

Memantine

Memantine is an uncompetitive antagonist of the NMDA-type glutamate receptor, used with the goal of blocking pathological NMDA receptor activation associated with excitotoxicity while permitting physiological activation associated with learning and memory. It is indicated for moderate-to-severe stages of dementia, as there is little evidence for its efficacy in earlier stages. Like the cholinesterase inhibitors, *memantine*

TABLE 21–1 ■ CHOLINESTERASE INHIBITORS USED FOR THE TREATMENT OF ALZHEIMER'S DISEASE

	DONEPEZIL	RIVASTIGMINE	GALANTAMINE
Enzymes inhibited[a]	AChE	AChE, BuChE	AChE
Mechanism	Noncompetitive	Noncompetitive	Competitive
Typical maintenance dose[b]	10 mg once daily	9.5 mg/24 h (transdermal) 3–6 mg twice daily (oral)	8–12 mg twice daily (immediate release) 16–24 mg/d (extended release)
FDA-approved indications	Mild-severe AD	Mild-moderate AD Mild-moderate PDD	Mild-moderate AD
Metabolism[c]	CYP2D6, CYP3A4	Esterases	CYP2D6, CYP3A4

[a] AChE is the major cholinesterase in the brain; BuChE is a serum and hepatic cholinesterase that is upregulated in AD brain.

[b] Typical starting doses are one-half of the maintenance dose and are given for the first month of therapy.

[c] Drugs metabolized by CYP2D6 and CYP3A4 are subject to increased serum levels when coadministered with drugs known to inhibit these enzymes, such as ketoconazole and paroxetine.

delays clinical deterioration temporarily. Adverse effects of *memantine* include mild headache or dizziness. The drug is excreted by the kidneys, and dosage should be reduced in patients with severe renal impairment.

Amyloid Immunotherapy

After 18 years without a new treatment option, the landscape of AD treatment changed dramatically in 2021 with FDA approval of the first disease-modifying therapy, *aducanumab*, a monoclonal antibody that reduces amyloid plaques (Sevigny et al., 2016). Passive immunotherapy targeting Aβ is based on the amyloid hypothesis, and FDA approval of *aducanumab* was via the Accelerated Approval Program, which allows early approval of treatments for serious conditions based on surrogate biomarkers, in this case amyloid plaque burden. Its approval was controversial because the data supporting clinical efficacy did not meet the level required for traditional FDA approval; accelerated approval requires a subsequent phase IV trial to establish clinical benefit. The labeled indication for *aducanumab* is AD at the mild cognitive impairment or mild dementia stage. Clinical trials of various amyloid immunotherapies suggest trends toward more effectiveness in earlier stages. This is consistent with the observation that amyloid plaques begin depositing approximately 15 years before symptom onset, and thus, by the moderate dementia stage when plaques may have been present for as long as 25 years, it may be too late to reverse Aβ-induced damage. The pivotal trials of *aducanumab* included patients with MCI or mild dementia due to AD and required a positive amyloid PET scan. In practice, CSF biomarker confirmation of amyloid positivity is also used as another means to identify potentially eligible patients. *Aducanumab* is given monthly by intravenous infusions and requires MRI safety scans before initiating treatment and at 6-month intervals. Amyloid-related imaging abnormalities (ARIAs) were observed in almost half of the patients in phase III trials and include localized areas of cerebral edema (ARIA-E) or microhemorrhages (ARIA-H). ARIAs are often asymptomatic and generally reversible with discontinuation of treatment but can cause headache, dizziness, nausea, and other neurological symptoms.

Treatment of Behavioral Symptoms

In addition to cognitive decline, behavioral and psychiatric symptoms in dementia (BPSD) are common, particularly in the middle stages of the disease. These symptoms include irritability and agitation, paranoia and delusional thinking, wandering, anxiety, and depression. Treatment can be difficult, and nonpharmacological approaches should generally be the first line.

A variety of pharmacological options are also available. Both cholinesterase inhibitors and *memantine* reduce some BPSD. However, their effects are modest and they do not treat some of the most troublesome symptoms, such as agitation. *Citalopram*, an SSRI (see Chapters 15 and 18), showed efficacy for agitation in a randomized clinical trial. Atypical antipsychotics, such as *risperidone*, *olanzapine*, *quetiapine*, and *ziprasidone* (see Chapter 19), are perhaps even more efficacious for agitation and psychosis in AD, but their use is often limited by adverse effects, including parkinsonism, sedation, and falls. In addition, the use of atypical antipsychotics in elderly patients with dementia-related psychosis has been associated with a higher risk of stroke and overall mortality, leading to an FDA black-box warning (Schneider et al., 2005). Benzodiazepines (see Chapter 22) can be used for occasional control of acute agitation but are not recommended for long-term management because of their adverse effects on cognition and other risks in the elderly population. The typical antipsychotic *haloperidol* (see Chapter 19) may be useful for aggression, but sedation and extrapyramidal symptoms limit its use to control acute episodes.

Clinical Summary

The typical patient with AD presenting in the early stages of disease should probably be treated with a cholinesterase inhibitor. Patients and families should be counseled that a realistic goal of therapy is to induce a temporary reprieve from progression, or at least a reduction in the rate of decline, rather than long-term recovery of cognition. If biomarkers indicate amyloid positivity, then amyloid immunotherapy can be considered. As the disease progresses, *memantine* can be added to the regimen. Behavioral symptoms are often treated with a serotonergic antidepressant or, if they are severe enough to warrant the risk of higher mortality, an atypical antipsychotic. Eliminating drugs likely to aggravate cognitive impairments, particularly anticholinergics, benzodiazepines, and other sedative/hypnotics, from the patient's regimen is another important aspect of AD pharmacotherapy.

Huntington's Disease

Clinical Overview

Huntington's disease is a dominantly inherited disorder characterized by the gradual onset of motor incoordination and cognitive decline in midlife (Bates et al., 2015). Symptoms develop insidiously, as a movement disorder manifested by brief, dance-like movements of the extremities, trunk, face, and neck (chorea), or psychiatric symptoms, or both. Fine-motor incoordination and impairment of rapid eye movements are early features. As the disorder progresses, the involuntary movements become more severe, dysarthria and dysphagia develop, and balance is impaired. The cognitive disorder manifests first as slowness of mental processing and difficulty in organizing complex tasks. Memory is impaired, but affected persons rarely lose their memory of family, friends, and the immediate situation. Patients usually lack insight into their own disease. Obsessive-compulsive behaviors and depression are common, and many patients have psychosis and behavioral disturbances. The risk of suicidality is high in HD. The outcome of HD is invariably fatal; over a course of 15 to 30 years, the affected person becomes totally disabled and unable to communicate, requiring full-time care; death ensues from the complications of immobility.

Genetics

Huntington's disease is an autosomal dominant disorder with nearly complete penetrance. The average age of onset is between 35 and 45 years, but the range varies from as early as age 2 to as late as the middle 80s. Although the disease is inherited equally from mother and father, more than 80% of those developing symptoms before age 20 inherit the defect from the father. Known homozygotes for HD show clinical characteristics identical to the typical HD heterozygote, indicating that the unaffected chromosome does not attenuate the disease symptomatology.

A region near the end of the short arm of chromosome 4 contains a polymorphic $(CAG)_n$ trinucleotide repeat that is significantly expanded in all individuals with HD. The expansion of this trinucleotide repeat is the genetic alteration responsible for HD. The range of CAG repeat length in normal individuals is between 9 and 34 triplets, with a median repeat length on normal chromosomes of 19. The repeat length in HD varies from 40 to over 100. Repeat length is correlated inversely with the age of onset of HD. The younger the age of onset, the higher is the probability of a large repeat number. The mechanism by which the expanded trinucleotide repeat leads to the clinical and pathological features of HD is unknown. The HD mutation lies within a large gene (10 kb) designated *HTT* (previously *IT15*) that encodes *huntingtin*, a protein of about 348,000 Da. The trinucleotide repeat, which encodes the amino acid glutamine, occurs at the 5′ end of HTT. Huntingtin has no sequence homology with other known proteins.

Pathophysiology

Huntington's disease is characterized by prominent neuronal loss in the striatum (caudate/putamen) of the brain. Atrophy of these structures proceeds in an orderly fashion, first affecting the tail of the caudate nucleus and then proceeding anteriorly from mediodorsal to ventrolateral. Interneurons and afferent terminals are largely spared, whereas the striatal projection neurons (the medium spiny neurons) are severely affected. This leads to large decreases in striatal GABA concentrations, whereas somatostatin and DA concentrations are relatively preserved. Significant loss of large pyramidal projection neurons in the deeper layers is also observed in the cortex.

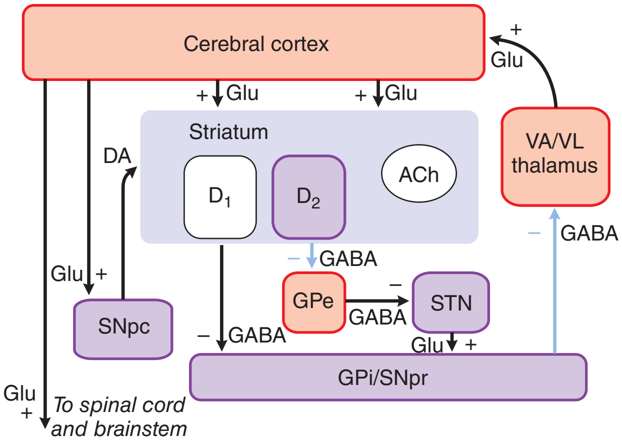


Figure 21–6 *The basal ganglia in Huntington's disease.* HD is characterized by loss of neurons from the striatum. The neurons that project from the striatum to the GPe and form the indirect pathway are affected earlier in the course of the disease than those that project to the GPi. This leads to a loss of inhibition of the GPe. The increased activity in this structure, in turn, inhibits the STN, SNpr, and GPi, resulting in a loss of inhibition to the VA/VL thalamus and increased thalamocortical excitatory drive. Structures in purple have reduced activity in HD, whereas structures in red have increased activity. Light blue lines indicate primary pathways of reduced activity. (See Abbreviations list for definitions of anatomical abbreviations.)

Selective vulnerability also appears to underlie the development of chorea. In most adult-onset cases, the medium spiny neurons that project to the GPi and SNpr (the indirect pathway) appear to be affected earlier than those projecting to the GPe (the direct pathway; see Figure 21–2). *The disproportionate impairment of the indirect pathway increases excitatory drive to the neocortex, producing involuntary choreiform movements* (Figure 21–6). In some individuals, rigidity rather than chorea is the predominant clinical feature; this is especially common in juvenile-onset cases. Here, the striatal neurons giving rise to both the direct and indirect pathways are impaired to a comparable degree.

Treatment

Symptomatic Treatment

None of the currently available medications slows the progression of the disease (Ross et al., 2014). Treatment is directed toward symptom management, particularly motor symptoms.

Tetrabenazine is used for the treatment of chorea associated with HD. *Tetrabenazine* and the related drug *reserpine* are inhibitors of VMAT2 and cause presynaptic depletion of catecholamines. *Tetrabenazine* is a reversible inhibitor; inhibition by *reserpine* is irreversible and may lead to long-lasting effects. Both drugs may cause hypotension and depression with suicidality; the shorter duration of effect of *tetrabenazine* simplifies clinical management. The recommended starting dose of *tetrabenazine* is 12.5 mg daily. Most patients can be managed with doses of 50 mg a day or less; however, *tetrabenazine* is extensively metabolized by CYP2D6. Genotyping for CYP2D6 may be needed to optimize therapy and is recommended for patients who require more than 50 mg daily. As might be expected with a drug that depletes DA stores, *tetrabenazine* can also cause parkinsonism. The recently approved deuterated tetrabenazine, *deutetrabenazine*, takes advantage of the stronger bonds that deuterium forms with carbon (the kinetic-isotope effect). The active deuterated dehydro-metabolites are VMAT2 inhibitors with longer half-lives than the corresponding products of *tetrabenazine* metabolism. *Deutetrabenazine* has therapeutic uses and an adverse effect profile similar to those of *tetrabenazine*, although *deutetrabenazine* may have a lower risk of depression.

Symptomatic treatment is needed for patients who are depressed, irritable, paranoid, excessively anxious, or psychotic. Depression can be treated effectively with standard antidepressant drugs with the caveat that drugs with substantial anticholinergic profiles can exacerbate chorea. SSRIs and selective serotonin and norepinephrine reuptake inhibitors (see Chapter 18) are effective treatments of depression and the irritability manifest in symptomatic HD. Paranoia, delusional states, and psychosis are treated with antipsychotic drugs, usually at lower doses than those used in primary psychiatric disorders (see Chapter 19). These agents also reduce cognitive function and impair mobility and thus should be used in the lowest doses possible and should be discontinued when the psychiatric symptoms resolve. In individuals with predominantly rigid HD, *clozapine, quetiapine* (see Chapter 19), or *carbamazepine* (see Chapter 20) may be more effective for the treatment of paranoia and psychosis. Acetylcholinesterase inhibitors have not been found to be effective for cognitive impairment in HD.

Many patients with HD exhibit worsening of involuntary movements as a result of anxiety or stress. In these situations, judicious use of sedative or anxiolytic benzodiazepines can be helpful. In juvenile-onset cases where rigidity rather than chorea predominates, DA agonists have had variable success in the improvement of rigidity. These individuals also occasionally develop myoclonus and seizures that can be responsive to *clonazepam*, *valproate*, and other anticonvulsants (see Chapter 20).

Encouraged by the success of genetic approaches for other neurogenetic disorders, such as spinal muscular atrophy, biomedical scientists are investigating genetic-based therapies for HD. Antisense oligonucleotides (ASO) to promote degradation of mutant and/or wild-type *HTT* RNA are in development in preclinical and clinical studies. A phase I/IIA clinical trial showed the safety of a non–allele-specific ASO and reduction of CSF huntingtin levels (Tabrizi et al., 2019). Other genetic approaches under investigation include RNA interference and splicing inhibitors.

Amyotrophic Lateral Sclerosis

Clinical Overview

Amyotrophic lateral sclerosis (ALS or Lou Gehrig disease) is a disorder of the motor neurons of the ventral horn of the spinal cord (lower motor neurons) and the cortical neurons that provide their afferent input (upper motor neurons). The disorder is characterized by rapidly progressive weakness, muscle atrophy and fasciculations, spasticity, dysarthria, dysphagia, and respiratory compromise. Many patients with ALS exhibit behavioral changes and cognitive dysfunction, and there is clinical, genetic, and neuropathological overlap between ALS and frontotemporal dementia spectrum disorders. ALS usually is progressive and fatal. Most patients die of respiratory compromise and pneumonia after 2 to 3 years, although some survive for many years.

Genetics and Pathophysiology

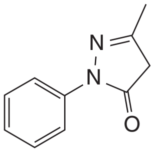
About 10% of ALS cases are familial (FALS), usually with an autosomal dominant pattern of inheritance. The most common genetic cause is a hexanucleotide repeat expansion in *C9ORF72*, which is responsible for up to 40% of FALS and around 5% of sporadic cases (Rohrer et al., 2015). Another 10% of FALS cases are due to mutations in the Cu/Zn *SOD1*. Mutations in the *TARDBP* gene encoding TDP-43 and in the *FUS/TLS* gene have been identified as causes of FALS. Both TDP-43 and FUS/TLS bind DNA and RNA and regulate transcription and alternative splicing. About 90% of ALS cases are sporadic. Of these, a few are caused by *de novo* mutations in *C9ORF72* (up to 7%), *SOD1*, *TDP-43*, *FUS/TLS*, or other genes, but for the majority of sporadic cases, the etiology remains unclear. The underlying pathophysiology remains under investigation, including roles for abnormal RNA processing, glutamate excitotoxicity, oxidative stress, and mitochondrial dysfunction (Mejzini et al., 2019).

Treatment

Riluzole

Riluzole (2-amino-6-[trifluoromethoxy] benzothiazole) is an agent with complex actions in the nervous system. *Riluzole* is absorbed orally and is highly protein bound. It undergoes extensive metabolism in the liver by CYP-mediated hydroxylation and glucuronidation, with a $t_{1/2}$ of approximately 12 h. *In vitro* studies show that *riluzole* has both presynaptic and postsynaptic effects; it inhibits glutamate release, blocks postsynaptic NMDA- and kainate-type glutamate receptors, and inhibits voltage-dependent Na^+ channels. The recommended dose is 50 mg twice daily, taken 1 h before or 2 h after a meal. *Riluzole* usually is well tolerated, although nausea or diarrhea may occur. Rarely, *riluzole* may produce hepatic injury with elevations of serum transaminases, and periodic monitoring of these is recommended. Meta-analyses of the available clinical trials indicated that *riluzole* extends survival by 2 to 3 months. Although the magnitude of the effect of *riluzole* on ALS is small, it represents a significant therapeutic milestone in the treatment of disease refractory to all previous treatments (Miller et al., 2012).

Edaravone



Edaravone

Edaravone was approved by the FDA in 2017 for the treatment of ALS, the first new drug approved for this indication since 1995. It is a small molecule with free radical scavenging properties that may reduce oxidative stress, although the exact mechanism of action is unknown. *Edaravone* has been used in Japan for acute stroke since 2001 and was FDA-approved for ALS under an orphan drug designation. A phase III study showed no benefit, but after *post hoc* subgroup analyses suggested an effect in early ALS, a subsequent trial enrolling only early-stage patients showed a smaller functional decline over 6 months in patients treated with *edaravone*. It is administered intravenously, with the first round daily for 14 days, followed by a 14-day holiday, then in subsequent cycles, 10 out of every 14 days followed by a 14-day holiday. The drug is metabolized to a glucuronide and a sulfate and excreted primarily in the urine as the glucuronide with a terminal $t_{1/2}$ of 4.5 to 6 h. At clinical doses, *edaravone* is not expected to inhibit major CYPs, UGTs, or drug transporters or to induce CYPs 1A2, 2B6, or 3A4; nor should inhibitors of these enzymes have substantial effects on the pharmacokinetics of *edaravone*. The infusion contains sodium bisulfite, which can cause hypersensitivity reactions. Other adverse effects include bruising, gait disorder, and headache.

Symptomatic Therapy of ALS: Spasticity

Spasticity is an important component of the clinical features of ALS and the feature most amenable to present forms of treatment. *Spasticity* is defined as an increase in muscle tone characterized by an initial resistance to passive movement of a joint, followed by a sudden relaxation (the so-called clasped-knife phenomenon). Spasticity results from loss of descending inputs to the spinal motor neurons, and the character of the spasticity depends on which nervous system pathways are affected. See further discussion of antispasticity agents in Chapter 13.

Baclofen. The best agent for the symptomatic treatment of spasticity in ALS is *baclofen*, a $GABA_B$ receptor agonist (see Figure 16–8). Initial doses of 5 to 10 mg/day are recommended, which can be increased to as much as 200 mg/day, if necessary. Alternatively, *baclofen* can be delivered directly into the space around the spinal cord using a surgically implanted pump and an intrathecal catheter. This approach minimizes the adverse effects of the drug, especially sedation, but it carries the risk of potentially life-threatening CNS depression.

Tizanidine. *Tizanidine* is an agonist of α_2 adrenergic receptors in the CNS. It reduces muscle spasticity, probably by increasing presynaptic inhibition of motor neurons. *Tizanidine* is primarily used in the treatment of spasticity in multiple sclerosis or after stroke, but it also may be effective in patients with ALS. Treatment should be initiated at a low dose of 2 to 4 mg at bedtime and titrated upward gradually. Drowsiness, asthenia, and dizziness may limit the dose that can be administered.

Other Agents. Benzodiazepines (see Chapter 22) such as *clonazepam* are effective antispasticity agents, but they may contribute to respiratory depression in patients with advanced ALS.

Dantrolene, approved in the U.S. for the treatment of muscle spasms, *is not used* in ALS because it can exacerbate muscular weakness. *Dantrolene* acts directly on skeletal muscle fibers, impairing Ca^{2+} release from the sarcoplasmic reticulum. It is effective in treating spasticity associated with stroke or spinal cord injury and in treating malignant hyperthermia (see Chapter 13). *Dantrolene* may cause hepatotoxicity, so it is important to monitor liver-associated enzymes before and during therapy with the drug.

Acknowledgment: *David G. Standaert contributed to this chapter in recent editions of this book. We have retained some of his text in the current edition.*

Drug Facts for Your Personal Formulary: *Drugs for Neurodegenerative Disease*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Anti-Parkinson: L-DOPA (DA precursor); Carbidopa (inhibits AADC, reduces the peripheral conversion of L-DOPA to DA)		
Carbidopa/levodopa	• Most effective symptomatic therapy for PD	• Therapeutic window narrows after several years of treatment: wearing off, dyskinesias, on/off phenomenon • Available as immediate-release tablets and orally disintegrated tablets (discontinued in the U.S.)
Carbidopa/levodopa sustained release	• Patients with PD with motor fluctuations on regular carbidopa/levodopa	• Bioavailability of immediate-release form, 75%
Carbidopa-levodopa extended-release capsules	• Patients with PD with motor fluctuations on regular carbidopa/levodopa	• Mixture of immediate- and extended-release beads
Carbidopa-levodopa intestinal gel	• Patients with PD with motor fluctuations on regular carbidopa/levodopa	• Requires placement of gastrostomy tube with jejunal extension • Useful for wearing-off issues
Levodopa inhalation formulation	• Patients with PD with motor fluctuations on regular carbidopa/levodopa	• Can be administered with carbidopa • Useful for wearing off
Anti-Parkinson: DA Agonists (longer acting than L-DOPA; can produce psychosis, impulse control disorder, sleepiness)		
Ropinirole	• PD • Restless legs syndrome	• Selective D2 receptor class agonist • Available in immediate release (3 times daily) and sustained release (once daily)
Pramipexole	• PD • Restless legs syndrome	• Selective D2 receptor class agonist • Available in immediate release (3 times daily) and sustained release (once daily)
Rotigotine	• PD • Restless legs syndrome	• D2 and D1 receptor class agonist • Transdermal formulation
Apomorphine	• Rescue therapy for acute intermittent treatment of off episodes	• Sublingual and subcutaneous formulations • Emetogenic, requires concurrent antiemetic • Sublingual form can cause oral pain and ulcers • Contraindicated with $5HT_3$ antagonists
Anti-Parkinson: COMT Inhibitors (reduce peripheral conversion of levodopa, increasing $t_{1/2}$ and CNS dose)		
Entacapone	• Adjunctive PD therapy given with each dose of levodopa, for wearing off	• Short $t_{1/2}$, inhibits peripheral COMT
Tolcapone	• Adjunctive PD therapy given with each dose of levodopa, for wearing off	• Long $t_{1/2}$, inhibits central and peripheral COMT • May be hepatotoxic; use only in patients not responding satisfactorily to other treatments; monitor liver function
Carbidopa/levodopa/entacapone	• PD, especially for wearing off on levodopa alone	• Fixed-dose combination formulation
Opicapone	• Adjunctive PD therapy for wearing off	• Once-a-day peripheral COMT inhibitor
Anti-Parkinson: MAO-B Inhibitors (reduce oxidative metabolism of dopamine in the CNS)		
Rasagiline	• PD, either as initial monotherapy or adjunct to levodopa	• Adjunct to reduce wearing off • Many drug interactions • Should not be given with meperidine • When administered with CYP1A2 inhibitors, C_p of rasagiline may double • Risk of serotonin syndrome
Selegiline	• PD, as adjunctive therapy in patients with deteriorating response to levodopa	• Generates amphetamine metabolites, which can cause anxiety and insomnia • MAO-B selectivity lost at doses >30–40 mg/day • Many drug interactions • Should not be given with meperidine • Risk of serotonin syndrome • Available in immediate-release, orally disintegrating tablet or transdermal patch
Safinamide	• PD, as adjunctive therapy to levodopa in patients with off periods	• Reversible MAO-B inhibition • Also has an effect on inhibiting glutamate release • Many drug interactions similar to rasagiline and selegiline • Risk of serotonin syndrome • Avoid in severe hepatic disease

Drug Facts for Your Personal Formulary: *Drugs for Neurodegenerative Disease (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Anti-Parkinson: Other		
Amantadine	• Early, mild PD • Levodopa-induced dyskinesias • Influenza	• Unclear mechanism of antiparkinsonian effects • Effective against dyskinesia • Available in immediate-release or long-acting formulation
Trihexyphenidyl	• PD, as adjunctive therapy	• Muscarinic receptor antagonist • Anticholinergic side effects
Benztropine	• PD, as adjunctive therapy	• Muscarinic receptor antagonist
Istradefylline	• Adjunctive treatment for off periods in PD	• Adenosine A_{2A} receptor antagonist • Side effects include hallucinations, nausea, and dizziness
Pimavanserin	• Treatment of delusions, hallucinations, and psychosis in PD	• Inverse serotonin $5HT2_A$ receptor agonist • QT interval monitoring required • Black-box label for increased rate of death in the elderly
Anti-Alzheimer: Acetylcholinesterase Inhibitors (boost cholinergic neurotransmission; first-line treatment)		
Donepezil	• Mild, moderate, severe AD dementia	• GI symptoms: main dose-limiting side effect • Bradycardia/syncope less common
Rivastigmine	• Mild-moderate AD dementia • Mild-moderate PD dementia	• Transdermal formulation available, with lower risk of GI side effects • Also inhibits BuChE
Galantamine	• Mild-moderate AD dementia	• GI symptoms: main dose-limiting side effect • Bradycardia/syncope less common than GI side effects
Anti-Alzheimer: Low-Affinity Uncompetitive NMDA Antagonist		
Memantine	• Moderate, severe AD dementia	• Reduces excitotoxicity through use-dependent blockade of NMDA receptors
Anti-Alzheimer: Amyloid Immunotherapy		
Aducanumab	• Reduces amyloid plaques; clinical efficacy uncertain	• Monthly intravenous infusion • Requires MRI monitoring for ARIA
Anti-Huntington		
Tetrabenazine Deutetrabenazine	• Chorea in HD	• Reversible VMAT2 inhibitor: depletes presynaptic catecholamines • Adverse effects: hypotension, depression with suicidality • Adjust dose for CYP2D6 status; 2D6 inhibitors (e.g., paroxetine, fluoxetine, quinidine, bupropion) ↑ exposure ~3-fold • Contraindications: concurrent or recent MAO inhibitor or reserpine
Anti-ALS		
Riluzole	• Extends survival in ALS up to 3 months	• Uncertain mechanism of action: inhibits glutamate release, blocks sodium channels and glutamate receptors
Edaravone	• Reduces progression in early stages of ALS	• Intensive intravenous administration regimen
Antispastic Agents		
Baclofen	• $GABA_B$ receptor agonist	• Sedation and CNS depression
Tizanidine	• α_2 Adrenergic receptor agonist	• Causes drowsiness; treatment is initiated with low dose and titrated upward
Benzodiazepines (e.g., clonazepam)	• See Chapter 22	• May contribute to respiratory depression
Dantrolene	• *Not used in ALS*, but for treating muscle spasm in stroke or spinal injury and for treating malignant hyperthermia	• May cause hepatotoxicity

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Chapter 22

Hypnotics and Sedatives

S. John Mihic and Jody Mayfield

BENZODIAZEPINES

- The Molecular Target for Benzodiazepines
- Pharmacological Properties of Benzodiazepines

NOVEL BENZODIAZEPINE RECEPTOR SITE AGONISTS

- Zaleplon
- Zolpidem
- Eszopiclone

MANAGEMENT OF PATIENTS AFTER LONG-TERM BENZODIAZEPINE THERAPY

FLUMAZENIL: A BENZODIAZEPINE RECEPTOR ANTAGONIST

MELATONIN CONGENERS

- Ramelteon
- Tasimelteon

BARBITURATES

- The Pharmacological Properties of Barbiturates
- ADME
- CNS Effects
- Systemic Effects
- Therapeutic Uses
- Untoward Effects
- Drug Interactions
- Barbiturate Poisoning

MISCELLANEOUS SEDATIVE-HYPNOTIC DRUGS

- Chloral Hydrate
- Meprobamate
- Other Agents

NONPRESCRIPTION HYPNOTIC DRUGS

NEW AND EMERGING AGENTS

- Suvorexant
- Lemborexant
- Doxepin
- Pregabalin
- Ritanserin
- Agomelatine

MANAGEMENT OF INSOMNIA

- Categories of Insomnia
- Prescribing Guidelines for Managing Insomnia

A *sedative* drug decreases activity, moderates excitement, and calms the recipient, whereas a *hypnotic* drug produces drowsiness and facilitates the onset and maintenance of a state of sleep that resembles natural sleep in its electroencephalographic characteristics and from which the recipient can be aroused easily. Sedation is a side effect of many drugs that are not considered general CNS depressants (e.g., antihistamines and antipsychotic agents). Although these and other agents can augment the effects of CNS depressants, they usually produce their desired therapeutic effects at concentrations lower than those causing substantial CNS depression. For example, benzodiazepine sedative-hypnotics do not produce generalized CNS depression. Although coma may occur at very high doses, neither surgical anesthesia nor fatal intoxication is produced by benzodiazepines unless other drugs with CNS depressant actions are concomitantly administered; an important exception is *midazolam*, which has been associated with decreased tidal volume and respiratory rate. Moreover, specific antagonists of benzodiazepines exist, such as *flumazenil*, which is used to treat cases of benzodiazepine overdose. This constellation of properties sets the benzodiazepine receptor agonists apart from other sedative-hypnotic drugs and imparts a measure of safety, such that benzodiazepines and the newer benzodiazepine receptor agonists (the "Z compounds") have largely displaced older agents for the treatment of insomnia and anxiety.

The CNS depressants discussed in this chapter include benzodiazepines, the Z compounds (e.g., *zolpidem* and *zaleplon*), barbiturates, and several sedative-hypnotic agents of diverse chemical structure. Many of the sedative-hypnotic drugs that do not specifically target the benzodiazepine receptor belong to a group of older, less-safe, sedative-hypnotic drugs that depress CNS function in a dose-dependent fashion, progressively producing a spectrum of responses from mild sedation to coma and death. These older sedative-hypnotic compounds share these properties with a large number of chemicals, including general anesthetics (see Chapter 21) and alcohols, most notably ethanol (see Chapter 27). The newer sedative-hypnotic agents, such as benzodiazepines and Z drugs, are safer in this regard.

HISTORICAL PERSPECTIVE

Humans have long sought sleep unburdened by worry and, to this end, have consumed many potions. In the mid-19th century, bromide was introduced specifically as a sedative-hypnotic. Chloral hydrate, paraldehyde, urethane, and sulfonal were used before the introduction of barbiturates (*barbital*, 1903; *phenobarbital*, 1912), of which about 50 were distributed commercially. Barbiturates attained such a high market share that fewer than a dozen other sedative-hypnotics were marketed successfully before 1960.

The partial separation of sedative-hypnotic-anesthetic properties from anticonvulsant properties characteristic of *phenobarbital* led to searches for agents with more selective effects on CNS functions. As a result, relatively nonsedating anticonvulsants, notably *phenytoin* and *trimethadione*, were developed in the late 1930s and early 1940s (see Chapter 20). The advent of *chlorpromazine* and *meprobamate* in the early 1950s, with their taming effects in animals, and the development of increasingly sophisticated methods for evaluating the behavioral effects of drugs, set the stage in the 1950s for the synthesis of *chlordiazepoxide*. Its introduction into clinical medicine in 1961 ushered in the era of benzodiazepines. Most benzodiazepines in the marketplace were selected for high anxiolytic potency relative to their depression of CNS function. However, all benzodiazepines possess sedative-hypnotic properties to varying degrees, which are exploited extensively clinically, especially to facilitate sleep. Mainly because of their remarkably low capacity to produce fatal CNS depression (i.e., high therapeutic indices), the benzodiazepines replaced barbiturates as the sedative-hypnotic agents of choice.

Abbreviations

ACh: acetylcholine
ALA: δ-aminolevulinic acid
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
COPD: chronic obstructive pulmonary disease
CYP: cytochrome P450
EEG: electroencephalogram
GABA: γ-aminobutyric acid
GI: gastrointestinal
GPCR: G protein-coupled receptor
MT: melatonin
OSA: obstructive sleep apnea
REM: rapid eye movement
SSRI: selective serotonin reuptake inhibitor

Benzodiazepines

All benzodiazepines in clinical use promote the binding of the major inhibitory neurotransmitter GABA to the GABA$_A$ receptor, a pentameric ligand-gated, anion-conducting channel (see Figures 16–5A and 16–11). Considerable heterogeneity exists among human GABA$_A$ receptors; this heterogeneity is thought to contribute to the myriad effects of these agents *in vivo*. Because receptor subunit composition governs the interactions of various allosteric modulators with these channels, considerable effort has been expended to find agents displaying selective actions on one or more subtypes of GABA$_A$ receptors. A number of distinct mechanisms of action, reflecting involvement of specific subunits of the GABA$_A$ receptor, likely contribute to distinct effects of various benzodiazepines—the sedative-hypnotic, muscle-relaxant, anxiolytic, amnesic, and anticonvulsant effects.

Although the benzodiazepines exert qualitatively similar clinical effects, quantitative differences in their pharmacodynamic spectra and pharmacokinetic properties have led to varying patterns of therapeutic application. While only the benzodiazepines used primarily for hypnosis are discussed in detail, this chapter describes the general properties of the group and important differences among individual agents (Figure 22–1) (see also Chapters 18 and 20).

The Molecular Target for Benzodiazepines

Benzodiazepines act at GABA$_A$ receptors by binding directly to a specific site that is distinct from the GABA binding site (see Figure 16–11).

The GABA$_A$ Receptor

The GABA$_A$ receptor is the major inhibitory receptor in the CNS. It is a transmembrane protein composed of five subunits that co-assemble around a central anion-conducting channel. Each subunit is composed of a large extracellular amino terminal domain, a transmembrane domain consisting of four transmembrane segments (M1–M4), an intracellular domain consisting of amino acids connecting M3 and M4, and a short carboxy terminus. The M2 segment of each subunit contributes to the formation of the central anion-conducting pore. GABA binds at the interfaces of α and β classes of subunits, while benzodiazepines bind at α/γ interfaces. The five subunits come from 19 isoforms, so the number of possible pentameric combinations is large. The number of pentamers actually expressed in nature is uncertain but likely numbers in the dozens. The GABA$_A$ receptor is a member of the cys-loop receptor superfamily, including also glycine, serotonin-3 (5HT$_3$), nicotinic acetylcholine (ACh) receptors, and the ACh binding protein.

The GABA$_A$ receptor pentamer contains a single benzodiazepine binding site, as well as other allosteric sites at which a variety of sedative-hypnotic-anesthetic agents exert modulatory effects on GABA$_A$ receptor function (see Chapter 16). The exact functional properties of the pentameric receptor depend on the subunit composition and arrangement of the individual subunits, and this heterogeneity likely contributes to the pharmacological diversity of benzodiazepine effects observed in behavioral, biochemical, and functional studies and to the selective effects of the Z compounds.

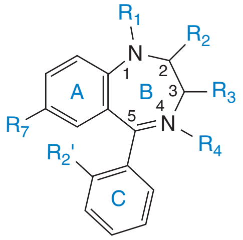


Figure 22–1 *Basic structure of benzodiazepines. Benzodiazepine* refers to the portion of this structure comprising the benzene ring (A) fused to a seven-member diazepine ring (B). Because all the important benzodiazepines contain a 5-aryl substituent (ring C) and a 1,4-diazepine ring, the term has come to mean the 5-aryl-1,4-benzodiazepines. Numerous modifications in the structure of the ring systems and substituents have yielded compounds with similar activities, including the benzodiazepine receptor antagonist *flumazenil*, in which ring C is replaced with a keto function at position 5 and a methyl substituent is added at position 4. A number of nonbenzodiazepine compounds (e.g., β-carbolines, *zolpidem*, *eszopiclone*) plus classic benzodiazepines and *flumazenil* bind to the benzodiazepine receptor, an allosteric site on the ionotropic GABA$_A$ receptor, a pentameric structure that forms a GABA-stimulated Cl$^-$ channel.

Effects of Benzodiazepines on GABA$_A$ Receptor–Mediated Events

Benzodiazepines are allosteric modulators of GABA$_A$ receptor function (Sieghart, 2015). They increase the affinity of the GABA$_A$ receptor for GABA and thereby enhance GABA-induced Cl$^-$ currents. Thus, in terms of channel kinetics, benzodiazepines increase the frequency of opening of the GABA$_A$ receptor Cl$^-$ channel in the presence of GABA (Nestler et al., 2020; Sigel and Steinmann, 2012). Inverse agonists at the benzodiazepine binding site do just the opposite, reducing both GABA binding and the frequency of channel opening. Benzodiazepine antagonists (e.g., *flumazenil*) competitively block benzodiazepine agonist and inverse agonist binding and effect, but do not on their own affect responses elicited by agents that bind at the GABA binding site (Nestler et al., 2020; Sigel and Steinmann, 2012).

In pharmacodynamic terms, agonists at the benzodiazepine binding site shift the GABA concentration-response curve to the left, whereas benzodiazepine inverse agonists shift the curve to the right. Both these effects are blocked by antagonists (e.g., *flumazenil*) that bind at the benzodiazepine binding site. Application of a benzodiazepine site antagonist, in the absence of either an agonist or antagonist at this same site, has no effect on the GABA concentration-response curve. The behavioral and electrophysiological effects of benzodiazepines can also be reduced or prevented by prior treatment with antagonists at the GABA binding site (e.g., *bicuculline*). The remarkable safety profile of the benzodiazepines likely relates to the fact that their effects *in vivo* depend on the presynaptic release of GABA; applied in the absence of GABA, benzodiazepines do not directly activate GABA$_A$ receptors. In other words, benzodiazepines act exclusively as modulators of GABA$_A$ receptor function.

The behavioral and sedative effects of benzodiazepines can be ascribed in part to potentiation of GABAergic pathways that regulate the firing of monoamine-containing neurons known to promote behavioral arousal and mediation of the inhibitory effects of fear and punishment on behavior. Inhibitory effects on muscular hypertonia or the spread of seizure activity can be attributed to potentiation of inhibitory GABAergic circuits at various levels of the neuraxis. The magnitude of the effects produced by benzodiazepines varies widely, depending on such factors as the types of inhibitory circuits that are operating, the sources and intensity of excitatory input, and the manner in which experimental manipulations are performed and assessed. Accordingly, benzodiazepines markedly prolong the period after brief activation of recurrent GABAergic pathways during which neither spontaneous nor applied excitatory stimuli can evoke neuronal discharge; this effect is reversed by the GABA_A receptor antagonist *bicuculline* (see Figure 16–8).

Benzodiazepines Versus Barbiturates at the GABA_A Receptor

The two classes of agents, barbiturates and benzodiazepines, differ markedly in their potencies. Barbiturates act to enhance GABA_A receptor function at low micromolar concentrations, while benzodiazepines are considerably more potent, binding with nanomolar affinity. Both benzodiazepines and barbiturates bind to allosteric sites on the GABA_A receptor pentamer and thereby enhance GABA-stimulated Cl^- channel function. However, barbiturates also have an additional effect: Higher concentrations of barbiturates directly activate GABA_A receptors. Furthermore, when tested using equieffective concentrations of GABA, maximally effective concentrations of barbiturates produce several times greater enhancement of GABA_A receptor function than maximally effective concentrations of benzodiazepines. These two phenomena almost certainly contribute to the profound and sometimes lethal CNS depression that barbiturates can cause. The lack of direct channel activation by benzodiazepines, their dependence on the presynaptic release of GABA at the GABA_A receptor, and their limited abilities to enhance GABA_A receptor function likely contribute to the safety of these agents as compared to barbiturates.

Pharmacological Properties of Benzodiazepines

The therapeutic effects of the benzodiazepines result from their actions on the CNS. The most prominent of these effects are sedation, hypnosis, decreased anxiety, muscle relaxation, anterograde amnesia, and anticonvulsant activity. Only two effects of these drugs result from peripheral actions: coronary vasodilation, seen after intravenous administration of therapeutic doses of certain benzodiazepines, and neuromuscular blockade, seen only with very high doses.

CNS Effects

While benzodiazepines depress activity at all levels of the neuraxis, some structures are affected differentially. All benzodiazepines have similar pharmacological profiles. Nevertheless, the drugs do differ in their pharmacokinetic and pharmacodynamic properties, and the clinical usefulness of individual benzodiazepines thus varies considerably. As the dose of a benzodiazepine is increased, sedation progresses to hypnosis and then to stupor. Although the clinical literature often refers to the "anesthetic" effects and uses of certain benzodiazepines, these drugs do not cause a true general anesthesia; awareness usually persists, and a failure to respond to a noxious stimulus sufficient to allow surgery cannot be achieved. Nonetheless, at "preanesthetic" doses, there is amnesia for events occurring subsequent to administration of the drug. Although many attempts have been made to separate the anxiolytic actions of benzodiazepines from their sedative-hypnotic effects, distinguishing between these behaviors is problematic. Accurate measurements of anxiety and sedation are difficult in humans, and the validity of animal models for measuring anxiety and sedation is uncertain.

Although analgesic effects of benzodiazepines have been observed in experimental animals, only transient analgesia is apparent in humans after intravenous administration. Such effects actually may involve the production of amnesia. Unlike barbiturates, benzodiazepines do not cause hyperalgesia.

Tolerance. Although most patients who chronically ingest benzodiazepines report that drowsiness wanes over a few days, tolerance to the impairment seen in some measures of psychomotor performance (e.g., visual tracking) is not usually observed. Whether tolerance develops to the anxiolytic effects of benzodiazepines remains debatable. Many patients use a fairly constant maintenance dose; increases or decreases in dosage appear to correspond with changes in their perceived problems or stresses. Conversely, other patients either do not reduce their dosages when stress is relieved or steadily escalate dosing. Such behavior may be associated with the development of drug dependence (see Chapter 28).

Some benzodiazepines induce muscle hypotonia without interfering with normal locomotion and can decrease rigidity in patients with cerebral palsy. *Clonazepam* in nonsedating doses causes muscle relaxation, but *diazepam* and most other benzodiazepines do not. Tolerance occurs to the muscle relaxant and ataxic effects of these drugs.

Experimentally, benzodiazepines inhibit seizure activity induced by either *pentylenetetrazol* or *picrotoxin* but suppress *strychnine*- and maximal electroshock–induced seizures only at doses that also severely impair locomotor activity. *Clonazepam, nitrazepam*, and *nordazepam* have greater selective anticonvulsant activity than do most other benzodiazepines. Benzodiazepines also suppress photic seizures in baboons and ethanol withdrawal seizures in humans. However, the development of tolerance to the anticonvulsant effects has limited the usefulness of benzodiazepines in the treatment of recurrent seizure disorders in humans (see Chapter 20).

Effects on the Electroencephalogram and Sleep Stages. The effects of benzodiazepines on the waking electroencephalogram (EEG) resemble those of other sedative-hypnotic drugs. Alpha rhythm activity is decreased, but there is an increase in low-voltage fast activity. Tolerance also occurs to these effects. With respect to sleep, some differences in the patterns of effects exerted by the various benzodiazepines have been noted, but benzodiazepine users usually report a sense of deep or refreshing sleep. Benzodiazepines decrease sleep latency, especially when first used, and diminish the number of awakenings and the time spent in stage 0 (a stage of wakefulness). They also produce an increased arousal threshold from sleep. Time in stage 1 (descending drowsiness) usually is decreased, and there is a prominent decrease in the time spent in slow-wave sleep (stages 3 and 4). Most benzodiazepines increase the latency from onset of spindle sleep to the first burst of rapid eye movement (REM) sleep. The time spent in REM sleep is usually shortened, but the number of cycles of REM sleep is typically increased, mostly late in the sleep time. *Zolpidem* and *zaleplon* suppress REM sleep less extensively than benzodiazepines and thus may be superior to benzodiazepines for use as hypnotics (Dujardin et al., 1998).

Despite the shortening of durations of stage 4 and REM sleep, benzodiazepine administration typically increases total sleep time, largely by increasing the time spent in stage 2, which is the major fraction of non-REM sleep. This effect is greatest in subjects with the shortest baseline total sleep time. In addition, despite the increased number of REM cycles, the number of shifts to lighter sleep stages (1 and 0) and the amount of body movement are diminished with benzodiazepine use. Nocturnal peaks in the secretion of growth hormone, prolactin, and luteinizing hormone are not affected. During chronic nocturnal use of benzodiazepines, the effects on the various stages of sleep usually decline within a few nights. When such use is discontinued, the pattern of drug-induced changes in sleep parameters may "rebound," and an increase in the amount and density of REM sleep may be especially prominent. If the dosage has not been excessive, patients usually will note only a shortening of sleep time rather than an exacerbation of insomnia.

Systemic Effects

Respiration. Hypnotic doses of benzodiazepines are without effect on respiration in normal subjects, but special care must be taken in the treatment of children and individuals with impaired hepatic or pulmonary function. At higher doses, such as those used for preanesthetic medication or for endoscopy, benzodiazepines slightly depress alveolar ventilation and cause respiratory acidosis as the result of a decrease in hypoxic rather than hypercapnic drive; these effects are exaggerated in patients with chronic obstructive pulmonary disease (COPD), and alveolar hypoxia, and CO_2 narcosis may result. These drugs can cause apnea during anesthesia or when given with opioids. Patients severely intoxicated with benzodiazepines require respiratory assistance only when they also have ingested another CNS depressant drug, most commonly ethanol.

Hypnotic doses of benzodiazepines may worsen sleep-related breathing disorders by adversely affecting control of the upper airway muscles or by decreasing the ventilatory response to CO_2. The latter effect may cause hypoventilation and hypoxemia in some patients with severe COPD. In patients with obstructive sleep apnea (OSA), hypnotic doses of benzodiazepines may decrease muscle tone in the upper airway and exaggerate the impact of apneic episodes on alveolar hypoxia, pulmonary hypertension, and cardiac ventricular load. Benzodiazepines may promote the appearance of episodes of apnea during REM sleep (associated with decreases in O_2 saturation) in patients recovering from a myocardial infarction; however, no impact of these drugs on survival of patients with cardiac disease has been reported.

Cardiovascular System. The cardiovascular effects of benzodiazepines are minor in normal subjects except in cases of severe intoxication (see previous discussion for adverse effects in patients with obstructive sleep disorders or cardiac disease). At preanesthetic doses, all benzodiazepines decrease blood pressure and increase heart rate. With *midazolam*, the effects appear to be secondary to a decrease in peripheral resistance; however, with *diazepam*, the effects are secondary to a decrease in left ventricular work and cardiac output. *Diazepam* increases coronary flow, possibly by an action to increase interstitial concentrations of adenosine, and the accumulation of this cardiodepressant metabolite also may explain the negative inotropic effects of the drug. In large doses, *midazolam* considerably decreases cerebral blood flow and O_2 assimilation.

Gastrointestinal (GI) Tract. Benzodiazepines are thought by some gastroenterologists to improve a variety of "anxiety-related" GI disorders. There is a paucity of evidence for direct actions. Although *diazepam* markedly decreases nocturnal gastric secretion in humans, other drug classes are considerably more effective in acid-peptic disorders (see Chapter 53).

ADME

All benzodiazepines are absorbed completely except *clorazepate*. *Clorazepate* is decarboxylated rapidly in gastric juice to *N*-desmethyldiazepam (nordazepam), which subsequently is absorbed completely. Drugs active at the benzodiazepine receptor may be divided into four categories based on their elimination $t_{1/2}$:

- Ultra-short-acting benzodiazepines
- Short-acting agents ($t_{1/2}$ <6 h), including *midazolam*, *triazolam*, *remimazolam*, the nonbenzodiazepine *zolpidem* ($t_{1/2}$ ~2 h), and *eszopiclone* ($t_{1/2}$, 5–6 h)
- Intermediate-acting agents ($t_{1/2}$, 6–24 h), including *estazolam* and *temazepam*
- Long-acting agents ($t_{1/2}$ >24 h), including *flurazepam*, *diazepam*, and *quazepam*

Flurazepam itself has a short $t_{1/2}$ (~2.3 h), but a major active metabolite, *N*-des-alkyl-flurazepam, is long-lived ($t_{1/2}$, 47–100 h); such features complicate the classification of certain benzodiazepines.

The benzodiazepines and their active metabolites bind to plasma proteins. The extent of binding correlates strongly with the oil:water partition coefficient and ranges from about 70% for *alprazolam* to nearly 99% for *diazepam*. The concentrations of these agents in the cerebrospinal fluid approximate the concentrations of free drugs in plasma. Uptake of benzodiazepines occurs rapidly into the brain and other highly perfused organs after intravenous administration (or oral administration of a rapidly absorbed compound); rapid uptake is followed by a phase of redistribution into tissues that are less well perfused but capacious, especially muscle and fat (see Table 2–2 and Figure 2–4). Redistribution is most rapid for benzodiazepines with the highest oil:water partition coefficients. The kinetics of redistribution of *diazepam* and other lipophilic benzodiazepines are complicated by enterohepatic circulation. These drugs cross the placental barrier and are also secreted into breast milk.

Most benzodiazepines are metabolized extensively by hepatic cytochrome P450 enzymes (CYPs), particularly CYPs 3A4 and 2C19. Some benzodiazepines, such as *oxazepam*, are not metabolized by CYPs but are conjugated directly by phase 2 enzymes. *Erythromycin, clarithromycin, ritonavir, itraconazole, ketoconazole, nefazodone*, and grapefruit juice are examples of CYP3A4 inhibitors (see Chapter 5) that can affect the rate of metabolism of benzodiazepines. Benzodiazepines do not significantly induce hepatic CYPs, so their chronic administration does not usually affect metabolism of benzodiazepines or other drugs. *Cimetidine* and oral contraceptives inhibit *N*-dealkylation and 3-hydroxylation of benzodiazepines. Ethanol, *isoniazid*, and *phenytoin* are less effective in this regard. These phase 1 reactions usually are reduced to a greater extent in elderly patients and in patients with chronic liver disease than are those reactions involving conjugation.

The active metabolites of some benzodiazepines are biotransformed more slowly than are the parent compounds; thus, the durations of action of many benzodiazepines bear little relationship to the $t_{1/2}$ of elimination of the parent drug. Conversely, the rate of biotransformation of drugs that are inactivated by the initial metabolic reaction is an important determinant of their durations of action; examples include *oxazepam*, *lorazepam*, *temazepam*, *triazolam*, and *midazolam*.

Benzodiazepine metabolism can seem daunting but can be organized around a few basic principles. Metabolism of the benzodiazepines occurs in three major stages. These stages and the relationships between the drugs and their metabolites are shown in Table 22–1.

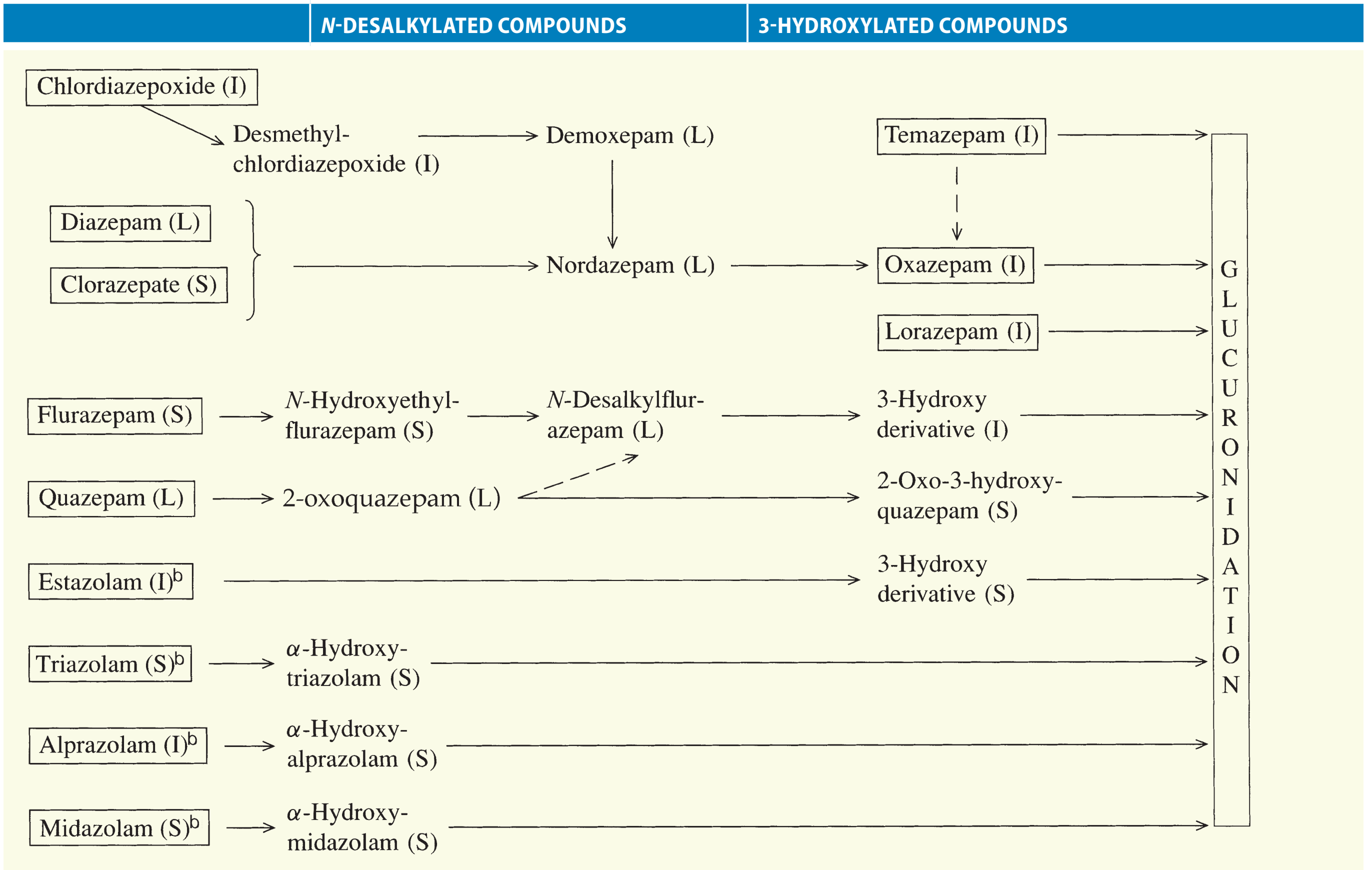
For benzodiazepines that bear a substituent at position 1 (or 2) of the diazepine ring, the *first phase* of metabolism involves modification or removal of the substituent. The eventual products are *N*-desalkylated compounds that are biologically active. Exceptions are *triazolam*, *alprazolam*, *estazolam*, and *midazolam*, which contain either a fused triazolo or an imidazolo ring and are α-hydroxylated.

The *second phase* of metabolism involves hydroxylation at position 3 and also usually yields an active derivative (e.g., *oxazepam* from *nordazepam*). The rates of these reactions are usually much slower than the first stage ($t_{1/2}$ >40–50 h), such that appreciable accumulation of hydroxylated products with intact substituents at position 1 does not occur. There are two significant exceptions to this rule: First, small amounts of temazepine accumulate during the chronic administration of *diazepam*; and second, following the replacement of S with O in *quazepam*, most of the resulting 2-oxoquazepam is hydroxylated slowly at position 3 without removal of the *N*-alkyl group. However, only small amounts of the 3-hydroxyl derivative accumulate during chronic administration of *quazepam* because this compound is conjugated at an unusually rapid rate. In contrast, the *N*-desalkylflurazepam that is formed by the "minor" metabolic pathway does accumulate during *quazepam* administration, and it contributes significantly to the overall clinical effect.

The *third major phase* of metabolism is the conjugation of the 3-hydroxyl compounds, principally with glucuronic acid; the $t_{1/2}$ values of these reactions usually are about 6 to 12 h, and the products invariably are inactive. Conjugation is the only major route of metabolism for *oxazepam* and *lorazepam* and is the preferred pathway for *temazepam* because of the slower conversion of this compound to *oxazepam*. *Triazolam* and *alprazolam* are metabolized principally by initial hydroxylation of the methyl group on the fused triazolo ring; the absence of a chlorine residue in ring C of *alprazolam* slows this reaction significantly. The products, sometimes referred to as *α-hydroxylated compounds,* are quite active but are metabolized rapidly, primarily by conjugation with glucuronic acid, such that there is no appreciable accumulation of active metabolites. The fused triazolo ring in *estazolam* lacks a methyl group and is hydroxylated to only a limited extent; the major route of metabolism involves the formation of the 3-hydroxyl derivative. The corresponding hydroxyl derivatives of *triazolam* and *alprazolam* also are formed to a significant extent. Compared with compounds without the triazolo ring, the rate of this reaction for all three drugs is unusually swift, and the 3-hydroxyl compounds are rapidly conjugated or oxidized further to benzophenone derivatives before excretion.

Midazolam is metabolized rapidly, primarily by hydroxylation of the methyl group on the fused imidazo ring; only small amounts of 3-hydroxyl compounds are formed. The α-hydroxylated compound, which has appreciable biological activity, is eliminated with a $t_{1/2}$ of 1 h

TABLE 22–1 ■ METABOLIC STAGES AND RELATIONSHIPS AMONG SOME OF THE BENZODIAZEPINES[a]



N-DESALKYLATED COMPOUNDS
3-HYDROXYLATED COMPOUNDS
Chlordiazepoxide (I)
Desmethyl-chlordiazepoxide (I)
Demoxepam (L)
Temazepam (I)
Diazepam (L)
Clorazepate (S)
Nordazepam (L)
Oxazepam (I)
Lorazepam (I)
GLUCURONIDATION
Flurazepam (S)
N-Hydroxyethyl-flurazepam (S)
N-Desalkylflur-azepam (L)
3-Hydroxy derivative (I)
Quazepam (L)
2-oxoquazepam (L)
2-Oxo-3-hydroxy-quazepam (S)
Estazolam (I)[b]
3-Hydroxy derivative (S)
Triazolam (S)[b]
α-Hydroxy-triazolam (S)
Alprazolam (I)[b]
α-Hydroxy-alprazolam (S)
Midazolam (S)[b]
α-Hydroxy-midazolam (S)

[a] Compounds enclosed in boxes are marketed in the U.S. The approximate half-lives of the various compounds are denoted in parentheses; S (short-acting), $t_{1/2}$ <6 h; I (intermediate-acting), $t_{1/2}$ = 6–24 h; L (long-acting), $t_{1/2}$ = >24 h. All compounds except clorazepate are biologically active; the activity of 3-hydroxydesalkylflurazepam has not been determined. Clonazepam (not shown) is an *N*-desalkyl compound, and it is metabolized primarily by reduction of the 7-NO_2 group to the corresponding amine (inactive), followed by acetylation; its $t_{1/2}$ is 20–40 h.

[b] See text for discussion of other pathways of metabolism.

after conjugation with glucuronic acid. Variable and sometimes substantial accumulation of this metabolite has been noted during intravenous infusion (Oldenhof et al., 1988).

The aromatic rings (A and C) of the benzodiazepines are hydroxylated only to a small extent. The only important metabolism at these sites is reduction of the 7-nitro substituents of *clonazepam*, *nitrazepam*, and *flunitrazepam*; the $t_{1/2}$ of these reactions are usually 20 to 40 h. The resulting amines are inactive and are acetylated to varying degrees before excretion.

Therapeutic Uses

Table 22–2 summarizes the therapeutic uses and routes of administration of benzodiazepines that are marketed in the U.S. Most benzodiazepines can be used interchangeably. For example, *diazepam* can be used to treat alcohol withdrawal symptoms, and most benzodiazepines work as hypnotics. Benzodiazepines that are useful as anticonvulsants have a long $t_{1/2}$, and rapid entry into the brain is required for efficacy in treatment of status epilepticus. Antianxiety agents, in contrast, should have a long $t_{1/2}$ despite the drawback of the risk of neuropsychological deficits caused by drug accumulation. For a hypnotic sleep medication, ideally there is a rapid onset of action when taken at bedtime, a sufficiently sustained action to maintain sleep throughout the night, and no residual action by the following morning. In practice, there are some disadvantages to the use of agents that have a relatively rapid rate of disappearance, such as *triazolam*, including the early morning insomnia experienced by some patients and a greater likelihood of rebound insomnia on drug discontinuation. With careful selection of dosage, *flurazepam* and other benzodiazepines with lower rates of elimination than *triazolam*'s can be used effectively.

Remimazolam is a benzodiazepine granted FDA approval in 2020 for the induction and maintenance of sedation during surgical procedures lasting 30 min or less. It has advantages over *midazolam*, another short-acting agent, possessing a faster onset and shorter duration of action; this shorter duration of action is due to rapid metabolism, yielding quicker patient recovery. Like other benzodiazepines, the effects of *remimazolam* can be reversed by *flumazenil*. Possible adverse reactions include hypoxia, diastolic hypotension, or diastolic or systolic hypertension.

Untoward Effects

At peak concentrations in plasma, hypnotic doses of benzodiazepines cause varying degrees of light-headedness, lassitude, increased reaction time, motor incoordination, impairment of mental and motor functions, confusion, and anterograde amnesia. Cognition appears to be affected less than motor performance. *All of these effects can greatly impair driving and other psychomotor skills, especially if combined with ethanol.* When the drug is given at the intended time of sleep, persistence of these effects into the following waking hours is adverse. These dose-related residual effects can be insidious because most subjects underestimate the degree of their impairment. Residual daytime sleepiness also may occur, even though successful drug therapy can reduce the daytime sleepiness resulting from chronic insomnia. The intensity and incidence of CNS toxicity generally increase with age (Monane, 1992). Other common side effects of benzodiazepines are weakness, headache, blurred vision, vertigo, nausea and vomiting, epigastric distress, and diarrhea; joint pains, chest pains, and incontinence are much rarer. Anticonvulsant benzodiazepines sometimes increase the frequency of seizures in patients with epilepsy.

AL GRAWANY

TABLE 22–2 ■ THERAPEUTIC USES OF BENZODIAZEPINES

COMPOUND	ROUTES OF ADMINISTRATION	THERAPEUTIC USES[a]	COMMENTS	$t_{1/2}$ (h)[b]	USUAL SEDATIVE-HYPNOTIC DOSE, mg[c]
Alprazolam	Oral	Anxiety disorders, agoraphobia (OL)	Withdrawal symptoms may be especially severe	12 ± 2	—
Chlordiazepoxide	Oral, IM, IV	Anxiety disorders, management of alcohol withdrawal, preanesthetic medication (OL)	Long-acting and self-tapering because of active metabolites	10 ± 3.4	50–100, 1–4× daily[d] (once daily for sleep)
Clobazam	Oral	Adjunctive treatment of seizures associated with Lennox-Gastaut syndrome (U.S. approved use), other types of epilepsies, anxiety disorders	Active metabolite $t_{1/2}$ 71–82 h; tolerance develops to anticonvulsant effects; not recommended in patients with severe hepatic impairment; decrease dose and titrate in CYP2C19-poor metabolizers	36–42	—
Clonazepam	Oral	Seizure disorders, panic disorder, adjunctive treatment in acute mania and certain movement disorders (OL)	Tolerance develops to anticonvulsant effects	23 ± 5	0.25–0.5 (hypnotic)
Clorazepate	Oral	Anxiety disorders, seizure disorders, management of alcohol withdrawal	Prodrug; activity due to formation of nordazepam during absorption	2.0 ± 0.9	3.75–20, 2–4× daily[d]
Diazepam	Oral, IM, IV, rectal	Anxiety disorders, alcohol withdrawal, status epilepticus, skeletal muscle relaxation, preanesthetic medication, Ménière disease (OL)	Prototypical benzodiazepine	43 ± 13	5–10, every 4 h
Estazolam	Oral	Insomnia	Contains triazolo ring; adverse effects may be similar to those of triazolam	10–24	1–2
Flurazepam	Oral	Insomnia	Active metabolites accumulate with chronic use	74 ± 24	15–30
Lorazepam	Oral, IM, IV	Anxiety disorders, alcohol withdrawal, preanesthetic medication, seizure disorders	Metabolized solely by conjugation	14 ± 5	1–4
Midazolam	Oral, IV, IM	Preanesthetic and intraoperative medication, anxiety disorders (agitation, alcohol withdrawal, seizure disorders, OL)	Rapidly inactivated	1.9 ± 0.6	1–5[e]
Oxazepam	Oral	Anxiety disorders, alcohol withdrawal	Metabolized solely by conjugation	8.0 ± 2.4	15–30, 3–4× daily[d]
Quazepam	Oral	Insomnia	Active metabolites accumulate with chronic use	39	7.5–15
Remimazolam	IV	Used as a sedative during medical procedures lasting 30 min or less	Very rapidly inactivated	0.6-0.9	5
Temazepam	Oral	Insomnia	Metabolized mainly by conjugation	11 ± 6	7.5–30
Triazolam	Oral	Insomnia	Rapidly inactivated; may cause disturbing daytime side effects	2.9 ± 1.0	0.125–0.5

OL, off-label.

[a] The therapeutic uses are examples to emphasize that most benzodiazepines can be used interchangeably. In general, the therapeutic uses of a given benzodiazepine are related to its $t_{1/2}$ and may not match the marketed indications. The issue is addressed more extensively in the text.

[b] Half-life of active metabolite may differ. See Appendix II for additional information.

[c] For additional dosage information, see Chapter 24 (anesthesia), Chapter 18 (anxiety), and Chapter 20 (seizure disorders).

[d] Approved as a sedative-hypnotic only for management of alcohol withdrawal; doses in a nontolerant individual would be smaller.

[e] Recommended doses vary considerably depending on specific use, condition of patient, and concomitant administration of other drugs.

A wide variety of serious allergic, hepatotoxic, and hematologic reactions to the benzodiazepines may occur, but the incidence is low; these reactions have been associated with the use of *flurazepam*, *triazolam*, and *temazepam*. Large doses taken just before or during labor may cause hypothermia, hypotonia, and mild respiratory depression in the neonate. Abuse by the pregnant mother can result in a withdrawal syndrome in the newborn.

Adverse Psychological Effects

Benzodiazepines may at times cause paradoxical effects. *Flurazepam* occasionally increases the incidence of nightmares—especially during the first week of use—and sometimes causes garrulousness, anxiety, irritability, tachycardia, and sweating. Amnesia, euphoria, restlessness, hallucinations, sleepwalking, sleep-talking, other complex behaviors, and hypomanic behavior have been reported to occur during use of various benzodiazepines. Bizarre uninhibited behavior may occur in some users, hostility and rage in others; collectively, these are sometimes referred to as *disinhibition* or *dyscontrol reactions*. Paranoia, depression, and suicidal ideation also occasionally may accompany the use of these agents. Such paradoxical or disinhibition reactions are rare and appear to be dose related. Because of reports of an increased incidence of confusion and abnormal behaviors, *triazolam* has been banned in the U.K. The FDA has declared *triazolam* to be safe and effective in low doses of 0.125 to 0.25 mg.

Chronic benzodiazepine use poses a risk for development of dependence and abuse (Woods et al., 1992). Mild dependence may develop in many patients who have taken therapeutic doses of benzodiazepines on a regular basis for prolonged periods, but not to the same extent as seen with older sedatives and other recognized drugs of abuse (see Chapter 28; Uhlenhuth et al., 1999). Withdrawal symptoms may include temporary intensification of the problems that originally prompted their use (e.g., insomnia or anxiety). Dysphoria, irritability, sweating, unpleasant dreams, tremors, anorexia, and faintness or dizziness also may occur, especially when withdrawal of the benzodiazepine occurs abruptly. Hence, it is prudent to taper the dosage gradually when therapy is to be discontinued. Despite their adverse effects, benzodiazepines are relatively safe drugs, and fatalities are rare unless other drugs are taken concomitantly. Ethanol is a common contributor to deaths involving benzodiazepines, but true coma is uncommon in the absence of another CNS depressant. Although overdosage with a benzodiazepine rarely causes severe cardiovascular or respiratory depression, therapeutic doses of benzodiazepines can further compromise respiration in patients with COPD or OSA. Benzodiazepine abuse of a different sort includes the use of *flunitrazepam* (Rohypnol; not licensed for use in the U.S.) as a "date rape drug."

Drug Interactions

Except for additive effects with other sedative or hypnotic drugs, reports of clinically important deleterious pharmacodynamic interactions between benzodiazepines and other drugs have been infrequent. In some cases, benzodiazepines are used in the premedication of surgical or dental patients before the induction of anesthesia, largely to decrease anxiety, but also to produce light sedation. In this case, the amnestic effects of benzodiazepines are desirable. *Midazolam*, with its very fast onset of action and an inactive metabolite, is the preoperative anxiolytic currently used predominantly. An unwanted pharmacodynamic interaction involves the use of *valproate* and benzodiazepines in combination, which may cause psychotic episodes. An example of a pharmacokinetic interaction is ethanol increasing the rate of absorption of benzodiazepines and thus the associated CNS depression.

Novel Benzodiazepine Receptor Site Agonists

Hypnotics in this class are commonly referred to as "Z compounds." They include *zolpidem*, *zaleplon*, *zopiclone* (not marketed in the U.S.), and *eszopiclone*, which is the S(+) enantiomer of *zopiclone* (Huedo-Medina et al., 2012) and is available in the U.S. Although the Z compounds are structurally unrelated to each other and to benzodiazepines, their therapeutic efficacy as hypnotics is due to agonist effects at the benzodiazepine site of the $GABA_A$ receptor (Hanson et al., 2008). Compared to benzodiazepines, Z compounds are less effective as anticonvulsants or muscle relaxants, which may be related to their relative selectivity for $GABA_A$ receptors containing the α_1 subunit. Over the last decade, Z compounds have largely replaced benzodiazepines in the treatment of insomnia. Z compounds were initially promoted as having less potential for dependence and abuse than traditional benzodiazepines. However, based on postmarketing clinical experience with *zopiclone* and *zolpidem*, tolerance and physical dependence can be expected during long-term use, especially with higher doses. The Z drugs are classified as schedule IV drugs in the U.S., indicating abuse liability concerns similar to those of benzodiazepines. The clinical presentation of overdose with Z compounds is similar to that of benzodiazepine overdose and can be treated with the benzodiazepine receptor site antagonist *flumazenil*.

Zaleplon

Zaleplon is a member of the pyrazolopyrimidine class. *Zaleplon* preferentially binds to the benzodiazepine binding site on $GABA_A$ receptors containing the α_1 receptor subunit. It is absorbed rapidly and reaches peak plasma concentrations in about 1 h. Its bioavailability is about 30% because of presystemic metabolism. *Zaleplon* is metabolized largely by aldehyde oxidase and to a lesser extent by CYP3A4. Its $t_{1/2}$ is short, about 1 h. *Zalepon*'s oxidative metabolites are converted to glucuronides and eliminated in urine. Less than 1% of *zaleplon* is excreted unchanged; none of *zaleplon*'s metabolites is pharmacologically active. *Zaleplon* is usually administered in 5-, 10-, or 20-mg doses (Dooley and Plosker, 2000). *Zaleplon*-treated subjects with either chronic or transient insomnia experience shorter periods of sleep onset latency.

Zolpidem

Zolpidem is an imidazopyridine sedative-hypnotic. The actions of *zolpidem* are also due to agonist effects at the benzodiazepine receptor site on $GABA_A$ receptors and generally resemble those of benzodiazepines. The drug normally has little effect on the stages of sleep in human subjects. It is effective in shortening sleep latency and prolonging total sleep time in patients with insomnia. After discontinuation of *zolpidem*, the beneficial effects on sleep reportedly persist for up to 1 week, but mild rebound insomnia on the first night of withdrawal may occur. *Zolpidem* is approved only for the short-term treatment of insomnia; however, tolerance and physical dependence are rare (Morselli, 1993). At therapeutic doses (5–10 mg), *zolpidem* infrequently produces residual daytime sedation or amnesia; the incidence of other adverse effects also is low. As with the benzodiazepines, overdoses of *zolpidem* do not produce severe respiratory depression unless other agents (e.g., ethanol) also are ingested. Hypnotic doses increase the hypoxia and hypercarbia of patients with OSA.

Zolpidem is absorbed readily from the GI tract; first-pass hepatic metabolism results in an oral bioavailability of about 70% (lower when the drug is ingested with food). *Zolpidem* is eliminated almost entirely by conversion to inactive products in the liver, largely through oxidation of the methyl groups on the phenyl and imidazopyridine rings to the corresponding carboxylic acids. Its plasma $t_{1/2}$ is typically about 2 h, but this value may increase 2-fold or more in those with cirrhosis and also tends to be greater in older patients, requiring adjustment of dosage. Although little or no unchanged *zolpidem* is found in the urine, elimination of the drug is slower in patients with chronic renal insufficiency; the increased elimination time is largely due to an increase in its apparent volume of distribution.

Zaleplon and Zolpidem Compared

Zaleplon and *zolpidem* are effective in relieving sleep-onset insomnia. Both drugs are FDA-approved for use for up to 7 to 10 days at a time. *Zaleplon* and *zolpidem* have sustained hypnotic efficacy without occurrence of rebound insomnia on abrupt discontinuation. *Zolpidem* has a $t_{1/2}$ of about 2 h, which is sufficient to cover most of a typical 8-h sleep period, and is presently approved for bedtime use only. *Zaleplon* has a shorter $t_{1/2}$ of about 1 h, which offers the possibility for safe dosing later in the night, within 4 h of the anticipated rising time. *Zaleplon* and *zolpidem* differ in residual side effects; late-night administration of *zolpidem* has been associated with morning sedation, delayed reaction time, and anterograde amnesia, whereas *zaleplon* does not differ from placebo in those respects.

Eszopiclone

Eszopiclone is the active S(+) enantiomer of *zopiclone*. It exerts its sleep-promoting effects by enhancing $GABA_A$ receptor function via the benzodiazepine binding site. *Eszopiclone* is used for the long-term (~12 months) treatment of insomnia, for sleep maintenance, and to decrease the latency to onset of sleep (Melton et al., 2005; Rosenberg et al., 2005). It is available in 1-, 2-, or 3-mg tablets. In clinical studies, no tolerance was observed, and no signs of serious withdrawal, such as seizures or rebound insomnia, were seen on discontinuation of the drug; however, there are such reports for *zopiclone*, the racemate used outside the U.S. Mild withdrawal signs, consisting of abnormal dreams, anxiety, nausea, and upset stomach can occur (≤2%). A minor reported adverse effect of *eszopiclone* is a bitter taste. *Eszopiclone* is absorbed rapidly after oral administration, with a bioavailability of about 80%, and shows wide distribution throughout the body. It is 50% to 60% bound to plasma proteins, is metabolized by CYPs 3A4 and 2E1, and has a $t_{1/2}$ of about 6 h.

Management of Patients After Long-Term Benzodiazepine Therapy

If a benzodiazepine has been used regularly for more than 2 weeks, its use should be tapered rather than discontinued abruptly. In some patients taking hypnotics with a short $t_{1/2}$, it is easier to switch first to a hypnotic with a long $t_{1/2}$ and then to taper. The onset of withdrawal symptoms from medications with a long $t_{1/2}$ may be delayed. Consequently, the patient should be warned about the symptoms associated with withdrawal effects.

Flumazenil: A Benzodiazepine Receptor Antagonist

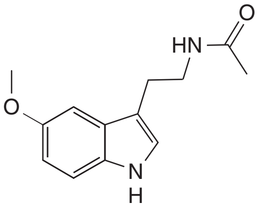
Flumazenil is an imidazobenzodiazepine that binds with high affinity to the benzodiazepine binding site on the $GABA_A$ receptor, where it competitively antagonizes the binding and allosteric effects of benzodiazepines and other ligands, including the Z drugs (Hoffman and Warren, 1993). *Flumazenil* antagonizes both the electrophysiological and behavioral effects of agonist and inverse-agonist benzodiazepines and β-carbolines.

Flumazenil is available only for intravenous administration. Administration of a series of small injections is preferred to a single bolus injection. A total of 1 mg *flumazenil* given over 1 to 3 min usually is sufficient to abolish the effects of therapeutic doses of benzodiazepines. Additional courses of treatment with *flumazenil* may be needed within 20 to 30 min should sedation reappear. The duration of clinical effects usually is only 30 to 60 min. Although absorbed rapidly after oral administration, less than 25% of the drug reaches the systemic circulation owing to extensive first-pass hepatic metabolism. *Flumazenil* is eliminated almost entirely by hepatic metabolism to inactive products with a $t_{1/2}$ of about 1 h. Oral doses are apt to cause headache and dizziness.

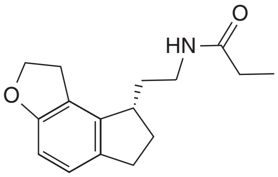
The primary indications for the use of *flumazenil* are the management of suspected benzodiazepine overdose and the reversal of sedative effects produced by benzodiazepines administered during general anesthesia and diagnostic or therapeutic procedures. *Flumazenil* administration should also be considered in cases in which the patient has consumed a combination of sedatives, as long as one of them is a benzodiazepine site agonist, such as in cases of combined ethanol and benzodiazepine overdose. However, *flumazenil* is not effective in treating single-drug overdoses with barbiturates, ethanol, opioids, or tricyclic antidepressants. Indeed, the administration of *flumazenil* in these settings may be associated with the onset of seizures, especially in patients poisoned with tricyclic antidepressants. *Flumazenil* may precipitate seizures or other withdrawal signs in patients taking benzodiazepines for protracted periods and in whom tolerance or dependence may have developed.

Melatonin Congeners

Melatonin is a circadian signaling molecule. In some fish and amphibians, melatonin modulates skin coloration through an action on melanin-containing pigment granules in melanophores. In humans, melatonin, not to be confused with the pigment melanin, is the principal indoleamine in the pineal gland, where it may be said to constitute a pigment of the imagination. The synthesis of melatonin in the pineal gland (by *N*-acetylation and *O*-methylation of serotonin; see Figure 15–2) is influenced by external factors, including environmental light. In mammals, melatonin induces pigment lightening in skin cells and suppresses ovarian functions; it also serves a role in regulating biological rhythms and has been studied as a treatment of jet lag and other sleep disturbances. Melatonin analogues have recently been approved for the treatment of insomnia.



MELATONIN



RAMELTEON

Ramelteon

Ramelteon is a synthetic tricyclic analogue of melatonin, approved in the U.S. for the treatment of insomnia, specifically difficulties of sleep onset (Spadoni et al., 2011).

Mechanism of Action

Melatonin levels in the suprachiasmatic nucleus rise and fall in a circadian fashion, with concentrations increasing in the evening as an individual prepares for sleep and then reaching a plateau and ultimately decreasing as the night progresses. Two G protein–coupled receptors (GPCRs) for melatonin, MT_1 and MT_2, in the suprachiasmatic nucleus, each play a different role in sleep. Binding of agonists such as melatonin to MT_1 receptors promotes the onset of sleep; melatonin binding to MT_2 receptors shifts the timing of the circadian system. *Ramelteon* binds to both MT_1 and MT_2 receptors with high affinity, but, unlike melatonin, it does not bind appreciably to quinone reductase 2, the structurally unrelated MT_3 receptor. *Ramelteon* is not known to bind to any other classes of receptors, such as nicotinic ACh, neuropeptide, dopamine, and opioid receptors, or the benzodiazepine binding site on $GABA_A$ receptors.

Clinical Pharmacology

Prescribing guidelines suggest that an 8-mg tablet be taken about 30 min before bedtime. *Ramelteon* is rapidly absorbed from the GI tract. Because of the significant first-pass metabolism that occurs after oral administration, *ramelteon* bioavailability is less than 2%. The drug is largely metabolized by hepatic CYPs 1A2, 2C, and 3A4, with a $t_{1/2}$ of about 2 h in humans. Of the four metabolites, M-II acts as an agonist at MT_1 and MT_2 receptors and may contribute to the sleep-promoting effects of *ramelteon*.

Ramelteon is efficacious in combating both transient and chronic insomnia, with no tolerance occurring in its reduction of sleep onset latency even after 6 months of drug administration (Mayer et al., 2009). It is generally well tolerated by patients and does not impair next-day cognitive function. Sleep latency was consistently found to be shorter in patients given *ramelteon* compared with placebo controls. No evidence of rebound insomnia or withdrawal effects were noted on *ramelteon* withdrawal. Unlike most agents mentioned in this chapter, *ramelteon* is not a controlled substance since there is no evidence of abuse liability. Dizziness, nausea, drowsiness, and headache have been reported as adverse effects in a small minority of patients.

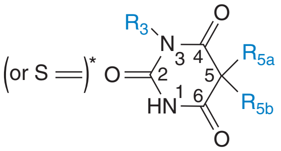
Tasimelteon

Tasimelteon is a selective agonist for MT_1 and MT_2 receptors. Although FDA approval was sought in the U.S. only for the treatment of non–24-h sleep-wake syndrome in totally blind patients experiencing circadian rhythm disorder (Johnsa and Neville, 2014), the FDA approved *tasimelteon* for use in both blind and nonblind individuals, although no clinical trials were performed in the latter group. In 2020, the FDA expanded the use of *tasimelteon* for the treatment of nighttime sleep disturbances in adults and children with Smith-Magenis syndrome, a rare neurodevelopmental disorder involving reversed circadian rhythm that makes nighttime sleep difficult. Discontinuation of *tasimelteon* use leads to a reversion to baseline sleep parameters within approximately 1 month.

Barbiturates

The barbiturates were once used extensively as sedative-hypnotic drugs. Except for a few specialized uses, they have been largely replaced by the much safer benzodiazepines and Z compounds. Table 22–3 lists the common barbiturates and their pharmacological properties.

Barbiturates are derivatives of this parent structure:



*O except in thiopental, where it is replaced by S.

Barbituric acid is 2,4,6-trioxohexahydropyrimidine. This compound lacks central depressant activity, but the presence of alkyl or aryl groups at position 5 confers sedative-hypnotic and sometimes other activities. Barbiturates in which the oxygen at C2 is replaced by sulfur are called *thiobarbiturates.* These compounds are more lipid soluble than the corresponding *oxybarbiturates.* In general, structural changes that increase lipid solubility decrease duration of action, decrease latency to onset of activity, accelerate metabolic degradation, and increase hypnotic potency.

The Pharmacological Properties of Barbiturates

The barbiturates reversibly depress the activity of all excitable tissues to varying extents. The CNS is particularly sensitive, but even when barbiturates are given in anesthetic concentrations, direct effects on peripheral excitable tissues are weak. However, serious deficits in cardiovascular and other peripheral functions occur in acute barbiturate intoxication.

ADME

For sedative-hypnotic use, the barbiturates usually are administered orally (see Table 22–2). Na^+ salts are absorbed more rapidly than the corresponding free acids, especially from liquid formulations. The onset of action varies from 10 to 60 min and is delayed by the presence of food. Intramuscular injections of solutions of the Na^+ salts should be placed deeply into large muscles to avoid the pain and possible necrosis that can result at more superficial sites. The intravenous route usually is reserved for the management of status epilepticus (*phenobarbital sodium*) or for the induction or maintenance of general anesthesia (e.g., *thiopental* or *methohexital*).

Barbiturates distribute widely in the body and readily cross the placenta. The highly lipid-soluble barbiturates such as *thiopental* and *methohexital*, used to induce anesthesia, undergo rapid redistribution after intravenous injection. Redistribution into less-vascular tissues, especially muscle and fat, leads to a decline in the concentration of barbiturate in the plasma and brain. With *thiopental* and *methohexital*, this results in the awakening of patients within 5 to 15 min of the injection of the usual anesthetic doses (see Figures 2–4 and 24–3).

Except for the less lipid-soluble *aprobarbital* and *phenobarbital*, nearly complete metabolism or conjugation of barbiturates in the liver precedes their renal excretion. The oxidation of radicals at C5 is the most important biotransformation that terminates biological activity. In some instances (e.g., *phenobarbital*), *N*-glycosylation is an important metabolic pathway. Other biotransformations include *N*-hydroxylation, desulfuration of thiobarbiturates to oxybarbiturates, opening of the barbituric acid ring, and *N*-dealkylation of *N*-alkyl barbiturates to active metabolites (e.g., *mephobarbital* to *phenobarbital*). About 25% of *phenobarbital* and nearly all of *aprobarbital* are excreted unchanged in the urine. Their renal excretion can be increased greatly by osmotic diuresis or alkalinization of the urine.

TABLE 22–3 ■ THERAPEUTIC USES OF BARBITURATES

COMPOUND	ROUTES OF ADMINISTRATION	THERAPEUTIC USES	COMMENTS	$t_{1/2}$ (h)
Amobarbital	IM, IV	Insomnia, preoperative sedation, emergency management of seizures	Only Na^+ salt for injection is sold in the U.S.	10–40
Butabarbital	Oral	Insomnia, preoperative sedation, daytime sedation	Redistribution shortens duration of action of single dose to 8 h	35–50
Mephobarbital (not licensed for use in the U.S.)	Oral	Seizure disorders, daytime sedation	Second-line anticonvulsant	10–70
Methohexital	IV	Induction and maintenance of anesthesia	Only Na^+ salt available; single dose provides 5–7 min of anesthesia	3–5
Pentobarbital	Oral, IM, IV, rectal (only injectable form is marketed in the U.S.)	Insomnia, preoperative and procedural sedation, emergency management of seizures	Administer only Na^+ salt parenterally	15–50
Phenobarbital	Oral, IM, IV	Seizure disorders, status epilepticus, daytime sedation (hyperbilirubinemia, off-label use)	First-line anticonvulsant; only Na^+ salt administered parenterally	80–120
Secobarbital	Oral	Insomnia, preoperative sedation	Only Na^+ salt available	15–40
Thiopental (not currently produced or marketed in the U.S.)	IV	Induction/maintenance of anesthesia, preoperative sedation, emergency management of seizures, intracranial pressure	Only Na^+ salt available; single dose provides brief period of anesthesia	8–10 ($t_{1/2}$ of anesthetic effects is short due to redistribution)

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The metabolic elimination of barbiturates is more rapid in young people than in the elderly and infants, and half-lives are increased during pregnancy partly because of the expanded volume of distribution. Chronic liver disease, especially cirrhosis, often increases the $t_{1/2}$ of the biotransformable barbiturates. Repeated administration, especially of *phenobarbital*, shortens the $t_{1/2}$ of barbiturates that are metabolized as a result of the induction of microsomal enzymes in the liver.

The barbiturates commonly used as hypnotics in the U.S. have $t_{1/2}$ *values such that the drugs are not fully eliminated in 24 h (see Table 22–3). Thus, these barbiturates will accumulate during repeated administration unless appropriate adjustments in dosage are made. Furthermore, the persistence of the drug in plasma during the day favors the development of tolerance and abuse.*

CNS Effects

Actions on the GABA$_A$ Receptor

Enhancement of inhibition occurs primarily at synapses where neurotransmission is mediated by GABA acting at GABA$_A$ receptors. Barbiturates bind to an allosteric site on the GABA$_A$ receptor distinct from the benzodiazepine site (see Figure 16–11); binding leads to an increase in the mean open time of the GABA-activated Cl$^-$ channel, with no effect on frequency. At higher concentrations, barbiturates directly activate channel opening, even in the absence of GABA (Nestler et al., 2020). Barbiturates also reportedly inhibit excitatory α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate receptors (Marszalec and Narahashi, 1993) and inhibit glutamate release via an effect on voltage-activated Ca^{2+} channels. Barbiturates also produce significantly greater enhancement of GABA$_A$ receptor function than even maximally effective concentrations of benzodiazepines.

Effects in the CNS

Barbiturates enhance GABA-mediated inhibitory transmission throughout the CNS; nonanesthetic doses preferentially suppress polysynaptic responses. Facilitation is diminished, and inhibition usually is enhanced. The site of inhibition is either postsynaptic, as at *cortical and cerebellar pyramidal cells* and in the *cuneate nucleus, substantia nigra*, and *thalamic relay neurons*, or presynaptic, as in the *spinal cord*.

Barbiturates can produce all degrees of depression of the CNS, ranging from mild sedation to general anesthesia in a dose-dependent manner (see Chapter 24). Certain barbiturates, particularly those containing a 5-phenyl substituent (e.g., *phenobarbital* and *mephobarbital*), have selective anticonvulsant activity (see Chapter 20). The antianxiety properties of the barbiturates are inferior to those exerted by the benzodiazepines.

Except for the anticonvulsant activities of *phenobarbital* and its congeners, the barbiturates possess a low degree of selectivity and a low therapeutic index. Pain perception and reaction are relatively unimpaired until the moment of unconsciousness, and in small doses, barbiturates increase reactions to painful stimuli. Hence, they cannot be relied on to produce sedation or sleep in the presence of even moderate pain.

Effects on Stages of Sleep

Hypnotic doses of barbiturates increase the total sleep time and alter the stages of sleep in a dose-dependent manner. Like the benzodiazepines, barbiturates decrease sleep latency, the number of awakenings, and the durations of REM and slow-wave sleep. During repetitive nightly administration, some tolerance to the effects on sleep occurs within a few days, and the effect on total sleep time may be reduced by as much as 50% after 2 weeks of use. Discontinuation leads to rebound increases in all the sleep parameters initially decreased by barbiturates.

Tolerance, Abuse, and Dependence

With chronic administration of gradually increasing doses, pharmacodynamic tolerance continues to develop over a period of weeks to months, depending on the dosage schedule, whereas pharmacokinetic tolerance reaches its peak in a few days to a week. Tolerance to the euphoric, sedative, and hypnotic effects occurs more readily and is greater than that to the anticonvulsant and lethal effects; thus, as tolerance increases, the therapeutic index decreases. Pharmacodynamic tolerance to barbiturates confers cross-tolerance to all general CNS depressant drugs, including ethanol. Like other CNS depressant drugs, barbiturates are abused, and some individuals develop physical dependence (see Chapter 28).

Effects on Peripheral Nerve Structures

Barbiturates selectively depress transmission in autonomic ganglia and reduce nicotinic excitation by choline esters. This effect may account, at least in part, for the fall in blood pressure produced by intravenous oxybarbiturates and by severe barbiturate intoxication. At skeletal neuromuscular junctions, the blocking effects of both *tubocurarine* and *decamethonium* are enhanced during barbiturate anesthesia. These actions probably result from the capacity of barbiturates at hypnotic or anesthetic concentrations to inhibit current flow through nicotinic ACh receptors. Several distinct mechanisms appear to be involved, and little stereoselectivity is evident.

Systemic Effects

Respiration

Barbiturates depress both the respiratory drive and the mechanisms responsible for the rhythmic character of respiration. The neurogenic drive is essentially eliminated by a dose three times greater than that used normally to induce sleep. Such doses also suppress the hypoxic drive and, to a lesser extent, the chemoreceptor drive. However, the margin between the lighter planes of surgical anesthesia and dangerous respiratory depression is sufficient to permit the ultra-short-acting barbiturates to be used, with suitable precautions, as anesthetic agents.

The barbiturates only slightly depress protective reflexes until the degree of intoxication is sufficient to produce severe respiratory depression. Coughing, sneezing, hiccoughing, and laryngospasm may occur when barbiturates are employed as intravenous anesthetic agents.

Cardiovascular System

When given orally in sedative or hypnotic doses, barbiturates do not produce significant overt cardiovascular effects. In general, the effects of *thiopental* anesthesia on the cardiovascular system are benign in comparison with those of the volatile anesthetic agents; there usually is either no change or a fall in mean arterial pressure (see Chapter 24). Barbiturates can blunt cardiovascular reflexes by partial inhibition of ganglionic transmission, most evident in patients with congestive heart failure or hypovolemic shock. Because barbiturates also impair reflex cardiovascular adjustments to inflation of the lung, positive-pressure respiration should be used cautiously and only when necessary to maintain adequate pulmonary ventilation in patients who are anesthetized or intoxicated with a barbiturate.

Additional cardiovascular changes often noted when *thiopental* and other intravenous thiobarbiturates are administered after conventional preanesthetic medication include decreased renal and cerebral blood flow with a marked fall in CSF pressure. Although cardiac arrhythmias are observed only infrequently, intravenous anesthesia with barbiturates can increase the incidence of ventricular arrhythmias, especially when *epinephrine* and *halothane* also are present. Anesthetic concentrations of barbiturates depress the function of Na$^+$ channels and at least two types of K$^+$ channels. However, direct depression of cardiac contractility occurs only when doses several times those required to cause anesthesia are administered.

GI Tract

The oxybarbiturates tend to decrease the tone of the GI musculature and the amplitude of rhythmic contractions; the locus of action is partly peripheral and partly central. A hypnotic dose does not significantly delay gastric emptying in humans. The relief of various GI symptoms by sedative doses is probably largely due to the central depressant action.

Liver

The effects vary with the duration of exposure to the barbiturate. *Acutely,* the barbiturates interact with several CYPs and inhibit the biotransformation of a number of other drugs and endogenous substrates, such as steroids; other substrates may reciprocally inhibit barbiturate biotransformations (see Chapter 5).

Chronic administration of barbiturates markedly increases the protein and lipid content of the hepatic smooth endoplasmic reticulum, as well as the activities of glucuronyl transferase and CYPs 1A2, 2C9, 2C19, and 3A4. The induction of these enzymes increases the metabolism of a number of drugs (including barbiturates) and endogenous substances, including steroid hormones, cholesterol, bile salts, and vitamins K and D. The self-induced increase in barbiturate metabolism partly accounts for tolerance to barbiturates. The inducing effect is not limited to the microsomal enzymes; for example, there are increases in δ-aminolevulinic acid (ALA) synthase, a mitochondrial enzyme, and aldehyde dehydrogenase, a cytosolic enzyme. The effect of barbiturates on ALA synthetase can cause dangerous disease exacerbations in persons with intermittent porphyria.

Kidneys

Severe oliguria or anuria may occur in acute barbiturate poisoning largely as a result of the marked hypotension.

Therapeutic Uses

The major uses of individual barbiturates are listed in Table 22–3. As with the benzodiazepines, the selection of a particular barbiturate for a given therapeutic indication is based primarily on pharmacokinetic considerations. Benzodiazepines and other compounds have largely replaced barbiturates as sedatives.

Untoward Effects

Aftereffects

Drowsiness may last for only a few hours after a hypnotic dose of barbiturate, but residual CNS depression sometimes is evident the following day, and subtle distortions of mood and impairment of judgment and fine motor skills may be demonstrable. Residual effects also may take the form of vertigo, nausea, vomiting, or diarrhea or sometimes may be manifested as overt excitement.

Paradoxical Excitement

In some persons, barbiturates produce excitement rather than depression, and the patient may appear to be inebriated. This type of idiosyncrasy is relatively common among geriatric and debilitated patients and occurs most frequently with *phenobarbital* and *N*-methylbarbiturates. Barbiturates may cause restlessness, excitement, and even delirium when given in the presence of pain, and may worsen a patient's perception of pain.

Hypersensitivity

Allergic reactions occur, especially in persons with asthma, urticaria, angioedema, or similar conditions. Hypersensitivity reactions include localized swellings, particularly of the eyelids, cheeks, or lips, and erythematous dermatitis. Rarely, exfoliative dermatitis may be caused by *phenobarbital* and can prove fatal; the skin eruption may be associated with fever, delirium, and marked degenerative changes in the liver and other parenchymatous organs.

Other

Because barbiturates enhance porphyrin synthesis, they are absolutely contraindicated in patients with acute intermittent porphyria or porphyria variegata. Hypnotic doses in the presence of pulmonary insufficiency are contraindicated. Rapid intravenous injection of a barbiturate may cause cardiovascular collapse before anesthesia ensues. Blood pressure can fall to shock levels; even slow intravenous injection of barbiturates often produces apnea and occasionally laryngospasm, coughing, and other respiratory difficulties.

Drug Interactions

Barbiturates combine with other CNS depressants to cause severe depression; interactions with ethanol and with first-generation antihistamines are common. *Isoniazid*, *methylphenidate*, and monoamine oxidase inhibitors also increase the CNS depressant effects of barbiturates.

Barbiturates competitively inhibit the metabolism of certain other drugs; however, the greatest number of drug interactions results from induction of hepatic CYPs (as described previously) and the accelerated disappearance of many drugs and endogenous substances from the body. Hepatic enzyme induction enhances metabolism of endogenous steroid hormones, which may cause endocrine disturbances, and enhances metabolism of oral contraceptives, which may increase the likelihood of unwanted pregnancy. Barbiturates also induce the hepatic generation of toxic metabolites of chlorocarbons (chloroform, trichloroethylene, carbon tetrachloride) and consequently promote lipid peroxidation, which facilitates periportal necrosis of the liver caused by these agents.

Barbiturate Poisoning

The incidence of barbiturate poisoning has declined markedly, largely as a result of their decreased use as sedative-hypnotic agents. Most of the cases are the result of attempts at suicide, but some are from accidental poisonings in children or drug abusers. The lethal dose of barbiturate varies, but severe poisoning is likely to occur when more than 10 times the full hypnotic dose has been ingested at once. The lethal dose becomes lower if alcohol or other depressant drugs are present. In severe intoxication, the patient is comatose; respiration is affected early. Breathing may be either slow or rapid and shallow. Eventually, blood pressure falls because the effect of the drug and of hypoxia on medullary vasomotor centers; depression of cardiac contractility and sympathetic ganglia also contributes. Pulmonary complications (e.g., atelectasis, edema, and bronchopneumonia) and renal failure are likely to be the fatal complications of severe barbiturate poisoning.

The treatment of acute barbiturate intoxication is based on general supportive measures, which are applicable in most respects to poisoning by any CNS depressant. The use of CNS stimulants is contraindicated. If renal and cardiac functions are satisfactory and the patient is hydrated, forced diuresis and alkalinization of the urine will hasten the excretion of *phenobarbital*. See Chapter 9, Drug Toxicity and Poisoning.

Miscellaneous Sedative-Hypnotic Drugs

Many drugs with diverse structures have been used for their sedative-hypnotic properties, including *ramelteon, chloral hydrate, meprobamate*, and *paraldehyde* (no longer licensed in the U.S.). With the exception of *ramelteon* and *meprobamate*, the pharmacological actions of these drugs generally resemble those of the barbiturates:

- They all are general CNS depressants that can produce profound hypnosis with little or no analgesia.
- Their effects on the stages of sleep are similar to those of the barbiturates.
- Their therapeutic indices are low, and acute intoxication, which produces respiratory depression and hypotension, is managed similarly to barbiturate poisoning.
- Their chronic use can result in tolerance and physical dependence.
- The syndrome after chronic use can be severe and life threatening.

Chloral Hydrate

Chloral hydrate may be used to treat patients with paradoxical reactions to benzodiazepines. *Chloral hydrate* is reduced rapidly to the active compound trichloroethanol (CCl_3CH_2OH), largely by hepatic alcohol dehydrogenase. Its pharmacological effects probably are caused by trichloroethanol, which can exert barbiturate-like effects on $GABA_A$ receptor channels *in vitro*. *Chloral hydrate* is regulated as a schedule IV controlled substance.

In the U.S., *chloral hydrate* is best known as a literary hypnotic, the "knockout drops" added to a strong alcoholic beverage to produce a "Mickey Finn" or "Mickey," a cocktail given to an unwitting imbiber to render the person malleable or unconscious, most famously Sam Spade in Dashiell Hammett's 1930 novel, *The Maltese Falcon*.

Meprobamate

Meprobamate, a *bis*-carbamate ester, was introduced as an antianxiety agent in 1955, and this remains its only approved use in the U.S.;

it has abuse liability and is regulated as a schedule IV drug. Marketing authorization for *meprobamate* was withdrawn in the European Union in 2012 and in Canada in 2013 due to concerns related to its side effects. *Meprobamate* also became popular as a sedative-hypnotic agent but was later largely replaced by benzodiazepines in this role. The pharmacological properties of *meprobamate* resemble those of the benzodiazepines in a number of ways. *Meprobamate* can release suppressed behaviors in experimental animals at doses that cause little impairment of locomotor activity, and although it can cause CNS depression, it cannot produce anesthesia. Large doses of *meprobamate* cause severe respiratory depression, hypotension, shock, and heart failure. *Meprobamate* appears to have a mild analgesic effect in patients with musculoskeletal pain, and it enhances the analgesic effects of other drugs.

Meprobamate is well absorbed when administered orally. Nevertheless, an important aspect of intoxication with *meprobamate* is the formation of gastric bezoars consisting of undissolved *meprobamate* tablets; treatment may require endoscopy, with mechanical removal of the bezoar. Most of the drug is metabolized in the liver by side-chain hydroxylation and glucuronidation; the kinetics of elimination may depend on dose. The $t_{1/2}$ of *meprobamate* may be prolonged during its chronic administration. The major unwanted effects of the usual sedative doses of *meprobamate* are drowsiness and ataxia; larger doses impair learning and motor coordination and prolong reaction time. *Meprobamate* enhances the CNS depression produced by other drugs. After long-term medication, abrupt discontinuation evokes a withdrawal syndrome usually characterized by anxiety, insomnia, tremors, and, frequently, hallucinations; generalized seizures occur in about 10% of cases.

Carisoprodol, a skeletal muscle relaxant whose active metabolite is *meprobamate*, also has abuse potential and has become a popular "street drug." *Carisoprodol* is designated as a schedule IV controlled substance in the U.S.

Other Agents

Etomidate is used in the U.S. and other countries as an intravenous anesthetic, often in combination with *fentanyl*. It is advantageous because it lacks pulmonary and vascular depressant activity, although it has a negative inotropic effect on the heart. Its pharmacology and anesthetic uses are described in Chapter 24.

Clomethiazole has sedative, muscle relaxant, and anticonvulsant properties. Given alone, its effects on respiration are slight, and the therapeutic index is high. However, deaths from adverse interactions with ethanol are relatively frequent.

Propofol is a rapidly acting and highly lipophilic diisopropylphenol used in the induction and maintenance of general anesthesia (see Chapter 24), as well as in the maintenance of long-term sedation. *Propofol* has found use in intensive care sedation in adults (McKeage and Perry, 2003), for sedation during GI endoscopy procedures, and during transvaginal oocyte retrieval.

Nonprescription Hypnotic Drugs

The antihistamines *diphenhydramine* and *doxylamine* are FDA approved as ingredients in over-the-counter nonprescription sleep aids. With an elimination $t_{1/2}$ of about 9 to 10 h, these antihistamines can be associated with prominent residual sleepiness the morning after when taken as a sleep aid the night before.

New and Emerging Agents

Suvorexant

Suvorexant, an inhibitor of orexin 1 and 2 receptors, is FDA-approved for the treatment of insomnia (Winrow and Renger, 2014). Orexins, produced by neurons in the lateral hypothalamus and projecting broadly throughout the CNS, play a major role in regulation of the sleep cycle. These neurons are quiescent during sleep but are active during wakefulness; thus, orexins promote wakefulness, while antagonists at orexin receptors enhance REM and non-REM sleep. *Suvorexant* decreases sleep onset latency and is superior to placebo in sleep maintenance. One 10-mg dose should be taken within 30 min of going to bed if at least 7 h remain until the projected time of awakening. The most common adverse reaction is daytime somnolence, and there is a possibility of the worsening of depression or suicidal ideation. *Suvorexant* is a schedule IV controlled substance.

Lemborexant

Lemborexant, another orexin 1/2 receptor antagonist, was granted FDA approval in 2019. Doses ranging from 2.5 to 10 mg are efficacious in the treatment of insomnia with minimal next-morning residual drowsiness in most patients (Murphy et al., 2017). *Lemborexant* has a $t_{1/2}$ of 17 to 19 h. As with *suvorexant*, the most common adverse reaction is somnolence, especially at higher doses. Like *suvorexant*, it is a schedule IV controlled drug. Importantly, orexin receptor antagonists do not produce the anterograde amnesia seen with benzodiazepines and the Z drugs.

Doxepin

Doxepin, a tricyclic antidepressant, enhances subjective measures of sleep quality and is indicated for the treatment of difficulties with sleep maintenance (Yeung et al., 2015). It acts presumably via antagonism of H_1 receptor function when administered in low doses, although it also may act as an antagonist of the norepinephrine transporter and an antagonist of muscarinic, α_1 adrenergic, and $5HT_{2A}$ receptors. *Doxepin* should be taken in initial doses of 6 mg (3 mg in elderly patients) within 30 min of bedtime. Abnormal thinking and behavior have been observed following its use, and it can worsen suicidal ideation and depression. *Doxepin* is FDA-approved for the treatment of sleep maintenance insomnia.

Pregabalin

Pregabalin, an anxiolytic agent that binds to Ca^{2+} channel $\alpha_2\delta$ subunits, has proved useful in clinical trials (Holsboer-Trachsler and Prieto, 2013); *pregabalin* slightly decreased sleep onset latency and increased the proportion of time spent in slow-wave sleep. *Pregabalin* appears to be an effective treatment of the insomnia seen in patients suffering from a generalized anxiety disorder. *Pregabalin* is designated as a schedule V controlled substance.

Ritanserin

Ritanserin and other $5HT_{2A/2C}$ receptor antagonists show a capacity to promote slow-wave sleep in patients with chronic primary insomnia or generalized anxiety disorder (Monti, 2010). *Ritanserin* is not licensed for use in the U.S.

Agomelatine

Agomelatine, a melatonin receptor agonist and a $5HT_{2C}$ receptor antagonist, is prescribed for the treatment of depression and may aid in ameliorating sleep disturbances often associated with depression. *Agomelatine* is not licensed for use in the U.S. but is approved for medical use in Europe and Australia.

Management of Insomnia

Insomnia is one of the most common complaints in general medical practice. A number of pharmacological agents are available for the treatment of insomnia. The "perfect" hypnotic would allow sleep to occur with normal sleep architecture. It would not cause next-day effects, either of rebound anxiety or of continued sedation. It would not interact with other medications. It could be used chronically without causing dependence or rebound insomnia on discontinuation. Controversy in the management of insomnia revolves around two issues:

- Pharmacological versus nonpharmacological treatment
- Use of short-acting versus long-acting hypnotics

The side effects of hypnotic medications must be weighed against the sequelae of chronic insomnia, which include a 4-fold increase in serious accidents (Balter and Uhlenhuth, 1992). Regular moderate exercise or even small amounts of exercise often are effective in promoting sleep. In addition to appropriate pharmacological treatment, the management of insomnia should correct identifiable causes, address inadequate sleep hygiene, eliminate performance anxiety related to falling asleep, provide entrainment of the biological clock so that maximum sleepiness occurs at the hour of attempted sleep, and suppress the use of alcohol and over-the-counter sleep medications.

Categories of Insomnia

- *Transient insomnia* lasts less than 3 days and usually is caused by a brief environmental or situational stressor. If hypnotics are prescribed, they should be used at the lowest dose and for only 2 to 3 nights. Note that benzodiazepines given acutely before important life events, such as examinations, may result in impaired performance.
- *Short-term insomnia* lasts from 3 days to 3 weeks and usually is caused by a personal stressor such as illness, grief, or job problems. Hypnotics may be used adjunctively for 7 to 10 nights and are best used intermittently during this time, with the patient skipping a dose after 1 to 2 nights of good sleep.
- *Long-term insomnia* lasts for more than 3 weeks; a specific stressor may not be identifiable.

Insomnia Accompanying Major Psychiatric Illnesses

The insomnia caused by major psychiatric illnesses often responds to specific pharmacological treatment of that illness. For example, in major depressive episodes with insomnia, selective serotonin reuptake inhibitors (SSRIs), which may cause insomnia as a side effect, usually will result in improved sleep because they treat the depressive syndrome. In a patient whose depression is responding to an SSRI but has persistent insomnia as a side effect of the medication, judicious use of evening *trazodone* may improve sleep, as well as augment the antidepressant effect of the reuptake inhibitor. However, the patient should be monitored for priapism, orthostatic hypotension, and arrhythmias.

Adequate control of anxiety disorders often produces adequate resolution of the accompanying insomnia. Sedative use in patients with anxiety disorders is decreasing because of a growing appreciation of the effectiveness of other agents, such as β adrenergic receptor antagonists (see Chapter 14) for performance anxiety and SSRIs for obsessive-compulsive disorder and perhaps generalized anxiety disorder. The profound insomnia in patients with acute psychosis owing to schizophrenia or mania usually responds to dopamine receptor antagonists (see Chapters 15 and 19). Benzodiazepines often are used adjunctively in this situation to reduce agitation and improve sleep.

Insomnia Accompanying Other Medical Illnesses

For long-term insomnia owing to other medical illnesses, adequate treatment of the underlying disorder, such as congestive heart failure, asthma, or COPD, may resolve the insomnia. Adequate pain management in conditions of chronic pain will treat both the pain and the insomnia and may make hypnotics unnecessary. *Adequate attention to sleep hygiene, including reduced caffeine intake, avoidance of alcohol, adequate exercise, and regular sleep and wake times, often will reduce the insomnia.*

Conditioned (Learned) Insomnia

In those who have no major psychiatric or other medical illness and in whom attention to sleep hygiene is ineffective, attention should be directed to conditioned (learned) insomnia. These patients have associated the bedroom with activities consistent with wakefulness rather than sleep. In such patients, all other activities associated with waking, even such quiescent activities as reading and watching television, should be done outside the bedroom.

Sleep-State Misperception

Some patients complain of poor sleep but have been shown to have no objective polysomnographic evidence of insomnia. They are difficult to treat.

Long-Term Insomnia

Nonpharmacological treatments are important for all patients with long-term insomnia. These include education about sleep hygiene, relaxation training, and behavioral modification approaches, such as sleep restriction and stimulus-control therapies.

Long-term hypnotic use leads to a decrease in effectiveness and may produce rebound insomnia on discontinuance. Almost all hypnotics change sleep architecture. The barbiturates reduce REM sleep; the benzodiazepines reduce slow-wave non-REM sleep and, to a lesser extent, REM sleep. While the significance of these findings is not clear, there is an emerging consensus that slow-wave sleep is particularly important for physical restorative processes. REM sleep may aid in the consolidation of learning. The blockade of slow-wave sleep by benzodiazepines may partly account for their diminishing effectiveness over the long term, and it also may explain their effectiveness in blocking sleep terrors, a disorder of arousal from slow-wave sleep.

Long-acting benzodiazepines can cause next-day confusion, whereas shorter-acting agents can produce rebound next-day anxiety. Paradoxically, the acute amnestic effects of benzodiazepines may be responsible for the patient's subsequent report of restful sleep. Anterograde amnesia may be more common with *triazolam*. Hypnotics should not be given to patients with sleep apnea, especially the obstructive type, because these agents decrease upper airway muscle tone while also decreasing the arousal response to hypoxia.

Insomnia in Older Patients

The elderly, like the very young, tend to sleep in a *polyphasic* (multiple sleep episodes per day) pattern rather than the *monophasic* pattern characteristic of younger adults. This pattern makes assessment of adequate sleep time difficult.

Changes in the pharmacokinetic profiles of hypnotic agents occur in the elderly because of reduced body water, reduced renal function, and increased body fat, leading to a longer $t_{1/2}$ for benzodiazepines. A dose that produces pleasant sleep and adequate daytime wakefulness during week 1 may produce daytime confusion and amnesia by week 3 as the drug level continues to rise, particularly with long-acting hypnotics. For example, the benzodiazepine *diazepam* is highly lipid soluble and is excreted by the kidney. Because of the increase in body fat and the decrease in renal excretion that typically occur from age 20 to 80, the $t_{1/2}$ of the drug may increase 4-fold over this span.

Injudicious use of hypnotics in the elderly can produce daytime cognitive impairment and thereby impair overall quality of life. Once an older patient has been taking benzodiazepines for an extended period, whether for daytime anxiety or for nighttime sedation, terminating the drug can be a long, involved process. Attempts at drug withdrawal may not be successful, and it may be necessary to leave the patient on the medication, with adequate attention to daytime side effects.

Prescribing Guidelines for Managing Insomnia

Hypnotics that act at $GABA_A$ receptors—benzodiazepine hypnotics and the newer agents *zolpidem*, *zopiclone*, and *zaleplon*—are preferred to barbiturates; the $GABA_A$ receptor agents have a higher therapeutic index, smaller effects on sleep architecture, and less abuse potential. Compounds with a shorter $t_{1/2}$ are favored in patients with sleep-onset insomnia but without significant daytime anxiety who need to function at full effectiveness during the day. These compounds also are appropriate for the elderly because of a decreased risk of falls and respiratory depression. However, the patient and physician should be aware that early morning awakening, rebound daytime anxiety, and amnestic episodes also may occur. These undesirable side effects are more common at higher doses of the benzodiazepines.

Benzodiazepines with longer $t_{1/2}$ values are favored for patients who have significant daytime anxiety. These benzodiazepines also are appropriate for patients receiving treatment of major depressive episodes because the short-acting agents can worsen early morning awakening. However, longer-acting benzodiazepines can be associated with next-day cognitive impairment or delayed daytime cognitive impairment (after 2–4 weeks of treatment) as a result of drug accumulation with repeated administration.

Older agents—barbiturates, chloral hydrate, *and* meprobamate—*should be avoided for the management of insomnia. They have high abuse potential and are dangerous in overdose.*

Drug Facts for Your Personal Formulary: *Sedative-Hypnotic Agents*

Drug	Therapeutic Uses	Clinical Pharmacology and Tips
Benzodiazepines—synergistic with other CNS depressants, especially ethanol; see Table 22–2		
Alprazolam	Anxiety disorders, agoraphobia	Withdrawal symptoms may be especially severe
Chlordiazepoxide	Anxiety disorders, alcohol withdrawal, preanesthetic medication	Long-acting and self-tapering because of active metabolites
Clobazam	Adjunctive treatment of seizures associated with Lennox-Gastaut syndrome, other epilepsy and anxiety disorders	Active metabolite has long half-life Decrease dose and titrate in CYP2C19-poor metabolizers Tolerance develops to anticonvulsant effects
Clonazepam	Seizure disorders, adjunctive treatment in acute mania and certain movement disorders	Tolerance develops to anticonvulsant effects
Clorazepate	Anxiety disorders, seizure disorders	Prodrug; activity due to formation of nordazepam during absorption
Diazepam	Anxiety disorders, alcohol withdrawal, status epilepticus, skeletal muscle relaxation, preanesthetic medication	Prototypical benzodiazepine
Estazolam	Insomnia	Contains triazolo ring; adverse effects may be similar to those of triazolam
Flurazepam	Insomnia	Active metabolites accumulate with chronic use
Lorazepam	Anxiety disorders, alcohol withdrawal, preanesthetic medication	Metabolized solely by conjugation
Midazolam	Preanesthetic and intraoperative medication	Rapidly inactivated
Oxazepam	Anxiety disorders, alcohol withdrawal	Metabolized solely by conjugation
Quazepam	Insomnia	Active metabolites accumulate with chronic use
Remimazolam	Preanesthetic and intraoperative medication	Very rapidly inactivated
Temazepam	Insomnia	Metabolized mainly by conjugation
Triazolam	Insomnia	Rapidly inactivated; may cause disturbing daytime side effects
"Z Compounds"—nonbenzodiazepines with agonist effects at the benzodiazepine site of $GABA_A$ receptors; these agents have largely replaced benzodiazepines for treating insomnia		
Zaleplon	Insomnia	Very short elimination half-life
Zolpidem	Insomnia	Short-term (2–6 week) treatment of insomnia
Eszopiclone	Insomnia	S(+) enantiomer of zopiclone
Benzodiazepine Antagonist		
Flumazenil	Benzodiazepine overdose (benzodiazepine and β-carboline antagonist)	Headache, dizziness; do not use in tricyclic antidepressant poisoning (seizures!)
Miscellaneous and Emerging Agents		
Ramelteon	Insomnia	Melatonin receptor agonist; significant first-pass effect
Tasimelteon	Circadian rhythm disorder in blind patients	Melatonin receptor agonist
Suvorexant	Insomnia	Orexin receptor antagonist; needs at least 7 h after 10-mg dose before awakening
Lemborexant	Insomnia	Orexin receptor antagonist
Doxepin	Depression, insomnia	Tricyclic antidepressant; sedating effects likely occur through H_1 receptor antagonism; beware of abnormal behavior, suicide ideation, depression; use half dose in the elderly
Propofol	Induction/maintenance of anesthesia, procedural sedation	Rapid recovery
Pregabalin (β-isobutyl–GABA)	Nerve/muscle pain, fibromyalgia, seizures	Schedule V substance, abuse potential; some concern for suicide ideation and angioedema

Drug Facts for Your Personal Formulary: *Sedative-Hypnotic Agents* (*continued*)

Drug	Therapeutic Uses	Clinical Pharmacology and Tips
Barbiturates—synergistic with other CNS depressants, especially ethanol; induce CYPs; respiratory depressants; see Table 22–3		
Amobarbital	Insomnia, preoperative sedation, emergency management of seizures	• IM and IV • Short-acting (3–8 h)
Butobarbital	Insomnia, preoperative sedation, daytime sedation	• Oral • Fast onset of action • Short-acting (3–8 h)
Mephobarbital (not licensed for use in U.S.)	Seizure disorders, daytime sedation	• Oral • Short-acting (3–8 h)
Methohexital	Induction and maintenance of anesthesia	• IV • Ultra-short-acting (5–15 min)
Pentobarbital	Insomnia, preoperative and procedural sedation, emergency management of seizures	• Oral, IM, IV, or rectal • Administer Na$^+$ salt parenterally • Short-acting (3–8 h)
Phenobarbital	Seizure disorders, status epilepticus, daytime sedation	• Oral, IM, IV • First-line anticonvulsant (see Chapter 20); administer Na$^+$ salt parenterally • Long-acting (days)
Secobarbital	Insomnia, preoperative sedation	• Oral • Short-acting (3–8 h)
Thiopental	Induction and maintenance of anesthesia, preoperative sedation, emergency management of seizures, intracranial hypertension	• IV single dose provides brief period of anesthesia • Ultra-short-acting (5–15 min)

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Chapter 23

Opioid Analgesics

Emily M. Jutkiewicz and John R. Traynor

INTRODUCTION TO OPIOIDS AND RECEPTORS

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- Opioid Receptor Distribution
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- Opioid Receptor Structure and Activation

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OVERALL SUMMARY AND CONCLUSIONS

Introduction to Opioids and Receptors

Pain is a common component of many clinical pathologies, and management of pain is a vital clinical need. Drugs such as *morphine* and *oxycodone* acting at opioid receptors remain the mainstay of pain treatment, despite the safety concerns associated with the long-term use of these drugs, which has led to addiction and death from their misuse and the worldwide opioid crisis. Morphine and related drugs exert their pharmacological effects by acting at opioid receptors. Opioid receptors are 7-transmembrane G protein-coupled receptors (GPCRs; see Chapter 3) and comprise a family of four types, the mu (μ), delta (δ), kappa (κ) opioid receptors, which we will refer to as the classical or canonical opioid receptors, and the nociceptin (NOP) receptor (NOPr), which has close structural homology to the classical opioid receptors but distinct ligands and pharmacology. In this chapter, we will use mu, delta, kappa, and NOPr to describe the receptors. The mu-opioid receptor is mainly responsible for the pain-relieving actions and, importantly, also the unwanted effects, of all clinically useful opioid analgesics, which generally mimic the pharmacology of morphine. Consequently, this chapter will focus mainly on this receptor and its ligands and their pharmacology, with some mention of the pharmacology of drugs acting at the delta, kappa, and NOPr receptors.

The original opioid drugs (*morphine* and *codeine*) are components of opium, the dried resin from the seed head of the opium poppy, *Papaver somniferum*. Opium also contains thebaine, which has no opioid activity but serves as a precursor for the synthesis of additional opioid drugs. Also present in opium are *papaverine* (1%), a smooth muscle relaxant, and *noscapine* (6%), which has been used as an antitussive. *Morphine*, *codeine*, and structurally related compounds found in opium, together with semisynthetic derivatives such as *oxycodone* that bind to the mu-opioid receptor, are termed opiates. In contrast, an opioid is any agent that binds to the ligand-binding (orthosteric) site of members of the opioid receptor family. Consequently, the term opioid is a broader definition and covers the opiates, fully synthetic drugs, such a *methadone* and *fentanyl*, and endogenous opioid peptides, including the enkephalins, endorphins, and dynorphins, which are the naturally occurring neurotransmitters acting at opioid receptors. Opioid drugs are often referred to as narcotic analgesics or narcotics, derived from the Greek word *narkotikos* for "benumbing" or "stupor," because of their sedative properties and ability to cause sleep in the presence of pain.

Opioid Receptors

Types of Opioid Receptors

The three types of classical opioid receptors, mu, delta, and kappa, share extensive sequence homology (55%–58%) and belong to the class A or rhodopsin family of GPCRs (see Figures 3–14 and 23–1). As such they

Abbreviations

AC: adenylyl cyclase
ACTH: corticotropin; formerly adrenocorticotropic hormone
ADH: antidiuretic hormone
COPD: chronic obstructive pulmonary disease
CSF: cerebrospinal fluid
CYP: cytochrome P450
DAMGO: [D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin
EEG: electroencephalogram
FSH: follicle-stimulating hormone
GABA: γ-aminobutyric acid
GI: gastrointestinal
GPCR: G protein-coupled receptor
GRK: GPCR kinase
HPA: hypothalamic-pituitary-adrenal
5HT: serotonin
LH: luteinizing hormone
6-MAM: 6-monoacetylmorphine
MAO: monoamine oxidase
MAPK: mitogen-activated protein kinase
NE: norepinephrine
NMDA: *N*-methyl-D-aspartate
NOP: nociceptin/orphanin FQ (N/OFQ)
NOPr: NOP receptor
NSAID: nonsteroidal anti-inflammatory drug
PAG: periaqueductal gray
PCA: patient-controlled analgesia
PKC: protein kinase C
POMC: proopiomelanocortin
TM: transmembrane

transduce agonist responses into the cell via members of the $G_{i/o}$ and G_z families of heterotrimeric G proteins. NOPr was added to the opioid receptor family based on its close sequence homology (48%–49%). However, neither opioid drugs nor the enkephalins, endorphins, or dynorphin bind to this receptor, and agonists for the NOPr do not exhibit the same pharmacology as the opioids. A previous definition of opioid receptors required that they be sensitive to the specific opioid antagonist *naloxone*, but with the addition of the NOPr to this family, this is no longer the case. The opioid receptors appear early in vertebrate evolution (Stevens, 2009). The gene for the human mu-opioid receptor (*OPRM1*) has been mapped to chromosome 6, for the delta-opioid receptor (*OPRD1*) to chromosome 1, for the kappa-opioid receptor (*OPRK1*) to chromosome 8, and for the NOPr (*OPRL1*) to chromosome 20 (Dreborg et al., 2009).

Opioid Receptor Distribution

As defined by the distribution of receptor protein, message, ligand binding, and the pharmacological effects initiated by opioids, opioid receptors are widely distributed in the periphery and central nervous system (Mansour et al., 1988) and are found both pre- and/or postsynaptically.

Mu-opioid receptors are found in regions of the brain involved in the control of both the sensory and affective components of pain as well as modulation of many other behaviors. This includes both superficial and deeper layers of the neocortex, caudate-putamen, nucleus accumbens, ventral tegmental area, thalamus, hippocampus, amygdala, raphe nucleus, periaqueductal gray (PAG), medulla and pons, and dorsal horn of the spinal cord.

The distribution of *kappa-opioid receptors* is consistent with the roles of the kappa-opioid system in regulation of diuresis, food intake, pain perception, and neuroendocrine functioning, including response to stress. Major regions expressing kappa-opioid receptors are the caudate-putamen, nucleus accumbens, amygdala, hypothalamus, and pituitary. Kappa-opioid receptors are also found in the found in the PAG, raphe nuclei, pons and medulla, and dorsal horn of the spinal cord, but there is very little expression in the cortex.

Delta-opioid receptors are expressed in regions related to olfactory areas of the brain as well as the neocortex, caudate-putamen, nucleus accumbens, and amygdala. There are low levels in the dorsal horn of the spinal cord. The delta-opioid receptor is involved in modulation of pain and mood.

NOPr receptors are the most widely distributed of the opioid receptors. The receptor is found in most regions of the brain and spinal cord, in areas related to the physiological actions of this system in the modulation of pain and reward as well as anxiety and stress, memory, and feeding behaviors (Ozawa et al., 2015).

Opioid receptors are also present on a variety of nonneuronal cells, including macrophages (peripheral and central microglia) and astrocytes (Dannals, 2013; Yaksh, 1987) and in the enteric nervous system of the gastrointestinal (GI) tract (Galligan and Akbarali, 2014). Delta-opioid receptors in the heart may afford cardioprotection.

Opioid Receptor Signaling

Following agonist occupation of opioid receptors and subsequent activation of heterotrimeric G proteins, both the α subunits and βγ dimers bind to downstream proteins to provide a complex pattern of intracellular signals (Figure 23–2). Signaling is similar for all members of the opioid receptor family (Al-Hasani and Bruchas, 2011).

The a_i subunits directly inhibit the enzyme adenylyl cyclase (AC) to reduce levels of cyclic AMP and so inhibit the phosphorylation of many proteins that are controlled by protein kinase A–dependent regulation as well as cyclic AMP–dependent calcium influx. Chronic treatment with opioid agonists leads to a loss of responsiveness of AC and an "overshoot" of cycle AMP production when the opioid is removed. The βγ dimer inhibits voltage-gated Ca^{2+} channels on presynaptic terminals, leading to reduced Ca^{2+} influx and an inhibition of transmitter release (Weiss and Zamponi, 2021). For example, inhibition of γ-aminobutyric acid (GABA) release in the ventrolateral periaqueductal gray leads to activation of descending antinociceptive pathways and, in the ventral tegmental area, enhances dopamine release in the nucleus accumbens, an important component of the reward pathway. The βγ dimers also act to open K^+ channels, including G protein-coupled inwardly rectifying potassium (GIRK) channels, which leads to hyperpolarization and reduced neuronal firing. Both effects are important for analgesic actions of the opioid drugs. The release of βγ subunits also lead to activation of the mitogen-activated protein kinase (MAPK) cascades, a diverse family of kinases that modulate many cellular responses by phosphorylation, including cell differentiation, ion channel function, and scaffolding of intracellular proteins. Other enzymes such as protein kinase C (PKC) and phospholipase C can be activated by βγ subunits. An important role for βγ dimers is the recruitment of G protein receptor kinases (GRKs), specifically GRK2 and 3, to phosphorylate the receptor. This is an initial step in receptor desensitization and leads to the recruitment of β-arrestin necessary for receptor internalization prior to degradation or as a prerequisite for recycling of refreshed receptors to the cell membrane to receive another signal. The receptor can be phosphorylated by members of the GRK family that do not require recruitment by the βγ or by PKC. However, β-arrestin also functions to scaffold and activate MAPK pathways.

Opioid Receptor Ligands

Endogenous Opioid Peptides

There are three families of endogenous opioid peptides, the enkephalins, endorphins (principally β-endorphin), and dynorphins, which act at the classical opioid receptors. These peptides share the common amino-terminal sequence of Tyr-Gly-Gly-Phe-(Met or Leu), which may be followed by various C-terminal extensions (Table 23–1). Thus, Leu- and Met-enkephalin are simple pentapeptides, whereas extended forms of these, as well as the dynorphins, contain up to 17 amino acids, and the endorphins are up to 31 amino acid residues in length. There

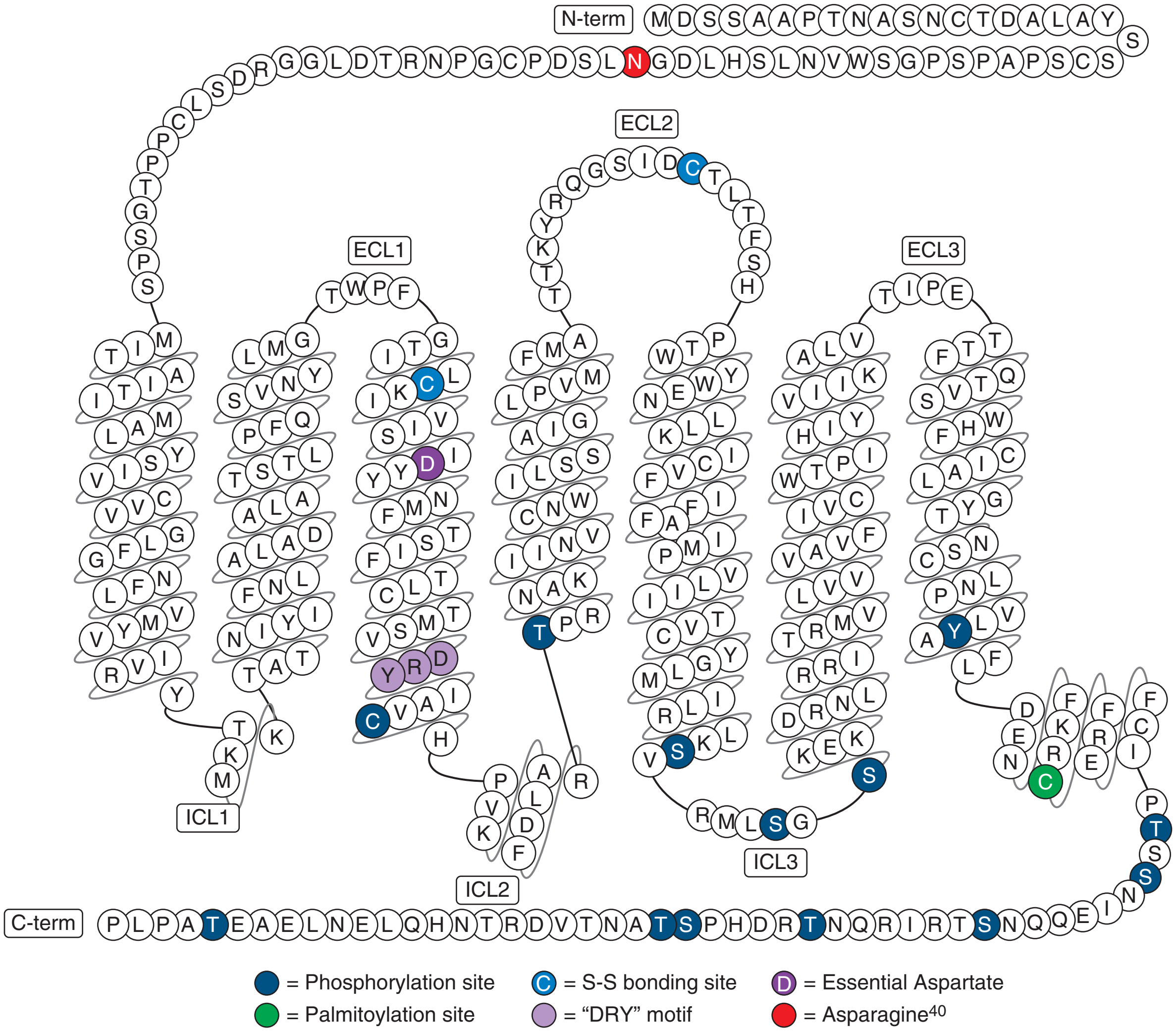


Figure 23–1 *General structure of an opioid receptor.* This diagram is of the mu-opioid receptor, but other opioid receptors have the same general structure and the typical characteristics of a G protein-coupled receptor (GPCR). Along the external amino terminus there are several aspartate (N) residues that are potential glycosylation sites. There are seven transmembrane (TM) regions joined by intracellular and extracellular loops (ICL and ECL, respectively), a long intracellular carboxy tail, and tyrosine (Y) and serine (S) phosphorylation sites in the areas where arrestins interact. There is a conserved aspartate (D) for binding to the tertiary N atom found in all opioid drugs and a disulfide linkage between two cysteine residues. The Na^+ binding site is in the TM bundle just below the orthosteric site.

is some specificity of the peptides for the different opioid receptors, but because of this structural similarity, there is a considerable degree of overlap. β-Endorphin is mu-opioid receptor preferring, and the dynorphins are considered the endogenous ligands for the kappa-opioid receptor. The simpler enkephalins are less selective for delta > mu receptors but do not bind to or activate the kappa-opioid receptor. None of these ligands bind to NOPr. Nociceptin (a 17-amino acid peptide), also known as orphanin F/Q or N/OFQ, which has an N-terminal amino acid sequence of Phe-Gly-Gly-Phe, is the endogenous ligand for NOPr, and has no activity at the classical opioid receptors (Lambert, 2008). The endomorphins are two opioid peptides with high affinity and selectivity for the mu-opioid receptor. Although originally discovered in mammalian brain tissues, the precursor peptides for these have never been identified, and the origin of the endomorphins remains unknown. It is therefore up for debate as to whether these are truly endogenous opioids. In total, there are more than 20 opioid peptides acting at the four opioid receptors.

The opioid peptides are derived from their large precursor proteins by complex cleavage with distinct trypsin-like enzymes (Figure 23–3). Preproopiomelanocortin (pre-POMC) provides POMC, which contains the sequence for β-endorphin. In addition, the POMC sequence is also processed into a variety of nonopioid peptides, including adrencorticotropin (ACTH), α-melanocyte-stimulating hormone, and β-lipotropin. Although β-endorphin contains the sequence for Met-enkephalin at its amino terminus, it is not converted to this peptide. Preproenkephalin contains the sequences for one copy of Leu-enkephalin and four copies of Met-enkephalin. Preprodynorphin contains three dynorphin peptides of differing lengths that all begin with the Leu-enkephalin sequence: dynorphin A, dynorphin B, and neoendorphin. Prepronociceptin contains the sequence of the 17-amino acid peptide nociceptin (also known as orphanin FQ or N/OFQ).

Not all cells that express a given opioid prohormone precursor store and release the same mixture of opioid peptides. This results from differential posttranslational processing of the prohormones into peptides of different lengths or even breakdown of larger opioid peptides into smaller fragments; for example, dynorphins contain the Leu-enkephalin sequence. Moreover, processing can be altered in response to physiological demands, leading to the release of a different mix of posttranslationally derived peptides.

Opioid peptides are present in areas of the central nervous system (CNS) related to the processing of pain information (e.g., spinal cord dorsal horn, the spinal trigeminal nucleus, and the PAG), to the modulation of affective behavior (e.g., amygdala, hippocampus, locus coeruleus, and frontal cerebral cortex), to the modulation of motor control (e.g., caudate nucleus and globus pallidus), to the regulation of the autonomic nervous system (e.g., medulla), and to neuroendocrinological functions (e.g., median eminence). Opioid peptides are found in the plasma, and this reflects release from secretory systems such as the pituitary, adrenals, and exocrine glands of the stomach and intestine.

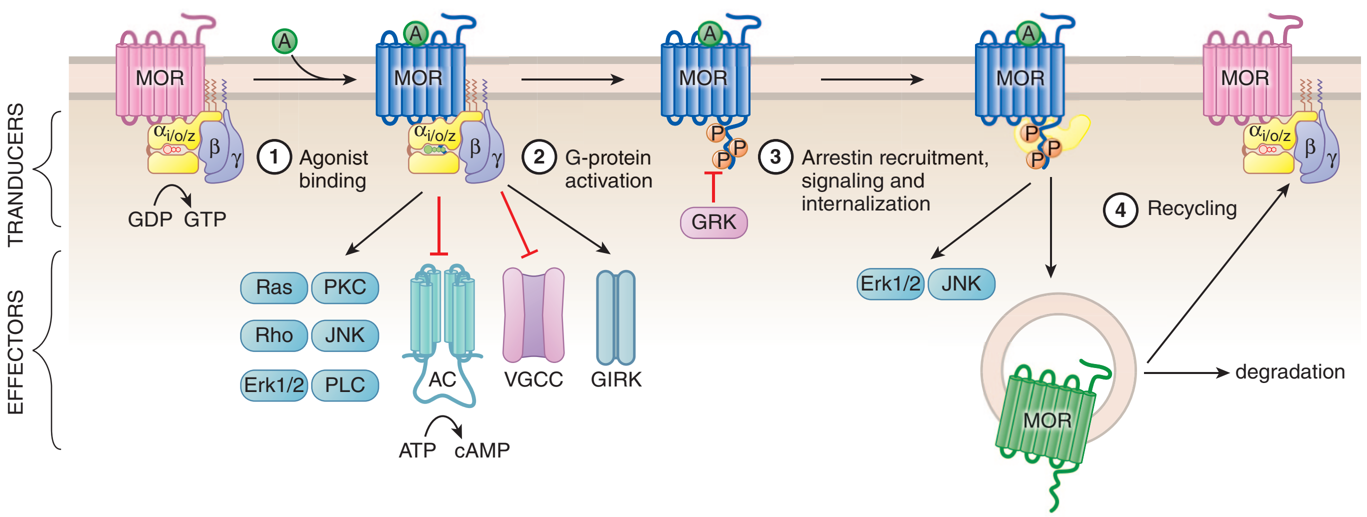


Figure 23–2 *Simplified scheme of opioid receptor signaling.* In the presence of an opioid agonist (green circle), inactive receptor (red, Ri) is converted to active receptor (blue, Ra) (Step 1). This causes the α subunit of the G protein heterotrimer to exchange GDP for GTP which leads to dissociation of the α and βγ subunits that go on to modulate various downstream effectors, including kinases (e.g., PKC, ERK, and JNK [c-Jun N-terminal kinase]), small GTPases (Ras and Rho), phospholipase C (PLC), ion channels (voltage-gated calcium channels [VGCC] and G protein-coupled K^+ channels [GIRKs]), enzymes, and their secondary messengers (step 2). Signaling is terminated by phosphorylation of the receptor by G protein receptor kinases (GRK), followed by arrestin recruitment, and internalization (step 3). In addition, arrestins can scaffold signaling pathways. Finally, internalized receptor (green) is targeted for either degradation or recycling to the plasma membrane (step 4). MOR, mu-opioid receptor.

Exogenous Opioid Receptor Ligands

Opioid receptor ligands are defined by their selectivity (or otherwise) for a particular opioid receptor and their functional properties as agonists, partial agonists, antagonists, biased agonists, or allosteric modulators, as defined below.

Agonists. Agonists bind to the orthosteric site to activate the receptors, leading to modulation of a wide variety of cell signaling cascades and resultant effects on physiology. Since clinically used drugs may have varying degrees of activity at other opioid receptors, highly selective agonists have been developed. For the classical opioid receptors, these are derivatives of the endogenous opioid peptides, DAMGO and DPDPE, for mu- and delta-opioid receptors, respectively, and dynorphin itself for the kappa-opioid receptor (see Table 23–1). Selective small-molecule agonists are also useful as tools for preclinical *in vivo* research. These include *morphine* for mu-, SNC80 for delta-, and U69593 and its derivatives for kappa-opioid receptors. Nociceptin itself and the small-molecule Ro64-6198 are used as selective agonists for the NOPr (Figure 23–4).

Partial Agonists. Partial agonists also bind to the orthosteric site of receptors but are not capable of eliciting the same level of response as seen with full agonists, even with escalating doses (i.e., these drugs have reduced level of efficacy or "ceiling" to the magnitude of their action). However, this definition is system dependent. For example, in preclinical pain models in rodents where the nociceptive insult can be varied in intensity, even *morphine* can be observed to be a partial agonist, compared to, for example, *fentanyl*. Similarly, *buprenorphine* acts as partial agonist at the mu-opioid receptor in preclinical models, but in the pain clinic, it is effective in managing postoperative pain. However, the partial agonist nature of *buprenorphine* does mean it has a ceiling effect and so inhibits respiration (a dangerous side effect of opioid full agonists) to a lesser extent than *morphine* or *oxycodone* and thus may be a safer alternative. Partial agonists used in the clinic are not selective for specific opioid receptors. For example, *buprenorphine* acts at all opioid receptors, including weak agonist activity at NOPr, and *pentazocine* is a partial agonist at both mu- and kappa-opioid receptors. Partial agonists are sometimes termed *agonist-antagonists* because they have agonist actions but also antagonize the effects of opioid agonists that have higher efficacy, such as hydromorphone. As such, partial agonists can precipitate withdrawal in opioid-dependent patients.

Antagonists. These drugs are competitive antagonists and prevent the binding of opioids to the orthosteric site on opioid receptors. They have no activity themselves, but their pharmacology derives solely from blocking the actions of opioid agonists. Commonly used opioid antagonists are *naloxone* and *naltrexone*. These compounds bind to all classical opioid receptors with similar affinity, although as mentioned above, they do not bind to the NOPr.

Antagonists for specific opioid receptors have been developed for research purposes. These include peptides, such as the somatostatin analogue CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2) as a mu-opioid receptor antagonist and a derivative of enkephalin (ICI-174864) as a selective delta-opioid receptor antagonist. In addition, chemical manipulation of *naltrexone* and its derivatives has given rise to the selective mu-opioid receptor antagonist *cyprodime*, the selective delta-opioid receptor antagonist *naltrindole*, and the selective kappa-opioid receptor antagonist nor-BNI (see Figure 23–4). JD-Tic is also a commonly used and highly selective antagonist at the kappa-opioid receptor.

Inverse Agonists. The opioid receptors, like other membrane-bound GPCRs, are not static structures but continually move through a series of conformations, including conformations that are able to activate intracellular signaling pathways. This gives the receptors a basal level of activity, in the absence of an agonist, and the receptors are said to be *constitutively active*. Inverse agonists are compounds that stabilize receptors in inactive conformations and so inhibit constitutive activity (see Chapter 3). Of the opioid receptors, the delta-opioid receptor shows high constitutive activity in cellular models. The selective delta-opioid receptor antagonist ICI-174864 also inhibits constitutive activity of the receptor and therefore is more accurately defined as an inverse agonist.

Biased Agonists. Intracellular signaling downstream of opioid receptors is highly complex (see Figure 23–2). Most agonists activate all of these downstream pathways equally. However, biased agonists are compounds that preferentially activate a particular pathway or pathways over other pathways. The most observed bias is between agonists that activate pathways downstream of G proteins as opposed to those downstream of β-arrestin. This became a hot area of research in opioid pharmacology following preclinical experiments that showed β-arrestin pathways might be responsible for certain unwanted effects of opioid agonists, particularly constipation and respiratory depression, while G protein pathways

TABLE 23–1 ■ MAJOR ENDOGENOUS OPIOID PEPTIDES AND DERIVATIVES

PEPTIDE	STRUCTURE[a]	RECEPTOR PREFERENCE
Classical opioid peptides	**Tyr-Gly-Gly-Phe-X**	
	X =	
Leu-Enkephalin	Leu	delta > mu
Met-Enkephalin	Met	delta > mu
Met-Enkephalin-Arg-Phe	Met-Arg-Phe	delta = mu
β-Endorphin	**Met**-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu	mu > delta
α-Neoendorphin	**Leu**-Arg-Lys-Tyr-Pro-Lys	mu > delta
Dynorphin 1-17	Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln	kappa
Dynorphin B	**Leu**-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr	kappa
Endomorphins	**Tyr-Pro-X-Phe-**$\mathbf{NH_2}$	
	X =	
Endomorphin 1	Trp	mu
Endomorphin 2	Phe	mu
Nociceptin[b]	**Phe-Phe-Gly-Thr**-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln	NOPr
Synthetic receptor selective peptides[c]		
DAMGO	Tyr-D-Ala-Gly-MePhe-Gly-ol	
DPDPE	Tyr-D-Pen-Gly-Phe-Pen S ——— S	

DPDPE, [D-Pen 2,D-Pen 5]enkephalin

[a] The first four amino acids of each peptide type are in bold. The substitution X is given for each peptide within the family.

[b] For nociceptin, the complete structure is provided, with the first four amino acids shown in bold.

[c] The complete structures of the synthetic peptides are shown. Pen = penicillamine.

were important for the analgesic effects. Although more recent studies have not supported this concept (Gillis et al., 2020), the idea that selective pharmacology could be gained in this way remains attractive. One compound designated as a G protein-biased agonist, namely *oliceridine*, has received approval for intravenous use in hospital settings in situations where other medications do not work, although the safety profile of *oliceridine* is no better than *morphine*.

Allosteric Modulators. These are compounds that act at opioid receptors but at a site distinct from the orthosteric site. Positive allosteric modulators quantitatively alter the action of opioids occupying the orthosteric binding site. As such, they can promote the activity of endogenous opioid peptides to provide antinociception and so may have the potential to provide analgesia without the side effects associated with traditional opioid agonists. Negative allosteric modulators inhibit the actions of opioid agonists but unlike *naloxone* and *naltrexone* are not competitive. Thus far, positive and negative allosteric modulators are only in the early preclinical stages of investigation (Livingston and Traynor, 2018).

Opioid Receptor Structure and Activation

The opioid receptors belong to the family of class A GPCRs (see Chapter 3) and comprise an extracellular N-terminus, seven transmembrane (TM) alpha-helical structures joined by two extracellular loops and three intracellular loops and an intracellular C-terminal domain (see Figure 23–1). Two conserved cysteine residues in the first and second extracellular loops form a disulfide bridge to stabilize ECL2. The extracellular loops are also N-glycosylated, which regulates export of newly synthesized receptors to the cell surface as well as internalization and degradation. These loops may also occlude access of ligands to the orthosteric binding site, thus altering ligand affinity. A short proximal section of the C-terminus is attached to the membrane by a palmitoyl group and forms an additional small alpha-helix. The intracellular loops and C-terminus interact with signaling partners within the cell membrane and inside the cell, in particular G proteins and β-arrestins, and also serve as substrates for phosphorylation by GRKs and other kinases, which is an important component of signal termination.

The structure of all four opioid receptor types has been solved using either X-ray crystallographic or single-particle cryogenic electron microscopy methods, although less information is available about the loops and C-terminus because of the flexibility of these regions. The amino acid residues making up the TM domains, which contain the orthosteric binding site, are similar across all of the receptors, although NOPr is more distant from the classical opioid receptors. The opioid receptor agonists and endogenous opioid peptides bind similarly in the orthosteric site of opioid receptors. For example, at the mu-opioid receptor the nitrogen atom and phenolic OH in morphine-like molecules or in the Tyr moiety of the endogenous opioid peptides, form a salt bridge with a negatively charged aspartate residue in TM3 that is conserved across GPCRs, and a water-mediated interaction with a histidine in TM6, respectively. These same residues are involved with ligands binding to the delta- and kappa-opioid receptors. Synthetic opioids that are not based on the enkephalin or morphine structure, for example, *methadone* and *fentanyl*, bind in the same orthosteric site but with different interactions between the ligand and the receptors. In addition, the larger endogenous peptides extend out of the orthosteric pocket toward the extracellular domains; these additional interactions determine the selectivity of the peptides. For example, the kappa-opioid receptor selective peptide dynorphin has several basic arginine residues that interact with negatively charged residues on the N-terminus of the kappa receptor but are absent on the N-terminus of the other opioid receptors.

Opioid receptors, like all GPCRs, are flexible proteins that exist in multiple conformational states, but in the simplest model, there is an inactive ensemble of conformations (R) and an ensemble of active conformations (R*). The inactive conformations, which do not activate intracellular partners, such as heterotrimer G proteins, are stabilized by the presence of an Na$^+$ ion, which forms a network with amino acids deep in TM domains beneath the orthosteric site, and a "DRY" motif (Asp [or Glu]-Arg-Tyr) at the junction of TM3 and ICL2, which forms an "ionic lock," and holds the receptor in an inactive conformation. Active receptor (R*) ensembles are formed in the presence of agonist and intracellular binding partner through changes in several conserved "molecular switch" regions, including opening of the DRY-mediated ionic lock as well as collapse of the Na$^+$ binding site. These changes lead to an outward movement of TM6 relative to other TM domains. This opens a binding site for the C-terminus of the G_α subunit of the heterotrimeric G protein, leading to activation of the G protein. The G protein and orthosteric binding sites are allosterically linked. Thus, agonist binding increases G protein affinity and G protein binding increases agonist affinity. Most of this mechanism has been determined through experiments with the β_2 adrenergic receptor but applies equally well to opioid receptors (Weis and Kobilka, 2018).

Opioid Receptor Variants and Receptor Complexes

There is growing evidence for increasing complexity of opioid receptor function. For example, there are several nonsynonymous variants of the mu-opioid receptor found in the human population (Ravindranathan et al., 2009). Of these, the single polynucleotide polymorphism in the gene of the opioid receptor (*OPRM1*) A118G, giving a receptor in which

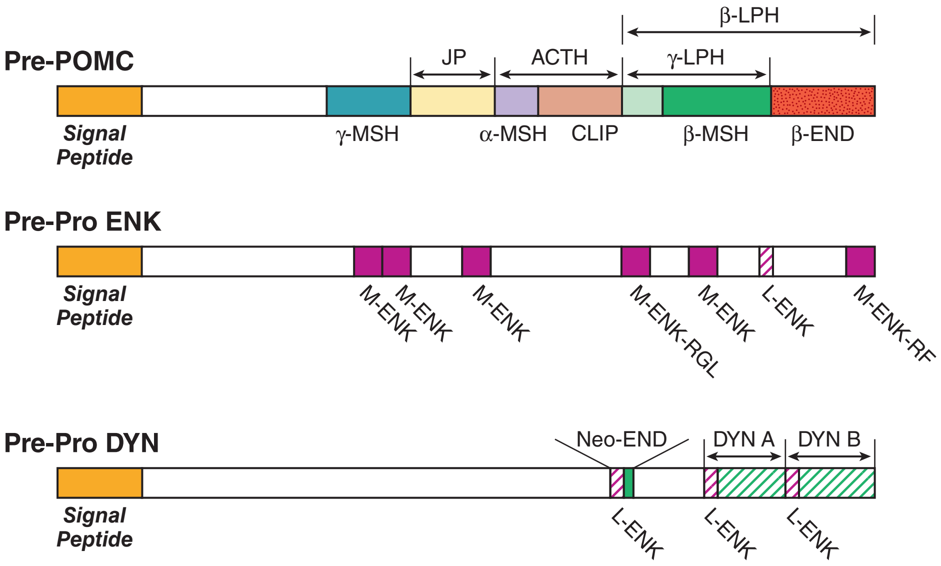

Pre-POMC
β-LPH
JP
ACTH
γ-LPH
Signal Peptide
γ-MSH
α-MSH
CLIP
β-MSH
β-END
Pre-Pro ENK
Signal Peptide
M-ENK
M-ENK
M-ENK
M-ENK-RGL
M-ENK
L-ENK
M-ENK-RF
Pre-Pro DYN
Neo-END
DYN A
DYN B
Signal Peptide
L-ENK
L-ENK
L-ENK

Figure 23–3 *Opioid peptide precursors.* Opioid peptides derive from precursor proteins that may also contain nonopioid peptides. *Pre-POMC* is a good example. Proteolytic processing of a pre-pro form by a signal peptidase removes the signal peptide; then, various prohormone convertases (endoproteases) attack at dibasic sequences, yielding α-, β-, and γ-melanocyte-stimulating hormone (MSH), ACTH, corticotropin-like intermediate lobe peptide (CLIP), β- and γ-lipotropin (LPH), and β-endorphin (β-END). In a similar manner, *pre-pro enkephalin (ENK)* yields Leu-enkephalin (L-ENK) and Met-enkephalin (M-ENK) and two extended derivatives, M-ENK-Arg-Gly-Leu and M-ENK-Arg-Phe. *Pre-pro dynorphin (DYN)* yields α neoendorphin (α-NEO) and dynorphin A and B (DYN A and DYN B), each of which contains the Leu-enkephalin sequence (Tyr-Gly-Gly-Phe-Leu) at its amino terminus. JP, joining peptide. *Pre-pronociceptin* (not shown) contains one copy of nociceptin peptide. For details of the proteolytic processing of POMC, see Figure 50–1.

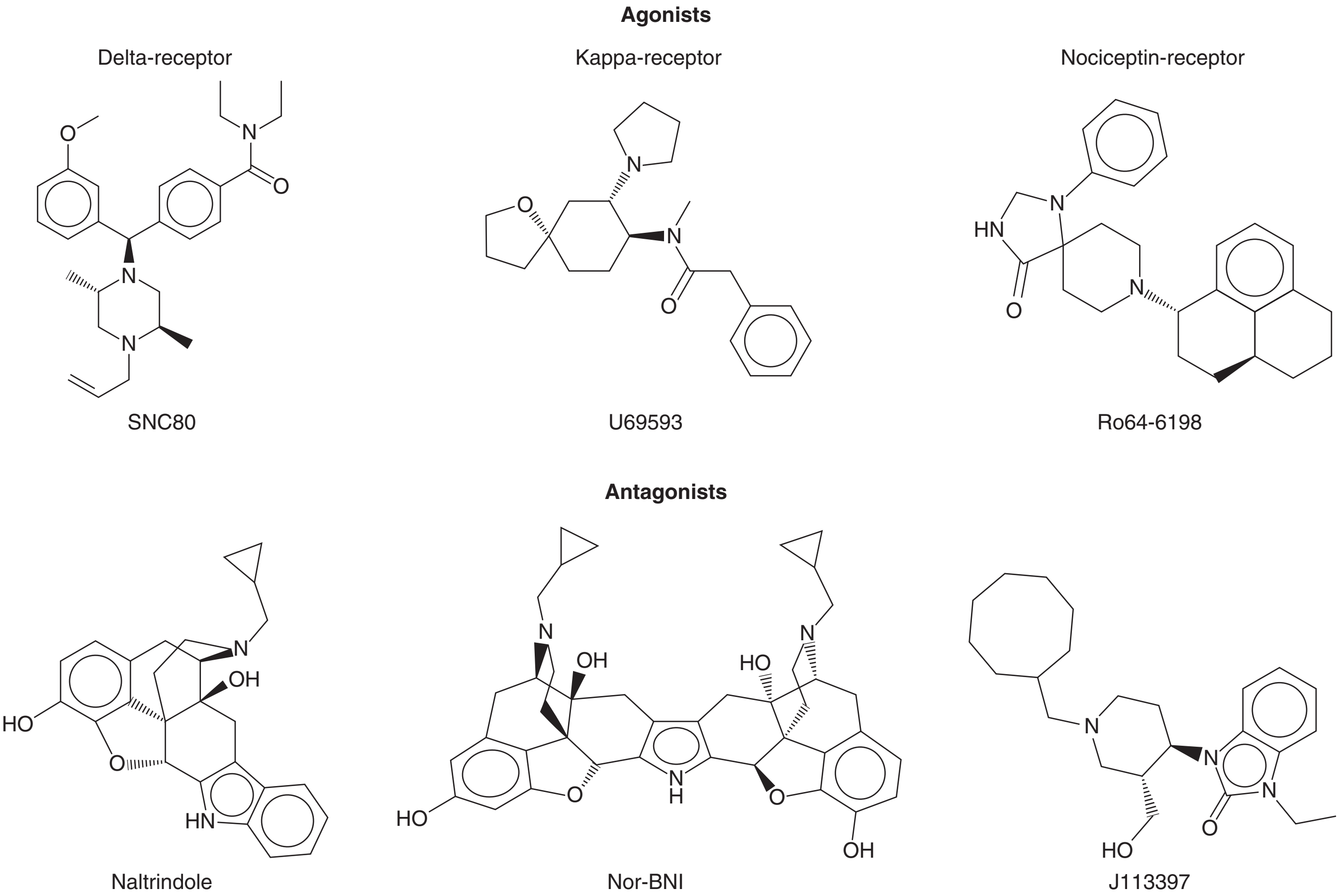

Agonists
Delta-receptor
Kappa-receptor
Nociceptin-receptor
SNC80
U69593
Ro64-6198
Antagonists
Naltrindole
Nor-BNI
J113397

Figure 23–4 *Examples of compounds acting at kappa, delta, and nociceptin receptor.*

BOX 23–1 ■ Pharmacology of Kappa-, Delta-, and NOP-Receptor Agonists

Kappa-opioid receptor agonists have analgesic effects (e.g., *butorphanol*, *pentazocine*) but are not typically employed for long-term analgesic therapy because they can produce dysphoric and psychotomimetic effects. Agonists acting at the kappa-opioid receptor inhibit the release of oxytocin and antidiuretic hormone and cause prominent diuresis. Some kappa-opioid receptor agonists, such as salvinorin A from *Salvia divinorum*, are used recreationally and have hallucinogenic effects in humans. There is evidence that kappa-opioid receptor antagonists may have antidepressant effects in depressed patients (Jacobson et al., 2020).

Agonists at delta-opioid receptors have antinociceptive effects in animal models of neuropathic and chronic pain but have not yet found clinical utility because of preclinical and clinical evidence of proconvulsant activity. Delta-opioid receptor agonists also have antidepressant-like, anxiolytic effects and anti-parkinsonian effects in preclinical models (Dripps and Jutkiewicz, 2018).

NOPr agonists can be pro- or anti-analgesic depending on their site of action and the animal species. NOPr agonists, unlike mu-opioid receptor agonists, are not rewarding and do not cause respiratory depression, so compounds with activity at both mu-opioid receptors and NOPrs are being investigated as potentially safer analgesics. The system has been implicated in regulation of body weight and stress-related mood disorders. Molecules targeting NOPr may find use in the management of anxiety and/or depression and substance abuse (Witkin et al., 2014).

Some selective agonists and antagonists for these receptors are shown in Figure 23–4.

asparagine in position 40 of the N-terminus (Fig 23–1) is replaced by aspartate, has received the most attention. This single nucleotide polymorphism is found in approximately 40% of Asian populations, 16% of Europeans, and 3% of African Americans (Zerbino et al., 2018) and has been linked to dependence on opioids and other drugs of abuse (Halikere et al., 2020).

Many splice variants of the mu-opioid receptor have been reported and may show a differential pharmacology, although the roles and level of expression of these are not well known (Gretton and Droney, 2014). In addition, opioid receptors can form receptor complexes with themselves (homomers) or with other opioid receptors or indeed many other GPCRs, giving receptor complexes with the potential for a very diverse pharmacology (Fujita et al., 2014). These aspects have potential clinical significance since drugs targeting splice variants or complex receptor heteromers could have safer pharmacological profiles. Although the basis of much research, no clinical candidates targeting these entities have yet emerged.

Pharmacology of Clinically Employed Opioid Drugs

Clinically used opioid agonists are generally selective for mu-opioid receptors and produce their therapeutic and adverse effects through activation of these receptors. Even so, the drugs have a highly complex pharmacology. The exact nature of observed physiological responses induced by opioid agonists is due to the wide distribution of mu-opioid receptors across the CNS and in the periphery, their degree of efficacy (e.g., whether they are full or partial agonists), and the level of selectivity of each drug for mu-opioid over other opioid receptors or, indeed, other targets. Opioid drugs are relatively receptor selective at lower doses but may interact with additional receptor types when given at high doses, especially as doses are escalated to overcome tolerance. Mu-opioid receptor agonists produce analgesia, affect mood and rewarding behavior, and alter respiratory, cardiovascular, GI, and neuroendocrine function. The pharmacology of the non-mu-opioid receptor systems is briefly highlighted in Box 23–1.

Pharmacology of the Prototypical Mu-Opioid Agonist Morphine

Analgesia

Opioid drugs acting at the mu-opioid receptor, as exemplified by *morphine*, are used to treat different types of pain (Box 23–2). When therapeutic doses of *morphine* are administered, patients report their pain to be less intense or entirely absent. Patients often report pain is still present, but they feel less discomfort. In addition to relief of distress, some patients may experience euphoria. Analgesia can be readily achieved without loss of consciousness, although drowsiness commonly occurs. *Morphine* at

BOX 23–2 ■ Pain States and Mechanisms

Meaningful discussion of the action of analgesic agents must recognize that all pain is not the same and that a number of variables contribute to a patient's pain reporting and therefore to the effect of the analgesic. Heuristically, one may think of pain as several distinct sets of events (Yaksh et al., 2015).

Acute Nociception

Acute activation of small, high-threshold sensory afferents (Aδ and C fibers) generates transient, stimulus-dependent inputs into the spinal cord, which in turn leads to activation of dorsal horn neurons that project contralaterally to the thalamus and thence to the somatosensory cortex. A parallel spinofugal projection runs through the medial thalamus and thence to portions of the limbic cortex, such as the anterior cingulate. The output produced by acutely activating these ascending systems is sufficient to evoke pain reports. Examples of such stimuli include a hot coffee cup, a needlestick, or an incision.

Tissue Injury

Following tissue injury or local inflammation (e.g., local skin burn, toothache, rheumatoid joint), an ongoing pain state arises that is characterized by burning, throbbing, or aching and an abnormal pain response termed *hyperalgesia*, which can be evoked by otherwise innocuous or mildly aversive stimuli (tepid bathwater on a sunburn; moderate extension of an injured joint). This pain typically reflects the effects of active factors such as prostaglandins, bradykinin, cytokines, serine proteases, and H^+ ions, among many mediators. Such mediators are released locally into the injury site and have the capacity, through eponymous receptors on the terminals of small, high-threshold afferents (Aδ and C fibers), to activate these sensory afferents and to reduce the stimulus intensity required for their activation (e.g., peripheral sensitization). In addition, the ongoing afferent traffic initiated by the tissue injury and inflammation leads to activation of spinal facilitatory cascades, yielding a greater output to the brain for any given afferent input. This facilitation is thought to underlie hyperalgesic states, for example, central sensitization. Such tissue injury/inflammation-evoked pain is often referred to as *nociceptive* pain (Figure 23–5) (Sorkin and Wallace, 1999). Examples of such states would be burn, postincision pain, abrasion of the skin, musculoskeletal injury, or inflammation of the joint.

Nerve Injury

Injury to a peripheral nerve yields complex anatomical and biochemical changes in the nerve and spinal cord that induce *spontaneous dysesthesias* (shooting, burning pain) and *allodynia* (hurt from a light touch). Nerve injury pain state may not depend on the activation of small afferents but may be initiated by low-threshold sensory afferents (e.g., Aβ fibers). Such nerve injuries result in the development of ectopic activity arising from neuromas formed by nerve injury and the dorsal root ganglia of the injured axons as well as changes in dorsal horn sensory processing. Such changes include activation of nonneuronal (glial) cells and loss of constitutive inhibitory circuits, such that low-threshold afferent input carried by Aβ fibers evokes a pain state (West et al., 2015). Examples of such nerve injury–inducing events include mononeuropathies secondary to nerve trauma or compression (carpal tunnel syndrome) and the postherpetic state (shingles). Polyneuropathies such as those occurring in diabetes or after chemotherapy (as for cancer) can also lead to ongoing dysesthesias and evoked hyperpathias. These pain states are said to be *neuropathic* (Figure 23–6). Many clinical pain syndromes, such as found in cancer, typically represent a combination of these inflammatory and neuropathic mechanisms.

Neuropathic pain is typically considered to respond less well to opioid analgesics than acute pain, and higher doses are required. There is a growing perception that, in the face of chronic tissue injury or inflammation (e.g., arthritis), there can be a transition from an inflammatory to a neuropathic pain phenotype.

Sensory Versus Affective Dimensions of Pain

Information generated by a high-intensity peripheral stimulus initiates activity in pathways activating higher-order systems that reflect the aversive magnitude of the stimulus. Painful stimuli have the certain ability to generate strong emotional components that reflect a distinction between pain as a specific sensation subserved by distinct neurophysiological structures (the *sensory discriminative* dimension) and pain such as suffering (the original sensation plus the reactions evoked by the sensation: the *affective motivational* dimension of the pain experience) (Melzack and Casey, 1968). Opioid drugs have potent effects on both components of the pain experience.

analgesic doses does not have anticonvulsant activity and usually does not cause slurred speech, emotional lability, or significant impairment of motor coordination. Lower doses of *morphine* can produce reductions in the affective response to pain but not the perceived intensity of the pain experience; higher, clinically effective doses reduce both perceived intensity and affective responses to the pain (Price et al., 1985).

The relief of pain by morphine-like opioid agonists is relatively selective in that other senses are generally not affected, including light touch and proprioception. Continuous dull pain (as generated by tissue injury and inflammation) is relieved more effectively than sharp intermittent pain, such as that associated with the movement of an inflamed joint. With sufficient amounts of opioid agonist, it is possible to relieve even the severe piercing pain associated with, for example, acute renal or biliary colic, although opioids can induce spasm in the sphincter of Oddi and may exacerbate the pain (see section on the GI tract, below).

When an analgesic dose of *morphine* is administered to normal, pain-free individuals, the experience may be unpleasant, and nausea and vomiting are common. Individuals may experience drowsiness, difficulty in mentation, apathy, and lessened physical activity. As the dose is increased, the subjective, analgesic, and toxic effects, including respiratory depression, become more pronounced.

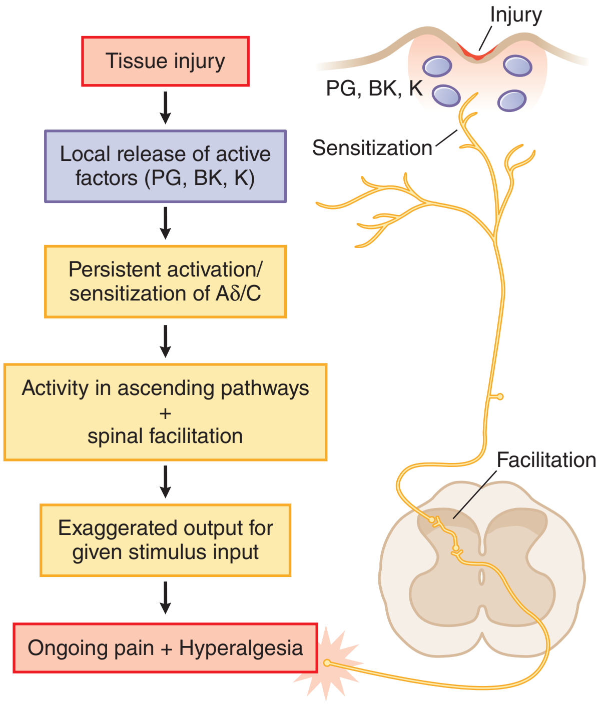

Injury
Tissue injury
PG, BK, K
Local release of active factors (PG, BK, K)
Sensitization
Persistent activation/ sensitization of Aδ/C
Activity in ascending pathways + spinal facilitation
Facilitation
Exaggerated output for given stimulus input
Ongoing pain + Hyperalgesia

Figure 23–5 *Mechanisms of tissue injury–evoked nociception.* BK, bradykinin; K, potassium; PG, prostaglandins.

The analgesic actions of opioid drugs are mediated by actions in the brain, spinal cord, and in some instances the periphery. These are summarized in Figure 23–7.

Supraspinal Actions. Direct microinjections of *morphine* into specific brain regions can produce potent analgesia that is reversible by the mu-opioid receptor antagonists, such a *naloxone*. The best characterized of these sites is the mesencephalic PAG region. *Morphine* and other mu-opioid receptor agonists inhibit release of the inhibitory transmitter GABA from tonically active PAG systems that regulate activity in projections to the medulla. PAG projections to the medulla activate medullospinal release of norepinephrine (NE) and serotonin (5HT) at the level of the spinal dorsal horn, which attenuate dorsal horn excitability (Yaksh, 1997).

Spinal Action. Opioid drugs delivered spinally (intrathecally or epidurally) induce powerful analgesia that is reversed by low doses of systemic *naloxone* (Yaksh, 1997). Mu-opioid receptors are largely limited to the *substantia gelatinosa* of the superficial dorsal horn, the region in which small, high-threshold sensory afferents terminate. A significant proportion of mu-opioid receptors are associated with small peptidergic primary afferent C fibers; the remainder are on local dorsal horn neurons. Mu-opioid receptor agonists act presynaptically on small, high-threshold primary afferents to *inhibit the opening of voltage-sensitive* Ca^{2+} *channels,*

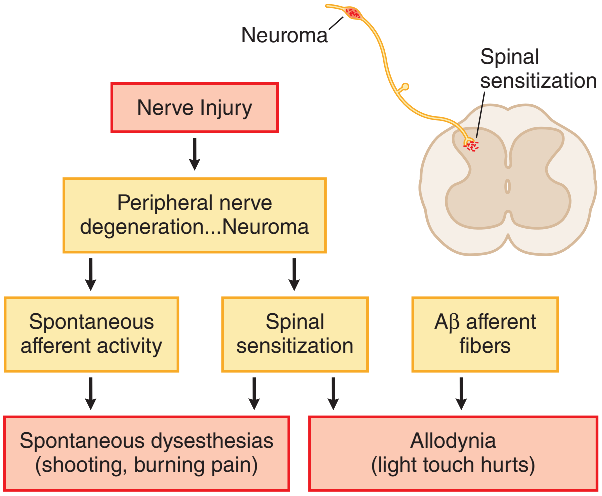

Neuroma
Spinal sensitization
Nerve Injury
Peripheral nerve degeneration...Neuroma
Spontaneous afferent activity
Spinal sensitization
Aβ afferent fibers
Spontaneous dysesthesias (shooting, burning pain)
Allodynia (light touch hurts)

Figure 23–6 *Mechanisms of nerve injury–evoked nociception.*

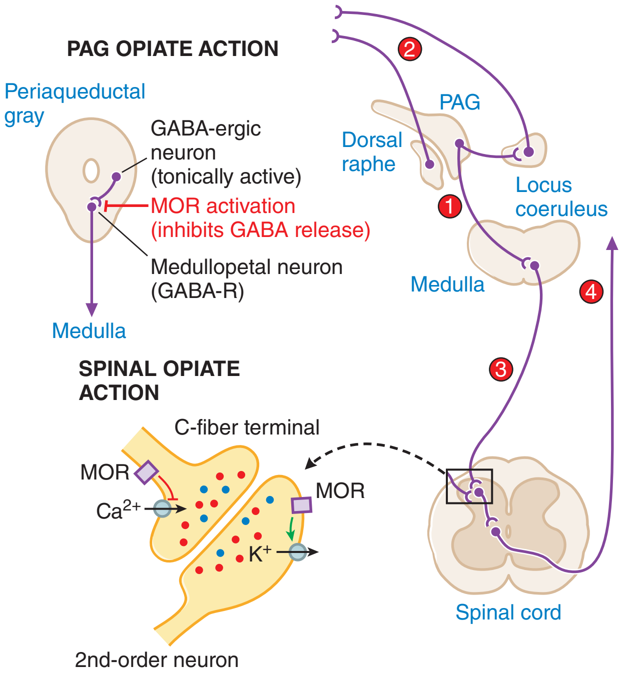


Figure 23–7 *Mechanisms of opioid action in producing analgesia*. **Top left:** Schematic of organization of opiate action in the periaqueductal gray area (PAG). **Top right:** Opioid-sensitive pathways in the PAG. Opioid actions via the mu-opioid receptor block the release of GABA from tonically active systems that otherwise regulate the projections to the medulla (1), leading to an activation of PAG outflow that results in activation of forebrain (2) and spinal (3) monoamine receptors that regulate spinal cord projections (4), which provide sensory input to higher centers and mood. **Bottom left:** Schematic of primary afferent synapse with second-order dorsal horn spinal neuron, showing pre- and postsynaptic opiate receptors coupled to Ca^{2+} and K^+ channels, respectively. Opioid receptor binding is highly expressed in the superficial spinal dorsal horn (substantia gelatinosa). These receptors are located presynaptically on the terminals of small primary afferents (C fibers) and postsynaptically on second-order neurons. Presynaptically, activation of mu-opioid receptors blocks the opening of the voltage-sensitive Ca^{2+} channel, which otherwise initiates transmitter release. Postsynaptically, mu-receptor activation enhances opening of K^+ channels, leading to hyperpolarization. Thus, an opioid agonist acting at these sites jointly serves to attenuate the afferent-evoked excitation of the second-order neuron.

thereby preventing transmitter release. A postsynaptic action is demonstrated by the ability of opioid analgesics to block excitation of dorsal horn neurons evoked by glutamate, partly by *hyperpolarizing the neurons through the activation of* K^+ *channels*, making them less likely to fire. Morphine selectively depresses the discharge of spinal dorsal horn neurons evoked by small (high-threshold) but not large (low-threshold) afferent nerve fibers. Overall, the capacity of spinal opioids to reduce the release of excitatory neurotransmitters from C fibers and to decrease the excitability of dorsal horn neurons accounts for the powerful and selective effect of the drugs on spinal nociceptive processing.

Peripheral Action. Direct application of high concentrations of opioid analgesics to a peripheral nerve can produce a local anesthetic-like action that is not reversed by *naloxone*. Conversely, at peripheral sites under conditions of inflammation where there is an increased terminal sensitivity leading to an exaggerated pain response (e.g., hyperalgesia), direct injection of opioids produces a local action that can exert a normalizing effect on the exaggerated thresholds. The effects may be on peripheral afferent terminals or on inflammatory cells that release products that sensitize the nerve terminal, or both (Stein and Machelska, 2011).

Opioid-Induced Hyperalgesia

A paradoxical increase in pain state can be observed following acute (hours to days) or chronic opioid drug exposure. This increase may be reflected by unexplained increases in pain reports, increased levels of pain with increasing drug dosages, or a diffuse sensitivity unassociated with the original pain (Lee et al., 2011). The mechanisms of this increased pain profile are not understood, although evidence suggests that inflammatory responses may be involved through Toll-like receptor 4 activation on microglia, upregulation of proinflammatory cytokines, chemokines, cyclooxygenase, and prostaglandin E_2, and other neuropeptides, such as cholecystokinin, neuropeptide FF, and nociceptin (Mercadante et al., 2019).

Respiratory Effects

Although effects of mu-opioid receptor agonists on respiration are readily demonstrated in preclinical models, significant respiratory depression rarely occurs in the clinic with standard analgesic doses, unless there are mitigating factors where opioid drugs should be used with caution, for example, in patients with asthma, chronic obstructive pulmonary disease (COPD), pulmonary hypertension, decreased respiratory reserve, preexisting respiratory depression, hypoxia, or hypercapnia. Moreover, it should be stressed that *respiratory depression is the primary cause of deaths in opioid overdose in individuals who misuse and abuse opioids.*

Mu-opioid receptor agonists depress all phases of respiratory activity, including rate, minute volume, and tidal exchange, and produce irregular and aperiodic breathing. The diminished respiratory volume is due primarily to a slower rate of breathing, which after ingestion of toxic amounts of opioids, may fall to three to four breaths per minute. Morphine-like opioid agonists depress respiration through several mechanisms involving mu-opioid receptors. Respiratory rate and tidal volume depend on intrinsic rhythm generators located in the ventrolateral medulla. These systems generate a "respiratory rhythm" that is driven by afferent input reflecting the partial pressure of arterial O_2 as measured by chemosensors in the carotid and aortic bodies and CO_2 as measured by chemosensors in the brainstem. Morphine-like opioid agonists reduce respiration in part by a direct depressant effect on rhythm generation, with changes in respiratory pattern and rate observed at lower doses than changes in tidal volume. A key factor is the depression of the ventilatory response to increased CO_2. This effect is mediated by depression of the excitability of brainstem chemosensory neurons. Also, opioids will depress ventilation otherwise driven by hypoxia through an effect on carotid and aortic body chemosensors. Importantly, hypoxic stimulation of chemoreceptors may still be effective when opioids have decreased the responsiveness to CO_2, and inhalation of O_2 may remove the residual drive resulting from the elevated PO_2 and produce apnea (Pattinson, 2008). In addition to the effect on respiratory rhythm and chemosensitivity, mu-opioid receptor agonists can have mechanical effects on airway function by increasing chest wall rigidity and diminishing upper airway patency (Lalley, 2008).

Studies comparing *morphine* and morphine-like opioids with respect to the ratio of analgesic versus respiratory-depressant activity have found that when equianalgesic doses are used, there is no significant difference. Maximal respiratory depression occurs within 5 to 10 min of intravenous administration of *morphine* or within 30 to 90 min of intramuscular or subcutaneous administration. Maximal respiratory-depressant effects occur more rapidly with more lipid-soluble agents. After therapeutic doses, respiratory minute volume may be reduced for as long as 4 to 5 h. Agents that have persistent kinetics, such as *methadone*, must be carefully monitored, particularly after dose incrementation. Respiratory depression produced by any mu-opioid receptor agonist can be reversed with an opioid antagonist. It is important to remember that most opioid antagonists have a relatively short duration of action as compared with agonists such as *morphine* or *methadone*, and fatal "re-narcotization" can occur if vigilance is not exercised and if more antagonist is not added as needed.

Factors Exacerbating Opioid-Induced Respiratory Depression. Several factors can increase the risk of opioid-related respiratory depression even at therapeutic doses. These include the following:

Other Medications. The combination of opioid drugs with other depressant medications, such as general anesthetics, tranquilizers, alcohol, or sedative-hypnotics, produces additive depression of respiratory activity.

Sleep. Natural sleep produces a decrease in the sensitivity of the medullary center to CO_2, and the depressant effects of *morphine* and sleep are at least additive. Obstructive sleep apnea is a risk factor for increasing the likelihood of fatal respiratory depression.

Age. Newborns can show significant respiratory depression and desaturation; this may be evident in lower Apgar scores if opioids are administered parenterally to women within 2 to 4 h of delivery because of transplacental passage of opioids. Elderly patients are at greater risk of depression because of reduced lung elasticity, chest wall stiffening, and decreased vital capacity.

Disease. Opioids may cause a greater depressant action in patients with chronic cardiopulmonary or renal diseases because they can manifest a desensitization of their response to increased CO_2.

COPD. Enhanced respiratory depression can be noted in patients with COPD and sleep apnea secondary to diminished hypoxic drive.

Pain Relief. Because pain stimulates respiration, removal of the painful condition (as with the analgesia resulting from the therapeutic use of the opioid) will reduce the ventilatory drive and lead to apparent respiratory depression.

Although respiratory depression is not considered to be a favorable therapeutic effect of opioids, their ability to suppress respiratory drive is used as therapeutic advantage to treat dyspnea resulting, for example, in patients with COPD, where air hunger leads to extreme agitation, discomfort, and gasping; opioid analgesics will suppress the gasping and decease the panic of the patient. Similarly, opioid drugs find use in patients who require artificial ventilation (Clemens and Klaschik, 2007).

Sedation

Mu-opioid receptor agonists can produce drowsiness and cognitive impairment. Such depression can augment respiratory impairment. These effects are most typically noted following initiation of opioid drug therapy or after dose incrementation. Importantly, these effects on arousal resolve over a few days due to the development of tolerance to the sedative effects. As with respiratory depression, the degree of drug effect can be enhanced by a variety of predisposing patient factors, including dementia, encephalopathies, brain tumors, and other depressant medications, including sleep aids, antihistamines, alcohol, antidepressants, and anxiolytics (Cherny, 1996).

Neuroendocrine Effects

The regulation of the release of hormones and factors from the pituitary is under complex regulation by opioid receptors in the hypothalamic-pituitary-adrenal (HPA) axis. Broadly considered, morphine-like opioid agonists reduce the release of a large number of HPA hormones (Armario, 2010). However, withdrawal from opioids stimulates the HPA axis (Houshyar et al., 2001; Nava et al., 2006).

Sex Hormones

In males, acute opioid agonist treatment reduces plasma cortisol, testosterone, and gonadotrophins. Inhibition of adrenal function is reflected by reduced cortisol production and reduced adrenal androgens (dehydroepiandrosterone). In females, morphine administration will produce lower luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release. In both males and females, chronic therapy can result in endocrinopathies, including hypogonadotropic hypogonadism. In men, this may cause decreased libido and, with extended exposure, reduced secondary sex characteristics. In women, these exposures are associated with menstrual cycle irregularities. These changes are reversible with removal of the drug.

Prolactin. Prolactin release from the anterior pituitary is under inhibitory control by dopamine released from neurons of the arcuate nucleus. Mu-opioid receptor agonists act presynaptically on these dopamine-releasing terminals to inhibit neurotransmitter release and thereby increase plasma prolactin levels.

ADH and Oxytocin. The effects of opioids on ADH and oxytocin release are complex. These hormones are synthesized in the perikarya of the magnocellular neurons in the paraventricular and supraoptic nuclei of the hypothalamus and released from the posterior pituitary (see Chapter 46). *Morphine* and morphine-like drugs bring about the liberation of ADH by acting on the hypothalamic-hypophysial system. In addition, mu-opioid receptor agonists may yield a hypotension secondary to histamine release, which would promote ADH release. Endogenous and exogenous mu-opioid receptor agonists modulate oxytocin release from the posterior pituitary, which is used to provide rest and sedation during stressed prodromal stages of labor (Morris et al., 2010).

Miosis

Mu-opioid receptor agonists induce pupillary constriction (miosis) in the awake state and block pupillary reflex dilation during anesthesia. The parasympathetic outflow from the *Edinger-Westphal nucleus* activates parasympathetic outflow through the ciliary ganglion to the pupil, producing constriction. This outflow is locally regulated by GABAergic interneurons. Opioid agonists block this GABAergic interneuron-mediated inhibition, leading to increased parasympathetic outflow (Larson, 2008). At high doses of agonists, the miosis is marked, and pinpoint pupils are pathognomonic; however, marked mydriasis will occur with the onset of asphyxia. While some tolerance to the miotic effect develops, regular opioid users with high circulating concentrations of opioids continue to have constricted pupils. Therapeutic doses of *morphine* increase accommodative power and lower intraocular tension in normal and glaucomatous eyes (Larson, 2008).

Seizures and Convulsions

In older children and adults, moderately high doses of mu-opioid receptor agonists produce electroencephalogram (EEG) slowing. In the newborn, *morphine* can produce epileptiform activity and occasionally seizure activity (Young and da Silva, 2000). Mechanisms likely involved in these excitatory actions include inhibition of inhibitory interneurons. Morphine-like drugs indirectly excite certain groups of neurons, such as hippocampal pyramidal cells, by inhibiting the inhibition (disinhibition) exerted by GABAergic interneurons (McGinty, 1988). It has been suggested that opioid receptors may couple through both inhibitory and stimulatory G proteins, with the inhibitory coupling but not the excitatory coupling showing tolerance with continued exposures (King et al., 2005).

The metabolites of several opioids (morphine-3-glucuronide, normeperidine) have been implicated in seizure activity (Seifert and Kennedy, 2004; Smith, 2000). Withdrawal syndrome from an opioid-dependent state in the adult and in the infant born to an opioid-dependent mother can lead to prominent EEG activation, tremor, and rigidity. Anticonvulsant agents may not always be effective in suppressing such opioid-induced seizures.

Cough

Cough is a protective reflex evoked by airway stimulation. It involves rapid expression of air against a transiently closed glottis. The reflex is complex, involving the central and peripheral nervous systems as well as the smooth muscle of the bronchial tree. *Morphine*, *codeine*, and related opioids depress the cough reflex at least in part by a direct effect on a cough center in the medulla; this cough suppression can be achieved without altering the protective glottal function (Chung and Pavord, 2008). There is no obligatory relationship between depression of respiration and depression of coughing, and effective antitussive agents are available that do not depress respiration (opioid- and opiate-based antitussives are discussed later in the chapter).

Nausea and Vomiting

These are side effects of morphine-like drugs caused by direct stimulation of the chemoreceptor trigger zone for emesis in the *area postrema* of the medulla. All clinically useful mu-opioid receptor agonists produce some degree of nausea and vomiting. Nausea and vomiting are relatively uncommon in recumbent patients given therapeutic doses of *morphine*, but nausea occurs in about 40% and vomiting in 15% of ambulatory patients given analgesic doses. *Morphine* and related analgesics produce an increase in vestibular sensitivity. A component of nausea is likely also due to the gastric stasis that occurs postoperatively and that is exacerbated by analgesic doses of *morphine* (Greenwood-Van Meerveld, 2007).

Cardiovascular System

In the supine patient, therapeutic doses of morphine-like opioids have no major effect on blood pressure or cardiac rate and rhythm. Such doses can, however, produce peripheral vasodilation, reduced peripheral resistance, and an inhibition of baroreceptor reflexes. Thus, when supine patients assume the head-up position, orthostatic hypotension and fainting may occur. The peripheral arteriolar and venous dilation produced by *morphine* involves several mechanisms, including morphine-induced *release of histamine* from mast cells and *blunting of reflex vasoconstriction* caused by increased P_{CO_2}. High doses of mu-opioid receptor agonists used as anesthetic induction agents, such as *fentanyl* and *sufentanil*, have only modest effects on hemodynamic stability, in part because they do not cause release of histamine (Monk et al., 1988).

Morphine may exert its therapeutic effect in the treatment of angina pectoris and acute myocardial infarction by decreasing preload, inotropy, and chronotropy, thus favorably altering determinants of myocardial O_2 consumption. *Morphine* also produces cardioprotective effects and can mimic the phenomenon of ischemic preconditioning, whereby a short ischemic episode paradoxically protects the heart against further ischemia. This effect appears to be mediated through a mitochondrial ATP-sensitive K^+ channel in cardiac myocytes. Morphine-like opioids should be used with caution in patients who have decreased blood volume because these agents can aggravate hypovolemic shock. *Morphine* should be used with great care in patients with cor pulmonale; deaths after therapeutic doses have been reported. Concurrent use of certain CNS depressants (phenothiazines, ethanol, benzodiazepines) may increase the risk of *morphine*-induced hypotension. Cerebral circulation is not affected directly by therapeutic doses of opioids. However, opioid-induced respiratory depression and CO_2 retention can result in cerebral vasodilation and an increase in cerebrospinal fluid (CSF) pressure. This pressure increase does not occur when P_{CO_2} is maintained at normal levels by artificial ventilation.

Skeletal Muscle Tone

At therapeutic doses required for analgesia, opioid agonists have little effect on motor tone or function. However, high doses of opioids, as used for anesthetic induction, produce muscular rigidity. Myoclonus, ranging from mild twitching to generalized spasm, is an occasional side effect that has been reported with all clinically used opioid agonists and is particularly prevalent in patients receiving high doses. The increased muscle tone is mediated centrally. High doses of spinal opioids can increase motor tone, possibly through an inhibition of inhibitory interneurons in the ventral horn of the spinal cord. Intracranial delivery of opioids can initiate rigidity in animal models, possibly reflecting increased extrapyramidal activity.

Gastrointestinal Tract

Opioids have effects on all aspects of GI function. Between 40% and 95% of patients treated with opioid agonists develop constipation and changes in bowel function (Benyamin et al., 2008). Opioid receptors are densely distributed in enteric neurons between the myenteric and submucosal plexuses and on a variety of secretory cells. The importance of these peripheral systems in altering GI motility is demonstrated by the therapeutic efficacy of peripherally limited agonists such as *loperamide* as antidiarrheals and the utility of peripherally limited opioid antagonists such as *methylnaltrexone* to reverse the constipation induced by systemic opioid agonists.

Esophagus. The esophageal sphincter is under control of brainstem reflexes that activate cholinergic motor neurons originating in the esophageal myenteric plexus. This system regulates passage of material from the esophagus to the stomach and prevents regurgitation; conversely, it allows relaxation in the act of emesis. *Morphine* inhibits lower esophageal sphincter relaxation induced by swallowing and by esophageal distension; the effect is believed to be centrally mediated because peripherally restricted opioids such as *loperamide* do not alter esophageal sphincter tone (Sidhu and Triadafilopoulos, 2008).

Stomach. *Morphine* increases tonic contracture of the antral musculature and upper duodenum and reduces resting tone in the musculature of the gastric reservoir, thereby prolonging gastric emptying time and increasing the likelihood of esophageal reflux. Passage of the gastric contents through the duodenum may be delayed by as much as 12 h. This also results in retardation of orally administered medications. *Morphine* and other opioid agonists usually decrease secretion of HCl. Activation of opioid receptors on parietal cells enhances secretion, but indirect effects, including increased secretion of somatostatin from the pancreas and reduced release of acetylcholine, appear to be dominant in most circumstances (Kromer, 1988).

Intestine. *Morphine* reduces propulsive activity in the small and large intestines and diminishes intestinal secretions. Opioid agonists suppress rhythmic inhibition of muscle tone, leading to concurrent increases in basal tone in the circular muscle of the small and large intestines. This results in enhanced high-amplitude phasic contractions, which are nonpropulsive (Wood and Galligan, 2004). The upper part of the small intestine, particularly the duodenum, is affected more than the ileum. A period of relative atony may follow the period of elevated basal tone. Intestinal secretion arises from activation of enterocytes by local cholinergic submucosal plexus secretomotor neurons. Opioids act on secretomotor neurons to inhibit their excitatory output to the enterocytes and thereby reduce intestinal secretion (Kromer, 1988). The reduced rate of passage of the intestinal contents, along with reduced intestinal secretion, leads to increased water absorption, increasing viscosity of the bowel contents, and constipation. The tone of the anal sphincter is augmented greatly, and reflex relaxation in response to rectal distension is reduced. Patients who take opioid agonists chronically remain constipated.

Biliary Tract. *Morphine* constricts the sphincter of Oddi, and the pressure in the common bile duct may rise more than 10-fold within 15 min. Fluid pressure also may increase in the gallbladder and produce symptoms that may vary from epigastric distress to typical biliary colic. All opioids can cause biliary spasm. Some patients with biliary colic experience exacerbation rather than relief of pain when given opioids. Spasm of the sphincter of Oddi probably is responsible for elevations of plasma amylase and lipase that sometimes occur after *morphine* administration. *Atropine* only partially prevents *morphine*-induced biliary spasm, but opioid antagonists prevent or relieve it.

Ureter and Urinary Bladder

Morphine inhibits the urinary voiding reflex and increases the tone of the external sphincter with a resultant increase in the volume of the bladder. Tolerance develops to these effects of opioids on the bladder. Clinically, opioid-mediated inhibition of micturition can be of such clinical severity that catheterization is sometimes required after therapeutic doses of *morphine*, particularly with spinal drug administration. The inhibition of systemic opioid effects on micturition is reversed by peripherally restricted antagonists (Rosow et al., 2007).

Uterus

Morphine may prolong labor. If the uterus has been made hyperactive by oxytocics, *morphine* tends to restore the contractions to normal.

Mast Cell Degranulation and Histamine Release

Many mu-opioid receptors agonists, including *morphine* and *meperidine*, evoke mast cell degranulation and histamine release. This action can cause bronchoconstriction, vasodilation, urticaria, other types of skin rashes, and pruritus. The effect on mast cells is not blocked by antagonists such as *naloxone* and so it not mu-opioid receptor mediated, but rather a due to their interaction with the Mas-related G protein-coupled receptor X2 (MRGPRX2; see Chapter 43 and McNeil, 2021a and 2021b) expressed in mast cells and small-diameter neurons in the dorsal root ganglia and trigeminal ganglia (Lansu et al., 2017). Mu-opioid agonists, such as *fentanyl*, do not activate this receptor and are associated with a lower incidence of histamine release.

Skin

Therapeutic doses of *morphine* cause dilation of cutaneous blood vessels. The skin of the face, neck, and upper thorax frequently becomes flushed. Pruritus commonly follows systemic administration of *morphine*. Itching is readily seen with *morphine* mainly due to histamine release. The systemic action is sensitive to antihistamines (*diphenhydramine*) and correlates with the mast cell degranulating properties of the opioid. Neither the pruritus nor the degranulation is reversed by opioid antagonists (Barke and Hough, 1993). Pruritus also can be seen following epidural or intrathecal opioid administration through a centrally mediated, *naloxone*-reversible mechanism (Kumar and Singh, 2013).

Immune System

Opioids modulate immune function by direct effects on immune cells and indirectly through centrally mediated neuronal mechanisms (Vallejo et al., 2004). The acute central immunomodulatory effects of opioids may be mediated by activation of the sympathetic nervous system; the chronic effects of opioids may involve modulation of the HPA axis. Direct effects on immune cells may involve variants of the classical neuronal opioid receptors. Proposed mechanisms for the immune-suppressive effects of *morphine* on neutrophils include nitric oxide–dependent inhibition of nuclear factor-κB activation and activation of MAPKs. Evidence suggests that several opioid drugs, including *morphine*, may interact with Toll-like receptor 4 to activate a variety of immunocytes (Hutchinson et al., 2007). Overall, however, opioid agonists are modestly immunosuppressive, and increased susceptibility to infection and tumor spread have been observed. In some situations, immune effects appear more prominent with acute administration than with chronic administration, which could have important implications for the care of the critically ill.

Temperature Regulation

Opioids alter the equilibrium point of the hypothalamic heat-regulatory mechanisms such that body temperature usually falls slightly. Mu-opioid receptor agonists acting in the CNS result in slightly increased thresholds for sweating and significantly lower the threshold temperatures for evoking vasoconstriction and shivering.

Chronic Effects of Mu-Opioid Drugs: Tolerance, Dependence, and Opioid Use Disorder

Acute or short-term opioid receptor activation produces the typical pharmacological pattern described above. However, repeated or prolonged mu-opioid receptor activation by agonists leads to the development of tolerance and physical dependence. Tolerance to opioids refers to the loss of the effectiveness of the opioid agonist with continuous or repeated administration (over days to weeks to months), requiring the administration of larger doses to produce the expected therapeutic effects. Physical dependence is most noticeable when an opioid agonist is withheld or in the presence of an antagonist, and symptoms of opioid withdrawal, such as diarrhea, hyperalgesia, restlessness, and increases in heart rate and respiration, are observed. There are a number of mechanisms thought to contribute to tolerance to and physical dependence on opioid agonists.

Tolerance

Mechanisms underlying tolerance may include loss of opioid receptors, changes in intracellular cascades (e.g., reduced inhibition of AC), as well as changes at the organ system level (e.g., loss of sedative and analgesic effects) (Christie, 2008). Different physiological responses can develop tolerance at markedly different rates. At the organ system level, some endpoints show little or no tolerance development (pupillary miosis); some show moderate tolerance (constipation, emesis, analgesia, sedation); and some show rapid tolerance (euphoria). The amount of tolerance that develops is generally thought to be due to the number of "spare" receptors present at the level of the organ or neural circuit mediating a response.

In general, cross-tolerance to different mu-opioid receptor agonists occurs. However, for reasons that are not clear, this cross-tolerance is neither absolute nor complete. This lack of complete cross-tolerance between agonists forms the basis for the clinical strategy of "opioid rotation" in pain therapy (Smith and Peppin, 2014).

Dependence

Dependence represents a state of adaptation manifested by a withdrawal syndrome produced by cessation of drug exposure (e.g., by drug abstinence) or administration of an antagonist (e.g., *naloxone*). Dependence is specific to the drug class and is receptor-mediated. At the organ system level, opioid withdrawal is manifested by significant somatomotor and autonomic outflow (reflected by agitation, hyperalgesia, hyperthermia, hypertension, diarrhea, pupillary dilation, and release of virtually all pituitary and adrenomedullary hormones) and by affective symptoms (dysphoria, anxiety, and depression). Consistent with the phenomenon of cross-tolerance, drugs interacting with the same opioid receptor will suppress the withdrawal observed in organisms dependent on another drug acting on the same receptor (e.g., *morphine* and *methadone*). A state of opioid withdrawal is highly aversive, and avoiding withdrawal is thought to contribute to persistent opioid-taking behavior.

Opioid Use Disorder

In the late 1990s and early 2000s, opioid misuse and abuse as well as overprescribing led to an opioid epidemic in which opioid overdose deaths increased dramatically. From 1999 to 2019, nearly half a million people have died from an opioid overdose, including prescription and illicit opioids. Opioid use disorder is a behavioral pattern characterized by compulsive use of a drug. The positive, rewarding effects of opioids are the driving component for initiating the recreational use of opioids and is subject to the development of tolerance. Given the aversive nature of withdrawal symptoms, avoidance and alleviation of withdrawal symptoms may become a primary motivation for continued drug-taking (Kreek and Koob, 1998). When the drive to acquire the drug leads to drug-seeking behaviors that occur despite the physical, emotional, or societal damage suffered by the drug seeker, then the obsession or compulsion to acquire and use the drug is considered to reflect an opioid use disorder. In animals, this may be manifested by a willingness to tolerate stressful conditions to acquire drug delivery. Importantly, drug dependence is not synonymous with drug addiction. Tolerance and dependence are physiological responses seen in all patients but are not necessarily predictors of opioid misuse and abuse. For example, cancer pain often requires prolonged treatment with high doses of opioids, leading to tolerance and dependence, but not necessarily opioid abuse.

Mechanisms of Tolerance, Dependence, Withdrawal, and Abuse Liability

The mechanisms underlying chronic tolerance and dependence/withdrawal are controversial. There are multiple molecular, cellular, and circuit-level mechanisms that may account for changes in homeostasis and the effects of mu-opioid receptor agonists. Figure 23–2 shows some of the receptor-effector signaling pathways that may contribute to the adaptive signaling changes that occur with immediate and long-term opioid agonist action (desensitization, tolerance, dependence, withdrawal).

Receptor Desensitization, Internalization, and Downregulation

In the face of a transient activation (minutes to hours), receptor desensitization occurs that is specific for that receptor and disappears with a time course parallel to the clearance of the agonist. Short-term desensitization probably involves phosphorylation of the receptors resulting in an uncoupling of the receptor from its G protein and/or internalization of the receptor (Williams et al., 2013). This process likely contributes to the termination of agonist activity.

Acute desensitization and/or receptor internalization may play a role in the initiation of chronic tolerance but is not sufficient to explain the persistent changes observed. Moreover, receptor desensitization and downregulation may be agonist specific. For instance, *morphine*, unlike other mu-opioid receptor agonists, does not promote significant mu-opioid receptor phosphorylation or internalization. Endocytosis and sequestration of receptors do not invariably lead to receptor degradation but can also result in receptor dephosphorylation and recycling to the surface of the cell. Agonists that cause rapid internalization of opioid receptors also rapidly desensitize signaling, but sensitivity can be at least partially restored by recycling of "reactivated" opioid receptors.

Adaptation of Intracellular Signaling Mechanisms

Assessment of the coupling of mu-opioid receptors to cellular effects, such as inhibition of AC, activation of inwardly rectifying K^+ channels, inhibition of Ca^{2+} currents (see Figure 23–2), and inhibition of neurotransmitter release demonstrates functional uncoupling of receptor occupancy from effector function. Importantly, chronic application of opioids initiates adaptive counterregulatory changes. A common example of such cellular processes is the rebound increase in cellular cyclic AMP levels produced by "superactivation" of AC and upregulation of the amount of enzyme as a result of long-term exposure to an opioid followed by its abrupt withdrawal (Williams et al., 2013).

System-Level Counteradaptation

The loss of analgesic effects with chronic opioid agonist exposure may reflect an enhanced excitability of linked systems. Thus, tolerance to the analgesic effects of chronically administered mu-opioid receptor agonists may result from an activation of bulbospinal pathways that increases the excitability of spinal dorsal horn pain transmission linkages. With chronic opioid exposure, opioid receptor occupancy will lead to the activation of PKC, which can phosphorylate and enhance the activation of local *N*-methyl-D-aspartate (NMDA) glutamate receptors. These receptors are considered to play an important role as an excitatory link in enhanced pain processing. Blockade of these receptors can at least partially attenuate the loss of analgesic efficacy with continued opioid exposure. Such system-level counteradaptation mechanisms may apply to specific circuits (e.g., pain modulation) but not necessarily to other effects of opioids (e.g., sedation or miosis) (Christie, 2008). These changes may be mechanistically important in opioid-induced hyperalgesia, by which higher doses of opioids may lead to a paradoxical increase in pain processing (Fletcher and Martinez, 2014).

Differential Tolerance Development and Fractional Occupancy Requirements

A difficulty in explaining tolerance relates to the differential rates of the development of tolerance. It is unclear why responses such as miosis show no tolerance development over extended exposure (indeed, miosis is considered symptomatic in drug overdose of highly tolerant patients), whereas analgesia and sedation are likely to show a reduction. One possibility is that tolerance represents a functional uncoupling of some fraction of the receptor population and that different physiological end points require activation of different fractions of their coupled receptors to produce a given physiological effect. Functional data suggest that opioid receptors may interact, forming homo- and heterodimers, and that such complexes may alter receptor signaling and trafficking and contribute to tolerance to *morphine* and possibly to disease states (Massotte, 2015; Zhang et al., 2015).

Mechanisms of the Reinforcing Effects of Mu-Opioid Receptor Agonists

The mechanisms by which opioids produce euphoria, tranquility, and other alterations of mood (including rewarding properties) are complex and not entirely understood. Neural systems that mediate opioid reinforcement overlap with, but are distinct from, those involved in physical dependence and analgesia (Koob and Le Moal, 2008). Behavioral and pharmacological data point to a pivotal role of the mesocorticolimbic dopamine system that projects to the nucleus accumbens in drug-induced reward and motivation (Figure 23–8). Increased dopamine release in this region is considered to underlie a positive reward state. The reinforcing effects of opioids are thought to be mediated partly via inhibition of local GABAergic neuronal activity in the ventral tegmental area, which otherwise acts to inhibit DA outflow, thus causing disinhibition of dopamine release.

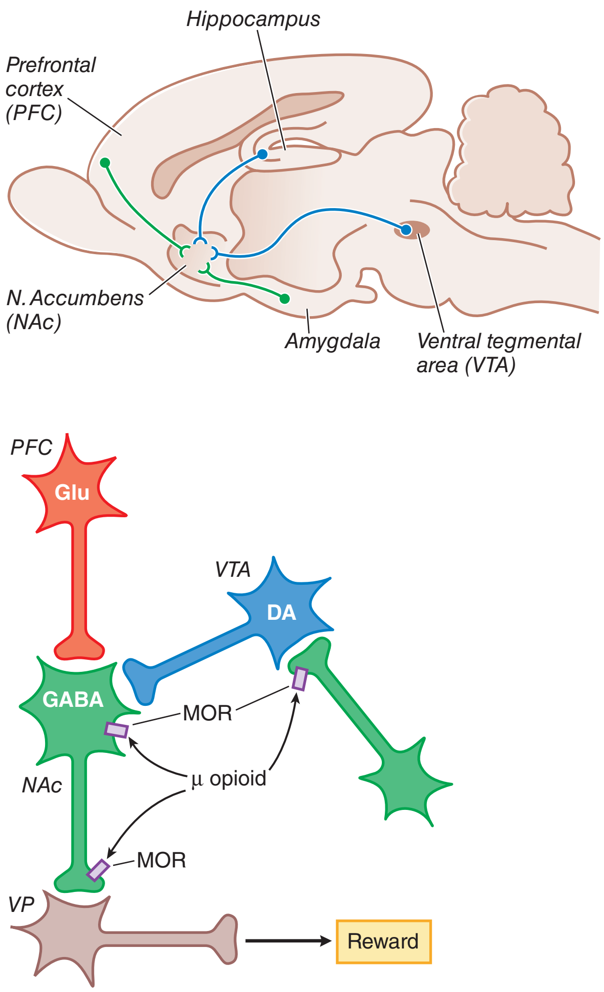


Figure 23–8 *Pathways underlying rewarding properties of opioid drugs.* **Upper panel:** This sagittal section of rat brain shows dopamine (DA) and GABA inputs from the ventral tegmental area (VTA) and prefrontal cortex (PFC), respectively, into the nucleus accumbens (NAc). **Lower panel:** Neurons are labeled with their primary neurotransmitters. At a cellular level, mu-opioid receptor agonists reduce excitability and transmitter release at the sites indicated by inhibiting Ca^{2+} influx and enhancing K^+ current. Thus, opioid-induced inhibition in the VTA on GABAergic interneurons or in the NAc reduce GABA-mediated inhibition and increase outflow from the ventral pallidum (VP), which appears to correlate with a positive reinforcing state (enhanced reward).

Adverse Effects and Precautions Affecting Patient Responses to Mu-Opioids

As can be seen from their complex pharmacology, *morphine* and related mu-opioid receptor agonists, aside from their action as analgesics, produce a wide spectrum of effects reflecting the wide distribution of opioid receptors across organ systems that can affect patient responses and susceptibilities.

Compromised Respiratory Function

Due to their respiratory-depressant actions, *morphine* and related opioid drugs must be used cautiously in patients with compromised respiratory function such as emphysema, kyphoscoliosis, severe obesity, or cor pulmonale. Although many patients with such conditions seem to be functioning within normal limits, they are already using compensatory mechanisms, such as increased respiratory rate. Many have chronically elevated levels of plasma carbon dioxide and may be less sensitive to its respiratory-stimulating actions.

Pain State

Patient in severe pain may tolerate larger doses of *morphine*. However, as the pain subsides, such patients may become sedated and even show respiratory depression as the stimulatory effects of pain on these parameters are diminished.

Asthma and Allergic Responses

Due to its histamine-releasing properties, *morphine* can precipitate or exacerbate asthmatic attacks and should be avoided in patients with a history of asthma. Other mu-opioid agonists, such as *fentanyl*, may be better choices for such patients.

Opioid analgesics may also evoke allergic phenomena, but a true allergic response is uncommon. The effects are manifested as urticaria and fixed eruptions; contact dermatitis in nurses and pharmaceutical workers also occurs. Wheals at the site of injection of *morphine*, *codeine*, and related drugs are likely secondary to histamine release. Anaphylactoid reactions have been reported after intravenous administration of *codeine* and *morphine*, but such reactions are rare. In people who use intravenous opioid *heroin*, such reactions may contribute to sudden death, episodes of pulmonary edema, and other complications.

Head Injury

While head injury per se does not constitute an absolute contraindication to the use of mu-opioid agonists, the possibility of exaggerated depression of respiration and the potential need to control ventilation of the patient must be considered. Because opioids may produce mental clouding and side effects such as miosis and vomiting, which are important signs in following the clinical course of patients with head injuries, the advisability of their use must be weighed carefully against these risks.

Liver or Kidney Disease

Most opioid analgesics are metabolized by the liver and should be used with caution in patients with hepatic disease. Renal disease significantly alters the pharmacokinetics of several drugs, including *morphine*, *codeine*, *dihydrocodeine*, and *meperidine*. Morphine-6-glucuronide may accumulate with continued dosing with *morphine* or *codeine*, and symptoms of opioid overdose may result. When repeated doses of *meperidine* or *propoxyphene* are given to such patients, the accumulation of metabolites may cause tremor and seizures and cardiac toxicity, respectively. Note, propoxyphene has been withdrawn in the U.S. and Europe due its narrow therapeutic window, in particular cardiac toxicity.

Blood-Brain Barrier

Morphine is hydrophilic, so proportionately less *morphine* normally crosses into the CNS than with more lipophilic opioids. In neonates or when the blood-brain barrier is compromised, lipophilic opioids may give more predictable clinical results than *morphine*.

Age and Sex

In adults, the duration of analgesia produced by *morphine* increases progressively with age; however, the degree of analgesia that is obtained with a given dose is similar. There is a growing body of data on gender differences in responses to pain and analgesics (Mogil, 2012). Females exhibit the majority of chronic pain syndromes, and surveys examining sex differences in acute pain models report either no sex difference or greater sensitivity in females (Loyd and Murphy, 2014).

Hypovolemia and Hypotension

Reduced blood volume causes patients to be considerably more susceptible to the vasodilating effects of *morphine* and related drugs. These agents must be used cautiously in patients with hypotension from any cause.

Morphine and Structurally Related Agonists

Chemistry and Structure-Activity Relationships

The structures of *morphine*, *codeine* (3-O-methylated morphine), and some of their analogues and antagonists are shown in Figure 23–9. *Heroin*, or 3,6-diacetylmorphine, is a semisynthetic derivative of *morphine*. Methylation of the phenolic hydroxyl at position 3, as in *codeine*, or acetylation of this hydroxyl, as in *heroin*, drastically reduces binding to the mu-opioid receptor; these compounds are converted *in vivo* to morphine and 6-acetyl morphine, respectively, to afford analgesia.

Thebaine, which is O-methylated at both the 3- and 6-hydroxyl groups and contains an additional double C=C bond, has no activity at opioid receptors but is used to synthesize 14-hydroxyl-derivatives, such as the potent agonists *oxymorphone* and *oxycodone* as well as the antagonists *naltrexone* and *naloxone*. *Thebaine* is also the precursor for the synthesis of the partial agonist *buprenorphine* and the highly potent full agonist *etorphine*, which is 1000 times more potent than *morphine* and restricted to veterinary use. The tertiary N atom in the morphine-like agonists is substituted with a methyl group (as in *morphine*); replacement of this with an allyl or cyclopropyl methyl group lowers efficacy (as with *buprenorphine*) and provides the antagonists *naloxone* and *naltrexone*.

In addition, structurally distinct, fully synthetic opioid analgesics have been developed to attain the holy grail of an opioid analgesic without respiratory depression or addiction liability (Figure 23–10). These include simplified versions of the *morphine* structure such as the morphinans (e.g., *levorphanol*) and benzomorphans (e.g., *pentazocine*), as well as the *methadone* and the *fentanyl* family of compounds, which lack a phenolic hydroxyl group. Despite these structural differences, all opioid analgesics bind to the same orthosteric site on the mu-opioid receptor as *morphine* and so have very similar pharmacology. On the other hand, structural modifications such as those described above can provide opioid drugs with different affinities for the various types of receptors, agonist versus antagonist activity, and altered distribution, metabolism, and pharmacokinetic profiles.

Morphine

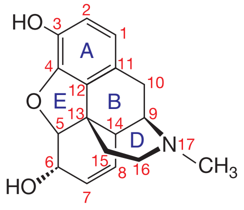
ADME

Absorption. In general opioid drugs are moderately well absorbed from the GI tract; absorption through the rectal mucosa also occurs, and a few opioid analgesics (e.g., *morphine, hydromorphone*) are available in suppositories. The more lipophilic opioids are absorbed readily through the nasal or buccal mucosa. Those with the greatest lipid solubility also can be absorbed transdermally, for example *fentanyl* and *buprenorphine* are available as patches. Opioids, particularly *morphine*, have been widely used for spinal delivery to produce direct analgesia at this site. These agents display useful transdural movement adequate to permit their use epidurally.

With many opioids, *morphine* being a prime example, the effect of a given dose is less after oral administration than after parenteral administration. This is due to a significant degree and variability of first-pass metabolism in the liver, particularly glucuronidation of the hydroxyl groups. For example, the bioavailability of oral preparations of *morphine* is only about 25%, but if this is taken into consideration together with the clearance rate, adequate relief of pain can be achieved with oral administration of *morphine*. Satisfactory analgesia in patients with cancer is associated with a broad range of steady-state concentrations of *morphine* in plasma (16–364 ng/mL) (Neumann et al., 1982). *Codeine*, in which the 3-hydroxy group is protected by O-methylation, has a much better oral bioavailability, although as mentioned above, it has to be converted to *morphine in vivo* to be effective as an analgesic. The duration of action is often longer with drugs given by the oral route.

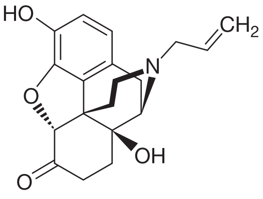
When *morphine* and most other opioids are given intravenously, they act promptly. However, after subcutaneous administration, the more lipid-soluble opioids act more rapidly than *morphine* because of differences in the rates of absorption and entry into the CNS. Overall, compared with more lipid-soluble opioids such as *codeine*, *heroin*, *methadone*, and *fentanyl*, *morphine* crosses the blood-brain barrier at a considerably lower rate.

Distribution and Metabolism. After a therapeutic dose, approximately one-third of *morphine* in the plasma is protein bound. *Morphine* itself does not persist in tissues, and 24 h after the last dose, tissue concentrations are low. The major pathway for the metabolism of *morphine* and related drugs is conjugation with glucuronic acid to give

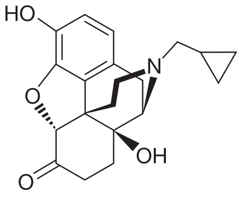


Morphine

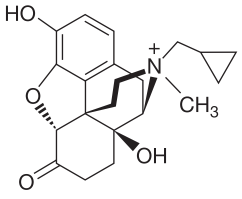
Nonproprietary name	Chemical radicals and position^a			Other changes†
	3	6	17	
Morphine	—OH	—OH	—CH$_3$	—
Heroin	—OCOCH$_3$	—OCOCH$_3$	—CH$_3$	—
Hydromorphone	—OH	=O	—CH$_3$	(1)
Oxymorphone	—OH	=O	—CH$_3$	(1), (2)
Levorphanol	—OH	—H	—CH$_3$	(1), (3)
Levallorphan	—OH	—H	—CH$_2$CH=CH$_2$	(1), (3)
Codeine	—OCH$_3$	—OH	—CH$_3$	—
Hydrocodone	—OCH$_3$	=O	—CH$_3$	(1)
Oxycodone	—OCH$_3$	=O	—CH$_3$	(1), (2)
Nalmefene	—OH	=CH$_2$	—CH$_2$—◁	(1), (2)
Nalorphine	—OH	—OH	—CH$_2$CH=CH$_2$	—
Naloxone	—OH	=O	—CH$_2$CH=CH$_2$	(1), (2)
Naltrexone	—OH	=O	—CH$_2$—◁	(1), (2)
Buprenorphine	—OH	—OCH$_3$	—CH$_2$—◁	(1), (4)
Butorphanol	—OH	—H	—CH$_2$—◇	(1), (2), (3)
Nalbuphine	—OH	—OH	—CH$_2$—◇	(1), (2)
Methylnaltrexone	—OH	=O	| —(N)—CH$_2$—◁ CH$_3$	(1), (2)



Naloxone



Naltrexone



Methylnaltrexone

^{a}The numbers 3, 6, and 17 refer to positions in the morphine molecule, as shown above. †Other changes in the morphine molecule are (1) Single instead of double bond between C7 and C8; (2) OH added to C14; (3) No oxygen between C4 and C5; (4) *Endo*etheno bridge between C6 and C14; 1-hydroxy-1,2,2-trimethylpropyl substitution on C7.

Figure 23–9 *Structures of morphine-related opioid agonists and antagonists.*

morphine-6-glucuronide and morphine-3-glucuronide; small amounts of morphine-3,6-diglucuronide are formed. N-demethylation of *morphine* to normorphine is a minor metabolic pathway. Although meant as a process for excretion, the more polar 3- and 6-glucuronides can cross the blood-brain barrier to exert significant clinical effects (Christrup, 1997). Indeed, morphine-6-glucuronide has pharmacological actions indistinguishable from those of *morphine* and if given systemically is reported to be approximately twice as potent as *morphine* in animal models and in humans (Osborne et al., 1992), although it has been reported that Chinese populations may be less sensitive to *morphine* as a result of decreased production of morphine-6-glucuronide (Caraco et al., 1999). In patients undergoing treatment with chronic oral *morphine*, plasma levels of morphine-6-glucuronide exceed those of *morphine* such that the 6-glucuronide accounts for a significant portion of analgesia and most of *morphine*'s effects. Morphine-6-glucuronide is excreted by the kidney, and in renal failure, the levels of this metabolite can accumulate, possibly explaining *morphine*'s potency and long duration in patients with compromised renal function.

In adults, the $t_{1/2}$ of *morphine* is about 2–3 h; the $t_{1/2}$ of morphine-6-glucuronide is somewhat longer. Children achieve adult renal function values by 6 months of age. In elderly patients, lower doses of *morphine* are recommended based on a smaller volume of distribution and the general decline in renal function (Owens et al., 1983). In contrast to the 6-glucuronide derivative, morphine-3-glucuronide, another important metabolite, has very-low-affinity opioid receptors, but it has been suggested this may contribute to the excitatory effects of *morphine* (Smith, 2000).

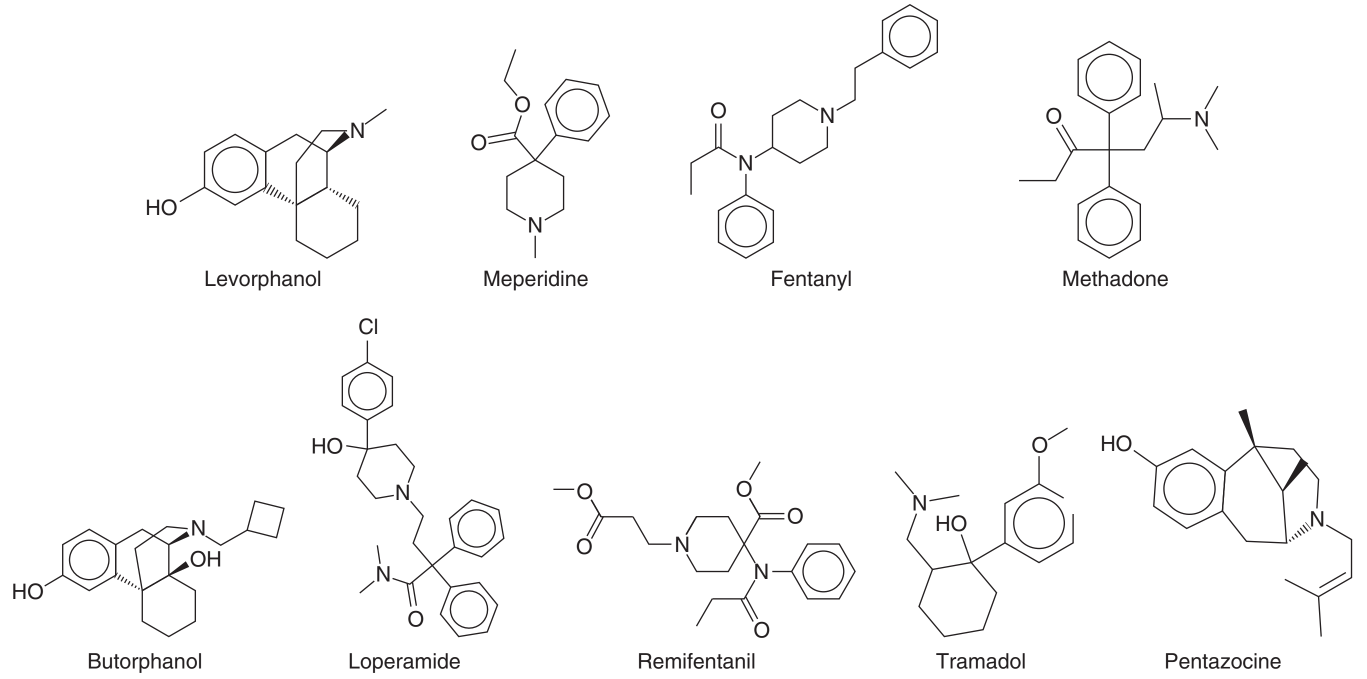


Figure 23–10 *Structures of opioid analgesics structurally different from morphine.*

Excretion. *Morphine* is eliminated by glomerular filtration, primarily as morphine-3-glucuronide; 90% of the total excretion of *morphine* takes place the first day after administration. Very little *morphine* is excreted unchanged. Enterohepatic circulation of *morphine* and its glucuronides accounts for the presence of small amounts of *morphine* in feces and urine for several days after the last dose.

Codeine

The half-life of *codeine* in plasma is 2 to 4 h. Metabolites of *codeine* are excreted chiefly as inactive forms in the urine. However, *codeine* in which the 3-hydroxy group is protected by O-methylation has a much better oral bioavailability (60%) due to lower first-pass metabolism, although as mentioned above, it has to be converted to *morphine in vivo* to be effective as an analgesic since the 3-methoxy group drastically reduces affinity for the mu-opioid receptor (see receptor structure above). Approximately 10% of administered *codeine* is O-demethylated to *morphine*, and so free and conjugated *morphine* can be found in the urine after therapeutic doses of *codeine*. The conversion of *codeine* to *morphine* is carried out by the enzyme CYP2D6. There are many known different polymorphisms of CYP2D6, resulting in poor, intermediate, and ultra-rapid metabolizers (Ingelman-Sunberg, 2004). Moreover, the frequency of variants differs across ethnic groups. This means caution is required in the use of *codeine* since an inability to convert *codeine* to *morphine* makes *codeine* ineffective as an analgesic for about 10% of the Caucasian population (Eichelbaum and Evert, 1996). Conversly, ultra-rapid metabolism can lead to problems due to higher than predicted serum *morphine* levels. In breastfeeding mothers, the ultra-rapid conversion of *codeine* can result in unsafe levels of *morphine* in breast milk, resulting in serious adverse effects in breastfed infants that can lead to death.

It is therefore vitally important to consider the possibility of metabolic enzyme polymorphism in any patient who experiences toxicity or does not receive adequate analgesia from *codeine* or other opioid prodrugs (e.g., *hydrocodone*, *oxycodone*, and *tramadol*) (Johansson and Ingelman-Sundberg, 2011).

Heroin

Heroin (diacetylmorphine) is rapidly hydrolyzed to 6-mono-acetylmorphine (6-MAM), which in turn is hydrolyzed to *morphine*. *Heroin* and 6-MAM are more lipid soluble than *morphine* and so enter the brain quicker. Since *heroin* binds very poorly to the mu-opioid receptor due to lack of a free 3-hydroxyl group, *morphine* and 6-MAM are responsible for the pharmacological actions of *heroin*, making *heroin* a prodrug. *Heroin* is excreted mainly in the urine, largely as free and conjugated *morphine* (Rook et al., 2006).

Hydromorphone

Hydromorphone is a semisynthetic derivative of *morphine* and displays all of the same actions. It is more lipid soluble than *morphine*, resulting in an increased onset of action, and is several times more potent. The drug is formulated in parenteral, rectal, subcutaneous, and oral preparations and as a nebulized formulation and is given off-label by epidural or intrathecal routes. *Hydromorphone* is metabolized in the liver to hydromorphone-3-glucoronide.

Oxymorphone, Oxycodone, and Hydrocodone

Oxymorphone is a potent mu-opioid receptor agonist with an onset of analgesia after parenteral dosing of about 5 to 10 min and a duration of action of 3 to 4 h. *Oxymorphone* is extensively metabolized in the liver and excreted as the 3- and 6-glucuronides. It is approximately equipotent to *oxycodone*, with an onset of action of 10 to 30 min and duration of 4 to 6 h. Hepatic CYPs 2D6 and 3A4 convert *hydrocodone* to hydromorphone and norhydrocodone, respectively. *Hydrocodone* has a serum half-life of about 4 h.

Oxycodone is available as immediate-release formulations with or without nonsteroidal anti-inflammatory drugs (NSAIDs) and as controlled-release preparations. Parenteral formulations are available in the U.K. for intravenous or intramuscular administration. *Oxycodone* undergoes hepatic metabolism to produce the more potent oxymorphone. *Oxycodone* is one of the most commonly abused opioid drugs.

Hydrocodone is used orally for relief of moderate-to-severe pain and is employed in a liquid formulation as a cough suppressant. It is approximately equipotent to *oxycodone*, with an onset of action of 10 to 30 min and duration of 4 to 6 h. Hepatic CYPs 2D6 and 3A4 convert *hydrocodone* to hydromorphone and norhydrocodone, respectively. *Hydrocodone* has a serum half-life of about 4 h.

Morphinans

Levorphanol

This drug is available for intravenous, intramuscular, and oral administration. The pharmacological effects of *levorphanol* closely parallel those of *morphine*. Compared to *morphine*, this agent is about seven times more potent and may produce less nausea and vomiting. *Levorphanol* is metabolized less rapidly than *morphine* and has a $t_{1/2}$ of 12 to 16 h; repeated administration at short intervals may thus lead to accumulation of the drug in plasma (Prommer, 2014). The D-isomer (dextrorphan) is devoid of analgesic action but has inhibitory effects at NMDA receptors.

Piperidine and Phenylpiperidine Analgesics

Meperidine

CNS Actions. *Meperidine* produces a pattern of effects similar but not identical to those already described for *morphine* (Latta et al., 2002), including analgesia and respiratory depression, leading to accumulation of CO$_2$, which in turn leads to cerebrovascular dilation, increased cerebral blood flow, and elevation of CSF pressure.

Meperidine can sometimes cause CNS excitation, characterized by tremors, muscle twitches, and seizures. These effects are due largely to accumulation of a metabolite, normeperidine. Meperidine has local anesthetic properties, particularly noted after epidural administration.

Cardiovascular Effects. The effects of *meperidine* on the cardiovascular system generally resemble those of *morphine*. Intramuscular administration of therapeutic doses of *meperidine* does not affect heart rate significantly, but intravenous administration frequently produces a marked increase in heart rate. Meperidine is a strong releaser of histamine.

Actions on Smooth Muscle, GI Tract, and Uterus. *Meperidine* does not cause as much constipation as *morphine*, even when given over prolonged periods; this may be related to its greater ability to enter the CNS, thereby producing analgesia at lower systemic concentrations. As with other opioids, clinical doses of *meperidine* slow gastric emptying sufficiently to delay absorption of other drugs significantly. The uterine muscles of a nonpregnant woman usually are mildly stimulated by *meperidine*. Administered before an oxytocic, *meperidine* does not exert any antagonistic effect. Therapeutic doses given during active labor do not delay the birth process; in fact, frequency, duration, and amplitude of uterine contractions may be increased.

ADME. *Meperidine* is absorbed by all routes of administration. The peak plasma concentration usually occurs at about 45 min, but the range is wide. After oral administration, only about 50% of the drug escapes first-pass metabolism to enter the circulation, and peak concentrations in plasma occur in 1 to 2 h. *Meperidine* is metabolized chiefly in the liver, with a $t_{1/2}$ of about 3 h. Metabolites are the *N*-demethyl product, normeperidine, and the hydrolysis product, meperidinate, both of which may be conjugated before excretion. In patients with cirrhosis, the bioavailability of *meperidine* is increased to as much as 80%, and the $t_{1/2}$ of both *meperidine* and the metabolite normeperidine ($t_{1/2}$ ~15–20 h) are prolonged. Only a small amount of *meperidine* is excreted unchanged.

Therapeutic Use. The major use of *meperidine* is for analgesia. The analgesic effects of *meperidine* are detectable about 15 min after oral administration, peak in 1 to 2 h, and subside gradually. The onset of analgesic effect is faster (within 10 min) after subcutaneous or intramuscular administration, and the effect reaches a peak in about 1 h, corresponding closely to peak concentrations in plasma. In clinical use, the duration of effective analgesia is about 1.5 to 3 h. Peak respiratory depression is observed within 1 h of intramuscular administration, and there is a return toward normal starting at about 2 h. In general, 75 to 100 mg *meperidine hydrochloride* given parenterally is approximately equivalent to 10 mg *morphine*. In terms of total analgesic effect, *meperidine* is about one-third as effective when given orally as when administered parenterally.

Single doses of *meperidine* can be effective in the treatment of postanesthetic shivering. *Meperidine*, 25 to 50 mg, is used frequently with antihistamines, corticosteroids, *acetaminophen*, or NSAIDs to prevent or ameliorate infusion-related rigors and shaking chills that accompany the intravenous administration of agents such as *amphotericin B*, *interleukin-2*, *trastuzumab*, and *alemtuzumab*.

Meperidine crosses the placental barrier and, even in reasonable analgesic doses, causes a significant increase in the percentage of babies who show delayed respiration, decreased respiratory minute volume, or decreased O$_2$ saturation or who require resuscitation. Fetal and maternal respiratory depression induced by *meperidine* can be treated with *naloxone*. *Meperidine* produces less respiratory depression in the newborn than does an equianalgesic dose of *morphine* or *methadone* (Fishburne, 1982).

Untoward Effects, Precautions, and Contraindications. The overall incidence of untoward effects is similar to that observed after equianalgesic doses of *morphine*, although constipation, urinary retention, and nausea may be less common. Patients who experience nausea and vomiting with *morphine* may not do so with *meperidine*; the converse also may be true. In patients or individuals who are tolerant to the depressant effects of *meperidine*, large doses repeated at short intervals may produce an excitatory syndrome that includes hallucinations, tremors, muscle twitches, dilated pupils, hyperactive reflexes, and convulsions. These excitatory symptoms are due to the accumulation of the long-lived metabolite normeperidine, which has a $t_{1/2}$ of 15 to 20 h, compared to 3 h for *meperidine*. Decreased renal or hepatic function increases the likelihood of toxicity. As a result of these properties, *meperidine* is not recommended for the treatment of chronic pain because of concerns over the toxicity of its metabolite. It should not be used for longer than 48 h or in doses greater than 600 mg/day.

Interactions With Other Drugs. Severe reactions may follow the administration of *meperidine* to patients being treated with monoamine oxidase (MAO) inhibitors. There are two basic types of interaction. The more prominent is an excitatory reaction ("serotonin syndrome") with delirium, hyperthermia, headache, hyper- or hypotension, rigidity, convulsions, coma, and death. This reaction may be due to the capacity of *meperidine* to block neuronal reuptake of serotonin, resulting in serotonergic overactivity. In the second type of interaction, several MAO inhibitors are substrates or inhibitors of hepatic CYPs and reduce *meperidine* metabolism, creating a condition resembling acute narcotic overdose. *Meperidine* and its congeners are contraindicated in patients taking MAO inhibitors or within 14 days after discontinuation of an MAO inhibitor.

Chlorpromazine increases the respiratory-depressant effects of *meperidine*, as do tricyclic antidepressants (but not *diazepam*). Concurrent administration of drugs such as *promethazine* or *chlorpromazine* also may greatly enhance *meperidine*-induced sedation without slowing clearance of the drug. Treatment with *phenobarbital* or *phenytoin* increases systemic clearance and decreases oral bioavailability of *meperidine*. As with *morphine*, concomitant administration of amphetamine has been reported to enhance the analgesic effects of *meperidine* and its congeners while counteracting sedation.

Diphenoxylate

Diphenoxylate is a *meperidine* congener that has a constipating effect in humans. Its only approved use is for the treatment of diarrhea, in combination with *atropine sulfate*. *Diphenoxylate* is unusual in that even its salts are virtually insoluble in aqueous solution, thus reducing the probability of abuse by the parenteral route. The recommended daily dosage of *diphenoxylate* for the treatment of diarrhea in adults is 20 mg in divided doses. *Difenoxin*, the main metabolite of *diphenoxylate*, is also marketed in a fixed dose with *atropine* for the management of diarrhea.

Loperamide

Like *diphenoxylate*, this agent slows GI motility by effects on the circular and longitudinal muscles of the intestine (Kromer, 1988). Part of its antidiarrheal effect may be due to a reduction of GI secretory processes (see Chapter 50). In controlling chronic diarrhea, *loperamide* is as effective as *diphenoxylate* and little tolerance develops to its constipating effect. Concentrations of drug in plasma peak about 4 h after ingestion. The apparent elimination $t_{1/2}$ is 7 to 14 h. *Loperamide* is poorly absorbed after oral administration and, in addition, does not penetrate well into the brain due to the exporting activity of P-glycoprotein, which is widely expressed in the brain endothelium. The usual dosage is 4 to 8 mg/day; the daily dose should not exceed 16 mg (Regnard et al., 2011). The most common side effect is abdominal cramps. *Loperamide* is unlikely to be abused parenterally because of its low solubility; large doses of *loperamide* given to human volunteers do not elicit pleasurable effects typical of opioids.

Fentanyl and Its Analogues

The beneficial and unwanted effects of *fentanyl* and its various analogues, such as *alfentanil*, *sufentanil*, and *remifentanil*, are similar to those of other mu-opioid receptor agonists. Differences between this class of drugs and other mu-opioids are highlighted below. In addition, the rapid pharmacokinetics of the compounds distinguishes them from other mu-opioid agonists and makes them important in anesthetic practice. As described below, they have a relatively short time to peak analgesic effect, rapid termination of effect after small bolus doses, cardiovascular safety, and capacity to significantly reduce the dosing requirement for the volatile anesthetics.

Pharmacology of Fentanyl-Like Compounds

Abuse Liability. *Fentanyl* and its derivatives have a high potential for misuse, and many illicit derivatives of *fentanyl* have found their way onto the street and in large part have fueled the opioid crisis and the high death rate from opioid overdose. Moreover, self-administration by chewing of *fentanyl* patches can be deadly. Practitioners must be aware of this potential and, as with all controlled substances, keep careful control of *fentanyl* stocks. Opioid use disorder is discussed later in the chapter.

Analgesia. *Fentanyl* and its congeners are extremely potent analgesics and typically exhibit a very short duration of action when given parenterally.

Respiratory Depression. Respiratory depression is similar to that observed with *morphine*, but onset is more rapid and of shorter duration that *morphine* but extended after large doses or long infusions. Delayed respiratory depression also can be seen after the use of *fentanyl* or *sufentanil*, possibly due to enterohepatic circulation. Life-threatening wooden chest syndrome caused by rigidity in the diaphragm, chest wall, and upper airways is common after the high doses of *fentanyl* and analogues used in anesthetic induction. Rigidity can be treated with depolarizing or nondepolarizing neuromuscular-blocking agents while controlling the patient's ventilation, but care must be taken to make sure that the patient is not simply immobilized and aware.

Cardiovascular System. *Fentanyl* and its derivatives decrease heart rate through vagal activation and may modestly decrease blood pressure. However, because the drugs do not release histamine directly, depressant effects on the myocardium are minimal. For this reason, high doses of *fentanyl* or *sufentanil* are commonly used as the primary anesthetic for patients undergoing cardiovascular surgery or for patients with poor cardiac function.

ADME

The fentanyl-like drugs are highly lipid soluble and rapidly cross the blood-brain barrier. This is reflected in the $t_{1/2}$ for equilibration between the plasma and CSF of about 5 min for *fentanyl* and *sufentanil*. As *fentanyl* is poorly absorbed from the GI tract, the optimal absorption is through buccal administration. The levels in plasma and CSF decline rapidly owing to redistribution of *fentanyl* from highly perfused tissue groups to other tissues, such as muscle and fat. As saturation of less well-perfused tissue occurs, the duration of effect of *fentanyl* and *sufentanil* approaches the length of their elimination $t_{1/2}$, 3 to 4 h. *Fentanyl* and *sufentanil* undergo hepatic metabolism and renal excretion. With the use of higher doses or prolonged infusions, the drugs accumulate, these clearance mechanisms become progressively saturated, and *fentanyl* and *sufentanil* become longer acting. *Remifentanil* is very rapidly metabolized by esterases in plasma and is discussed separately below.

Therapeutic Uses

Fentanyl and *sufentanil* have wide popularity as anesthetic adjuvants (see Chapter 24), administered intravenously and epidurally. After systemic delivery, *fentanyl* is about 100 times more potent than *morphine*; *sufentanil* is about 1000 times more potent than *morphine*. The time to peak analgesic effect after intravenous administration of *fentanyl* and *sufentanil* (~5 min) is notably less than that for *morphine* and *meperidine* (~15 min). Recovery from analgesic effects also occurs more quickly. However, with larger doses or prolonged infusions, the duration of action is similar to that of longer-acting opioids.

The use of *fentanyl* in chronic pain treatment has become widespread. Transdermal patches that provide sustained release of *fentanyl* for 48 to 72 h are available. However, factors promoting increased absorption (e.g., fever) can lead to relative overdosage and increased side effects. Transbuccal absorption from buccal tablets or lollipop-like lozenges permits rapid absorption and has found use in the management of acute incident pain and for the relief of breakthrough cancer pain. *Fentanyl* should only be used in opioid-tolerant patients, defined as consuming more than 60 mg of oral *morphine* equivalent.

Epidural use of *fentanyl* and *sufentanil* for postoperative or labor analgesia is popular. A combination of epidural opioids with local anesthetics permits reduction in the dosage of both components.

Remifentanil

Remifentanil has a more rapid onset of analgesic action than *fentanyl* or *sufentanil*. Analgesic effects occur within 1 to 1.5 min following intravenous administration. Peak respiratory depression after bolus doses of *remifentanil* occurs after 5 min. *Remifentanil* is metabolized by plasma esterases, with a $t_{1/2}$ of 8 to 20 min; thus, elimination is independent of hepatic metabolism or renal excretion. Age and weight can affect clearance of *remifentanil*. After 3- to 5-h infusions of *remifentanil*, recovery of respiratory function can be seen within 3 to 5 min; full recovery from all effects of *remifentanil* occurs within 15 min. The primary metabolite, remifentanil acid, has 0.05% to 0.025% of the potency of the parent compound and is excreted renally.

Remifentanil hydrochloride is useful for short, painful procedures that require intense analgesia and blunting of stress responses; the drug is routinely given by continuous intravenous infusion because of its short duration of action, which allows for easy management of drug levels. When postprocedural analgesia is required, *remifentanil* alone is a poor choice. In this situation, either a longer-acting opioid or another analgesic modality should be combined with *remifentanil* for prolonged analgesia, or another opioid should be used. *Remifentanil* is not used intraspinally (epidural or intrathecal administration) because of its formulation with glycine, an inhibitory neurotransmitter in the dorsal horn of the spinal cord (Stroumpos et al., 2010).

Methadone

Methadone is a long-acting mu-opioid receptor agonist with pharmacological properties qualitatively similar to those of *morphine*. *Methadone* has a chiral center and is used clinically as the racemate, although the analgesic activity is almost entirely the result of its content of l-methadone, which is 8 to 50 times more potent than the d-isomer. d-Methadone binds very poorly to the mu-opioid receptor and so also lacks significant respiratory depressant action and addiction liability, although it does possesses antitussive activity (Fredheim et al., 2008).

Propoxyphene is a methadone analogue that was used to treat mild-to-moderate pain. As mentioned earlier, the U.S. FDA recommended removal of the drug (trade name: Darvon) from the U.S. market in 2010 due to reports of cardiac toxicity.

Pharmacological Effects

Important properties of *methadone* are its analgesic activity, its efficacy by the oral route, its extended duration of action in suppressing withdrawal symptoms in physically dependent individuals, and its tendency to show persistent effects with repeated administration. Miotic and respiratory-depressant effects can be detected for more than 24 h after a single dose; on repeated administration, marked sedation is seen in some patients. Effects on cough, bowel motility, biliary tone, and the secretion of pituitary hormones are qualitatively similar to those of *morphine*.

ADME

Methadone is absorbed well from the GI tract and can be detected in plasma within 30 min of oral ingestion; it reaches peak concentrations at about 4 h. Peak concentrations occur in brain within 1 to 2 h of subcutaneous or intramuscular administration, and this correlates well with the intensity and duration of analgesia. *Methadone* also can be absorbed from the buccal mucosa. *Methadone* undergoes extensive biotransformation in the liver. The major metabolites are pyrrolidine and pyrroline derivatives that result from N-demethylation and cyclization and are excreted in the urine and the bile along with small amounts of unchanged drug. The amount of *methadone* excreted in the urine is increased when the urine is acidified. The $t_{1/2}$ of *methadone* is long, 15 to 40 h. *Methadone* appears to be firmly bound to protein in various tissues, including brain. After repeated administration, there is gradual accumulation in tissues. When administration is discontinued, low concentrations are maintained in plasma by slow release from extravascular binding sites. This process may explain why withdrawal symptoms following *methadone* are less severe than with *morphine* but last longer, resulting in a relatively mild but protracted withdrawal syndrome.

Therapeutic Uses

Although an effective analgesic, the primary use of *methadone hydrochloride* is detoxification and maintenance therapy treatment for opioid use disorder. Because it is a full agonist with all the properties of *morphine*, this takes place within certified treatment programs. Outside treatment programs, *methadone* is used for the management of chronic pain. The onset of analgesia occurs 10 to 20 min after parenteral administration and 30 to 60 min after oral medication. The typical oral dose is 2.5 to 10 mg repeated every 8 to 12 h as needed depending on the severity of the pain and the response of the patient. Care must be taken when increasing the dosage because of the prolonged $t_{1/2}$ of the drug and its tendency to accumulate over a period of several days with repeated dosing. The peak respiratory-depressant effects of *methadone* typically occur later and persist longer than peak analgesia, so it is necessary to exercise vigilance and strongly caution patients against self-medicating with CNS depressants, particularly during treatment initiation and dose titration. *Methadone* should not be used in labor. Despite its longer plasma $t_{1/2}$, the duration of the analgesic action of single doses is essentially the same as that of *morphine*. With repeated use, cumulative effects are seen, so either lower dosages or longer intervals between doses become possible. *Methadone*, like other opioids, will produce tolerance and dependence. Development of physical dependence during the long-term administration of *methadone* can be demonstrated following abrupt drug withdrawal or by administration of an opioid antagonist. Likewise, subcutaneous administration of *methadone* to those with an opioid use disorder produces euphoria equal in duration to that caused by *morphine*, and its overall abuse potential is comparable with that of *morphine*.

Adverse Effects

Side effects are similar to those described for *morphine*. *Rifampin* and *phenytoin* accelerate the metabolism of *methadone* and can precipitate withdrawal symptoms. Unlike other opioids, *methadone* is associated with the prolonged QT syndrome and is additive with agents known to prolong the QT interval.

Partial Agonists

These compounds exhibit clinically useful analgesia but with less respiratory depression and addictive potential. The compounds are generally not selective for the mu-opioid receptor over delta- or kappa-opioid receptors as compared with high-efficacy mu-opioid receptor agonists.

Buprenorphine

Pharmacology. *Buprenorphine* is a highly lipophilic mu-opioid agonist that is 25 to 50 times more potent than *morphine* but less efficacious. The pharmacology of *buprenorphine* is qualitatively similar to *morphine*. Administered sublingually, *buprenorphine* (0.4–0.8 mg) produces satisfactory analgesia in postoperative patients. The main therapeutic use of *buprenorphine* is as for the management of opioid use disorder. *Buprenorphine* also functions as a delta- and kappa-opioid antagonist and a weak partial agonist at NOPr.

ADME. Concentrations of *buprenorphine* in blood peak within 5 min of intramuscular injection and within 1 to 2 h of oral or sublingual administration. The plasma $t_{1/2}$ is about 3 h. However, the $t_{1/2}$ for dissociation of *buprenorphine* from the mu receptor is approximately 170 min, as opposed to 7 min for *fentanyl*, so plasma levels of *buprenorphine* may not parallel clinical effects.

Buprenorphine is metabolized to norbuprenorphine by CYP3A4 and should not be taken with known inhibitors of CYP3A4 (e.g., azole antifungals, macrolide antibiotics, and HIV protease inhibitors) or drugs that induce CYP3A4 activity (e.g., certain anticonvulsants and *rifampin*). Both N-dealkylated and conjugated metabolites are detected in the urine, but most of the drug is excreted unchanged in the feces. When *buprenorphine* is discontinued, a withdrawal syndrome develops that is delayed in onset for 2 to 14 days and persists for 1 to 2 weeks.

Therapeutic Uses

Analgesia. *Buprenorphine*, formulated as an injection, sublingual tablets, or skin patch, is used as an analgesic. About 0.3 mg IM *buprenorphine* is equianalgesic to 10 mg IM *morphine*. Some of the subjective and respiratory-depressant effects are unequivocally slower in onset and last longer than those of *morphine*. The respiratory depression and other effects of *buprenorphine* can be prevented by prior administration of *naloxone*, but they are not readily reversed by *naloxone* once the effects have been produced, probably due to the high affinity of buprenorphine and its slow dissociation from mu-opioid receptors. As a partial agonist, *buprenorphine* antagonizes the respiratory depression produced by anesthetic doses of *fentanyl* without completely reversing opioid pain relief. *Buprenorphine* can also precipitate withdrawal in patients who have been receiving higher efficacy mu-opioid agonists for several weeks.

Opioid Use Disorder. Due to its extended length of action at the mu-opioid receptor and its partial mu-opioid agonist activity, *buprenorphine* is used for the management of patients suffering from opioid use disorder (Greenwald et al., 2014). The drug controls opioid craving and also serves to block the actions of higher-efficacy opioids the addicted patient might take. For this use, *buprenorphine* is administered in a fixed-dose combination with *naloxone* to prevent misuse and diversion but may be given alone to pregnant women or patients with *naloxone* allergy. Induction doses start at 2 to 4 mg daily and stabilize at approximately 12 to 16 mg/day. Because *buprenorphine* can precipitate withdrawal in individuals with an opioid use disorder, detoxification is needed before starting treatment. The complex pharmacology of *buprenorphine* with actions at mu-, delta-, and kappa-opioid receptors and nociceptin receptors may contribute to its effectiveness for the management of opioid dependence. Because it is safer than *methadone*, U.S. physicians can treat up to 275 patients with the *buprenorphine-naloxone* combination in an outpatient setting after completing a short training course.

Pentazocine

Pentazocine was synthesized in an effort to develop an effective analgesic with little or no abuse potential.

Pentazocine is a partial agonist at both mu- and kappa-opioid receptors. The pattern of CNS effects produced by *pentazocine* is similar to those of the morphine-like opioids, including analgesia, sedation, and respiratory depression. An oral dose of about 50 mg *pentazocine* results in analgesia equivalent to that produced by a 60-mg oral dose of *codeine*. Due to its partial agonist nature, ceiling effects for analgesia and respiratory depression are observed at doses above 50 to 100 mg of *pentazocine*. However, the analgesic actions of the drug are thought to be mediated by its kappa-opioid receptor action. Consistent with kappa-opioid receptor activation, high doses of *pentazocine* (60–90 mg) elicit dysphoric and psychotomimetic effects. The cardiovascular responses to *pentazocine* differ from those seen with typical mu-opioid receptor agonists in that high doses cause an increase in blood pressure and heart rate. *Pentazocine* does not antagonize the respiratory depression produced by *morphine*. However, when given to patients who are dependent on *morphine* or other mu-opioid receptor agonists, *pentazocine* may precipitate withdrawal.

Therapeutic Use. *Pentazocine lactate* injection is indicated for the relief of mild-to-moderate pain and is also used as a preoperative medication

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and as a supplement to anesthesia. *Pentazocine* tablets for oral use are only available in fixed-dose combinations with *acetaminophen* or *naloxone*. Combination of *pentazocine* with *naloxone* reduces the potential misuse of tablets as a source of injectable *pentazocine*.

Nalbuphine

Like *pentazocine, nalbuphine* is a partial agonist at kappa- and mu-opioid receptors with effects that qualitatively resemble those of *pentazocine*; however, *nalbuphine* produces fewer dysphoric side effects than *pentazocine* (Schmidt et al., 1985).

An intramuscular dose of 10 mg *nalbuphine* is equianalgesic to 10 mg *morphine*, with similar onset and duration of analgesic and subjective effects. *Nalbuphine* depresses respiration as much as equianalgesic doses of *morphine*; however, like *pentazocine*, *nalbuphine* exhibits a ceiling effect such that increases in dosage beyond 30 mg produce no further respiratory depression or analgesia. In contrast to *pentazocine* and *butorphanol* (see below), 10 mg *nalbuphine* given to patients with stable coronary artery disease does not produce an increase in cardiac index, pulmonary arterial pressure, or cardiac work, and systemic blood pressure is not significantly altered; these indices also are relatively stable when *nalbuphine* is given to patients with acute myocardial infarction. *Nalbuphine* produces few side effects at doses of 10 mg or less; sedation, sweating, and headache are the most common. At much higher doses (70 mg), psychotomimetic side effects (e.g., dysphoria, racing thoughts, and distortions of body image) can occur. *Nalbuphine* is metabolized in the liver and has a plasma $t_{1/2}$ of 2 to 3 h. *Nalbuphine* is 20% to 25% as potent when administered orally as when given intramuscularly. Prolonged administration of *nalbuphine* can produce physical dependence. The withdrawal syndrome is similar in intensity to that seen with *pentazocine*.

Therapeutic Use. *Nalbuphine* is used to produce analgesia. Because it is a partial agonist, administration to patients who have been receiving morphine-like opioids may create difficulties unless a brief drug-free interval is interposed.

Butorphanol

Butorphanol has a profile of actions similar to those of *pentazocine* and *nalbuphine*.

In postoperative patients, a parenteral dose of 2 to 3 mg *butorphanol* produces analgesia and respiratory depression approximately equal to that produced by 10 mg *morphine* or 80 to 100 mg *meperidine*. The plasma $t_{1/2}$ of *butorphanol* is about 3 h. Like *pentazocine*, analgesic doses of *butorphanol* produce an increase in pulmonary arterial pressure and in the work of the heart; systemic arterial pressure is slightly decreased. The major side effects of *butorphanol* are drowsiness, weakness, sweating, feelings of floating, and nausea. While the incidence of psychotomimetic side effects is lower than that with equianalgesic doses of *pentazocine*, they are qualitatively similar. Physical dependence can occur.

Therapeutic Use. *Butorphanol* is used for the relief of acute pain (e.g., postoperative) and, because of its potential to antagonize other mu-opioid receptor agonists, should not be used in combination with higher-efficacy agents. Because of its side effects on the heart, it is less useful than *morphine* or *meperidine* in patients with congestive heart failure or myocardial infarction. A nasal formulation is available and has proven to be effective in pain relief, including migraine pain (Gillis et al., 1995); this method of administration is associated with drowsiness and dizziness.

Other Opioid Agonists

Tramadol

Pharmacology and Therapeutic Use. The analgesic effect of *tramadol* is due to weak mu-opioid receptor activity and inhibition of uptake of NE and 5HT. The affinity of *tramadol* for the mu-opioid receptor is only 1/6000 that of *morphine*, but the O-demethylated metabolite of *tramadol* is two to four times more potent than the parent drug and may account for part of the analgesic effect. *Tramadol* is supplied as a racemate that is more effective than either enantiomer alone. The (+)-enantiomer binds with higher affinity to the mu-opioid receptor and inhibits 5HT uptake. The (−)-enantiomer is more active on NE uptake and stimulates α_2 adrenergic receptors.

In the treatment of mild-to-moderate pain, *tramadol* is as effective as *morphine*. However, for the treatment of severe or chronic pain, *tramadol* is less effective. *Tramadol* is as helpful as *meperidine* in the treatment of labor pain and may cause less neonatal respiratory depression (Grond and Sablotzki, 2004). *Tramadol* is also available as long-acting formulations and in a fixed-dose combination with *acetaminophen*.

Side effects of *tramadol* include nausea, vomiting, dizziness, dry mouth, sedation, and headache. Respiratory depression appears to be less than with equianalgesic doses of *morphine* and is reversed by *naloxone*; the degree of constipation is less than that seen after equivalent doses of *codeine*. *Tramadol* can cause seizures and possibly exacerbate seizures in patients with predisposing factors. *Tramadol* should not be used in patients taking MAO inhibitors, selective serotonin reuptake inhibitors, or other drugs that lower the seizure threshold. Precipitation of withdrawal necessitates that *tramadol* be tapered prior to discontinuation.

ADME. *Tramadol* is 68% bioavailable after a single oral dose. *Tramadol* undergoes extensive hepatic metabolism by a number of enzymes, including CYPs 2D6 and 3A4, and by conjugation with subsequent renal excretion. The elimination $t_{1/2}$ is 6 h for *tramadol* and 7.5 h for its active metabolite. Analgesia begins within an hour of oral dosing and peaks within 2 to 3 h. The duration of analgesia is about 6 h.

Tapentadol

Tapentadol is structurally and mechanistically similar to *tramadol*. It is a weak inhibitor of monoamine reuptake but has a significantly more potent activity at mu-opioid receptors. Serotonin syndrome is a risk, especially when *tapentadol* is used concomitantly with selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, tricyclic antidepressants, or MAO inhibitors that impair serotonin metabolism. *Tapentadol* is metabolized largely by glucuronidation.

Oliceridine

This is the first mu-opioid receptor biased agonist to be approved by the FDA. However, although showing bias in preclinical studies, the compound has the same pharmacological profile and contraindications as *morphine*. It is approved only for use in adults to manage severe acute pain that requires an intravenous opioid and where alternative treatments do not provide adequate pain relief.

Dosage and Routes of Opioid Analgesic Administration

Dosing information for commonly used opioid drugs is provided in Tables 23–2 and 23–3. Information on *morphine* equivalent doses for various opioid drugs is provided in Table 23–4.

In addition to the traditional oral and parenteral formulations for opioids, many other methods of administration have been developed to improve therapeutic efficacy while minimizing side effects. These include the following:

Patient-Controlled Analgesia (PCA). The patient has limited control of the dosing of opioid from an infusion pump programmed within tightly mandated parameters. This permits better alignment between pain control and individual differences in pain perception and responsiveness to opioids and gives the patient a greater sense of control over the pain.

Spinal Delivery. Administration of opioids into the epidural or intrathecal spaces provides more direct access to the first pain-processing synapse in the dorsal horn of the spinal cord. The management of chronic pain with spinal opioids has been addressed by the use of chronically implanted intrathecal catheters connected to subcutaneously implanted refillable pumps (Yaksh et al., 2017). Epidural administration of opioids is popular in the management of postoperative

TABLE 23–2 ■ DOSING DATA FOR CLINICALLY EMPLOYED OPIOID ANALGESICS

DRUG	APPROXIMATE EQUIANALGESIC ORAL DOSE	APPROXIMATE EQUIANALGESIC PARENTERAL DOSE	RECOMMENDED STARTING DOSE (Adults >50 kg)		RECOMMENDED STARTING DOSE (Children and Adults <50 kg)	
			ORAL	PARENTERAL	ORAL	PARENTERAL
Morphine	30 mg/3–4 h	10 mg/3–4 h	15 mg/3–4 h	5 mg/3–4 h	0.3 mg/kg/3–4 h	0.1 mg/kg/3–4 h
Codeine	130 mg/3–4 h	75 mg/3–4 h	30 mg/3–4 h	30 mg/2 h (IM/SC)	0.5 mg/kg/3–4 h	Not recommended
Hydromorphone	6 mg/3–4 h	1.5 mg/3–4 h	2 mg/3–4 h	0.5 mg/3–4 h	0.03 mg/kg/3–4 h	0.005 mg/kg/3–4 h
Hydrocodone (typically with acetaminophen)	30 mg/3–4 h	Not available	5 mg/3–4 h	Not available	0.1 mg/kg/3–4 h	Not available
Levorphanol	4 mg/6–8 h	2 mg/6–8 h	4 mg/6–8 h	2 mg/6–8 h	0.04 mg/kg/6–8 h	0.02 mg/kg/6–8 h
Meperidine	300 mg/2–3 h	100 mg/3 h	Not recommended	50 mg/3 h	Not recommended	0.75 mg/kg/2–3 h
Methadone	10 mg/6–8 h	10 mg/6–8 h	5 mg/12 h	Not recommended	0.1 mg/kg/12 h	Not recommended
Oxycodone	20 mg/3–4 h	Not available	5 mg/3–4 h	Not available	0.1 mg/kg/3–4 h	Not available
Oxymorphone	10 mg/3–4 h	1 mg/3–4 h	5 mg/3–4 h	1 mg/3–4 h	0.1 mg/kg/3–4 h	Not recommended
Tramadol	100 mg	100 mg	50–100 mg/6 h	50–100 mg/6 h	Not recommended	Not recommended
Fentanyl Transdermal 72-h patch (25 µg/h) = morphine 50 mg/24 h						
Buprenorphine	Not available	0.3–0.4 mg/6–8 h	Not available	0.4 mg/6–8 h	Not available	0.004 mg/kg/6–8 h
Butorphanol	Not available	2 mg/3–4 h	Not available	2 mg/3–4 h	Not available	Not recommended
Nalbuphine	Not available	10 mg/3–4 h	Not available	10 mg/3–4 h	Not available	0.1 mg/kg/3–4 h
Oliceridine	Not available	1–3mg/1–3h	Not available	1–2 mg	Not available	

These data are merely guidelines. Clinical response must be the guide for each patient, with consideration to hepatic and renal function, disease, age, concurrent medications (their effects and dose limitations [acetaminophen, 3 g/day for adults]), and other factors that could modify pharmacokinetics and drug response. Recommended start doses are approximately but not precisely equianalgesic and are driven by doses available from manufacturers. Transdermal fentanyl is contraindicated for acute pain and in patients receiving <60 mg oral morphine equivalent per day. Use Table 23–4 for converting morphine to methadone dosing.

For morphine, hydromorphone, and oxymorphone, rectal administration is an alternate route for patients unable to take oral medications, but equianalgesic doses may differ from oral and parenteral doses because of pharmacokinetic differences.

Doses listed for patients with body weight less than 50 kg cannot be used as initial starting doses in babies less than 6 months of age; consult the *Clinical Practice Guideline #1, Acute Pain Management: Operative or Medical Procedures and Trauma* (cited below), section on neonates, for recommendations.

Source: Modified and updated from Agency for Healthcare Policy and Research, 1992. Acute Pain Management Guideline Panel. AHCPR Clinical Practice Guidelines, No. 1: Acute Pain Management: Operative or Medical Procedures and Trauma. Agency for Health Care Policy and Research, Rockville, MD, **1992**.

TABLE 23–3 ■ EPIDURAL OR INTRATHECAL OPIOIDS FOR THE TREATMENT OF ACUTE (BOLUS) OR CHRONIC (INFUSION) PAIN

DRUG	SINGLE DOSE (mg)[a]	INFUSION RATE (mg/h)[b]	ONSET (min)	DURATION OF EFFECT OF SINGLE DOSE (h)[c]
Epidural				
Morphine	1–6	0.1–1.0	30	6–24
Meperidine	20–150	5–20	5	4–8
Methadone	1–10	0.3–0.5	10	6–10
Hydromorphone	1–2	0.1–0.2	15	10–16
Fentanyl	0.025–0.1	0.025–0.10	5	2–4
Sufentanil	0.01–0.06	0.01–0.05	5	2–4
Alfentanil	0.5–1	0.2	15	1–3
Subarachnoid (Intrathecal)				
Morphine	0.1–0.3		15	8–24+
Fentanyl	0.005–0.025		5	3–6

[a] Low doses may be effective when administered to the elderly or when injected in the thoracic region.

[b] If combining with a local anesthetic, consider using 0.0625% bupivacaine.

[c] Duration of analgesia varies widely; higher doses produce longer duration. With the exception of epidural/intrathecal morphine or epidural sufentanil, all other spinal opioid use is considered to be off-label.

Source: Adapted and updated from Ready LB, Edwards WT, eds. *Management of Acute Pain: A Practical Guide*. International Association for Study of Pain, Seattle, **1992**.

TABLE 23–4 ■ MORPHINE MILLIGRAM EQUIVALENT (MME) DOSES FOR COMMONLY PRESCRIBED OPIOIDS

OPIOID	CONVERSION FACTOR[a]
Codeine	0.15
Fentanyl transdermal (in μg/h)	2.4
Hydrocodone	1
Hydromorphone	4
Methadone	
1–20 mg/day	4
21–40 mg/day	8
41–60 mg/day	10
≥61–80 mg/day	12
Morphine	1
Oxycodone	1.5
Oxymorphone	3

[a] Multiply the dose for each opioid by the conversion factor to determine the dose in MMEs. For example, tablets containing hydrocodone 5 mg and acetaminophen 300 mg taken four times a day would contain a total of 20 mg of hydrocodone daily, equivalent to 20 MME daily; extended-release tablets containing oxycodone 10 mg taken twice a day would contain a total of 20 mg of oxycodone daily, equivalent to 30 MME daily. Note the following precautions: (1) All doses are in milligrams/day except for fentanyl, which is micrograms/hour. (2) Equianalgesic dose conversions are only estimates and cannot account for individual variability in genetics and pharmacokinetics. (3) Do not use the calculated dose in MMEs to determine the doses to use when converting one opioid to another; when converting opioids, the new opioid is typically dosed at substantially lower than the calculated MME dose to avoid accidental overdose due to incomplete cross-tolerance and individual variability in opioid pharmacokinetics. (4) Use particular caution with methadone dose conversions because the conversion factor increases at higher doses. (5) Use particular caution with fentanyl because it is dosed in micrograms/hour instead of milligrams/day, and its absorption is affected by heat and other factors.

Source: Dowell D, et al. CDC guideline for prescribing opioids for chronic pain—United States, 2016. *MMWR Recomm Rep* **2016**, *65*(No. RR-1):1–49. doi:http://dx.doi.org/10.15585/mmwr.rr6501e1. Accessed May 4, 2017. Adapted by the Centers for Disease Control and Prevention from Von Korff M, et al. *Clin J Pain,* **2008**, *24*:521–527 and Washington State Interagency Guideline on Prescribing Opioids for Pain (http://www.agencymeddirectors.wa.gov/Files/2015AMDGOpioidGuideline.pdf).

pain and causes less respiratory depression due to lower systemic levels. Similarly, this delivery method is popular for providing analgesia during labor and delivery because of reduced placental transfer and less potential for respiratory depression of the newborn. In addition, intraspinal narcotics often are combined with other agents that include local anesthetics, N-type Ca^{2+} channel blockers, α_2 adrenergic agonists, and $GABA_B$ agonists, allowing the use of lower concentrations of these agents (Yaksh et al., 2017). However, epidural and intrathecal opioids have their own dose-dependent side effects, such as pruritus, nausea, vomiting, respiratory depression, and urinary retention.

Rectal Administration. This is an alternative for patients with difficulty swallowing or other oral pathology and who prefer a less invasive route than parenteral administration. This route is not well tolerated by most children.

Oral Transmucosal Administration. Opioids can be absorbed through the oral mucosa more rapidly than through the stomach. Bioavailability is greater owing to avoidance of first-pass metabolism, and lipophilic opioids are absorbed better by this route than are more hydrophilic compounds such as *morphine*. For example, a variety of formulations of *fentanyl* are available for this route.

Transnasal Administration. A transnasal *fentanyl* spray is FDA approved for the treatment of breakthrough cancer pain. Administration is well tolerated, and pain relief occurs rapidly

Butorphanol has been employed intranasally. *Naloxone* is commonly given by this route for overdose rescue, as described above.

Transdermal Administration. Transdermal *fentanyl* patches are approved for use in sustained pain. This modality is well suited for cancer pain treatment because of its ease of use, prolonged duration of action, and stable blood levels. Dermatological side effects from the patches, such as rash and itching, usually are mild. Individuals with an opioid use disorder have been known to chew the patches and receive an overdose, sometimes with fatal outcomes, following rapid and efficient buccal and sublingual absorption. Fever and external heat sources (heating pads, hot baths) can increase absorption of *fentanyl* and potentially lead to an overdose.

Opioid Rotation

Changing to a different opioid when the patient fails to achieve benefit, or side effects become limiting before analgesia is sufficient, is widely employed. Failure or intolerance of one opioid cannot necessarily predict the patient's response or acceptance to another (Quang-Cantagrel et al., 2000). Practically, opioid rotation involves incrementing the dose of a given opioid agonist (e.g., *morphine*) to a level limited by side effects and insufficient analgesia and then substituting an alternate opioid medication at an equieffective dose. Care must be taken to titrate the doses and monitor the patient closely during such drug transitions.

Combination Therapy

In general, the use of combinations of drugs with the same pharmacokinetic profile is not warranted (e.g., *morphine* plus *methadone*). The same holds if the drugs have overlapping targets and opposing effects (e.g., combining an agonist with an agent with a partial agonist). On the other hand, certain opioid combinations are useful. For example, in a chronic pain state with periodic incident or breakthrough pain, the patient might receive a slow-release formulation of *morphine* for baseline pain relief, and the acute incident (breakthrough) pain may be managed with a rapid-onset/short-lasting formulation such as buccal *fentanyl*. For inflammatory or nociceptive pain, opioids may be usefully combined with other analgesic agents, such as *acetaminophen* or other NSAIDs. In the case of neuropathic pain, other drug classes may be useful alone or in combination with an opioid. For example, antidepressants that block amine reuptake, such as *amitriptyline* or *duloxetine*, and anticonvulsants such as *gabapentin* may enhance the analgesic effect and may be synergistic in some pain states.

Opioid Antagonists

A variety of agents bind competitively to one or more of the opioid receptors, display little or no intrinsic activity, and robustly antagonize the effects of receptor agonists. Relatively minor changes in the structure of an opioid ligand can convert an agonist into an antagonist at one or more types of opioid receptors. Simple substitutions transform *morphine* to *nalorphine*, *levorphanol* to *levallorphan*, and *oxymorphone* to *naloxone* or *naltrexone*. Other congeners, especially *naloxone* and *naltrexone*, appear to be devoid of agonist actions and interact with the canonical opioid receptors types (mu-, delta-, and kappa-opioid receptors), albeit with somewhat different affinities. Importantly, *naloxone* and *naltrexone* do not bind to the nociceptin receptor. The majority of these agents are relatively lipid soluble and have excellent CNS penetration after systemic delivery (Barnett et al., 2014). However, a number of antagonists only bind to opioid receptors mainly at peripheral (non-CNS) sites due to their poor bioavailability, such as *methylnaltrexone*. These opioid receptor antagonists are used to manage opioid-induced constipation (Becker et al., 2007).

Alvimopan is a mu-opioid receptor antagonist with distribution restricted to the periphery. Following oral administration, a deamidated metabolite of *alvimopan* slowly and variably appears in the bloodstream and is attributed to activity of the intestinal microbiome. Like *alvimopan*, the metabolite is an antagonist with high affinity for the mu-opioid receptor. Both parent drug and metabolite have terminal half-lives of 10 to 18 h. *Alvimopan* is FDA-approved for treatment of postoperative ileus in patients with less than 7 days of opioid exposure immediately prior to beginning *alvimopan*. Due to increased incidence of myocardial infarction with prolonged use, *alvimopan* is for short-term use (15 doses) only.

Nalmefene (not marketed in the U.S.) is a relatively pure mu-opioid receptor antagonist that is more potent than *naloxone*.

Therapeutic Uses

Opioid antagonists have obvious therapeutic utility in the treatment of opioid overdose but are also used to treat opioid-induced constipation and alcohol use disorder. Opioid antagonists displace opioid agonists from the mu-opioid receptor and thus reverse the effects of agonists. Under ordinary circumstances, these opioid antagonists produce few effects in the absence of an exogenous agonist. However, under certain conditions (e.g., pain, shock, alcohol use), when the endogenous opioid systems are activated, the administration of an opioid antagonist alone may have effects.

Treatment of Opioid Overdoses

Opioid antagonists, particularly *naloxone*, have an established use in the treatment of opioid-induced toxicity, especially respiratory depression. Its specificity is such that reversal by *naloxone* is virtually diagnostic for the involvement of an opioid agonist. *Naloxone* acts rapidly to reverse the respiratory depression. The duration of action of *naloxone* is relatively short, and it often must be given repeatedly or by continuous infusion to prevent re-narcotization. *Naloxone* nasal spray (Narcan®) is widely available for emergency use in a nonhospital setting. It should be noted that *naloxone* or any opioid antagonist may precipitate withdrawal in dependent subjects, causing undesirable effects such as diarrhea, hypertension/tachycardia, and pain. Opioid antagonists also have been employed effectively to decrease neonatal respiratory depression secondary to the intravenous or intramuscular administration of opioids to the mother. In the neonate, the initial dose is 10 μg/kg given intravenously, intramuscularly, or subcutaneously.

Management of Constipation

The peripherally limited antagonists *methylnaltrexone* and *naloxegol* have important roles in the management of constipation and reduced GI motility present in the patient undergoing chronic opioid therapy for chronic pain, *methadone* maintenance, and following abdominal surgery. Treatment with such agents facilitates recovery of normal bowel function and leaves the analgesic activity of the postoperative opioid intact, which is mostly mediated by the CNS (Vaughan-Shaw et al., 2012).

Management of Opioid and Alcohol Use Disorders

An extended-release formulation of *naltrexone* is approved for the treatment of opioid and alcohol use disorder. The extended-release matrix is delivered by intramuscular injection and given by a practitioner once per month. To reduce the risk of eliciting withdrawal symptoms, patients should wait 7 to 14 days since their last dose of an opioid agonist before starting *naltrexone* treatment. In opioid use disorder, *naltrexone* will prevent opioid agonists from binding to and activating mu-opioid receptors, preventing opioid-induced euphoria and, hopefully, relapse to opioid use. In all patients taking this medication, *naltrexone* will block the centrally mediated analgesic effects and peripherally mediated GI slowing produced by mu-opioid receptor agonists.

Miscellaneous

Naltrexone in combination with *bupropion* is also FDA-approved as an adjunct for weight management in patients with obesity. *Samidorphan*, an analogue of *naltrexone*, has been recently approved as a combination with *olanzapine* for the management of schizophrenia. The antagonist has been shown to reduce weight gain in patients on *olanzapine*.

Pharmacological Properties

Effects in the Absence of Opioid Agonist

Subcutaneous doses of *naloxone* up to 12 mg produce no discernible effects in humans, and doses of 24 mg cause only slight drowsiness. *Naltrexone* also is a relatively pure antagonist but with a longer duration of action and greater bioavailability following oral administration. The effects of opioid receptor antagonists are usually both subtle and limited, likely reflecting the low levels of tonic activity and organizational complexity of the opioid systems in various physiological systems. Opioid receptor antagonism in humans is associated with variable effects, ranging from no effect to mild hyperalgesia. In some, but not all, studies, opioid antagonists are reported to block the analgesic effects produced by acupuncture, exercise, mindfulness meditation, positive mood, magnetic stimulation, and placebo medications, suggesting that the endogenous opioid system may play a role in these analgesic effects (Bruehl et al., 2020; Ciampi de Andrade et al., 2011; Flaten, 2014; Frangos et al., 2021; Staud and Price, 2006).

Endogenous opioid peptides participate in the regulation of pituitary secretion by exerting tonic inhibitory effects on the release of certain hypothalamic hormones (see Chapter 46). Thus, the administration of *naloxone* or *naltrexone* increases the secretion of gonadotropin-releasing hormone and corticotropin-releasing hormone and elevates the plasma concentrations of LH, FSH, and ACTH, as well as the steroid hormones produced by their target organs. *Naloxone* stimulates the release of prolactin in women. Endogenous opioid peptides probably have some role in the regulation of feeding or energy metabolism; however, *naltrexone* does not accelerate weight loss in very obese subjects, even though short-term administration of opioid antagonists reduces food intake in lean and obese individuals. Long-term administration of antagonists may increase the density of opioid receptors in the brain and causes a temporary exaggeration of responses to the subsequent administration of opioid agonists.

Effects in the Presence of Opioid Agonists

Antagonistic Effects. Small doses (0.4–0.8 mg) of *naloxone* given intramuscularly or intravenously prevent or promptly reverse the effects of receptor agonists. In patients with respiratory depression, an increase in respiratory rate is seen within 1 to 2 min. Sedative effects are reversed, and blood pressure, if depressed, returns to normal. Higher doses of *naloxone* are required to antagonize the respiratory-depressant effects of *buprenorphine*, *fentanyl*, and other high-affinity opioid receptor agonists; 1 mg *naloxone* intravenously completely blocks the effects of 25 mg *heroin*. *Naloxone* reverses the psychotomimetic and dysphoric effects of agonist-antagonist agents such as *pentazocine*, but much higher doses (10–15 mg) are required. The duration of antagonistic effects depends on the dose but usually is 1 to 4 h. Antagonism of opioid effects by *naloxone* often is accompanied by an "overshoot" phenomenon. For example, respiratory rates depressed by opioids transiently become higher than before the period of depression. Rebound release of catecholamines may cause hypertension, tachycardia, and ventricular arrhythmias. Pulmonary edema also has been reported after *naloxone* administration.

Effects in Opioid-Dependent Patients

In subjects who are dependent on morphine-like opioids, small subcutaneous doses of *naloxone* (0.5 mg) precipitate a moderate-to-severe withdrawal syndrome similar to that seen after abrupt withdrawal of opioids, except that the syndrome appears within minutes of administration and subsides in about 2 h. The severity and duration of the syndrome are related to the dose of the antagonist and to the degree and type of dependence. Higher doses of *naloxone* will precipitate a withdrawal syndrome in patients dependent on *pentazocine*, *butorphanol*, or *nalbuphine*. In dependent patients, peripheral side effects of opioids, notably reduced GI motility and constipation, can be reversed by *methylnaltrexone*, with subcutaneous doses (0.15 mg/kg) producing reliable bowel movements and no evidence of centrally mediated withdrawal signs (Thomas et al., 2008). *Naloxone* produces an overshoot phenomenon suggestive of early acute physical dependence 6 to 24 h after even a single dose of a mu-opioid receptor agonist.

ADME

Although absorbed readily from the GI tract, *naloxone* undergoes extensive first-pass metabolism and is almost completely metabolized by the liver (primarily by conjugation with glucuronic acid) before reaching the systemic circulation and thus must be administered parenterally. The $t_{1/2}$ of *naloxone* is about 1 h, but its clinically effective duration of action can be even less.

Compared with *naloxone*, *naltrexone* is more effective by the oral route, and has a duration of action that approaches 24 h after moderate oral doses. Peak concentrations in plasma are reached within 1 to 2 h and then decline with an apparent $t_{1/2}$ of about 3 h. *Naltrexone* is metabolized to 6-naltrexol, which is a weaker antagonist with a longer $t_{1/2}$, about 13 h. *Naltrexone* is more potent than *naloxone*, and 100-mg oral doses given to patients dependent on opioids produce concentrations in tissues sufficient to block the euphorigenic effects of 25-mg IV doses of *heroin* for 48 h. *Methylnaltrexone* is similar to *naltrexone*; it is converted to methyl-6-naltrexol isomers and eliminated primarily via active renal secretion. The $t_{1/2}$ of *methylnaltrexone* is about 8 h.

Acute Opioid Toxicity

Acute opioid toxicity may result from clinical overdosage, accidental overdosage, or attempts at suicide. Occasionally, a delayed toxicity may occur from the injection of an opioid into chilled skin areas or in patients with low blood pressure and shock. The drug is not fully absorbed; therefore, a subsequent dose may be given. When normal circulation is restored, an excessive amount may be absorbed suddenly.

The triad of coma, pinpoint pupils, and depressed respiration suggests opioid poisoning. The patient is stuporous or, if a large overdose has been taken, may be in a profound coma. The respiratory rate will be very low, or the patient may be apneic and possibly cyanotic. Body temperature falls, and the skin becomes cold and clammy. The skeletal muscles are flaccid, the jaw is relaxed, and the tongue may fall back and block the airway. Convulsions occasionally may be noted in infants and children. When death occurs, it is nearly always from respiratory failure.

The first step in treatment is to establish a patent airway and ventilate the patient. *Naloxone* is used to reverse the severe respiratory depression. *Naloxone* can be given IM, SC, or IV or by first responders and the public using a nasal spray (Narcan®) containing 4 mg *naloxone*. Administration can be repeated every 2 to 3 min if the person does not respond after the first dose. This may be particularly needed with very potent opioids such as the *fentanyl* derivatives. The duration of action of *naloxone* is shorter than that of many opioid agonists; hence, patients can slip back into coma. Steps for responding to an opioid overdose can be found in the Substance Abuse and Mental Health Administration's *Opioid Overdose Prevention Toolkit* (https://store.samhsa.gov/product/Opioid-Overdose-Prevention-Toolkit/SMA18-4742; accessed June 29, 2022).

Additional Therapeutic Uses of Opioids

Dyspnea

Morphine is used to alleviate the dyspnea of acute left ventricular failure and pulmonary edema, and the patient's response to intravenous *morphine* may be dramatic. The mechanism underlying this pronounced relief is not clear. It may involve an alteration of the patient's reaction to impaired respiratory function and an indirect reduction of the work of the heart owing to reduced fear and apprehension. However, it is more probable that the major benefit is due to cardiovascular effects, such as decreased peripheral resistance secondary to histamine release and an increased capacity of the peripheral and splanchnic vascular compartments. *Nitroglycerin*, which also causes vasodilation, may be superior to *morphine*. Nonetheless, opioids generally are contraindicated in pulmonary edema unless severe pain is also present.

Anesthetic Adjuvants

High doses of opioids, notably *fentanyl* and analogues, are widely used as the primary anesthetic agents in many surgical procedures. They have powerful minimum alveolar concentration ("MAC")-sparing effects; for example, they reduce the concentrations of volatile anesthetic otherwise required to produce an adequate anesthetic depth. Although respiration is so depressed that physical assistance is required, patients can retain consciousness. Therefore, when an opioid is the primary anesthetic, an agent that results in unconsciousness and produces amnesia, such as the benzodiazepines or lower concentrations of volatile anesthetics, is also employed. High doses of opioid as employed in the operating room setting also result in prominent rigidity of the chest wall and masseters, requiring concurrent treatment with muscle relaxants to permit intubations and ventilation.

Opioid-Related Antitussive Agents

As mentioned earlier, mu-opioid receptor agonists, particularly *codeine*, are effective in suppressing the cough reflex, although for *codeine*, this action may not involve opioid receptors. In addition, *dextromethorphan* and *pholcodine* are structurally compounds that do not bind to or have activity at opioid receptors and produce antitussive activity through other mechanisms.

Dextromethorphan (D-3-methoxy-*N*-methylmorphinan) as the D-isomer has no analgesic or addictive properties and does not act through mu-opioid receptors. Rather, the drug acts centrally to elevate the threshold for coughing. Its effectiveness in patients with pathological cough has been demonstrated in controlled studies; its potency is nearly equal to that of *codeine*, but *dextromethorphan* produces fewer subjective and GI side effects. In therapeutic dosages, the drug does not inhibit ciliary activity, and its antitussive effects persist for 5 to 6 h. The average adult dosage of *dextromethorphan hydrobromide* is 10 to 20 mg every 4 h or 30 mg every 6 to 8 h, not to exceed 120 mg daily. The drug is marketed for over-the-counter sale in liquids, syrups, capsules, soluble strips, lozenges, and freezer pops or in combinations with antihistamines, expectorants, and decongestants. An extended-release *dextromethorphan* suspension is approved for twice-daily administration. The toxicity of *dextromethorphan* is low, although the compound does inhibit neuronal serotonin uptake and must be avoided in patients taking MAO inhibitors as it can cause "serotonin syndrome."

Extremely high doses of *dextromethorphan* are misused because it acts at NMDA receptors and can cause hallucinations, euphoria, significant perceptual distortions, and even dissociative effects, as well as significantly impair motor functioning and coordination.

Pholcodine [3-*O*-(2-morpholinoethyl) morphine] is used clinically in many countries outside the U.S. *Pholcodine* is at least as effective as *codeine* as an antitussive; it has a long $t_{1/2}$ and can be given once or twice daily.

Overall Summary and Conclusions

Failure to adequately manage pain can have important negative consequences on physiological function, such as autonomic hyperreactivity (increased blood pressure, increased heart rate, suppression of GI motility, and reduced secretions) and reduced mobility (leading to deconditioning, muscle wasting, joint stiffening, and decalcification), and can contribute to deleterious changes in the psychological state (depression, helplessness syndromes, and anxiety). By many hospital-accrediting organizations, and by law in many states, appropriate pain assessment and adequate pain management are standard of care, with pain considered the "fifth vital sign."

Mu-opioid receptor agonists are highly effective for treating moderate-to-severe, acute pain. Chronic use of mu-opioid receptor agonists leads to the development of tolerance, physical dependence, and potentially, opioid use disorder. The extensive overuse of mu-opioid receptor agonists for chronic pain and other medical conditions has contributed to the opioid epidemic that began at the start of the

21st century. Drug overdose has become the leading cause of accidental death in the U.S., driven by opioid misuse and abuse (National Institute on Drug Abuse, 2021; Rudd et al., 2016). Thus, there has been a growing concern over the appropriate use of opioids in pain management. The three-step ladder promoted by the World Health Organization encourages the use of more conservative therapies before initiating opioid therapy (Table 23–5). Weaker opioids can be supplanted by stronger opioids in cases of moderate and severe pain. The Centers for Disease Control and Prevention (CDC) also provides guidelines for chronic opioid treatment (Dowell et al., 2016; see *Guidelines for Prescribing Opiates for Chronic Pain*, available at: https://www.cdc.gov/drugoverdose/pdf/prescribing/Guidelines_Factsheet-a.pdf) and for the treatment of acute pain conditions (see *Clinical Guidance for Selected Common Acute Pain Conditions*, available at: https://www.cdc.gov/acute-pain/index.html).

While all of the currently available, clinically used opioid analgesics activate the orthosteric binding site on the mu-opioid receptor, targeting other opioid receptor types, combinations of opioid receptor types, or allosteric binding sites on the mu-opioid receptor may produce analgesia with less liability for opioid misuse and abuse. In addition, endogenous opioids and opioid receptors may be involved in modulating homeostatic and disease states other than pain, such as mood, reward, obesity, and movement disorders. This is becoming an active area of research and discovery.

TABLE 23–5 ■ WORLD HEALTH ORGANIZATION ANALGESIC LADDER

Step 1: Mild-to-Moderate Pain

Nonopioid ± adjuvant agent

- Acetaminophen or an NSAID should be used, unless contraindicated. Adjuvant agents are those that enhance analgesic efficacy, treat concurrent symptoms that exacerbate pain, or provide independent analgesic activity for specific types of pain.

Step 2: Mild-to-Moderate Pain or Pain Uncontrolled After Step 1

Short-acting opioid as required ± nonopioid ATC ± adjuvant agent

- Morphine, oxycodone, or hydromorphone should be added to acetaminophen or an NSAID for maximum flexibility of opioid dose.

Step 3: Moderate-to-Severe Pain or Pain Uncontrolled After Step 2

Sustained-release/long-acting opioid ATC or continuous infusion + short-acting opioid as required ± nonopioid ± adjuvant agent

- Sustained-release oxycodone, morphine, oxymorphone, or transdermal fentanyl is indicated.

ATC, around the clock.

Source: Adapted from http://www.who.int/cancer/palliative/painladder/en/.

Drug Facts for Your Personal Formulary: *Opioid Agonists and Antagonists*

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Agonists		
Morphine Hydromorphone Oxycodone Hydrocodone	• Potent mu-agonists • Strong analgesic in moderate-to-severe pain states • Morphine is a useful adjunct in pulmonary edema and general anesthesia	• ↓ GI motility ⇒ constipation • Hydrocodone, oxycodone formulated with NSAIDs • Hydrocodone, oxycodone, and fentanyl are more potent than morphine • Oxycodone and hydrocodone popular drugs of abuse
Fentanyl	• Potent mu-agonist analgesic • Administered orally (buccal tablet, sublingual tablet/spray, oral lozenge), intravenous (push/infusion), intramuscular, topical, topical iontophoretic, spinal	• Rapid onset, short duration of action • Longer effective $t_{1/2}$ than sufentanil > alfentanil > remifentanil
Sufentanil Alfentanil Remifentanil	• Like fentanyl • Rapid onset, short duration of action • Administered intravenously	• Sufentanil and alfentanil also given epidurally • Remifentanil: ultrashort acting
Meperidine	• Mu-agonist analgesic • Rapid onset, intermediate duration of action	• Not for extended use due to accumulation of seizure-inducing metabolite • Potent histamine releaser
Methadone	• Potent mu-agonist analgesic like morphine • Rapid onset, long duration of action • Maintenance therapy for opioid use disorder in specialized clinics/rehab programs	• Long oral $t_{1/2}$, ~27 h ⇒ potential for accumulation with too frequent repeated delivery • NMDA receptor antagonist
Codeine	• Low potency; prodrug for morphine • Useful for mild-to-moderate pain • Administered orally	• Useful antitussive • Activated *in vivo* to morphine by CYP2D6 • CYP2D6 polymorphisms cause large individual differences in analgesia, no analgesic effect in 10% of Caucasians
Levorphanol	• Opioid agonist • Rapid onset, modest duration of analgesia • Administered orally	• Less selective for mu over delta or kappa receptors than morphine • Long elimination $t_{1/2}$, ~14 h ⇒ potential for accumulation with too frequent repeated delivery • 5HT/NE reuptake inhibitor; NMDA receptor antagonist • Adverse effects: delirium, hallucinations
Peripherally Restricted Agonist		
Loperamide	• Mu-opioid agonist • Effective antidiarrheal • Administered orally	• Loperamide crosses blood-brain barrier poorly; can be formulated with simethicone

Drug Facts for Your Personal Formulary: *Opioid Agonists and Antagonists (continued)*

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Agonist Restricted by Coformulation		
Diphenoxylate	• Mu-opioid agonist • Effective antidiarrheal • Administered orally	• Diphenoxylate will cross the blood-brain barrier, so it is formulated with atropine, the anticholinergic effects of which (weakness, nausea) discourage abuse
Partial Agonists; Agonist/Antagonist Combinations		
Buprenorphine	• Mild-to-moderate pain • Less respiratory depression than full agonists • Administered by intramuscular, intravenous, sublingual, transdermal, buccal film, transdermal patch • Coformulated with naloxone for maintenance therapy for opioid use disorder (to prevent diversion). Prescribed from clinicians' offices with appropriate training	• Partial agonist at mu receptor, antagonist at kappa and delta receptors • Slow dissociation from mu-opioid receptors • Delivery to a patient on a full opioid agonist may initiate withdrawal (may be done therapeutically in management of heroin addiction)
Butorphanol Nalbuphine Pentazocine	• Kappa/mu partial agonists • Analgesia to mild-to-moderate pain	• Delivery to patient on a full opioid agonist may initiate withdrawal • Pentazocine is also formulated with naloxone
Other Agonists		
Tramadol	• Analgesia for moderate pain • Weak mu-agonist and a 5HT/NE uptake inhibitor • Available as a fixed-dose combination with acetaminophen	• Potential for seizures • Serotonin syndrome risk • As an adjunct to other opioids for chronic pain
Tapentadol	• Analgesia for moderate pain • Weak mu-agonist and a 5HT/NE uptake inhibitor	• Serotonin syndrome risk
Oliceridine	• Analgesia • IV use in hospital setting only	• Partial agonist • G protein bias
Central Antitussives		
Dextromethorphan	• ↓ Cough reflex; receptor mechanisms unclear • Administered orally • Available as an extended-release formulation	• Serotonin syndrome risk • Has no analgesic or addictive properties
Codeine	• See codeine listing, above	• See codeine listing, above
Antagonists		
Naloxone	• Antagonist at mu, delta, and kappa receptors • Rapid onset, moderately short acting • Rapidly reverses central and peripheral opioid effects • Used in treating opioid overdose • Autoinjector and nasal sprays available for emergency administration	• $t_{1/2}$ ~64 min • Re-narcotization may occur with long-lasting agonists as naloxone is metabolized • May induce moderate hyperalgesia • Known as Narcan®; used by emergency medical technicians to revive comatose opioid abusers • Nasally administered version can be used by nonspecialists (i.e., the general public) to reverse overdose
Naltrexone Nalmefene	• Antagonists at mu, delta, and kappa receptors • Rapid onset, longer acting than naloxone • Reverse central and peripheral opioid effects • Used in treating alcohol and opioid dependence	• Start only after 7–10 days of abstinence from opioids to reduce risk withdrawal symptoms • Long-term use of naltrexone ⇒ hypersensitivity to opioids • Naltrexone: formulated with bupropion for managing obesity and with morphine for severe pain; contraindicated in hepatitis and liver failure (black-box warning: excessive doses cause hepatocellular injury)
Samidorphan	• Mu-opioid receptor antagonist • Only approved for use with olanzapine in treatment of schizophrenia	• Higher affinity that naltrexone • Improved oral bioavailability • Kappa- and delta-opioid receptor partial agonism
Peripherally Restricted Antagonists		
Methylnaltrexone	• Antagonist at mu, delta, and kappa receptors • Reverses peripheral opioid effects (e.g., opioid-induced constipation) but not analgesia	• Does not cross blood-brain barrier; thus, not useful in treating addiction or reversing CNS effects of opioids
Alvimopan	• Antagonist at mu, delta, and kappa receptors • Penetrates poorly into CNS • FDA approved for ileus	• Reverses peripheral opioid effects only

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Chapter 24

General Anesthetics and Therapeutic Gases

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GENERAL PRINCIPLES OF SURGICAL ANESTHESIA
- Hemodynamic Effects of General Anesthesia
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THERAPEUTIC GASES
- Oxygen
- Carbon Dioxide
- Nitric Oxide
- Helium
- Hydrogen Sulfide

General anesthetics depress the CNS to a sufficient degree to permit the performance of surgery and unpleasant procedures. General anesthetics have low therapeutic indices and thus require great care in administration. The selection of specific drugs and routes of administration to produce general anesthesia is based on the pharmacokinetic properties and on the secondary effects of the various drugs. The practitioner should consider the context of the proposed diagnostic or surgical procedure and the individual patient's characteristics and associated medical conditions when choosing appropriate anesthetic agents.

General Principles of Surgical Anesthesia

The administration of general anesthesia is driven by three general objectives:

1. *Minimizing the potentially deleterious direct and indirect effects of anesthetic agents and techniques.*
2. *Sustaining physiologic homeostasis during surgical procedures* that may involve major blood loss, tissue ischemia, reperfusion of ischemic tissue, fluid shifts, exposure to a cold environment, and impaired coagulation.
3. *Improving postoperative outcomes* by choosing techniques that block or treat components of the surgical stress response that may lead to short- or long-term sequelae.

Hemodynamic Effects of General Anesthesia

The most prominent physiological effect of anesthesia induction is a decrease in systemic arterial blood pressure. The causes include direct vasodilation, myocardial depression, or both; a blunting of baroreceptor control; and a generalized decrease in central sympathetic tone. Agents vary in the magnitude of their specific effects, but in all cases, the hypotensive response is enhanced by underlying volume depletion or preexisting myocardial dysfunction.

Respiratory Effects of General Anesthesia

Nearly all general anesthetics reduce or eliminate both ventilatory drive and their reflexes that maintain airway patency. Therefore, ventilation generally must be assisted or controlled for at least some period during surgery. The gag reflex is lost, and the stimulus to cough is blunted. Lower esophageal sphincter tone also is reduced, so both passive and active regurgitation may occur. Endotracheal intubation has been a major reason for a decline in the number of aspiration deaths during general anesthesia. Muscle relaxation is valuable during the induction of general anesthesia where it facilitates management of the airway, including endotracheal intubation. Neuromuscular blocking agents commonly are used to effect such relaxation (see Chapter 13). Alternatives to an endotracheal tube include a face mask and a laryngeal mask, an inflatable mask placed in the oropharynx forming a seal around the glottis. Airway management techniques are based on the anesthetic procedure, the need for neuromuscular relaxation, and the physical characteristics of the patient.

Hypothermia

Patients commonly develop hypothermia (body temperature <36°C) during surgery. The reasons include low ambient temperature, exposed body cavities, cold intravenous fluids, altered thermoregulatory control, and reduced metabolic rate. Metabolic rate and total body O_2 consumption decrease with general anesthesia by about 30%, reducing heat generation. Hypothermia may lead to an increase in perioperative morbidity. Prevention of hypothermia is a major goal of anesthetic care.

Nausea and Vomiting

Nausea and vomiting continue to be significant problems following general anesthesia and are caused by the action of anesthetics on the chemoreceptor trigger zone and the brainstem vomiting center, which are modulated by 5-hydroxytryptamine (5HT), histamine, acetylcholine (ACh), dopamine (DA), and neurokinin 1 (NK1) (see Figure 54–6). The $5HT_3$ receptor antagonists *ondansetron*, *dolasetron*, and *palonosetron* (see Chapters 15 and 54) are effective in suppressing nausea and vomiting. Common preventive strategies include anesthetic induction with *propofol*; the combined use of *droperidol*, *metoclopramide*, and *dexamethasone*; and avoidance of nitrous oxide (N_2O). A new subclass of antiemetic drugs includes NK1 antagonists (e.g., *aprepitant*, *rolapitant*).

Abbreviations

ACh: acetylcholine
ADME: absorption, distribution, metabolism, excretion
CBF: cerebral blood flow
CMR: cerebral metabolic rate
CMRO$_2$: cerebral metabolic rate of O_2 consumption
CNS: central nervous system
DA: dopamine
ED$_{50}$: median effective dose
EEG: electroencephalogram
FIO$_2$: inspired O_2 fraction
GABA: γ-aminobutyric acid
GFR: glomerular filtration rate
Hb: hemoglobin
5HT: 5-hydroxytryptamine (serotonin)
ICP: intracranial pressure
IV: intravenous
LD$_{50}$: median lethal dose
MAC: minimum alveolar concentration
MI: myocardial infarction
MOC: methoxycarbonyl
NK1: neurokinin 1
NMDA: *N*-methyl-D-aspartate
Paco$_2$: arterial CO_2 tension
Po$_2$: partial pressure of O_2
PRIS: propofol infusion syndrome
RBF: renal blood flow
VLPO: ventrolateral preoptic

Other Emergent and Postoperative Phenomena

Hypertension and tachycardia are common during emergence from anesthesia as the sympathetic nervous system regains its tone and is enhanced by pain. Myocardial ischemia can appear or worsen during emergence in patients with coronary artery disease. Emergence excitement occurs in 5% to 30% of patients and is characterized by tachycardia, restlessness, crying, moaning, and thrashing. Neurological signs, including delirium, spasticity, hyperreflexia, and Babinski sign, are often manifest in the patient emerging from anesthesia. Postanesthesia shivering occurs frequently because of core hypothermia. A small dose of *meperidine* (12.5 mg) lowers the shivering trigger temperature and effectively stops the activity. The incidence of all these emergence phenomena is greatly reduced with opioids and α_2 adrenergic agonists (*dexmedetomidine*).

Airway obstruction may occur during the postoperative period because of residual anesthetic effects. Pulmonary function is reduced following all types of anesthesia and surgery, and hypoxemia may occur. In the immediate postoperative period, pulmonary function reduction can be compounded by the respiratory suppression associated with opioids used for pain control. Regional anesthetic techniques are an important part of a perioperative approach that employs local anesthetic wound infiltration; epidural, spinal, and plexus blocks; and nonsteroidal anti-inflammatory drugs, opioids, α_2 adrenergic receptor agonists, and NMDA (*N*-methyl-D-aspartate) receptor antagonists.

Actions and Mechanisms of General Anesthetics

The Anesthetic State

The components of the anesthetic state include:

- *Amnesia*
- *Analgesia*
- *Unconsciousness*
- *Immobility* in response to noxious stimulation
- *Attenuation of autonomic responses* to noxious stimulation

The potency of general anesthetic agents is measured by determining the concentration of general anesthetic that *prevents* movement in response to surgical stimulation. For inhalational anesthetics, anesthetic potency is measured in *minimum alveolar concentration* (*MAC*) *units*, with *1 MAC* defined as *the minimum alveolar concentration* that prevents movement in response to surgical stimulation in 50% of subjects. The strengths of MAC as a measurement are the following:

- Alveolar concentrations can be monitored continuously by measuring end-tidal anesthetic concentration using infrared spectroscopy or mass spectrometry.
- MAC provides a direct correlate of the free concentration of the anesthetic at its site(s) of action in the central nervous system (CNS).
- MAC is a simple-to-measure end point that reflects an important clinical goal.

End points other than immobilization also can be used to measure anesthetic potency. For example, the ability to respond to verbal commands (MAC$_{awake}$) and the ability to form memories also have been correlated with alveolar anesthetic concentration. Verbal response and memory formation are suppressed at a fraction of MAC. The ratio of the anesthetic concentrations required to produce amnesia and immobility vary significantly among different inhalational anesthetic agents.

Generally, the potency of intravenous agents is defined as the free plasma concentration (at equilibrium) that produces loss of response to surgical incision (or other end points) in 50% of subjects.

Mechanisms of Anesthesia

The molecular and cellular mechanisms by which general anesthetics produce their effects have remained one of the great mysteries of pharmacology. The leading unitary theory was that anesthesia is produced by perturbation of the physical properties of cell membranes. This thinking was based largely on the observation that the anesthetic potency of a gas correlated with its solubility in olive oil. This correlation is referred to as the Meyer-Overton rule. Clear exceptions to the Meyer-Overton rule (Franks, 2006) suggest protein targets that may account for anesthetic effect. Increasing evidence supports the hypothesis that different anesthetic agents produce specific components of anesthesia by actions at different molecular targets. Given these insights, the unitary theory of anesthesia has been largely discarded.

Molecular Mechanisms of General Anesthetics

Most intravenous general anesthetics act predominantly through GABA$_A$ receptors and perhaps through some interactions with other ligand-gated ion channels such as NMDA receptors and two-pore K$^+$ channels. GABA$_A$ receptors (GABA-gated chloride channels; see Figures 16–5 and 16–11) are sensitive to a wide variety of anesthetics, including the halogenated inhalational agents, many intravenous agents (*propofol*, barbiturates, and *etomidate*; see Figure 24–1), and neurosteroids. At clinical concentrations, general anesthetics increase the sensitivity of the GABA$_A$ receptor to GABA (γ-aminobutyric acid), thereby enhancing inhibitory neurotransmission and depressing nervous system activity. The action of anesthetics on the GABA$_A$ receptor probably is mediated by binding of the anesthetics to specific allosteric sites on the GABA$_A$ receptor protein (but they do not compete with GABA for its binding site). The capacity of *propofol* and *etomidate* to inhibit the response to noxious stimuli is mediated by a specific site on β_3 subunits of certain GABA$_A$ receptors, whereas the sedative effects of these anesthetics represent effects on channels containing β_2 subunits.

Structurally related to the GABA$_A$ receptors are other ligand-gated ion channels, including *glycine receptors* and neuronal *nicotinic ACh receptors* (see Figures 16–5, 16–6, and 16–9). Glycine-gated Cl$^-$ channels (glycine receptors) may play a role in mediating inhibition by anesthetics of responses to noxious stimuli. Inhalational anesthetics enhance the capacity of glycine to activate glycine receptors, which play an important role in inhibitory neurotransmission in the spinal cord and brainstem. *Propofol*, neurosteroids, and barbiturates also potentiate glycine-activated currents, whereas *etomidate* and *ketamine* do not. Subanesthetic concentrations of the inhalational anesthetics inhibit some classes of neuronal

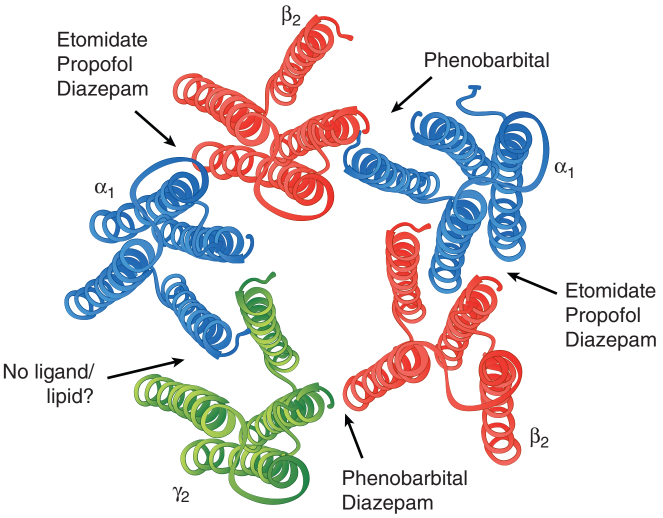


Figure 24–1 *Anesthetics and GABA$_A$ interaction sites.* Structure of a predominant synaptic isoform of the GABAA receptor obtained by cryo-electron microscopy (Kim et al, 2020). View is from the synaptic perspective looking down the ion channel axis. Five subunits (two α_1, two β_2 and one γ_2) arrange like barrel staves to form a central chloride-conducting pore. The neurotransmitter GABA binds at β-α subunit interfaces in the extracellular domain. Shown here is a cross-section of the transmembrane domain, which houses interaction sites for common intravenous general anesthetics and diazepam. Binding of these anesthetics potentiates the receptor's response to GABA, thereby increasing fast synaptic inhibitory signaling. (Figure provided by Professor Ryan Hibbs, Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX) For a side view of the interaction sites, see Figure 16-11. For details about the subunit organization of pentameric ligand-gated ion channels, see Figure 13-1.

nicotinic ACh receptors, which seem to mediate other components of anesthesia such as analgesia or amnesia.

Ketamine, nitrous oxide, *cyclopropane*, and *xenon* are the only general anesthetics that do not have significant effects on GABA$_A$ or glycine receptors. These agents inhibit a different type of ligand-gated ion channel, the NMDA receptor (see Figure 16–9 and Table 16–2). NMDA receptors are glutamate-gated cation channels that are somewhat selective for Ca^{2+} and are involved in long-term modulation of synaptic responses (long-term potentiation) and glutamate-mediated neurotoxicity.

Halogenated inhalational anesthetics activate some members of a class of K^+ channels known as *two-pore domain channels*; other two-pore domain channel family members are activated by xenon, N_2O, and *cyclopropane*. These channels are located in both presynaptic and postsynaptic sites. The postsynaptic channels may be the molecular locus through which these agents hyperpolarize neurons.

Cellular Mechanisms of Anesthesia

General anesthetics produce two important physiological effects at the cellular level:

1. Inhalational anesthetics can hyperpolarize neurons. Neuronal hyperpolarization may affect pacemaker activity and pattern-generating circuits.
2. Both inhalational and intravenous anesthetics have substantial effects on synaptic transmission and much smaller effects on action potential generation or propagation.

Inhalational anesthetics inhibit excitatory synapses and enhance inhibitory synapses in various preparations. The inhalational anesthetics inhibit neurotransmitter release. Inhalational anesthetics also can act postsynaptically, altering the response to released neurotransmitter. These actions are thought to be due to specific interactions of anesthetic agents with neurotransmitter receptors.

Intravenous anesthetics produce a narrower range of physiological effects. Their predominant actions are at the synapse, where they have profound and relatively specific effects on the postsynaptic response to released neurotransmitter. Most of the intravenous agents act predominantly by enhancing inhibitory neurotransmission, whereas ketamine predominantly inhibits excitatory neurotransmission at glutamatergic synapses.

Anatomic Sites of Anesthetic Action

In principle, general anesthetics could interrupt nervous system function at myriad levels, including peripheral sensory neurons, the spinal cord, the brainstem, and the cerebral cortex. Most anesthetics cause, with some exceptions, a global reduction in cerebral metabolic rate (CMR) and in cerebral blood flow (CBF). A consistent feature of general anesthesia is a suppression of metabolism in the thalamus (Alkire et al., 2008), which serves as a major relay by which sensory input from the periphery ascends to the cortex. Suppression of thalamic activity may serve as a switch between the awake and anesthetized states (Franks, 2008). General anesthesia also suppresses activity in specific regions of the cortex, including the mesial parietal cortex, posterior cingulate cortex, precuneus, and inferior parietal cortex.

Similarities between natural sleep and the anesthetized state suggest that anesthetics might also modulate endogenous sleep-regulating pathways, which include ventrolateral preoptic (VLPO) and tuberomammillary nuclei. VLPO projects inhibitory GABAergic fibers to ascending arousal nuclei, which in turn project to the cortex, forebrain, and subcortical areas and release histamine, 5HT, orexin, norepinephrine, and ACh to mediate wakefulness. Intravenous and inhalational agents with activity at GABA$_A$ receptors can increase the inhibitory effects of VLPO, thereby suppressing consciousness. *Dexmedetomidine*, an α_2 adrenergic agonist, also increases VLPO-mediated inhibition by suppressing the inhibitory effect of locus ceruleus neurons on VLPO. Finally, both intravenous and inhalational anesthetics depress hippocampal neurotransmission, a probable locus for their amnestic effects.

Parenteral Anesthetics

Parenteral anesthetics are the most common drugs used for anesthetic induction of adults. Their lipophilicity, coupled with the relatively high perfusion of the brain and spinal cord, results in rapid onset and short duration after a single bolus dose. These drugs ultimately accumulate in fatty tissue. Each of these anesthetics has its own unique properties and side effects (Tables 24–1 and 24–2). *Propofol* is advantageous for procedures where rapid return to a preoperative mental status is desirable. *Etomidate* usually is reserved for patients at risk for hypotension or myocardial ischemia. *Ketamine* is best suited for patients with asthma

TABLE 24–1 ■ PHARMACOLOGICAL PROPERTIES OF PARENTERAL ANESTHETICS

DRUG	IV INDUCTION DOSE (mg/kg)	MINIMAL HYPNOTIC LEVEL (μg/mL)	INDUCTION DOSE DURATION (min)	$t_{1/2}\beta$ (h)	*CL* (mL/min/kg)	PROTEIN BINDING (%)	V_{SS} (L/kg)
Propofol	1.5–2.5	1.1	4–8	1.8	30	98	2.3
Etomidate	0.2–0.4	0.3	4–8	2.9	17.9	76	2.5
Ketamine	1.0–4.5	1	5–10	2.5	19.1	27	3.1
Thiopental	3–5	15.6	5–8	12.1	3.4	85	2.3
Methohexital	1.0–1.5	10	4–7	3.9	10.9	85	2.2

CL, clearance; $t_{1/2}\beta$, β-phase (tissue elimination) half-life; V_{ss}, volume of distribution at steady state

TABLE 24–2 ■ SOME PHARMACOLOGICAL EFFECTS OF PARENTERAL ANESTHETICS[a]

DRUG	CBF	CMRo$_2$	ICP	MAP	HR	CO	RR	$\dot{V}_E$
Propofol	– – –	– – –	– – –	– –	+	–	– –	– – –
Etomidate	– – –	– – –	– – –	0	0	0	–	–
Ketamine	++	0	++	+	++	+	0	0
Thiopental	– – –	– – –	– – –	–	+	–	–	– – –

[a] Typical effects of a single induction dose in humans; see text for references. Qualitative scale from – – – to +++ signifies slight, moderate, or large decrease or increase, respectively; 0 indicates no significant change. CBF, cerebral blood flow; CMRo$_2$, cerebral metabolic rate of O_2 consumption; ICP, intracranial pressure; MAP, mean arterial pressure; HR, heart rate; CO, cardiac output; RR, respiratory rate; $\dot{V}_E$, minute ventilation.

or for children undergoing short, painful procedures. *Thiopental* has a long-established track record of safety; however, clinical use is limited currently by availability.

Pharmacokinetic Principles

Parenteral anesthetics are small, hydrophobic, substituted aromatic or heterocyclic compounds (Figure 24–2). Hydrophobicity is the key factor governing their pharmacokinetics. After a single intravenous bolus, these drugs preferentially partition into the highly perfused and lipophilic tissues of the brain and spinal cord, where they produce anesthesia within a single circulation time. Subsequently, blood levels fall rapidly, resulting in drug redistribution out of the CNS back into the blood. The anesthetic then diffuses into less-perfused tissues, such as muscle and viscera, and at a slower rate into the poorly perfused but very hydrophobic adipose tissue. Termination of anesthesia after single boluses of parenteral anesthetics primarily reflects redistribution out of the CNS rather than metabolism (see Figure 2–4).

After redistribution, anesthetic blood levels fall according to a complex interaction between the metabolic rate and the amount and lipophilicity of the drug stored in the peripheral compartments. Thus, parenteral anesthetic half-lives are "context sensitive," and the degree to which a $t_{1/2}$ is contextual varies greatly from drug to drug, as might be predicted based on their differing hydrophobicities and metabolic clearances (Figure 24–3; Table 24–1). For example, after a single bolus of *thiopental*, patients usually emerge from anesthesia within 10 min; however, a patient may require more than a day to awaken from a prolonged *thiopental* infusion. Most individual variability in sensitivity to parenteral anesthetics can be accounted for by pharmacokinetic factors. For example, in patients with lower cardiac output, the relative perfusion of the brain and the fraction of anesthetic dose delivered to the brain are higher; thus, patients in septic shock or with cardiomyopathy usually require lower doses of parenteral anesthetics. The elderly also typically require a smaller parenteral anesthetic dose, primarily because of a smaller initial volume of distribution.

Specific Parenteral Agents

Propofol

Propofol is the most used parenteral anesthetic in the U.S. The clinical pharmacological properties of *propofol* are summarized in Table 24–1.

The active ingredient in *propofol*, 2,6-diisopropylphenol, is an oil at room temperature and insoluble in aqueous solutions. *Propofol* is formulated for intravenous administration as a 1% (10-mg/mL) emulsion in 10% soybean oil, 2.25% glycerol, and 1.2% purified egg phosphatide. In the U.S., disodium EDTA (0.05 mg/mL) or sodium metabisulfite (0.25 mg/mL) is added to inhibit bacterial growth. *Propofol* should be administered within 4 h of its removal from sterile packaging; unused drug should be discarded. The lipid emulsion formulation of *propofol* is associated with significant pain on injection and with hyperlipidemia.

Clinical Use and ADME. The induction dose of *propofol* in a healthy adult is 2 to 2.5 mg/kg. Dosages should be reduced in the elderly and in the presence of other sedatives and increased in young children. Because of its reasonably short elimination $t_{1/2}$, *propofol* often is used for maintenance of anesthesia as well as for induction. For short procedures, small boluses (10%–50% of the induction dose) every 5 min or as needed are effective. An infusion of *propofol* (50–200 μg/kg/min) produces a more stable drug level and is better suited for longer-term anesthetic maintenance. Sedating doses of *propofol* are 20% to 50% of those required for general anesthesia.

Propofol has a context-sensitive $t_{1/2}$ of about 10 min with an infusion lasting 3 h and about 30 min for infusions lasting up to 8 h (see Figure 24–3). *Propofol*'s shorter duration of action after infusion can be explained by its very high clearance, coupled with the slow diffusion of drug from the peripheral to the central compartment. *Propofol* is metabolized in the liver by conjugation to sulfate and glucuronide to less-active metabolites that are renally excreted. *Propofol* is highly protein bound, and its pharmacokinetics, like those of the barbiturates, may be affected by conditions that alter serum protein levels. Clearance of *propofol* is reduced in the elderly. In neonates, *propofol* clearance is also reduced. By contrast, in young children, a more rapid clearance in combination with a larger central volume may necessitate larger doses of *propofol* for induction and maintenance of anesthesia.

Side Effects

Nervous System. The sedation and hypnotic actions of *propofol* are mediated by its action on GABA$_A$ receptors; agonism at these receptors results in an increased Cl^- conduction and hyperpolarization of neurons. *Propofol* suppresses the electroencephalogram (EEG) and, in sufficient doses, can produce burst suppression of the EEG. *Propofol* decreases the cerebral metabolic rate of O_2 consumption (CMRo$_2$), CBF, and intracranial and intraocular pressures by about the same amount as *thiopental*. *Propofol* can be used in patients at risk for cerebral ischemia; however, no human outcome studies have been performed to determine its efficacy as a neuroprotectant.

Cardiovascular System. *Propofol* produces a dose-dependent decrease in blood pressure that is significantly greater than that produced by *thiopental*. The fall in blood pressure can be explained by both vasodilation and possibly mild depression of myocardial contractility. *Propofol* appears to blunt the baroreceptor reflex and reduce sympathetic nerve activity. *Propofol* should be used with caution in patients at risk for, or intolerant of, decreases in blood pressure.

Respiratory System. *Propofol* produces a slightly greater degree of respiratory depression than *thiopental*. Patients given *propofol* should be monitored to ensure adequate oxygenation and ventilation. *Propofol* appears to

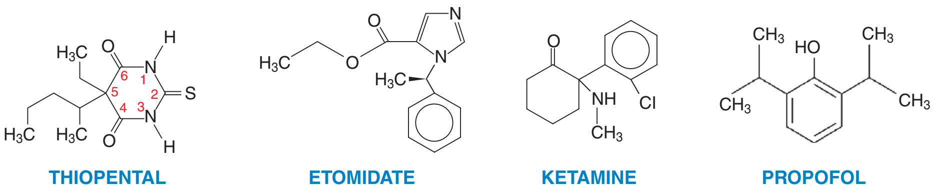


Figure 24–2 *Structures of some parenteral anesthetics.*

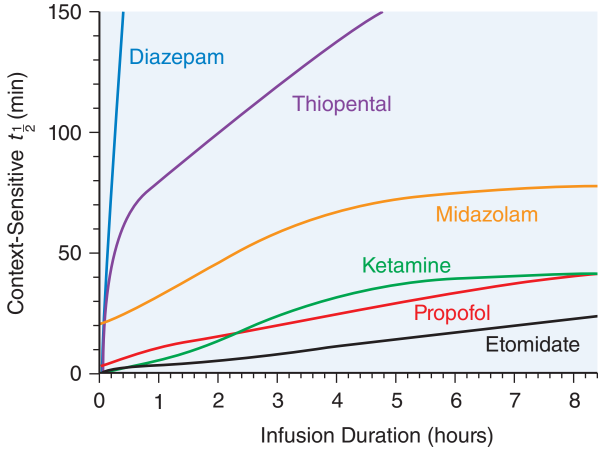


Figure 24–3 *Context-sensitive half-time of general anesthetics.* The duration of action of single intravenous doses of anesthetic/hypnotic drugs is similarly short for all and is determined by redistribution of the drugs away from their active sites (see Figure 2–4). However, after prolonged infusions, drug half-lives and durations of action are dependent on a complex interaction between the rate of redistribution of the drug, the amount of drug accumulated in fat, and the drug's metabolic rate. This phenomenon has been termed the *context-sensitive half-time*; that is, the $t_{1/2}$ of a drug can be estimated only if one knows the context—the total dose and over what time period it has been given. Note that the half-times of some drugs such as *etomidate*, *propofol*, and *ketamine* increase only modestly with prolonged infusions; others (e.g., *diazepam* and *thiopental*) increase dramatically. (Reproduced with permission from Reves JG, Glass PSA, Lubarsky DA, et al. Intravenous anesthetics. In: Miller RD, et al., eds. *Miller's Anesthesia*. 7th ed. Churchill Livingstone, Philadelphia, **2010**, 718. Copyright © Elsevier.)

be less likely than barbiturates to provoke bronchospasm and may be the induction agent of choice in patients with asthma. The bronchodilator properties of *propofol* may be attenuated by the metabisulfite preservative in some *propofol* formulations.

Other Side Effects. *Propofol* has a significant antiemetic action. *Propofol* elicits pain on injection that can be reduced with *lidocaine* and the use of larger arm and antecubital veins. A rare but potentially fatal complication, *propofol infusion syndrome* (PRIS), has been described primarily in prolonged, higher-dose infusions of *propofol* in young or head-injured patients (Kam and Cardone, 2007). PRIS is characterized by metabolic acidosis, hyperlipidemia, rhabdomyolysis, and liver enlargement.

Etomidate

Etomidate is a substituted imidazole that is supplied as the active d-isomer. *Etomidate* is poorly soluble in water and is formulated as a 2-mg/mL solution in 35% propylene glycol. Unlike *thiopental*, *etomidate* does not induce precipitation of neuromuscular blockers or other drugs frequently given during anesthetic induction.

Clinical Use and ADME.

Etomidate is primarily used for anesthetic induction of patients at risk for hypotension. Induction doses of *etomidate* (see Table 24–1) are accompanied by a high incidence of pain on injection and myoclonic movements. *Lidocaine* effectively reduces the pain of injection, while myoclonic movements can be reduced by premedication with either benzodiazepines or opiates. *Etomidate* is pharmacokinetically suitable for off-label infusion for anesthetic maintenance (10 μg/kg/min) or sedation (5 μg/kg/min); however, long-term infusions are not recommended because of side effects.

An induction dose of *etomidate* has a rapid onset; redistribution limits the duration of action. Metabolism occurs in the liver, primarily to inactive compounds. Elimination is both renal (78%) and biliary (22%). Compared to *thiopental*, the duration of action of *etomidate* increases less with repeated doses (see Figure 24–3).

Side Effects

Nervous System. *Etomidate* produces hypnosis and has no analgesic effects. The effects of *etomidate* on CBF, metabolism, and intracranial and intraocular pressures are similar to those of *thiopental* (without dropping mean arterial blood pressure). *Etomidate* produces increased EEG activity in epileptogenic foci and has been associated with seizures.

Cardiovascular System. Cardiovascular stability after induction is a major advantage of *etomidate* over either *propofol* or barbiturates. Induction doses of *etomidate* typically produce a small increase in heart rate and little or no decrease in blood pressure or cardiac output. *Etomidate* has little effect on coronary perfusion pressure while reducing myocardial O_2 consumption.

Respiratory and Other Side Effects. The degree of respiratory depression due to *etomidate* appears to be less than that due to *thiopental*. Like *methohexital*, *etomidate* may induce hiccups; it does not significantly stimulate histamine release. *Etomidate* has been associated with nausea and vomiting. The drug also inhibits adrenal biosynthetic enzymes required for the production of cortisol and some other steroids. Although the hemodynamic profile of *etomidate* may be advantageous, potential negative effects on steroid synthesis raise concerns about its use in trauma and critically ill patients (van den Heuvel et al., 2013) and obviate *etomidate* use for long-term infusion. A rapidly metabolized and ultra-short-acting analogue, *methoxycarbonyl-etomidate*, retains the favorable pharmacological properties of *etomidate* but does not produce adrenocortical suppression after bolus dosing (Cotton and Claing, 2009).

Ketamine

Ketamine is an arylcyclohexylamine and congener of *phencyclidine*. *Ketamine* is supplied as a mixture of the R+ and S– isomers even though the S– isomer is more potent and has fewer side effects. Although more lipophilic than *thiopental*, *ketamine* is water-soluble.

Clinical Use and ADME.

Ketamine is useful for anesthetizing patients at risk for hypotension and bronchospasm and for certain pediatric procedures. However, significant side effects limit its routine use. *Ketamine* rapidly produces a hypnotic state quite distinct from that of other anesthetics. Patients have profound analgesia, unresponsiveness to commands, and amnesia but may have their eyes open, move their limbs involuntarily, and breathe spontaneously. This cataleptic state has been termed *dissociative anesthesia*. The administration of *ketamine* has been shown to reduce the development of tolerance to long-term opioid use. *Ketamine* typically is administered intravenously but also is effective by intramuscular, oral, and rectal routes. *Ketamine* does not elicit pain on injection or true excitatory behavior as described for *methohexital*, although involuntary movements produced by *ketamine* can be mistaken for anesthetic excitement. Low-dose *ketamine* has potential use in depression (Rasmussen et al., 2013). Subanesthetic doses of *ketamine* can desensitize central pain pathways and modulate opioid receptors. It may be used perioperatively to reduce opioid requirements as part of a multimodal analgesic regimen (Bell et al., 2006). Currently, *ketamine* is also being explored as an antidepressant (see Figure 18–2).

The onset and duration of an induction dose of *ketamine* are determined by the same distribution/redistribution mechanisms operant for all the other parenteral anesthetics. *Ketamine* is metabolized to norketamine by hepatic CYPs (mainly by 3A4; less by 2B6 and 2D9). Norketamine, with approximately 20% of the activity of *ketamine*, is hydroxylated and excreted in urine and bile. *Ketamine's* large volume of distribution and rapid clearance make it suitable for continuous infusion (see Table 24–1 and Figure 24–3).

Side Effects

Nervous System. *Ketamine* has indirect sympathomimetic activity and produces distinct behavioral effects. The *ketamine*-induced cataleptic state is accompanied by nystagmus with pupillary dilation, salivation, lacrimation, and spontaneous limb movements with increased overall muscle tone. Patients are amnestic and unresponsive to painful stimuli. *Ketamine* produces profound analgesia, a distinct advantage over other parenteral anesthetics. Unlike other parenteral anesthetics, *ketamine* increases CBF and intracranial pressure (ICP) with minimal alteration of cerebral metabolism. The effects of *ketamine* on CBF can be readily attenuated by the simultaneous administration of sedative-hypnotic agents.

Emergence delirium, characterized by hallucinations, vivid dreams, and delusions is a frequent complication of *ketamine* that can result in serious patient dissatisfaction and can complicate postoperative management. Benzodiazepines reduce the incidence of emergence delirium.

Cardiovascular System. Unlike other anesthetics, induction doses of *ketamine* typically increase blood pressure, heart rate, and cardiac output. The cardiovascular effects are indirect and are most likely mediated by inhibition of both central and peripheral catecholamine reuptake. *Ketamine* has direct negative inotropic and vasodilating activity, but these effects usually are overwhelmed by the indirect sympathomimetic action. Thus, *ketamine* is a useful drug, along with *etomidate*, for patients at risk for hypotension during anesthesia. However, it is important to note that in chronically ill patients with depleted sympathetic tone, the direct negative inotropic and vasodilating actions of *ketamine* may be unmasked. While not arrhythmogenic, *ketamine* increases myocardial O_2 consumption and is not an ideal drug for patients at risk for myocardial ischemia.

Respiratory System. The respiratory effects of *ketamine* are perhaps the best indication for its use. Induction doses of *ketamine* produce small and transient decreases in minute ventilation, but respiratory depression is less severe than with other parenteral anesthetics. *Ketamine* is a potent bronchodilator and is particularly well suited for anesthetizing patients at high risk for bronchospasm.

Barbiturates

Barbiturates are derivatives of barbituric acid with either an oxygen or a sulfur at the 2-position (see Figure 24–2 and Chapters 20 and 22). The three barbiturates most commonly used in clinical anesthesia are *sodium thiopental* (not currently marketed in the U.S.), *thiamylal* (currently licensed in the U.S. only for veterinary use), and *methohexital*. *Sodium thiopental* was used most frequently for inducing anesthesia.

Barbiturates are supplied as racemic mixtures despite enantioselectivity in their anesthetic potency. Barbiturates are formulated as the sodium salts with 6% sodium carbonate and reconstituted in water or isotonic saline to alkaline solutions ($10 < \text{pH} < 11$). *Mixing barbiturates with drugs in acidic solutions during anesthetic induction can result in precipitation of the barbiturate as the free acid; thus, standard practice is to delay the administration of other drugs until the barbiturate has cleared the intravenous tubing.*

The pharmacological properties and other therapeutic uses of the barbiturates are presented in Chapter 22. Table 22–3 lists the common barbiturates with their clinical pharmacological properties.

Clinical Use and ADME. Recommended intravenous dosing for parenteral barbiturates in a healthy young adult is given in Table 24–1. The availability of *thiopental* is limited currently by the lack of an FDA-licensed product and the prohibition of its import due to controversy over its use in administration of the death penalty by lethal injection.

The principal mechanism limiting anesthetic duration after single doses is redistribution of these hydrophobic drugs from the brain to other tissues. However, after multiple doses or infusions, the duration of action of the barbiturates varies considerably depending on their clearances. See Table 24–1 for pharmacokinetic parameters.

Methohexital differs from the other two intravenous barbiturates in its much more rapid clearance; thus, it accumulates less during prolonged infusions. Because of their slow elimination and large volumes of distribution, prolonged infusions or very large doses of *thiopental* and *thiamylal* can produce unconsciousness lasting several days. All three barbiturates are primarily eliminated by hepatic metabolism and subsequent renal excretion of inactive metabolites; a small fraction of *thiopental* undergoes desulfuration to the longer-acting hypnotic *pentobarbital*. Hepatic disease or other conditions that reduce serum protein concentrations will increase the initial free concentration and hypnotic effect of an induction dose.

Side Effects

Nervous System. Barbiturates suppress the EEG and can produce EEG burst suppression. They reduce the CMR, as measured by $CMRo_2$, in a dose-dependent manner. As a consequence of the decrease in $CMRo_2$, CBF and ICP are similarly reduced. Presumably, their CNS depressant activity contributes to their anticonvulsant effects (see Chapter 20). *Methohexital* can increase ictal activity, and seizures have been described in patients who received doses sufficient to produce burst suppression of the EEG, properties that make *methohexital* a good choice for anesthesia in patients who undergo electroconvulsive therapy.

Cardiovascular System. The anesthetic barbiturates produce dose-dependent decreases in blood pressure. The effect is due primarily to vasodilation, particularly venodilation, and to a lesser degree to a direct decrease in cardiac contractility. Typically, heart rate increases as a compensatory response to a lower blood pressure, although barbiturates also blunt the baroreceptor reflex. *Thiopental* maintains the ratio of myocardial O_2 supply to demand in patients with coronary artery disease within a normal blood pressure range. Hypotension can be severe in patients with an impaired ability to compensate for venodilation, such as those with hypovolemia, cardiomyopathy, valvular heart disease, coronary artery disease, cardiac tamponade, or β adrenergic blockade. None of the barbiturates has been shown to be arrhythmogenic.

Respiratory System. Barbiturates are respiratory depressants. Induction doses of *thiopental* decrease minute ventilation and tidal volume, with a smaller and inconsistent decrease in respiratory rate. Reflex responses to hypercarbia and hypoxia are diminished by anesthetic barbiturates; at higher doses or in the presence of other respiratory depressants such as opiates, apnea can result. Compared to *propofol*, barbiturates produce a higher incidence of wheezing in asthmatics, attributed to histamine release from mast cells during induction of anesthesia.

Other Side Effects. Short-term administration of barbiturates has no clinically significant effect on the hepatic, renal, or endocrine systems. True allergies to barbiturates are rare; however, direct drug-induced histamine release is occasionally seen. Barbiturates can induce fatal attacks of porphyria in patients with acute intermittent or variegate porphyria and are contraindicated in such patients. *Methohexital* can produce pain on injection to a greater degree than *thiopental*. Inadvertent intra-arterial injection of thiobarbiturates can induce a severe inflammatory and potentially necrotic reaction that can threaten limb survival. *Methohexital*, and to a lesser degree other barbiturates, can produce excitatory symptoms on induction, such as cough, hiccup, muscle tremors, twitching, and hypertonus.

Novel Parenteral Anesthetics

Remimazolam

Remimazolam is an intravenous sedative-hypnotic that was recently approved by the FDA. The basic chemical structure is that of *midazolam*, with an attached carboxylic ester linkage. The ester linkage results in rapid metabolism by nonspecific esterases in the plasma, much like what occurs with *remifentanil*. The drug therefore combines the pharmacological effects of *midazolam* and metabolic kinetics of *remifentanil*. *Remimazolam* is administered for procedural sedation. Like other benzodiazepines, *remimazolam* is an agonist of the $GABA_A$ receptor. At low doses, the drug acts as an anxiolytic; higher doses result in sedation.

Like other intravenously administered sedative-hypnotics, the distribution of *remimazolam* is highly dependent on cardiac output, with the majority of drug being distributed to the vessel-rich regions. *Remimazolam* is rapidly metabolized via ester hydrolysis, a process that follows first-order kinetics, and does not become saturated at clinically used concentrations of the drug. There is no accumulation of drug. The drug has an extremely short context sensitivity, and prolonged infusions do not result in prolonged, residual effects. Because elimination is organ-independent (occurs via plasma esterase activity), the drug can be safely used in patients with hepatic and/or renal impairment.

The recommended dose of *remimazolam* when given as a single injection for procedural sedation is 2.5 to 5 mg IV. Peak sedative effects should occur 3 to 5 min following a single IV dose. Infusion rates ranging from 1 to 5 mg/min can be used for maintenance of sedation. Alternatively, repeated boluses of 1.25 to 2.5 mg every 2 min can be given.

Use of *remimazolam* as the sole hypnotic agent as part of balanced general anesthesia is under investigation. Similarly, while the short context sensitivity of *remimazolam* may make it an ideal drug for prolonged sedation (i.e., intensive care unit sedation), there are few studies outlining its use for such an indication. Current manufacturer recommendations indicate that the drug should be used for procedures lasting 30 min or less. The side effect profile of *remimazolam* mimics that of other benzodiazepines.

Propofol Derivatives

Modifications of *propofol* and/or its emulsion are being investigated to mitigate *propofol*-induced venoirritation after a bolus. Emulsions with a higher ratio of medium-chain triglycerides to long-chain triglycerides have been shown to alleviate pain on injection.

Fospropofol is a water-soluble ester prodrug of *propofol* that is hydrolyzed by alkaline phosphatase to yield *propofol*, phosphate, and formaldehyde. It produces less pain on injection compared to *propofol*. Furthermore, since it does not contain lipid, egg products, or preservatives, it does not have the concerns of bacterial infection or hyperlipidemia associated with *propofol* emulsions. *Fospropofol* produces dose-dependent sedation and can be administered in otherwise-healthy individuals at 2 to 8 mg/kg IV (delivered either as a bolus or by a short infusion over 5–10 min). The optimum dose for sedation is about 6.5 mg/kg. This results in a loss of consciousness in about 10 min. The duration of the sedative effect is approximately 45 min. *Fospropofol* was recently discontinued for clinical use in the U.S. due to its prolonged onset of action (time to peak effect, 8–13 min), slow recovery, and significant perianal pruritis associated with its administration.

Etomidate Derivatives

Derivatives of *etomidate* have been investigated that either lesson or eliminate adrenocortical suppression. *Methoxycarbonyl etomidate* (MOC-etomidate) contains an ester moiety that is rapidly metabolized. MOC-etomidate does not produce prolonged adrenocortical suppression. Its metabolite is metabolically active and has an adrenocortical inhibition potency that is approximately 300-fold lower than its parent compound (Mahmoud and Mason, 2018).

The substitution of nitrogen on the *etomidate* imidazole ring with a methylene group creates *carboetomidate*. *Carboetomidate* maintains the same hypnotic efficacy associated with *etomidate*; however, there is an approximately 2000-fold lower adrenocortical inhibition associated with *carboetomidate*. This drug also inhibits 5HT receptors, and therefore, may also have decreased emetogenic potential.

Inhalational Anesthetics

A wide variety of gases and volatile liquids can produce anesthesia. The structures of the currently used inhalational anesthetics are shown in Figure 24–4. The inhalational anesthetics have therapeutic indices (median lethal dose [LD_{50}]/median effective dose [ED_{50}]) that range from 2 to 4, making these among the most dangerous drugs in clinical use. The toxicity of these drugs is largely a function of their side effects, and each of the inhalational anesthetics has a unique side effect profile. Hence, the selection of an inhalational anesthetic often is based on balancing a patient's pathophysiology with drug side effect profiles.

Table 24–3 lists the widely varying physical properties of the inhalational agents in clinical use. Ideally, an inhalational agent would produce rapid induction of anesthesia and rapid recovery following discontinuation.

Pharmacokinetic Principles

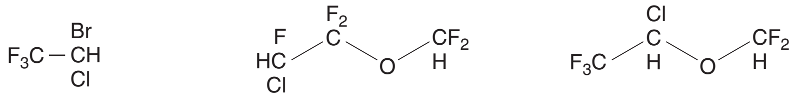
Inhalational agents behave as gases rather than as liquids and thus require different pharmacokinetic constructs for analyzing their uptake and distribution. Inhalational anesthetics distribute between tissues (or between blood and gas) such that equilibrium is achieved when the partial pressure of anesthetic gas is equal in the two tissues. When a person has breathed an inhalational anesthetic for a sufficiently long time that all tissues are equilibrated with the anesthetic, the partial pressure of the anesthetic in all tissues will be equal to the partial pressure of the anesthetic in inspired gas. While the partial pressure of the anesthetic may be equal in all tissues, the concentration of anesthetic in each tissue will be different. Indeed, anesthetic partition coefficients are defined as the ratio of anesthetic concentration in two tissues when the partial pressures of anesthetic are equal in the two tissues. Blood:gas, brain:blood, and fat:blood partition coefficients for the various inhalational agents are listed in Table 24–3. These partition coefficients show that inhalational anesthetics are more soluble in some tissues (e.g., fat) than they are in others (e.g., blood). In clinical practice, equilibrium is achieved when the partial pressure in inspired gas is equal to the partial pressure in end-tidal (alveolar) gas. For inhalational agents that are not very soluble in blood or any other tissue, equilibrium is achieved quickly, as illustrated for nitrous oxide in Figure 24–5. If an agent is more soluble in a tissue such as fat, equilibrium may take many hours to reach. This occurs because fat represents a huge anesthetic reservoir that will be filled slowly because of the modest blood flow to fat. Anesthesia is produced when anesthetic partial pressure in brain is equal to or greater than MAC. Because the brain is well perfused, anesthetic partial pressure in brain becomes equal to the partial pressure in alveolar gas (and in blood) over the course of several minutes. Therefore, anesthesia is achieved shortly after alveolar partial pressure reaches MAC.

Elimination of inhalational anesthetics is largely a reversal of uptake. For inhalational agents with high blood and tissue solubility, recovery will be a function of the duration of anesthetic administration. This occurs because the accumulated amounts of anesthetic in the fat reservoir will prevent blood (and therefore alveolar) partial pressures from falling rapidly. Patients will be arousable when alveolar partial pressure reaches MAC_{awake}, a partial pressure somewhat lower than MAC (see Table 24–3).

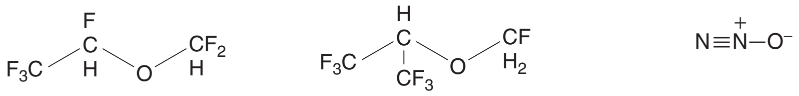
Specific Inhalational Agents

Isoflurane

Isoflurane is a volatile liquid at room temperature and is neither flammable nor explosive in mixtures of air or O_2. *Isoflurane* is a commonly used inhalational anesthetic worldwide.



Halothane **Enflurane** **Isoflurane**



Desflurane **Sevoflurane** **Nitrous Oxide**

Figure 24–4 *Structures of inhalational general anesthetics.* Note that all inhalational general anesthetic agents except *nitrous oxide* and *halothane* are ethers and that fluorine replaces chlorine in the development of the halogenated agents. All structural differences are associated with important differences in pharmacological properties.

TABLE 24–3 ■ PROPERTIES OF INHALATIONAL ANESTHETIC AGENTS

AGENT	MAC[a] (vol%)	MAC_{AWAKE}[b] (vol%)	VAPOR PRESSURE (mmHg, 20°C)	PARTITION COEFFICIENT AT 37°C: BLOOD/GAS	PARTITION COEFFICIENT AT 37°C: BRAIN/BLOOD (Brain/Gas)	PARTITION COEFFICIENT AT 37°C: FAT/BLOOD (Fat/Gas)	% RECOVERED AS METABOLITES
Isoflurane[c]	1.05–1.28	0.4	238	1.43	2.6	45 (91)	0.17
Enflurane	1.68	0.4	175	1.91	1.4	36 (98)	2.4
Sevoflurane	1.4–3.3	0.6	157	0.63–0.69	1.7 (1.2)	48 (50)	3.5
Desflurane	5.2–9.2	2.4	669	0.424	1.3 (0.54)	27 (19)	<0.02
N_2O[c]	105	60.0	Gas	0.47	1.1	2.3	0.004
Xe	55–71	32.6	Gas	0.115	—	(1.9)	0

[a] MAC values are expressed as volume percent, the percentage of the atmosphere that is anesthetic. An MAC value greater than 100% means that hyperbaric conditions would be required.

[b] MAC_{AWAKE} is the concentration at which appropriate responses to commands are lost.

[c] EC_{50} for memory suppression (vol%): isoflurane, 0.24; N_2O, 52.5; values not available for other agents.

Clinical Use and ADME. *Isoflurane* is typically used for maintenance of anesthesia *after induction* with other agents because of its pungent odor. Induction of anesthesia can be achieved in less than 10 min with an inhaled concentration of 1.5% to 3% *isoflurane* in O_2; this concentration is reduced to 1% to 2% (~1–2 MAC) for maintenance of anesthesia. The use of adjunct agents such as opioids or nitrous oxide reduces the concentration of *isoflurane* required for surgical anesthesia.

Isoflurane has a blood:gas partition coefficient substantially lower than that of *enflurane*. Consequently, induction with *isoflurane* and recovery from *isoflurane* are relatively faster. More than 99% of inhaled *isoflurane* is excreted unchanged by the lungs. *Isoflurane* does not appear to be a mutagen, teratogen, or carcinogen.

Side Effects

Cardiovascular System. *Isoflurane* produces a concentration-dependent decrease in arterial blood pressure; cardiac output is well maintained; hypotension is the result of decreased systemic vascular resistance. *Isoflurane* produces vasodilation in most vascular beds, with pronounced effects in skin and muscle, and is a potent coronary vasodilator, simultaneously producing increased coronary blood flow and decreased myocardial O_2 consumption. *Isoflurane* significantly attenuates baroreceptor function. Patients anesthetized with *isoflurane* generally have mildly elevated heart rates as a compensatory response to reduced blood pressure; however, rapid changes in *isoflurane* concentration can produce both transient tachycardia and hypertension due to *isoflurane*-induced sympathetic stimulation.

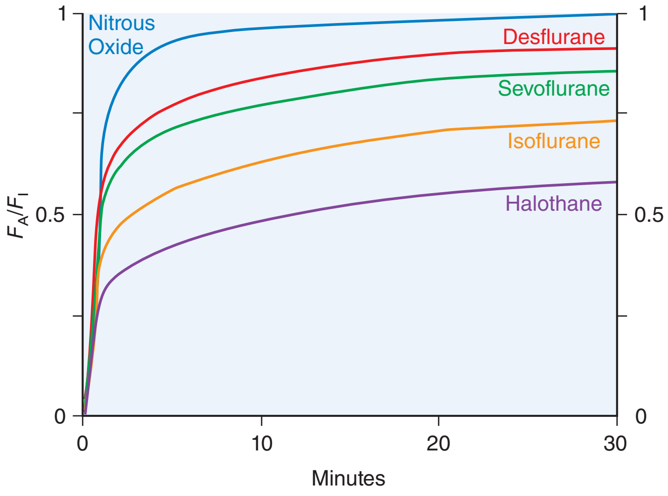


Figure 24–5 *Uptake of inhalational general anesthetics.* The rise in end-tidal alveolar (F_A) anesthetic concentration toward the inspired (F_I) concentration is most rapid with the least-soluble anesthetics (*nitrous oxide* and *desflurane*) and slowest with the most soluble anesthetic, *halothane*. All data are from human studies. (Reproduced with permission from Eger EI II. Inhaled anesthetics: uptake and distribution. In: Miller RD et al., eds. *Miller's Anesthesia*. 7th ed. Churchill Livingstone, Philadelphia, **2010**, 540. Copyright © Elsevier.)

Respiratory System. *Isoflurane* produces concentration-dependent depression of ventilation. This drug is particularly effective at depressing the ventilatory response to hypercapnia and hypoxia. Although *isoflurane* is a bronchodilator, it also is an airway irritant and can stimulate airway reflexes during induction of anesthesia, producing coughing and laryngospasm.

Nervous System. *Isoflurane* dilates the cerebral vasculature, producing increased CBF (Drummond et al., 1983). There is a modest risk of an increase in ICP in patients with preexisting intracranial hypertension. *Isoflurane* reduces $CMRo_2$ in a dose-dependent manner.

Muscle. *Isoflurane* produces some relaxation of skeletal muscle by its central effects. It also enhances the effects of both depolarizing and nondepolarizing muscle relaxants. Like other halogenated inhalational anesthetics, *isoflurane* relaxes uterine smooth muscle and is not recommended for analgesia or anesthesia for labor and vaginal delivery.

Kidney. *Isoflurane* reduces renal blood flow and glomerular filtration rate (GFR), resulting in a small volume of concentrated urine.

Liver and Gastrointestinal Tract. Splanchnic and hepatic blood flows are reduced with increasing doses of *isoflurane* as systemic arterial pressure decreases. There are no reported incidences of hepatic toxicity.

Sevoflurane

Sevoflurane is a clear, colorless, volatile liquid at room temperature and must be stored in a sealed bottle. It is nonflammable and nonexplosive in mixtures of air or O_2. However, *sevoflurane* can undergo an exothermic reaction with desiccated CO_2 absorbent to produce airway burns or spontaneous ignition, explosion, and fire. *Sevoflurane must not be used with an anesthesia machine in which the CO_2 absorbent has been dried by prolonged gas flow through the absorbent. The reaction of sevoflurane with desiccated CO_2 absorbent also can produce CO, which can result in serious patient injury.*

Clinical Use and ADME. *Sevoflurane* is widely used for maintenance of anesthesia after intravenous induction. *Sevoflurane* is the preferred volatile anesthetic for inhalational induction of anesthesia due to its pleasant smell, rapid onset, and lack of irritation to the airway. Thus, it has largely replaced *halothane* (not available in the U.S.) as the preferred agent for anesthetic induction in adult and pediatric patients. Induction of anesthesia is rapidly achieved using inhaled concentrations of 2% to 4% *sevoflurane*. *Sevoflurane* has properties that make it an ideal induction agent.

The low solubility of *sevoflurane* in blood and other tissues provides for rapid induction of anesthesia and rapid changes in anesthetic depth following changes in delivered concentration. Approximately 5% of absorbed *sevoflurane* is metabolized by hepatic CYP2E1, with the predominant product being hexafluoroisopropanol. Hepatic metabolism of *sevoflurane* also produces inorganic fluoride. Interaction of *sevoflurane* with soda lime produces decomposition products that may be toxic, such as compound A, pentafluoroisopropenyl fluoromethyl ether (see kidney discussion under Side Effects).

Side Effects

Cardiovascular System. *Sevoflurane* produces concentration-dependent decreases in arterial blood pressure (due to systemic vasodilation) and cardiac output. *Sevoflurane* does not produce tachycardia and thus may be a preferable agent in patients prone to myocardial ischemia.

Respiratory System. *Sevoflurane* produces a concentration-dependent reduction in tidal volume and increase in respiratory rate in spontaneously breathing patients. The increased respiratory frequency does not compensate for reduced tidal volume, with the net effect being a reduction in minute ventilation and an increase in $Paco_2$. *Sevoflurane* is not irritating to the airway and is a potent bronchodilator. As a result, *sevoflurane* is the most effective clinical bronchodilator of the inhalational anesthetics.

Nervous System. *Sevoflurane* produces effects on cerebral vascular resistance, $CMRo_2$, and CBF that are similar to those produced by *isoflurane* and *desflurane*. *Sevoflurane* can increase ICP in patients with poor intracranial compliance, the response to hypocapnia is preserved during sevoflurane anesthesia, and increases in ICP can be prevented by hyperventilation. In children, *sevoflurane* is associated with delirium on emergence from anesthesia. This delirium is short lived and without any reported adverse long-term sequelae.

Muscle. *Sevoflurane* produces skeletal muscle relaxation and enhances the effects of nondepolarizing and depolarizing neuromuscular blocking agents.

Kidney. Controversy has surrounded the potential nephrotoxicity of compound A, which is produced by interaction of *sevoflurane* with the CO_2 absorbent soda lime. Biochemical evidence of transient renal injury has been reported in human volunteers (Eger et al., 1997). Large clinical studies have shown no evidence of increased serum creatinine, blood urea nitrogen, or any other evidence of renal impairment following *sevoflurane* administration. *The FDA recommends that sevoflurane be administered with fresh gas flows of 1 to 2 L/min, with sevoflurane exposures not exceeding 2 MAC-hours to minimize exposure to compound A*.

Liver and Gastrointestinal Tract. *Sevoflurane* is not known to cause hepatotoxicity or alterations of hepatic function tests.

Desflurane

Desflurane is a highly volatile liquid at room temperature (vapor pressure = 669 mmHg) and must be stored in tightly sealed bottles. Delivery of a precise concentration of *desflurane* requires the use of a specially heated vaporizer that delivers pure vapor that then is diluted appropriately with other gases (O_2, air, or N_2O). *Desflurane* is nonflammable and nonexplosive in mixtures of air or O_2.

Clinical Use and ADME. *Desflurane* irritates the tracheobronchial tree and can provoke coughing, salivation, and bronchospasm. Anesthesia therefore usually is induced with an intravenous agent, with *desflurane* subsequently administered for maintenance of anesthesia. Maintenance of anesthesia usually requires inhaled concentrations of 6% to 8% (~1 MAC). Lower concentrations of *desflurane* are required if it is coadministered with nitrous oxide or opioids.

Desflurane has a very low blood:gas partition coefficient (0.42) and also is not very soluble in fat or other peripheral tissues. Thus, the alveolar and blood concentrations rapidly rise to the level of inspired concentration, providing rapid induction of anesthesia and rapid changes in depth of anesthesia following changes in the inspired concentration. Emergence from *desflurane* anesthesia also is rapid. *Desflurane* is minimally metabolized; more than 99% of absorbed *desflurane* is eliminated unchanged through the lungs.

Side Effects

Cardiovascular System. *Desflurane* produces hypotension primarily by decreasing systemic vascular resistance. Cardiac output is well preserved, as is blood flow to the major organ beds (splanchnic, renal, cerebral, and coronary) (Eger, 1994). Transient tachycardia is often noted with abrupt increases in *desflurane*'s delivered concentration, a result of this *desflurane*-induced stimulation of the sympathetic nervous system. The hypotensive effects of *desflurane* do not wane with increasing duration of administration.

Respiratory System. *Desflurane* causes a concentration-dependent increase in respiratory rate and a decrease in tidal volume. At low concentrations (<1 MAC), the net effect is to preserve minute ventilation. *Desflurane* concentrations greater than 1 MAC depress minute ventilation, resulting in elevated arterial CO_2 tension ($Paco_2$). *Desflurane* is a bronchodilator. However, it also is a strong airway irritant and can cause coughing, breath-holding, laryngospasm, and excessive respiratory secretions. *Because of its irritant properties, desflurane is not used as the primary anesthetic for induction of anesthesia.*

Nervous System. *Desflurane* decreases cerebral vascular resistance and $CMRo_2$. Burst suppression of the EEG is achieved with approximately 2 MAC; at this level, $CMRo_2$ is reduced by approximately 50%. Under conditions of normocapnia and normotension, *desflurane* produces an increase in CBF and can increase ICP in patients with poor intracranial compliance. The vasoconstrictive response to hypocapnia is preserved during *desflurane* anesthesia; increases in ICP thus can be prevented by hyperventilation.

Muscle, Kidney, Liver, and Gastrointestinal Tract. *Desflurane* produces direct skeletal muscle relaxation as well as enhances the effects of nondepolarizing and depolarizing neuromuscular blocking agents. Consistent with its minimal metabolic degradation, *desflurane* has no reported nephrotoxicity or hepatotoxicity.

Desflurane and Carbon Monoxide. Inhaled anesthetics are administered via a system that permits unidirectional flow of gas and rebreathing of exhaled gases. To prevent rebreathing of CO_2 (which can lead to hypercarbia), CO_2 absorbers are incorporated into the anesthesia delivery circuits. With almost complete desiccation of the CO_2 absorbents, substantial quantities of carbon monoxide can be produced. This effect is greatest with *desflurane* and can be prevented by the use of well-hydrated, fresh CO_2 absorbent.

Halothane

Halothane is a volatile liquid at room temperature and must be stored in a sealed container. Because *halothane* is a light-sensitive compound, it is marketed in amber bottles with thymol added as a preservative. Mixtures of *halothane* with O_2 or air are neither flammable nor explosive.

Clinical Use and ADME. *Halothane* has been used for maintenance of anesthesia. Concerns over hepatic toxicity have limited its use in developed countries. *Halothane* can produce fulminant hepatic necrosis (*halothane hepatitis*) in approximately 1 in 10,000 patients receiving *halothane* and "is referred to as halothane *hepatitis*" (Summary of the National Halothane Study, 1966). This syndrome (with a 50% fatality rate) is characterized by fever, anorexia, nausea, and vomiting, developing several days after anesthesia, and can be accompanied by a rash and peripheral eosinophilia. *Halothane* hepatitis may be the result of an immune response to hepatic proteins that become trifluoroacetylated as a consequence of *halothane* metabolism. *Halothane* has a low cost and is still widely used in developing countries. Due to its side effect profile and the availability of safer agents with more favorable pharmacokinetic profiles, *halothane* is no longer marketed in the U.S. Those interested in further information on *halothane* should consult previous recent editions of this book.

Enflurane

Enflurane is a clear, colorless liquid at room temperature and has a mild, sweet odor. Like other inhalational anesthetics, it is volatile and must be stored in a sealed bottle. It is nonflammable and nonexplosive in mixtures of air or oxygen.

Clinical Use and ADME. *Enflurane* is primarily utilized for maintenance rather than induction of anesthesia. Surgical anesthesia can be induced with *enflurane* in less than 10 min with an inhaled concentration of 2%

to 4.5% in oxygen and maintained with concentrations from 0.5% to 3%. *Enflurane* concentrations required to produce anesthesia are reduced when it is coadministered with nitrous oxide or opioids. Concerns over *enflurane*'s ability to decrease seizure threshold and potentially produce nephrotoxicity have limited its clinical utility in developed countries (Mazze et al., 1977).

Because of its relatively high blood:gas partition coefficient, induction of anesthesia and recovery from *enflurane* are relatively slow. *Enflurane* is metabolized to a modest extent, with 2% to 8% of absorbed *enflurane* undergoing oxidative metabolism by hepatic CYP2E1. Fluoride ions are a by-product of *enflurane* metabolism, but plasma fluoride levels are low and nontoxic. Patients taking *isoniazid* exhibit enhanced metabolism of *enflurane* with consequent elevation of serum fluoride.

Side Effects. *Enflurane* causes a decrease in arterial blood pressure, the result of vasodilation and depression of myocardial contractility, with minimal effects on heart rate. The drug is an effective bronchodilator and produces a pattern of rapid shallow breathing. Due to its actions as a cerebral vasodilator, *enflurane* can increase ICP. It can produce seizure activity and should not be used in patients with seizure disorders. *Enflurane* relaxes skeletal and uterine muscle. As with other anesthetic gases, *enflurane* reduces renal blood flow, GFR, and urinary output.

Nitrous Oxide

Nitrous oxide is a colorless, odorless gas at room temperature. N_2O is sold in steel cylinders and must be delivered through calibrated flowmeters provided on all anesthesia machines. N_2O is neither flammable nor explosive, but it does support combustion as actively as oxygen does when it is present in proper concentration with a flammable anesthetic or material.

Clinical Use and ADME. N_2O is a weak anesthetic agent that has significant analgesic effects. Surgical anesthetic depth is only achieved under hyperbaric conditions. By contrast, analgesia is produced at concentrations as low as 20%. The analgesic property of N_2O is a function of the activation of opioidergic neurons in the periaqueductal gray matter and the adrenergic neurons in the locus ceruleus. N_2O is frequently used in concentrations of approximately 50% to provide analgesia and mild sedation in outpatient dentistry. N_2O cannot be used at concentrations above 80% because this limits the delivery of adequate O_2. Because of this limitation, N_2O is used primarily as an adjunct to other inhalational or intravenous anesthetics.

Nitrous oxide is very insoluble in blood and other tissues. This results in rapid equilibration between delivered and alveolar anesthetic concentrations and provides for rapid induction of anesthesia and rapid emergence following discontinuation of administration. The rapid uptake of N_2O from alveolar gas serves to concentrate coadministered halogenated anesthetics; this effect (the "second gas effect") speeds induction of anesthesia. On discontinuation of N_2O administration, N_2O gas can diffuse from blood to the alveoli, diluting O_2 in the lung. This can produce an effect called *diffusional hypoxia. To avoid hypoxia, 100% O_2 rather than air should be administered when N_2O is discontinued*. Almost all (99.9%) of the absorbed N_2O is eliminated unchanged by the lungs.

Side Effects

Cardiovascular System. Although N_2O produces a negative inotropic effect on heart muscle *in vitro*, depressant effects on cardiac function generally are not observed in patients because of the stimulatory effects of N_2O on the sympathetic nervous system. The cardiovascular effects of N_2O also are heavily influenced by the concomitant administration of other anesthetic agents. When N_2O is coadministered with halogenated inhalational anesthetics, one observes increases in heart rate, arterial blood pressure, and cardiac output. In contrast, when N_2O is coadministered with an opioid, one generally sees decreases in arterial blood pressure and cardiac output. N_2O also increases venous tone in both the peripheral and the pulmonary vasculature. The effects of N_2O on pulmonary vascular resistance can be exaggerated in patients with preexisting pulmonary hypertension; thus, the drug generally is not used in these patients.

Respiratory System. N_2O causes modest increases in respiratory rate and decreases in tidal volume in spontaneously breathing patients. Even modest concentrations of N_2O markedly depress the ventilatory response to hypoxia. Thus, it is prudent to monitor arterial O_2 saturation directly in patients receiving or recovering from N_2O.

Nervous System. N_2O can significantly increase CBF and ICP. This cerebral vasodilatory capacity of N_2O is significantly attenuated by the simultaneous administration of intravenous agents such as opiates and *propofol*. By contrast, the combination of N_2O and inhaled agents results in greater vasodilation than the administration of the inhaled agent alone at equivalent anesthetic depth.

Muscle. N_2O does not relax skeletal muscle and does not enhance the effects of neuromuscular blocking drugs.

Kidney, Liver, and Gastrointestinal Tract. N_2O is not known to have nephrotoxic or hepatotoxic effects.

Other Adverse Effects. A major problem with N_2O is that it will exchange with N_2 in any air-containing cavity in the body. Moreover, because of their differential blood:gas partition coefficients, N_2O will enter the cavity faster than N_2 escapes, thereby increasing the volume or pressure in this cavity. Examples of air collections that can be expanded by N_2O include a pneumothorax, an obstructed middle ear, an air embolus, an obstructed loop of bowel, an intraocular air bubble, a pulmonary bulla, and intracranial air. N_2O should be avoided in these clinical settings.

Nitrous oxide interacts with the cobalt of vitamin B_{12}, thereby preventing vitamin B_{12} from acting as a cofactor for methionine synthase (Sanders and Maze, 2007). Inactivation of methionine synthase can produce signs of vitamin B_{12} deficiency, including megaloblastic anemia and peripheral neuropathy, a particular concern in patients with malnutrition, vitamin B_{12} deficiency, or alcoholism. The clinical use of N_2O is controversial due to its potential metabolic effects related to increased homocysteine and changes in DNA and protein synthesis (Ko et al., 2014). For this reason, N_2O is not used as a chronic analgesic or as a sedative in critical care settings.

Anesthetic Adjuncts

A general anesthetic is usually given with adjuncts to augment specific components of anesthesia, permitting lower doses of general anesthetics with fewer side effects.

Benzodiazepines

Benzodiazepines (see Chapters 18 and 22) can produce anesthesia similar to that of barbiturates; they are more commonly used for sedation rather than general anesthesia because prolonged amnesia and sedation may result from anesthetizing doses. As adjuncts, benzodiazepines are used for anxiolysis, amnesia, and sedation prior to induction of anesthesia or for sedation during procedures not requiring general anesthesia. The benzodiazepine most frequently used in the perioperative period is *midazolam*, followed distantly by *diazepam* and *lorazepam*.

Midazolam is water soluble and typically is administered intravenously but also can be given orally, intramuscularly, or rectally; oral *midazolam* is particularly useful for sedation of young children. *Midazolam* produces minimal venous irritation (as opposed to *diazepam* and *lorazepam*, which are formulated in propylene glycol and are painful on injection, sometimes producing thrombophlebitis). *Midazolam* has the pharmacokinetic advantage, particularly over lorazepam, of being more rapid in onset and shorter in duration of effect. Sedative doses of *midazolam* (0.01–0.05 mg/kg IV) reach peak effect in about 2 min and provide sedation for approximately 30 min. Elderly patients tend to be more sensitive to and have a slower recovery from benzodiazepines. *Midazolam* is metabolized principally by hepatic CYP3A4, and drug interactions with inducers, inhibitors, and substrates of that CYP are predictable. Either for prolonged sedation or for general anesthetic maintenance, *midazolam* is more suitable than other benzodiazepines for infusion, although *midazolam*'s duration of action ($t_{1/2}$) does significantly increase with prolonged infusions (see Figure 24–3). Benzodiazepines reduce CBF and cerebral metabolism, but at equianesthetic doses are less effective than barbiturates in this respect. Benzodiazepines modestly decrease blood pressure and respiratory drive, occasionally resulting in apnea.

α_2 Adrenergic Agonists

Dexmedetomidine is a selective α_2 adrenergic receptor agonist (Kamibayashi and Maze, 2000) used for short-term (<24 h) sedation of critically ill adults and for sedation prior to and during surgical or other medical procedures in nonintubated patients. Activation of α_{2A} adrenergic receptors by *dexmedetomidine* produces both sedation and analgesia.

The recommended loading dose is 1 μg/kg given over 10 min, followed by infusion at a rate of 0.2 to 0.7 μg/kg/h. Reduced doses should be considered in patients with risk factors for severe hypotension. *Dexmedetomidine* is highly protein bound and is metabolized primarily in the liver; the glucuronide and methyl conjugates are excreted in the urine. Common side effects of *dexmedetomidine* include hypotension and bradycardia, attributed to decreased catecholamine release by activation peripherally and in the CNS of the α_{2A} receptor. Nausea and dry mouth also are common untoward reactions. At higher drug concentrations, the α_{2B} subtype is activated, resulting in hypertension and a further decrease in heart rate and cardiac output. *Dexmedetomidine* provides sedation and analgesia with minimal respiratory depression. However, *dexmedetomidine* does not appear to provide reliable amnesia, and additional agents may be needed.

Analgesics

Analgesics typically are administered with general anesthetics to reduce anesthetic requirements and minimize hemodynamic changes produced by painful stimuli. Nonsteroidal anti-inflammatory drugs, cyclooxygenase 2 inhibitors, and acetaminophen (see Chapter 42) sometimes provide adequate analgesia for minor surgical procedures. However, opioids are the primary analgesics used during the perioperative period because of the rapid and profound analgesia they produce. *Fentanyl*, *sufentanil*, *alfentanil*, *remifentanil*, *meperidine*, *hydromorphone*, and *morphine* are the major parenteral opioids used in the perioperative period. The primary analgesic activity of each of these drugs is produced by agonist activity at μ opioid receptors (see Chapter 23).

The choice of a perioperative opioid is based primarily on duration of action because, at appropriate doses, all produce similar analgesia and side effects. *Remifentanil* has an ultrashort duration of action (<10 min), accumulates minimally with repeated doses, and is particularly well suited for procedures that are briefly painful. Single doses of *fentanyl*, *sufentanil*, and *alfentanil* all have similar intermediate durations of action (40, 60, and 15 min, respectively), but recovery after prolonged administration varies considerably.

The frequency and severity of nausea, vomiting, and pruritus after emergence from anesthesia are increased by all opioids to about the same degree. A useful side effect of *meperidine* is its capacity to reduce shivering, a common problem during emergence from anesthesia; other opioids are not as efficacious against shivering, perhaps due to less κ receptor agonism. Finally, opioids often are administered intrathecally and epidurally for management of acute and chronic pain (see Chapter 23). Neuraxial opioids with or without local anesthetics can provide profound analgesia for many surgical procedures; however, respiratory depression and pruritus usually limit their use to major operations.

Neuromuscular Blocking Agents

The practical aspects of the use of neuromuscular blockers as anesthetic adjuncts are briefly described here. The detailed pharmacology of this drug class is presented in Chapter 13.

Depolarizing (e.g., *succinylcholine*) and nondepolarizing (e.g., *vecuronium*) muscle relaxants often are administered during the induction of anesthesia to relax muscles of the jaw, neck, and airway and thereby facilitate laryngoscopy and endotracheal intubation. Barbiturates will precipitate when mixed with muscle relaxants and should be allowed to clear from the intravenous line prior to injection of a muscle relaxant. The action of nondepolarizing muscle relaxants usually is antagonized, once muscle paralysis is no longer desired, with an acetylcholinesterase inhibitor, such as *neostigmine* or *edrophonium* (see Chapter 12), in combination with a muscarinic receptor antagonist (e.g., *glycopyrrolate* or *atropine*; see Chapter 11) to offset the muscarinic activation resulting from esterase inhibition. *Sugammadex* is a modified gamma cyclodextrin molecule that specifically binds and encapsulates *rocuronium* and *vecuronium*, resulting in a rapid reversal of *rocuronium*- and *vecuronium*-induced neuromuscular block (Ledowski, 2015).

Anesthetic Administration in Special Populations

Obesity

Obesity is associated with numerous physiological and anthropometric changes that affect the pharmacokinetics of anesthetic drugs, namely increases in cardiac output, fat mass, and lean body mass. Increases in adipose tissue serve to increase the apparent volume of distribution at steady state, thereby increasing context sensitivity of anesthetics after prolonged administration. Emergence from anesthesia may be delayed after repeated drug administration or long infusion times.

However, the initial distribution and dilution of drug in the first minutes of drug administration (i.e., the front-end kinetics) are affected by cardiac output and regional blood flow. Since cardiac output is preferentially distributed to the metabolically active lean tissue, lean body weight rather than total body weight should be used to calculate initial loading doses (Ingrande et al., 2011). In fact, elegant physiological pharmacokinetic models have demonstrated that dose adjustments of *thiopental* based on lean body weight or based on cardiac output result in the same peak plasma concentration (Wada et al., 1997).

The excess adiposity associated with obesity alters the pharmacodynamics of anesthetics. Obese patients may be more sensitive to upper airway obstruction after the administration of sedatives and/or opioids, especially given the high prevalence of obstructive sleep apnea and obesity hypoventilation syndrome in this population. Doses of sedatives and opioids should be decreased accordingly when these drugs are used for conscious sedation. Extreme caution should be given if these classes of drugs are given concurrently due to drug synergism.

Advanced Age

Physiological changes associated with aging alter the pharmacokinetics of anesthetics in elderly patients. Advanced age is associated with reduced lean body mass and increased body fat, both of which affect volume of distribution. Hepatic blood flow and hepatic mass are reduced, thereby reducing hepatic clearance and impairing anesthetic drug metabolism. Kidney function may be impaired, reducing drug elimination. Reduced drug metabolism coupled with depressed drug elimination means loading should be reduced and drug readministration should occur less frequently in this population.

There is a decrease in cardiac output associated with aging. This reduces drug distribution and clearance of anesthetics. The age-related changes in cardiovascular function increase the cardio-depressant risk associated with anesthetics in this population. The reduction in cardiac output may delay the onset of action of most anesthetic agents and also may reduce peripheral drug distribution with lower apparent volume of distribution.

In addition to these pharmacokinetic changes, the pharmacodynamics of anesthetics are altered in the elderly. Elderly subjects are more sensitive to the sedative-hypnotic effects of benzodiazepines and intravenous induction agents (Vuyk, 2003). A single study showed that induction doses of *propofol* can be up to 44% lower in elderly patients compared to their younger counterparts (Peacock et al., 1992). Hence, doses of anesthetics may need to be lowered in the elderly.

Pediatrics

Changes in body composition affect the pharmacokinetics of anesthetics in pediatric patients. In general, neonates and infants have a higher amount of adipose tissue and extracellular water compared to children of older ages. Therefore, the apparent volumes of distribution of both lipophilic drugs and hydrophilic drugs in children are inversely proportional to age. Total protein, albumin, and alpha-1 acid glycoprotein levels are decreased in neonates. Free fractions of anesthetics are therefore higher in this population. The high amount of free drug and the higher ratio of cerebral to systemic blood flow may increase the CNS concentration of a given drug in neonates compared to older children (Ku and Smith, 2015). After approximately 1 year of age, the concentrations of albumin and alpha-1 acid glycoprotein reach adult levels.

Compared to adults, infants and children have an increased liver blood flow, which may increase hepatic metabolism. However, drug metabolism and elimination are generally slower in neonates owing to immature expression of CYPs and reduced GFR. Expression of CYPs is lowest at birth and increases with increasing age. Likewise, GFR is lowest at birth and rises to adult values by 6 to 12 months of age. However, reduced renal clearance secondary to the decreased GFR may be offset due to the reduced protein binding and increased free fraction of drug.

Therapeutic Gases

Oxygen

Oxygen is essential to life. Hypoxia is a life-threatening condition in which O_2 delivery is inadequate to meet the metabolic demands of the tissues. Hypoxia may result from alterations in tissue perfusion, decreased O_2 tension in the blood, or decreased O_2 carrying capacity. In addition, hypoxia may result from restricted O_2 transport from the microvasculature to cells or impaired utilization within the cell. An inadequate supply of O_2 ultimately results in the cessation of aerobic metabolism and oxidative phosphorylation, depletion of high-energy compounds, cellular dysfunction, and death.

Normal Oxygenation

Oxygen makes up 21% of air, which at sea level represents a partial pressure of 21 kPa (158 mmHg). While the fraction (percentage) of O_2 remains constant regardless of atmospheric pressure, the partial pressure of O_2 (Po_2) decreases with lower atmospheric pressure. Ascent to elevated altitude reduces the uptake and delivery of O_2 to the tissues, whereas increases in atmospheric pressure (e.g., hyperbaric therapy or breathing at depth) raise the Po_2 in inspired air and increase gas uptake. As the air is delivered to the distal airways and alveoli, the Po_2 decreases by dilution with CO_2 and water vapor and by uptake into the blood.

Under ideal conditions, when ventilation and perfusion are well matched, the alveolar Po_2 will be about 14.6 kPa (110 mmHg). The corresponding alveolar partial pressures of water and CO_2 are 6.2 kPa (47 mmHg) and 5.3 kPa (40 mmHg), respectively. Under normal conditions, there is complete equilibration of alveolar gas and lung capillary blood, and the Po_2 in end-capillary blood is typically within a fraction of a kilopascal of that in the alveoli. The Po_2 in arterial blood, however, is further reduced by venous admixture (shunt), the addition of mixed venous blood from the pulmonary artery, which has a Po_2 of about 5.3 kPa (40 mmHg). Together, the diffusional barrier, ventilation-perfusion mismatches, and the shunt fraction are the major causes of the alveolar-to-arterial O_2 gradient, which is normally 1.3 to 1.6 kPa (10–12 mmHg) when air is breathed and 4.0 to 6.6 kPa (30–50 mmHg) when 100% O_2 is breathed. O_2 is delivered to the tissue capillary beds by the circulation and again follows a gradient out of the blood and into cells. Tissue extraction of O_2 typically reduces the Po_2 of venous blood by an additional 7.3 kPa (55 mmHg). Although the Po_2 at the site of cellular O_2 utilization—the mitochondria—is not known, oxidative phosphorylation can continue at a Po_2 of only a few millimeters of mercury.

In the blood, O_2 is carried primarily in chemical combination with hemoglobin and is to a small extent dissolved in solution. The quantity of O_2 combined with hemoglobin depends on the Po_2, as illustrated by the sigmoidal oxyhemoglobin dissociation curve (Figure 24–6). Hemoglobin is about 98% saturated with O_2 when air is breathed under normal circumstances, and it binds 1.3 mL of O_2 per gram when fully saturated. The steep slope of this curve with falling Po_2 facilitates unloading of O_2 from hemoglobin at the tissue level and reloading when desaturated mixed venous blood arrives at the lung. Shifting of the curve to the right with increasing temperature, increasing Pco_2, and decreasing pH, as is found in metabolically active tissues, lowers the O_2 saturation for the same Po_2 and thus delivers additional O_2 where and when it is most needed. However, the flattening of the curve with higher Po_2 indicates that increasing blood Po_2 by inspiring O_2-enriched mixtures can increase the amount of O_2 carried by hemoglobin only minimally. Because of the low solubility of O_2 (0.226 mL/L per kPa or 0.03 mL/L per mmHg at 37°C), breathing 100% O_2 can increase the amount of O_2 dissolved in blood by only 15 mL/L, less than one-third of normal metabolic demands. However, if the inspired Po_2 is increased to 3 atm (304 kPa) in a hyperbaric chamber, the amount of dissolved O_2 is sufficient to meet normal metabolic demands even in the absence of hemoglobin (Table 24–4).

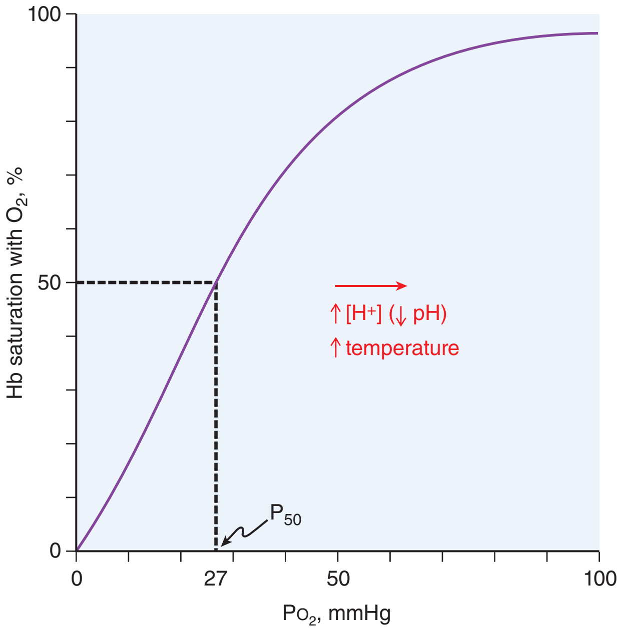


Figure 24–6 *Oxyhemoglobin dissociation curve for whole blood.* The relationship between Po_2 and hemoglobin (Hb) saturation is shown. The P_{50}, or the Po_2 resulting in 50% saturation, is indicated. An increase in temperature or a decrease in pH (as in working muscle) shifts this relationship to the right, reducing the hemoglobin saturation at the same Po_2 and thus aiding in the delivery of O_2 to the tissues.

Oxygen Deprivation. *Hypoxemia* generally implies a failure of the respiratory system to oxygenate arterial blood. Classically, there are five causes of hypoxemia:

- Low Fio_2(inspired O_2 fraction)
- Hypoventilation
- Ventilation-perfusion mismatch
- Shunt or venous admixture
- Increased diffusion barrier

The term *hypoxia* denotes insufficient oxygenation of the tissues. In addition to failure of the respiratory system to oxygenate the blood adequately, a number of other factors can contribute to hypoxia at the tissue level. These may be divided into categories of O_2 delivery and O_2 utilization. O_2 delivery decreases globally when cardiac output falls or locally when regional blood flow is compromised, such as from a vascular occlusion (e.g., stenosis, thrombosis, or microvascular occlusion) or increased downstream pressure (e.g., compartment syndrome, venous stasis, or venous hypertension). Decreased O_2 carrying capacity of the blood likewise will reduce O_2 delivery, such as occurs with anemia, carbon monoxide poisoning, or hemoglobinopathy. Finally, hypoxia may occur when transport of O_2 from the capillaries to the tissues is decreased (edema) or utilization of the O_2 by the cells is impaired (CN^- toxicity).

TABLE 24–4 ■ THE CARRIAGE OF OXYGEN IN BLOOD[a]

ARTERIAL Po_2 kPa (mmHg)	ARTERIAL O_2 CONTENT ($mL\ O_2/L$)			MIXED VENOUS Po_2 kPa (mmHg)	MIXED VENOUS O_2 CONTENT ($mL\ O_2/L$)			EXAMPLES
	DISSOLVED	BOUND TO Hb	TOTAL		DISSOLVED	BOUND TO Hb	TOTAL	
4.0 (30)	0.9	109	109.9	2.7 (20)	0.6	59	59.6	High altitude; respiratory failure breathing air
12.0 (90)	2.7	192	194.7	5.5 (41)	1.2	144	145.2	Normal person breathing air
39.9 (300)	9.0	195	204	5.9 (44)	1.3	153	154.3	Normal person breathing 50% O_2
79.7 (600)	18	196	214	6.5 (49)	1.5	163	164.5	Normal person breathing 100% O_2
239 (1800)	54	196	250	20.0 (150)	4.5	196	200.5	Normal person breathing hyperbaric O_2

[a] This table illustrates the carriage of oxygen in the blood under a variety of circumstances. As arterial O_2 tension increases, the amount of dissolved O_2 increases in direct proportion to the Po_2, but the amount of oxygen bound to Hb reaches a maximum of 196 mL O_2 /L (100% saturation of Hb at 15 g/dL). Further increases in O_2 content require increases in dissolved oxygen. At 100% inspired O_2, dissolved O_2 still provides only a small fraction of total demand. Hyperbaric oxygen therapy is required to increase the amount of dissolved oxygen to supply all or a large part of metabolic requirements. Note that, during hyperbaric oxygen therapy, the hemoglobin in the mixed venous blood remains fully saturated with O_2. The figures in this table are approximate and are based on the assumptions of 15 g/dL Hb, 50 mL O_2/L whole-body oxygen extraction, and constant cardiac output. When severe anemia is present, arterial Po_2 remains the same, but arterial content is lower; oxygen extraction continues, resulting in lower O_2 content and tension in mixed venous blood. Similarly, as cardiac output falls significantly, the same oxygen extraction occurs from a smaller volume of blood and results in lower mixed venous oxygen content and tension.

Effects of Hypoxia

Cellular and Metabolic Effects. At the molecular level, nonlethal hypoxia produces a marked alteration in gene expression, mediated in part by hypoxia-inducible factor 1α (Guimarães-Camboa et al., 2015).

When the mitochondrial Po_2 falls below about 0.13 kPa (1 mmHg), aerobic metabolism stops, and the less-efficient anaerobic pathways of glycolysis become responsible for the production of cellular energy. End products of anaerobic metabolism, such as lactic acid, are released into the circulation in measurable quantities. Energy-dependent ion pumps slow, and transmembrane ion gradients dissipate. Intracellular concentrations of Na^+, Ca^{2+}, and H^+ increase, finally leading to cell death. The time course of cellular demise depends on the relative metabolic demands, oxygen storage capacity, and anaerobic capacity of the individual organs. Restoration of perfusion and oxygenation prior to hypoxic cell death paradoxically can result in an accelerated form of cell injury (ischemia-reperfusion syndrome), which is thought to result from the increased generation of highly reactive oxygen free radicals.

Cell and Organ Survival. Ultimately, hypoxia results in the cessation of aerobic metabolism, exhaustion of high-energy intracellular stores, cellular dysfunction, and death. The time course of cellular demise depends on the tissue's relative metabolic requirements, O_2 and energy stores, and anaerobic capacity. Survival times (the time from the onset of circulatory arrest to significant organ dysfunction) range from 1 to 2 min in the cerebral cortex to around 5 min in the heart and 10 min in the kidneys and liver, with the potential for some degree of recovery if reperfused. Revival times (the duration of hypoxia beyond which recovery is no longer possible) are about four to five times longer.

Organ System Effects. Less-severe degrees of hypoxia have progressive physiological effects on different organ systems (Nunn, 2005).

Respiratory System. Hypoxia stimulates the carotid and aortic baroreceptors to cause increases in both the rate and the depth of ventilation. Minute volume almost doubles when normal individuals inspire gas with a Po_2 of 6.6 kPa (50 mmHg). Dyspnea is not always experienced with simple hypoxia but occurs when the respiratory minute volume approaches half the maximal breathing capacity; this may occur with minimum exertion in patients in whom maximal breathing capacity is reduced by lung disease. In general, little warning precedes the loss of consciousness resulting from hypoxia.

Cardiovascular System. Hypoxia causes reflex activation of the sympathetic nervous system by both autonomic and humoral mechanisms, resulting in tachycardia and increased cardiac output. Peripheral vascular resistance, however, decreases primarily through local autoregulatory mechanisms, with the net result that blood pressure generally is maintained unless hypoxia is prolonged or severe. In contrast to the systemic circulation, hypoxia causes pulmonary vasoconstriction and hypertension, an extension of the normal regional vascular response that matches perfusion with ventilation to optimize gas exchange in the lung (hypoxic pulmonary vasoconstriction).

CNS. The CNS is least able to tolerate hypoxia. Hypoxia is manifest initially by decreased intellectual capacity and by impairment of judgment and psychomotor ability. This state progresses to confusion and restlessness and ultimately to stupor, coma, and death as the arterial Po_2 decreases below 4 to 5.3 kPa (30–40 mmHg). Victims often are unaware of this progression.

Adaptation to Hypoxia

Long-term hypoxia results in adaptive physiological changes; these have been studied most thoroughly in persons exposed to high altitude. Adaptations include increased numbers of pulmonary alveoli, increased concentrations of hemoglobin in blood and myoglobin in muscle, and a decreased ventilatory response to hypoxia. Short-term exposure to high altitude produces similar adaptive changes. In susceptible individuals, however, acute exposure to high altitude may produce *acute mountain sickness*, a syndrome characterized by headache, nausea, dyspnea, sleep disturbances, and impaired judgment progressing to pulmonary and cerebral edema. Mountain sickness is treated with rest and analgesics when mild or supplemental O_2, descent to lower altitude, or an increase in ambient pressure when more severe. *Acetazolamide* (a carbonic anhydrase inhibitor) and *dexamethasone* also may be helpful. The syndrome usually can be avoided by a slow ascent to altitude, adequate hydration, and prophylactic use of *acetazolamide* or *dexamethasone*.

Examples of "normal" hypoxia are widespread, and the comparative physiology of hypoxic tolerance offers clues to the mechanisms involved. Aspects of fetal and newborn physiology are strongly reminiscent of adaptive mechanisms found in hypoxia-tolerant animals (Guimarães-Camboa et al., 2015; Mortola, 1999), including shifts in the oxyhemoglobin dissociation curve (fetal hemoglobin), reductions in metabolic rate and body temperature (hibernation-like mode), reductions in heart rate and circulatory redistribution (as in diving mammals), and redirection of energy utilization from growth to maintenance metabolism. These adaptations probably account for the relative tolerance of the fetus and neonate to both chronic (uterine insufficiency) and short-term hypoxia.

Oxygen Inhalation

Physiological Effects. O_2 inhalation is used primarily to reverse or prevent the development of hypoxia. However, when O_2 is breathed in excessive amounts or for prolonged periods, secondary physiological changes and toxic effects can occur.

Respiratory System. Inhalation of O_2 at 1 atm or above causes a small degree of respiratory depression in normal subjects, presumably as a result of loss of tonic chemoreceptor activity. However, ventilation typically increases within a few minutes of O_2 inhalation because of a paradoxical increase in the tension of CO_2 in tissues. This increase results from the increased concentration of oxyhemoglobin in venous blood, which causes less-efficient removal of carbon dioxide from the tissues. Expansion of poorly ventilated alveoli is maintained in part by the nitrogen content of alveolar gas; nitrogen is poorly soluble and thus remains in the air spaces while O_2 is absorbed. High O_2 concentrations delivered to poorly ventilated lung regions dilute the nitrogen content and can promote absorption atelectasis (partial or complete collapse of the lung), occasionally resulting in an increase in shunt and a paradoxical worsening of hypoxemia after a period of O_2 administration.

Cardiovascular System. Heart rate and cardiac output are slightly reduced when 100% O_2 is breathed; blood pressure changes little. Elevated pulmonary artery pressures in patients living at high altitude who have chronic hypoxic pulmonary hypertension may reverse with O_2 therapy or return to sea level. In neonates with congenital heart disease and left-to-right shunting of cardiac output, O_2 supplementation must be regulated carefully because of the risk of further reducing pulmonary vascular resistance and increasing pulmonary blood flow.

Metabolism. Inhalation of 100% O_2 does not produce detectable changes in O_2 consumption, CO_2 production, respiratory quotient, or glucose utilization.

Oxygen Administration

Oxygen is supplied as a compressed gas in steel cylinders; purity of 99% is *medical grade*. For safety, O_2 cylinders and piping are color coded (green in the U.S.), and some form of mechanical indexing of valve connections is used to prevent the connection of other gases to O_2 systems.

Oxygen is delivered by inhalation except during extracorporeal circulation, when it is dissolved directly into the circulating blood. A closed delivery system with an endotracheal tube produces an airtight seal to the patient's airway, and complete separation of inspired from expired gases can precisely control $F\textsc{io}_2$. In all other systems, such as nasal cannulas and face masks, the actual delivered $F\textsc{io}_2$ will depend on the ventilatory pattern (i.e., rate, tidal volume, inspiratory-expiratory time ratio, and inspiratory flow) and delivery system characteristics.

Monitoring of Oxygenation. Monitoring and titration are required to meet the therapeutic goal of O_2 therapy and to avoid complications and side effects. Although cyanosis is a physical finding of substantial clinical importance, it is not an early, sensitive, or reliable index of oxygenation. Noninvasive monitoring of arterial O_2 saturation can be achieved using transcutaneous pulse oximetry, in which O_2 saturation is measured from the differential absorption of light by oxyhemoglobin and deoxyhemoglobin and the arterial saturation determined from the pulsatile component of this signal. Pulse oximetry measures hemoglobin saturation and not $P\textsc{o}_2$. It is not sensitive to increases in $P\textsc{o}_2$ that exceed levels required to saturate the blood fully. Pulse oximetry is useful for monitoring the adequacy of oxygenation during procedures requiring sedation or anesthesia, rapid evaluation and monitoring of potentially compromised patients, and titrating O_2 therapy in situations where toxicity from O_2 or side effects of excess O_2 are of concern. A specific tool for measuring cerebral oxygenation is near-infrared spectroscopy (Guarracino, 2008).

Complications of Oxygen Therapy. In addition to the potential to promote absorption atelectasis and depress ventilation, high flows of dry O_2 can dry out and irritate mucosal surfaces of the airway and the eyes, as well as decrease mucociliary transport and clearance of secretions. Humidified O_2 thus should be used when prolonged therapy (>1 h) is required. Finally, any O_2-enriched atmosphere constitutes a fire hazard, and appropriate precautions must be taken. Hypoxemia can occur despite the administration of supplemental O_2. Therefore, it is essential that both O_2 saturation and adequacy of ventilation be assessed frequently.

Therapeutic Uses of Oxygen

Correction of Hypoxia. The primary therapeutic use of O_2 is to correct hypoxia. Hypoxia is most commonly a manifestation of an underlying disease, and administration of O_2 thus should be viewed as temporizing therapy. Efforts must be directed at correcting the cause of the hypoxia. Hypoxia resulting from most pulmonary diseases can be alleviated at least partially by administration of O_2, allowing time for definitive therapy to reverse the primary process.

Reduction of Partial Pressure of an Inert Gas. Because nitrogen constitutes some 79% of ambient air, it also is the predominant gas in most gas-filled spaces in the body. In situations such as bowel distension from obstruction or ileus, intravascular air embolism, or pneumothorax, it is desirable to reduce the volume of air-filled spaces. Because nitrogen is relatively insoluble, inhalation of high concentrations of O_2 (and thus low concentrations of nitrogen) rapidly lowers the total-body partial pressure of nitrogen and provides a substantial gradient for the removal of nitrogen from gas spaces. Administration of O_2 for air embolism is also beneficial because it helps to relieve localized hypoxia distal to the vascular obstruction. In the case of *decompression sickness*, or *bends*, lowering the inert gas tension in blood and tissues by O_2 inhalation prior to or during barometric decompression reduces the supersaturation that occurs after decompression so that bubbles do not form.

Hyperbaric Oxygen Therapy. O_2 can be administered at greater than atmospheric pressure in hyperbaric chambers (Thom, 2009). Clinical uses of hyperbaric O_2 therapy include the treatment of trauma, burns, radiation damage, infections, nonhealing ulcers, skin grafts, spasticity, and other neurological conditions. Hyperbaric O_2 may be useful in generalized hypoxia. In carbon monoxide poisoning, hemoglobin and myoglobin become unavailable for O_2 binding because of the high affinity of these proteins for carbon monoxide. High $P\textsc{o}_2$ facilitates competition of O_2 for hemoglobin binding sites as carbon monoxide is exchanged in the alveoli. In addition, hyperbaric O_2 increases the availability of dissolved O_2 in the blood (see Table 24–4). Adverse effects of hyperbaric O_2 therapy include middle ear barotrauma, CNS toxicity, seizures, lung toxicity, and aspiration pneumonia.

Hyperbaric O_2 therapy has two components: increased hydrostatic pressure and increased O_2 pressure. Both factors are necessary for the treatment of decompression sickness and air embolism. The hydrostatic pressure reduces bubble volume, and the absence of inspired nitrogen increases the gradient for elimination of nitrogen and reduces hypoxia in downstream tissues. Increased O_2 pressure at the tissue is the primary therapeutic goal for other indications for hyperbaric O_2. A small increase in $P\textsc{o}_2$ in ischemic areas enhances the bactericidal activity of leukocytes and increases angiogenesis. Repetitive brief exposures to hyperbaric O_2 may enhance therapy for chronic refractory osteomyelitis, osteoradionecrosis, crush injury, or the recovery of compromised skin and tissue grafts. Increased O_2 tension can be bacteriostatic and useful in the treatment of the spread of infection with *Clostridium perfringens* and clostridial myonecrosis (gas gangrene).

Oxygen Toxicity

Oxygen can have deleterious actions at the cellular level. O_2 toxicity may result from increased production of hydrogen peroxide and reactive intermediates such as superoxide anion, singlet oxygen, and hydroxyl radicals that attack and damage lipids, proteins, and other macromolecules, especially those in biological membranes. A number of factors limit the toxicity of oxygen-derived reactive agents, including enzymes such as superoxide dismutase, glutathione peroxidase, and catalase, which scavenge toxic oxygen by-products, and reducing agents such as iron, glutathione, and ascorbate. These factors, however, are insufficient to limit the destructive actions of oxygen when patients are exposed to high concentrations over an extended time period. Tissues show differential sensitivity to oxygen toxicity, which is likely the result of differences in both their production of reactive compounds and their protective mechanisms.

Respiratory Tract. The pulmonary system is usually the first to exhibit toxicity, a function of its continuous exposure to the highest O_2 tensions in the body. Subtle changes in pulmonary function can occur within 8 to 12 h of exposure to 100% O_2. Increases in capillary permeability, which will increase the alveolar/arterial O_2 gradient and ultimately lead to further hypoxemia, and decreased pulmonary function can be seen after only 18 h of exposure. Serious injury and death, however, require much longer exposures. Pulmonary damage is directly related to the inspired O_2 tension, and concentrations of less than 0.5 atm appear to be safe over long time periods. The capillary endothelium is the most sensitive tissue of the lung. Endothelial injury results in loss of surface area from interstitial edema and leaks into the alveoli.

Nervous System. Retinopathy of prematurity is an eye disease in premature infants involving abnormal vascularization of the developing retina that can result from O_2 toxicity or relative hypoxia. CNS problems are rare, and toxicity occurs only under hyperbaric conditions where exposure exceeds 200 kPa (2 atm). Symptoms include seizures and visual changes, which resolve when O_2 tension is returned to normal. In premature neonates and those who have sustained *in utero* asphyxia, hyperoxia and hypocapnia are associated with worse neurological outcomes.

Carbon Dioxide

Carbon dioxide is produced by metabolism at approximately the same rate as O_2 is consumed. At rest, this value is about 3 mL/kg/min, but it may increase dramatically with exercise. CO_2 diffuses readily from the cells into the blood, where it is carried partly as bicarbonate ion (HCO_3^-), partly in chemical combination with hemoglobin and plasma proteins, and partly in solution at a partial pressure of about 6 kPa (46 mmHg) in mixed venous blood. CO_2 is transported to the lung, where it is normally exhaled at the rate it is produced, leaving a partial pressure of about 5.2 kPa (40 mmHg) in the alveoli and in arterial blood. An increase in $P\text{CO}_2$ results in respiratory acidosis and may be due to decreased ventilation or the inhalation of CO_2, whereas an increase in ventilation results in decreased $P\text{CO}_2$ and respiratory alkalosis. Because CO_2 is freely diffusible, the changes in blood $P\text{CO}_2$ and pH soon are reflected by intracellular changes in $P\text{CO}_2$ and pH and by widespread effects in the body, especially on respiration, circulation, and the CNS.

Respiration

Carbon dioxide is a rapid, potent stimulus to ventilation in direct proportion to the inspired CO_2. CO_2 stimulates breathing by acidifying central chemoreceptors and the peripheral carotid bodies. Elevated $P\text{CO}_2$ causes bronchodilation, whereas hypocarbia causes constriction of airway smooth muscle; these responses may play a role in matching pulmonary ventilation and perfusion.

Circulation

The circulatory effects of CO_2 result from the combination of its direct local effects and its centrally mediated effects on the autonomic nervous system. The direct effect of CO_2 on the heart, diminished contractility, results from pH changes and a decreased myofilament Ca^{2+} responsiveness. The direct effect on systemic blood vessels results in vasodilation. CO_2 causes widespread activation of the sympathetic nervous system. The results of sympathetic nervous system activation generally are opposite to the local effects of carbon dioxide. The sympathetic effects consist of increases in cardiac contractility, heart rate, and vasoconstriction (see Chapter 12). The balance of opposing local and sympathetic effects, therefore, determines the total circulatory response to CO_2. The net effect of CO_2 inhalation is an increase in cardiac output, heart rate, and blood pressure. In blood vessels, however, the direct vasodilating actions of CO_2 appear more important, and total peripheral resistance decreases when the $P\text{CO}_2$ is increased. CO_2 also is a potent coronary vasodilator. Cardiac arrhythmias associated with increased $P\text{CO}_2$ are due to the release of catecholamines.

Hypocarbia results in opposite effects: decreased blood pressure and vasoconstriction in skin, intestine, brain, kidney, and heart. These actions are exploited clinically in the use of hyperventilation to diminish intracranial hypertension.

CNS

Hypercarbia depresses the excitability of the cerebral cortex and increases the cutaneous pain threshold through a central action. This central depression has therapeutic importance. For example, in patients who are hypoventilating from narcotics or anesthetics, increasing $P\text{CO}_2$ may result in further CNS depression, which in turn may worsen the respiratory depression. This positive-feedback cycle can have lethal consequences.

Methods of Administration

Carbon dioxide is marketed in gray metal cylinders as the pure gas or as CO_2 mixed with O_2. It usually is administered at a concentration of 5% to 10% in combination with O_2 by means of a face mask. Another method for the temporary administration of CO_2 is by rebreathing, such as from an anesthesia breathing circuit or from something as simple as a paper bag.

Therapeutic Uses

Carbon dioxide is used for insufflation during endoscopic procedures (e.g., laparoscopic surgery) because it is highly soluble and does not support combustion. CO_2 can be used to flood the surgical field during cardiac surgery. Because of its density, CO_2 displaces the air surrounding the open heart so that any gas bubbles trapped in the heart are CO_2 rather than insoluble N_2. It is used to adjust pH during cardiopulmonary bypass procedures when a patient is cooled.

Hypocarbia still has some uses in anesthesia; it constricts cerebral vessels, decreasing brain size slightly, and thus may facilitate the performance of neurosurgical operations. While short-term hypocarbia is effective for this purpose, sustained hypocarbia has been associated with worse outcomes in patients with head injury. Hypocarbia should be instituted with a clearly defined indication and normocarbia should be reestablished as soon the indication for hypocarbia no longer applies.

Nitric Oxide

Nitric oxide (NO) is a free radical gas now known as a critical endogenous cell-signaling molecule with an increasing number of potential therapeutic applications.

Endogenous NO is produced from L-arginine by NO synthases (neural, inducible, and endothelial) (see Chapter 3). In the vasculature, basal production of NO by endothelial cells is a primary determinant of resting vascular tone. NO causes vasodilation of smooth muscle cells and inhibition of platelet aggregation and adhesion. Impaired NO production is implicated in atherosclerosis, hypertension, cerebral and coronary vasospasm, ischemia-reperfusion injury, and inflammation and in mediating central nociceptive pathways. NO is rapidly inactivated in the circulation by oxyhemoglobin and by the reaction of NO with the heme iron, leading to the formation of nitrosyl-hemoglobin. Small quantities of methemoglobin are also produced, and these are converted to the ferrous form of heme iron by cytochrome b5 reductase. The majority of inhaled NO is excreted in the urine in the form of nitrate.

Therapeutic Uses

Inhaled NO selectively dilates the pulmonary vasculature (Cooper, 1999) and has potential as a therapy for numerous diseases associated with increased pulmonary vascular resistance. Inhaled NO is FDA-approved for only one indication, persistent pulmonary hypertension of the newborn.

Diagnostic Uses

Inhaled NO can be used during cardiac catheterization to evaluate the pulmonary vasodilating capacity of patients with heart failure and infants with congenital heart disease. Inhaled NO also is used to determine the diffusion capacity (D_L) across the alveolar-capillary unit. NO is more effective than CO_2 in this regard because of its greater affinity for hemoglobin and its higher water solubility at body temperature. NO is produced from the nasal passages and from the lungs of normal human subjects and can be detected in exhaled gas. The measurement of fractional exhaled NO is a noninvasive marker for airway inflammation with utility in the assessment of respiratory tract diseases, including asthma, respiratory tract infection, and chronic lung disease.

Toxicity

Administered at low concentrations (0.1–50 ppm), inhaled NO appears to be safe and without significant side effects. Pulmonary toxicity can occur with levels higher than 50 to 100 ppm. NO is an atmospheric pollutant; the Occupational Safety and Health Administration places the 7-hour exposure limit at 50 ppm. Part of the toxicity of NO may be related to its further oxidation to NO_2 in the presence of high concentrations of O_2.

The development of methemoglobinemia is a significant complication of inhaled NO at higher concentrations, and rare deaths have been reported with overdoses of NO. Methemoglobin concentrations should be monitored intermittently during NO inhalation. Inhaled NO can inhibit platelet function and has been shown to increase bleeding time in some clinical studies, although bleeding complications have not been reported. In patients with impaired function of the left ventricle, NO has a potential to further impair left ventricular performance by dilating the pulmonary circulation and increasing the blood flow to the left ventricle, thereby increasing left atrial pressure and promoting pulmonary edema formation.

The most important requirements for safe NO inhalation therapy include:

- Continuous measurement of NO and NO_2 concentrations using either chemiluminescence or electrochemical analyzers
- Frequent calibration of monitoring equipment
- Intermittent analysis of blood methemoglobin levels
- The use of certified tanks of NO
- Administration of the lowest NO concentration required for therapeutic effect

Methods of Administration

Courses of treatment of patients with inhaled NO are highly varied, extending from 0.1 to 40 ppm in dose and for periods of a few hours to several weeks in duration. The determination of a dose-response relationship on a frequent basis should assist in the titration of the optimum dose of NO. Commercial NO systems are available that will accurately deliver inspired NO concentrations between 0.1 and 80 ppm and simultaneously measure NO and NO_2 concentrations.

Helium

Helium is an inert gas whose low density, low solubility, and high thermal conductivity provide the basis for its medical and diagnostic uses. Helium can be mixed with O_2 and administered by mask or endotracheal tube. Under hyperbaric conditions, it can be substituted for the bulk of other gases, resulting in a mixture of much lower density that is easier to breathe.

The primary uses of helium are in pulmonary function testing, the treatment of respiratory obstruction, laser airway surgery, as a label in imaging studies, and for diving at depth. Helium is also suited for determinations of residual lung volume, functional residual capacity, and related lung volumes. These measurements require a highly diffusible nontoxic gas that is insoluble and does not leave the lung by the bloodstream so that, by dilution, the lung volume can be measured. Helium can be added to O_2 to reduce turbulence due to airway obstruction because the density of helium is less than that of air, and the viscosity of helium is greater than that of air. Mixtures of helium and O_2 reduce the work of breathing. Helium has high thermal conductivity, making it useful during laser surgery on the airway. Laser-polarized helium is used as an inhalational contrast agent for pulmonary magnetic resonance imaging. Helium also has potential as a cytoprotective agent (Smit et al., 2015).

Hydrogen Sulfide

Hydrogen sulfide (H_2S), which has a characteristic rotten egg smell, is a colorless, flammable, water-soluble gas that is primarily considered as a toxin due to its capacity to inhibit mitochondrial respiration through blockade of cytochrome c oxidase. Inhibition of respiration can be toxic; however, if depression of respiration occurs in a controlled manner, it may allow nonhibernating species exposed to inhaled H_2S to enter a state akin to suspended animation (i.e., a slowing of cellular activity to a point at which metabolic processes are inhibited but not terminal) and thereby increase tolerance to stress. H_2S activates ATP-dependent K^+ channels, has vasodilating properties, and serves as a free radical scavenger. H_2S can protect against whole-body hypoxia, lethal hemorrhage, and ischemia-reperfusion injury in various organs, including the kidney, lung, liver, and heart. Currently, effort is under way for development of gas-releasing molecules that could deliver H_2S and other therapeutic gases to diseased tissue. H_2S in low quantities may have the potential to limit cell death (Lefer, 2007).

Drug Facts for Your Personal Formulary: *General Anesthetics and Therapeutic Gases*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Parenteral Anesthetics		
Propofol Etomidate Ketamine Thiopental	• Anesthetic induction • Rapid-onset and short-duration anesthetics used in procedures for rapid return to preoperative mental status	• Highly lipophilic → entry to brain and spinal cord, accumulation in fatty tissues • Propofol dosage: ↓ in elderly due to reduced clearance, ↑ in young children due to rapid clearance • PRIS: rare complication associated with prolonged and high-dose propofol infusion in young or head-injured patients • Etomidate: preferred for patients at risk of hypotension or MI; produces hypnosis, no analgesic effects; ↑ EEG activity, associated with seizures • Ketamine: suited for patients at risk for hypotension and asthma and for pediatric procedures; increases HR, BP, cardiac output, CBF, and ICP; emergence delirium, hallucinations, vivid dreams limit use
Barbiturates Methohexital Thiopental	• Anesthetic induction	• Respiratory and EEG depressants • Methohexital: more rapid clearance than other barbiturates • Thiopental: action terminated by redistribution; good safety record; not available in the U.S. • Intra-arterial injection of thiobarbiturates → severe inflammatory and potentially necrotic reaction

Drug Facts for Your Personal Formulary: *General Anesthetics and Therapeutic Gases (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Inhalational Anesthetics		
Isoflurane	• Maintenance of anesthesia • Commonly used inhalational anesthetic	• Highly volatile at RT; not flammable in air or O_2 • ↓ Ventilation and RBF (renal blood flow); ↑ CBF • Induces hypotension and ↑ coronary blood flow, thus ↓ myocardial O_2 consumption • ↓ Baroreceptor function • Excreted unchanged by the lungs
Enflurane	• Maintenance of anesthesia	• Volatile at RT; store in tightly sealed bottles • Slow induction and recovery • ↓ Arterial BP due to vasodilation and ↓ myocardial contractility • Possible effects: ↑ ICP, seizure activity
Sevoflurane	• Preferred agent for anesthetic induction • Used for outpatient anesthesia (not irritating airway; induction and recovery are rapid)	• Reacts exothermically with desiccated CO_2 absorbent • Ideal induction agent (pleasant smell, rapid onset) • ↓ Arterial pressure and cardiac output; potent bronchodilator • Preferred for patients with myocardial ischemia • Compound A, product of interaction of sevoflurane with the CO_2-absorbent soda lime, is nephrotoxic
Desflurane	• Used for outpatient surgery (rapid onset, rapid recovery)	• Highly volatile at RT; store in tightly sealed bottles • Nonflammable in mixtures of air or O_2 • An airway irritant
Halothane	• Maintenance of anesthesia	• Highly volatile at RT, light sensitive; store in tightly sealed amber bottles with thymol (preservative) • Possible hepatic toxicity has limited its use and is no longer available in the U.S.
Nitrous oxide (N_2O)	• Weak anesthetic agent used for its significant analgesic effects	• Colorless and odorless gas at RT; used as adjunct to other anesthetics • Will expand volume of air-containing cavities → avoid use in obstructions of ear and bowel and in intraocular and intracranial air bubbles, etc. • To avoid diffusional hypoxia, administer 100% O_2 rather than air when discontinuing N_2O • Can increase CBF and ICP • Clinical use controversial due to potential metabolic effects related to ↑ homocysteine and changes in DNA and protein synthesis
Anesthetic Adjuncts • Augment anesthetic effects of general anesthesia		
Benzodiazepines Midazolam, diazepam, lorazepam	• Used for anxiolysis, amnesia, preanesthetic sedation, and sedation during procedures not requiring general anesthesia	• Midazolam most commonly used, followed distantly by diazepam and lorazepam (see Chapters 18 and 22)
α_2 Adrenergic agonists Dexmedetomidine	• Short-term (<24 h) sedation of critically ill adults • Sedation prior to and during surgical or medical procedures in nonintubated patients	• Activation of the α_{2A} adrenergic receptor by dexmedetomidine → sedation and analgesia • Side effects: hypotension and bradycardia due to decreased catecholamine release in the CNS; nausea and dry mouth
Analgesics *Opioids* Fentanyl, sufentanil, alfentanil, remifentanil, meperidine, morphine	• To reduce anesthetic requirement and minimize hemodynamic changes due to painful stimuli	• Opioids are the primary analgesics during perioperative period; the choice of opioid is based on duration of action (see Chapter 23) • Opioids often are administered intrathecally and epidurally for management of acute and chronic pain
NSAIDs Acetaminophen		• NSAIDs and acetaminophen are used for minor surgical procedures to control postoperative pain
Neuromuscular Blocking Agents Succinylcholine (depolarizing) Atracurium, vecuronium, et al. (nondepolarizing, competitive)	• Skeletal muscle relaxant	• Action of nondepolarizing muscle relaxants usually is antagonized, once muscle paralysis is no longer desired, with an AChE inhibitor (e.g., neostigmine or edrophonium; see Chapter 12), in combination with a muscarinic receptor antagonist

Drug Facts for Your Personal Formulary: *General Anesthetics and Therapeutic Gases (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Therapeutic Gases		
Oxygen	• Used primarily to reverse or prevent the development of hypoxia	• Excessive O_2 ↓ ventilation • Monitoring and titration are required to avoid complications and side effects • HR and cardiac output are slightly ↓ when 100% O_2 is breathed • High flows of dry O_2 can dry out and irritate mucosal surfaces of the airway and the eyes; humidified O_2 should be used for prolonged therapy (>1 h) • O_2-enriched atmosphere constitutes a fire hazard; take precautions
Carbon dioxide	• Insufflation during endoscopic procedures • Flooding the surgical field during cardiac surgery • Adjusting pH during cardiopulmonary bypass	• CO_2 is highly soluble, noncombustible, denser than air • ↑ P_{CO_2} → respiratory acidosis • Effects on cardiovascular system: combination of direct CNS and reflex sympathetic effects; net effect: ↑ cardiac output, HR, and BP
Nitric oxide	• Inhaled NO is used to dilate pulmonary vasculature in persistent pulmonary hypertension of the newborn	• Cell-signaling molecule; induces vasodilation • Pulmonary toxicity can occur with levels >50–100 ppm • Use lowest NO concentration required for therapeutic effect • Monitor blood methemoglobin levels intermittently during inhalation therapy
Helium	• Pulmonary function testing, treatment of respiratory obstruction, laser airway surgery • As a label in imaging studies	• Mixtures of He and O_2 reduce the work of breathing • Potential as a cytoprotective agent • For diving at depth
Hydrogen sulfide	• Potential therapeutic use for protection against effects of hypoxia	

AChE, acetylcholinesterase; BP, blood pressure; HR, heart rate; MI, myocardial infarction; NSAID, nonsteroidal anti-inflammatory drug; RT, room temperature.

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Chapter 25

Local Anesthetics

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Local anesthetics bind reversibly to a specific receptor site within the pore of the Na^+ channels in nerves and block ion movement through this pore. When applied locally to nerve tissue in appropriate concentrations, local anesthetics can act on any part of the nervous system and on every type of nerve fiber, reversibly blocking the action potentials responsible for nerve conduction. Thus, a local anesthetic in contact with a nerve trunk can cause both sensory and motor paralysis in the area innervated. These effects of clinically relevant concentrations of local anesthetics are reversible with recovery of nerve function and no evidence of damage to nerve fibers or cells in most clinical applications.

HISTORY

The first local anesthetic, *cocaine*, was serendipitously discovered to have anesthetic properties in the late 19th century. *Cocaine* occurs in abundance in the leaves of the coca shrub (*Erythroxylon coca*). For centuries, Andean natives have chewed an alkali extract of these leaves for its stimulatory and euphoric actions. When, in 1860, Albert Niemann isolated *cocaine*, he tasted his newly isolated compound, noted that it numbed his tongue, and a new era began. Sigmund Freud studied *cocaine*'s physiological actions, and Carl Koller introduced *cocaine* into clinical practice in 1884 as a topical anesthetic for ophthalmological surgery. Shortly thereafter, Halstead popularized its use in infiltration and conduction block anesthesia. Subsequently, Einhorn and others developed synthetic substitutes in an attempt to avoid the toxic and addictive qualities of *cocaine*.

Chemistry and Structure-Activity Relationship

Cocaine is an ester of benzoic acid and the complex alcohol 2-carbomethoxy, 3-hydroxytropane (Figure 25–1). Because of its toxicity and addictive properties (see Chapter 28), a search for synthetic substitutes for *cocaine* began in 1892 with the work of Einhorn and colleagues, resulting in the synthesis of *procaine*, which became the prototype for local anesthetics for nearly half a century. The most widely used agents today are *lidocaine*, *bupivacaine*, and *tetracaine*.

Typical local anesthetics contain hydrophilic and hydrophobic moieties that are separated by an intermediate ester or amide linkage. A broad range of compounds containing these minimal structural features can satisfy the requirements for action as local anesthetics. The hydrophilic group usually is a tertiary amine but also may be a secondary amine; the hydrophobic moiety must be aromatic. The nature of the linking group determines some of the pharmacological properties of these agents. For example, plasma esterases readily hydrolyze local anesthetics with an ester link.

The structure-activity relationship and the physicochemical properties of local anesthetics have been well reviewed (Courtney and Strichartz, 1987). Hydrophobicity increases both the potency and the duration of action of the local anesthetics; association of the drug at hydrophobic sites enhances the partitioning of the drug to its sites of action and decreases the rate of metabolism by plasma esterases and hepatic enzymes. In addition, the receptor site for these drugs on Na^+ channels is thought to be hydrophobic (see Mechanism of Action), so that receptor affinity for anesthetic agents is greater for the more hydrophobic drugs. Hydrophobicity also increases toxicity, so that the therapeutic index is decreased for more hydrophobic drugs.

Molecular size influences the rate of dissociation of local anesthetics from their receptor sites. Smaller drug molecules can escape from the receptor site more rapidly. This characteristic is important in rapidly firing cells, in which local anesthetics bind during action potentials and dissociate during the period of membrane repolarization. Rapid binding of local anesthetics during action potentials causes the frequency and voltage dependence of their action.

Abbreviations

ACh: acetylcholine
CSF: cerebrospinal fluid
CYP: cytochrome P450
EDTA: ethylenediaminetetraacetic acid
NE: norepinephrine
NET: norepinephrine transporter
TRP: transient receptor potential
TRPV channel: TRP vanilloid subtype channel

Mechanism of Action

Cellular Site of Action

Local anesthetics act at the cell membrane to prevent the generation and the conduction of nerve impulses. Conduction block can be demonstrated in squid giant axons from which the axoplasm has been removed.

Local anesthetics block conduction by decreasing or preventing the large transient increase in the permeability of excitable membranes to Na$^+$ that normally is produced by a slight depolarization of the membrane (see Chapters 10, 13, and 16; Strichartz and Ritchie, 1987). This action of local anesthetics is due to their direct interaction with voltage-gated Na$^+$ channels. As the anesthetic action progressively develops in a nerve, the threshold for electrical excitability gradually increases, the rate of rise of the action potential declines, impulse conduction slows, and the safety factor for conduction decreases. These factors decrease the probability of propagation of the action potential, and nerve conduction eventually fails.

Local anesthetics can bind to other membrane proteins (Butterworth and Strichartz, 1990). In particular, they can block K$^+$ channels (Strichartz and Ritchie, 1987). However, because the interaction of local anesthetics with K$^+$ channels requires higher concentrations of drug, blockade of conduction is not accompanied by any large or consistent change in resting membrane potential.

Quaternary analogues of local anesthetics block conduction when applied internally to perfused giant axons of squid but are relatively ineffective when applied externally. These observations suggest that the site at which local anesthetics act, at least in their charged form, is accessible only from the inner surface of the membrane (Narahashi and Frazier, 1971; Strichartz and Ritchie, 1987). Therefore, local anesthetics applied externally first must cross the membrane before they can exert a blocking action.

The Local Anesthetic Receptor Site on Na$^+$ Channels

The major mechanism of action of these drugs involves their interaction with a specific binding site within the Na$^+$ channel (Butterworth and Strichartz, 1990). The Na$^+$ channels of the mammalian brain are complexes of glycosylated transmembrane proteins with an aggregate molecular size in excess of 300,000 Da; the individual subunits are designated α (260,000 Da) and β$_1$ to β$_4$ (33,000–38,000 Da). The large α subunit of the Na$^+$ channel contains four homologous domains (I–IV); each domain consists of six transmembrane segments in α-helical conformation (S1–S6; Figure 25–2) and an additional, membrane-reentrant pore (*P*) loop. The Na$^+$-selective transmembrane pore of the channel resides in the center of a nearly symmetrical structure formed by the four homologous domains. The voltage dependence of channel opening reflects conformational changes that result from the movement of "gating charges" within the voltage sensor module of the Na$^+$ channel in response to changes in the transmembrane potential. The gating charges are located in the S4 transmembrane helices, which are hydrophobic and positively charged, containing arginine or occasionally lysine residues at every third position. These residues are thought to move perpendicular to the plane of the membrane under the influence of the transmembrane potential, initiating a series of conformational changes in all four domains, which leads to the open state of the channel (Figure 25–2) (Catterall et al., 2000, 2020).

The transmembrane pore of the Na$^+$ channel is surrounded by the S5 and S6 transmembrane helices and the short membrane-associated segments between them that form the *P* loop. Amino acid residues in these short segments are the most critical determinants of the ion conductance and selectivity of the channel.

After it opens, the Na$^+$ channel inactivates within a few milliseconds due to closure of an inactivation gate. This functional gate is formed by the short intracellular loop of protein that connects homologous domains III and IV. This loop folds over the intracellular mouth of the transmembrane pore during the process of inactivation and binds to an inactivation gate "receptor" on the intracellular surface of the pore module.

Amino acid residues important for local anesthetic binding are found in the S6 segments in domains I, III, and IV (Ragsdale et al., 1994; Yarov-Yarovoy et al., 2002). Hydrophobic amino acid residues near the center of the S6 segments interact directly with bound local anesthetics, locating

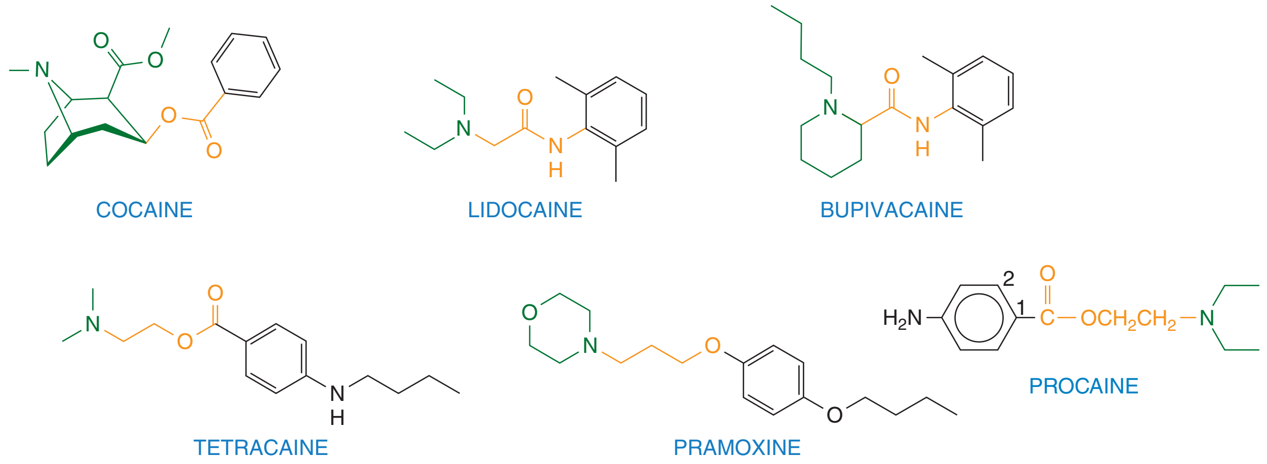


Figure 25–1 *Structural formulas of selected local anesthetics.* Most local anesthetics consist of a hydrophobic (aromatic) moiety (black), a linker region (orange), and a substituted amine (hydrophilic region, green). The structures at the top are grouped by the nature of the linker region. *Procaine* is a prototypic ester-type local anesthetic; esters generally are rapidly hydrolyzed by plasma esterases, contributing to the relatively short duration of action of drugs in this group. *Lidocaine* is a prototypic amide-type local anesthetic; these structures generally are more resistant to clearance and have longer durations of action. There are exceptions, including *benzocaine* (poorly water soluble; used only topically) and the structures with a ketone, an amidine, and an ether linkage. *Chloroprocaine* has a chlorine atom on C2 of the aromatic ring of *procaine*.

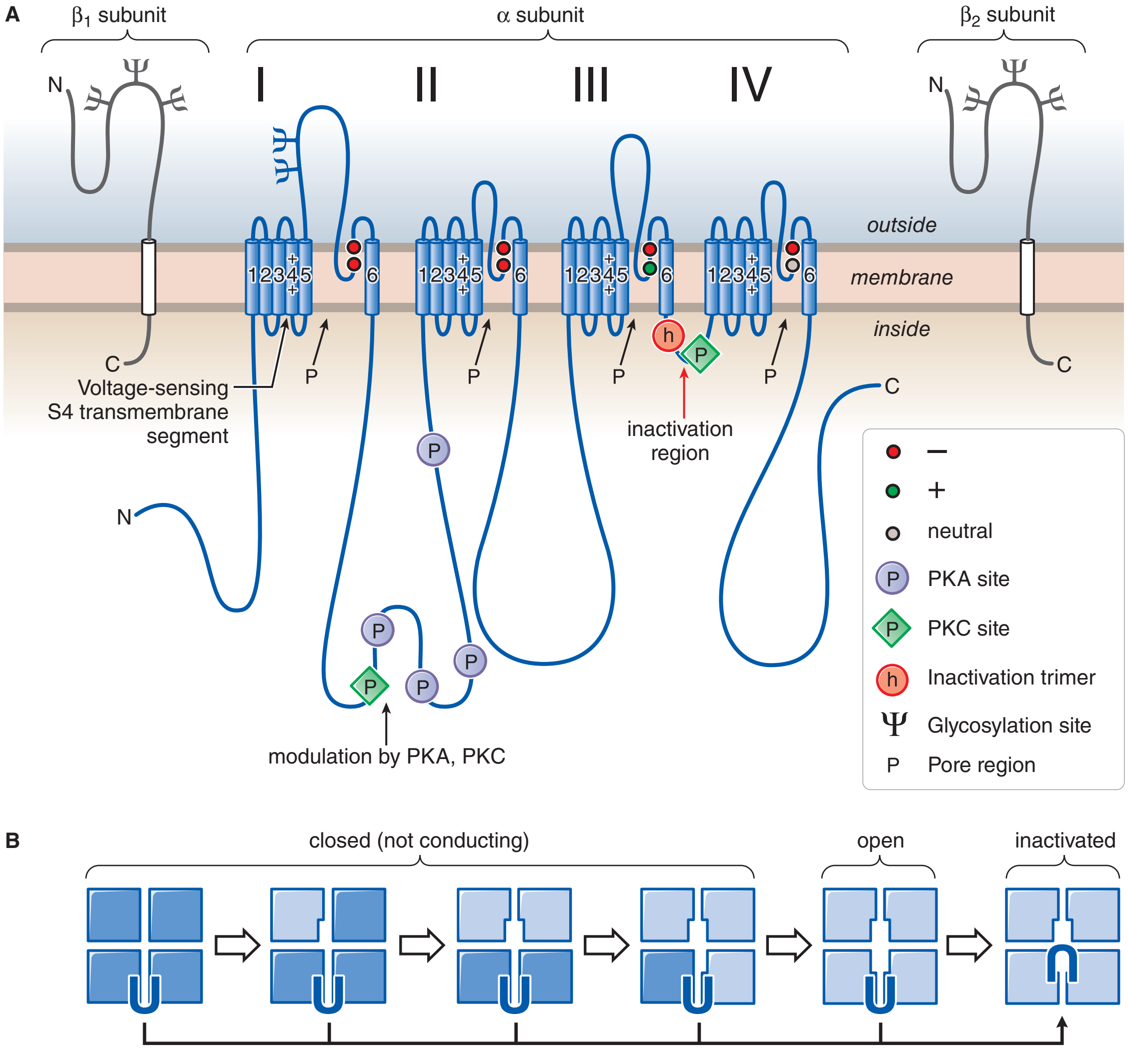


Figure 25–2 *Molecular organization and function of voltage-gated Na^+ channels.* **A.** A two-dimensional representation of the α (center), β_1 (left), and β_2 (right) subunits of the voltage-gated Na^+ channel from mammalian brain. The polypeptide chains are represented by continuous lines with length approximately proportional to the actual length of each segment of the channel protein. Cylinders represent regions of transmembrane α helices. ψ indicates sites of demonstrated *N*-linked glycosylation. Note the repeated structure of the four homologous domains (I–IV) of the α subunit. **Voltage Sensing**. The S4 transmembrane segments in each homologous domain of the α subunit serve as voltage sensors. (+) represents the positively charged amino acid residues at every third position within these segments. Electrical field (negative inside) exerts a force on these charged amino acid residues, pulling them toward the intracellular side of the membrane; depolarization allows them to move outward and initiate a conformational change that opens the pore. **Pore**. The S5 and S6 transmembrane segments and the short membrane-associated loop between them (*P* loop) form the walls of the pore in the center of an approximately symmetrical square array of the four homologous domains (see **B**). The amino acid residues indicated by circles in the *P* loop are critical for determining the conductance and ion selectivity of the Na^+ channel and its ability to bind the extracellular pore-blocking toxin tetrodotoxin (TTX) and saxitoxin. **Inactivation**. The short intracellular loop connecting homologous domains III and IV serves as the fast inactivation gate of the Na^+ channel. It is thought to fold into the intracellular surface of the pore domain and occlude the pore within a few milliseconds after it opens. Three hydrophobic residues (isoleucine-phenylalanine-methionine [IFM]) at the position marked **h** appear to serve as an inactivation particle, binding to the intracellular surface of the pore module and binding to an inactivation gate receptor there. **Modulation**. The gating of the Na^+ channel can be modulated by protein phosphorylation. Phosphorylation of the inactivation gate between homologous domains III and IV by protein kinase C (PKC) slows inactivation. Phosphorylation of sites in the intracellular loop between homologous domains I and II by either PKC or protein kinase A (PKA) reduces Na^+ channel activation. (Adapted with permission from Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron*, **2000**, *26*:13–25. Copyright © Elsevier). **B.** The four homologous domains of the Na^+ channel α subunit are illustrated as a square array, as viewed looking down on the membrane. The sequence of conformational changes that the Na^+ channel undergoes during activation and inactivation is diagrammed. On depolarization, each of the four homologous domains sequentially undergoes a conformational change to an activated state. After all four domains have activated, the Na^+ channel can open. Within a few milliseconds after opening, the inactivation gate between domains III and IV closes into the intracellular surface of the pore module and binds there, preventing further ion conductance (see Catterall, 2000).

the local anesthetic receptor site in the center of the transmembrane pore of the Na^+ channel, with part of its structure contributed by amino acids in the S6 segments of domains I, III, and IV (Figure 25–3A). Ancestral Na^+ channels in bacteria comprise four identical subunits, each similar to one of the four domains of the mammalian Na^+ channel α subunit and containing a similar voltage sensor and pore-lining segment. In schematic terms, a voltage-gated Na^+ channel is composed of a funnel-shaped extracellular *vestibule* that feeds sodium ions into a narrow *selectivity filter*, which opens onto a large, water-filled *central cavity* that has an intracellular exit *gate* (Figure 25–3B). Functionally, the channel can exist in a cycle of multiple states, controlled by the local effects of the membrane potential on the positive gating charges in the S4 transmembrane segments in domains I–IV of the channel protein, as shown in Figure 25–2A. These states are *resting/closed, intermediate/closed, open,* and *inactivated*.

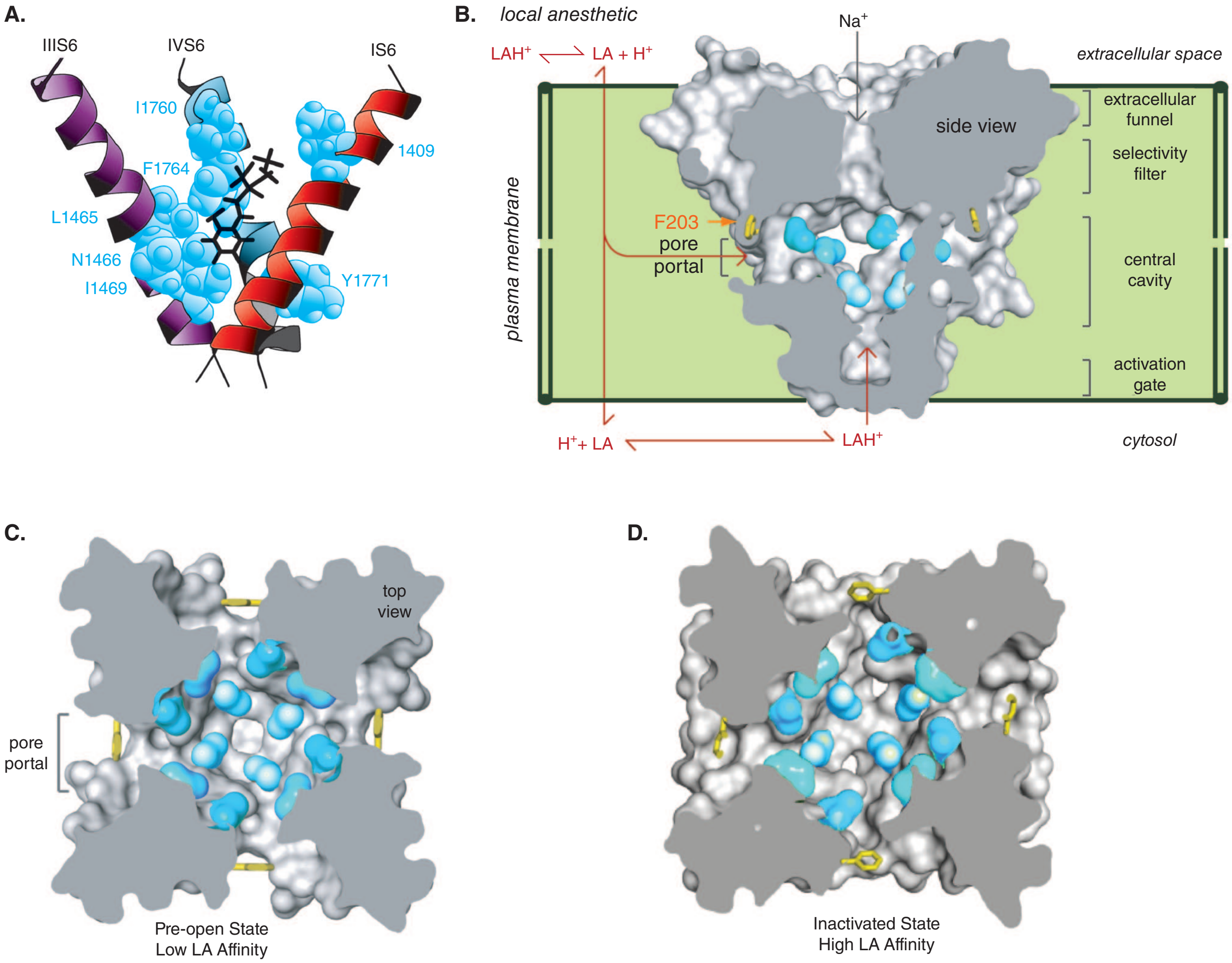


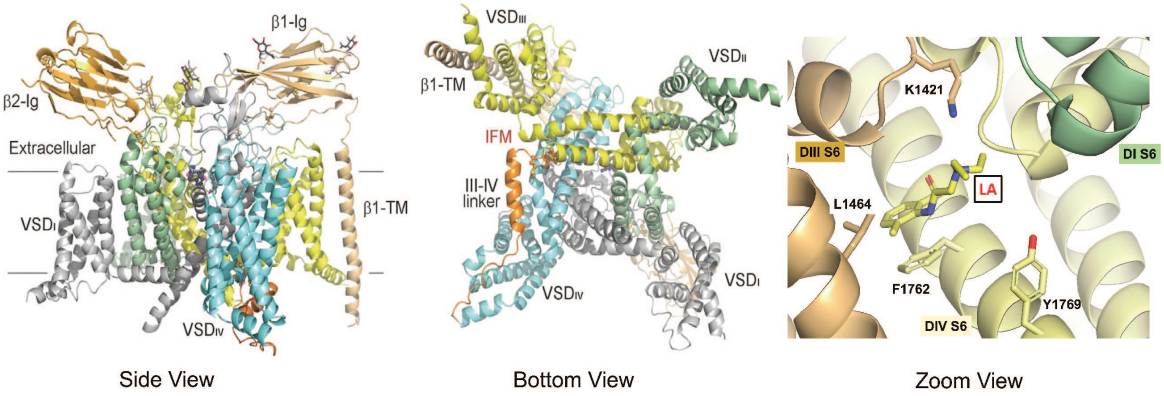
Figure 25–3 *A structural view of the interaction of a local anesthetic (LA) with a voltage-gated Na⁺ channel.* **A.** Functional model of the receptor site for LAs with *etidocaine* bound (black sticks) and amino acid residues in the pore-lining S6 segments in domains I, III, and IV (light blue balls), whose mutation causes loss of LA block. **B.** LA access and binding. LAs bind in the center of the pore region depicted by the light blue balls. LAs exist in charged and uncharged forms at physiological pH, in accordance with the Henderson-Hasselbalch relationship (see Figure 2–3). The uncharged species, LA, can diffuse across the membrane, possibly interacting with the channel protein en route. Within the cell, LA equilibrates with H⁺; the charged form, LAH⁺, binds in the channel with greater affinity than does the uncharged species. The resting/closed conformation of the channel has a relatively low affinity for LA. Depolarization initiates a conformational change that opens the intracellular activation gate, allowing rapid inward sodium conductance through the pore and rapid access of LAs to their receptor site in a high-affinity conformation. Thus, stimulation of a nerve by an action potential enhances LA binding. With a low frequency of stimulation, LA has time to dissociate and the channels reliably return to their resting state (low affinity for LA). With a high frequency of stimulation, as in nociceptive sensory afferents after a wound, there is insufficient time for LA to fully dissociate; thus, the fraction of channels liganded by LA increases in the continued presence of LA, leading to greater and greater conduction blockade. **C.** Top view of a cross-section through the bacterial Na⁺ channel $\mathrm{Na_VAb}$ highlighting the positions of amino acid residues that bind LAs in light blue (Payandeh et al., 2011). A portal or fenestration in the side of the pore provides a slow route of drug access from the membrane. **D.** Top view. The LA receptor site (light blue) undergoes a substantial conformational change in the inactivated state, which increases binding of LAs.

The three-dimensional structure of an ancestral Na⁺ channel (Payandeh et al., 2011) revealed the arrangement of its transmembrane segments and the positions of the amino acid residues in the local anesthetic binding site in the pore, as represented by light blue balls (Figure 25–3C, D).

Recently, the complete structures of mammalian Na⁺ channels have been determined at high resolution by cryogenic electron microscopy, including the peripheral nerve Na⁺ channel $\mathrm{Na_V1.7}$ (Figure 25–4). This structure reveals the functional components of the peripheral nerve Na⁺ channel in atomic detail and shows a conservation of structure of the core transmembrane region of Na⁺ channels from bacteria to mammals (Payandeh et al., 2011; Shen et al., 2019).

Frequency and Voltage Dependence

The degree of block produced by a given concentration of local anesthetic depends on how the nerve has been stimulated and on its resting membrane potential. Thus, a resting nerve is much less sensitive to a local anesthetic than one that is repetitively stimulated; higher frequency of stimulation and more positive membrane potential cause a greater degree of anesthetic block. These frequency- and voltage-dependent effects of local anesthetics occur because the charged form of the local anesthetic molecule gains access to its binding site within the pore primarily when the Na⁺ channel is open and because the local anesthetic binds more tightly to and stabilizes the inactivated state of the Na⁺ channel (Butterworth and Strichartz, 1990; Courtney and Strichartz, 1987; Hille, 1977). Remarkably, the conformation of the local anesthetic receptor site is changed considerably in the inactivated state (Payandeh et al., 2012; Figure 25–3D), revealing how preferential binding to inactivated Na⁺ channels may occur. The frequency and voltage dependence of local anesthetic inhibition of Na⁺ channels contribute to the selectivity of drug action because rapidly fired action potentials that signal intense pain are blocked preferentially compared to more slowly fired action potentials that communicate other sensory information.



Side View Bottom View Zoom View

Figure 25–4 *Three-dimensional structure of a Na$^+$ channel.* The three-dimensional structure of the sensory nerve Na$^+$ channel Na$_V$1.7 determined by cryogenic electron microscopy (Shen et al., 2019). **Left panel:** This side view shows the organization of the 24 transmembrane segments of the four homologous domains of the Na$^+$ channel α subunits (domain I, gray; domain II, green; domain III, yellow; domain IV, blue) and the binding positions of the β1 and β2 subunits (β1, light tan; β2, dark tan). **Center panel:** This bottom view highlights the domain III–IV linker and the pore-blocking IFM motif of the fast inactivation gate (dark tan). **Right panel:** This zoom view is a model of *lidocaine* (local anesthetic [LA]) bound in its receptor site in the pore, based on structures of Na$_V$1.7 and Na$_V$1.5, the primary cardiac Na$^+$ channel (Jiang et al., 2020; Shen et al., 2019). Amino acid numbering is that from Na$_V$1.5. In contrast to this binding position of LAs, the potent marine neurotoxins tetrodotoxin and saxitoxin bind in the extracellular funnel with high affinity ($K_d \sim 10^{-9}$ M) and block the pore (Jiang et al., 2020; Shen et al., 2019). β2-Ig, beta-2 subunit immunoglobulin-like domain; D# S6, Domain # (Roman numerals I through IV) transmembrane segment 6; IFM, fast inactivation Ile/Phe/Met (IFM) motif; VSD, voltage-sensing domain. Side and bottom views: From Shen H, et al. Structures of human Na$_V$1.7 channel in complex with auxiliary subunits and animal toxins. *Science*, **2019**, *363*:1303-1308. Reprinted with permission from AAAS.

Local anesthetics exhibit frequency and voltage dependence to different extents depending on their pK_a, lipid solubility, molecular size, and binding to different channel states. In general, the frequency dependence of local anesthetic action depends critically on the rate of dissociation from the receptor site in the pore of the Na$^+$ channel. A high frequency of stimulation is required for rapidly dissociating drugs so that drug binding during the action potential exceeds drug dissociation between action potentials. Dissociation of smaller and more hydrophobic drugs is more rapid, so a higher frequency of stimulation is required to yield frequency-dependent block. Frequency-dependent block of ion channels is also important for the actions of antiarrhythmic drugs (see Chapter 34).

Differential Sensitivity of Nerve Fibers

For most patients, treatment with local anesthetics causes the sensation of pain to disappear first, followed by loss of the sensations of temperature, touch, deep pressure, and finally motor function (Table 25–1). Classical experiments with intact nerves showed that the δ wave in the compound action potential, which represents slowly conducting, small-diameter myelinated fibers, was reduced more rapidly and at lower concentrations of cocaine than was the α wave, which represents rapidly conducting, large-diameter fibers (Gasser and Erlanger, 1929). In general, autonomic fibers, small unmyelinated C fibers (mediating pain sensations), and small myelinated Aδ fibers (mediating pain and temperature sensations) are blocked before the larger myelinated Aγ, Aβ, and Aα fibers (mediating postural, touch, pressure, and motor information) (Raymond and Gissen, 1987). *The differential rate of block exhibited by fibers mediating different sensations is of considerable practical importance in the use of local anesthetics.*

The precise mechanisms responsible for this apparent specificity of local anesthetic action on pain fibers are not fully known, but several factors may contribute. Although sensitivity to local anesthetic block

TABLE 25–1 ■ SUSCEPTIBILITY OF NERVE TYPES TO LOCAL ANESTHETICS

CLASSIFICATION	ANATOMIC LOCATION	MYELIN	DIAMETER (μm)	CONDUCTION VELOCITY (m/s)	FUNCTION	CLINICAL SENSITIVITY TO BLOCK
A fibers						
A α	Afferent to and efferent from muscles and joints	Yes	6–22	10–85	Motor and proprioception	+
A β						++
A γ	Efferent to muscle spindles	Yes	3–6	15–35	Muscle tone	++
A δ	Sensory roots and afferent peripheral nerves	Yes	1–4	5–25	Pain, temperature, touch	+++
B fibers	Preganglionic sympathetic	Yes	<3	3–15	Vasomotor, visceromotor, sudomotor, pilomotor	++++
C fibers						
Sympathetic	Postganglionic sympathetic	No	0.3–1.3	0.7–1.3	Vasomotor, visceromotor, sudomotor, pilomotor	++++
Dorsal root	Sensory roots and afferent peripheral nerves	No	0.4–1.2	0.1–2	Pain, temperature, touch	++++

Adapted with permission from Carpenter RL, Mackey DC. Local anesthetics. In: Barash PG, Cullen BF, Stoelting RK, eds. *Clinical Anesthesia*. 2nd ed. Lippincott, Philadelphia, **1992**, 509–541. http://lww.com.

increases with decreasing fiber size, consistent with high sensitivity for pain sensation mediated by small fibers (Gasser and Erlanger, 1929), no clear correlation of the concentration dependence of local anesthetic block with fiber diameter is observed when the orientation of nerve fibers allows direct measurement of action potentials (Fink and Cairns, 1984; Franz and Perry, 1974; Huang et al., 1997). Therefore, it is unlikely that the fiber size *per se* determines the sensitivity to local anesthetic block under steady-state conditions. However, the spacing of nodes of Ranvier increases with the size of nerve fibers. Because a fixed number of nodes must be blocked to prevent conduction, small fibers with closely spaced nodes of Ranvier may be blocked more rapidly during treatment of intact nerves because the local anesthetic reaches a critical length of nerve more rapidly. Differences in tissue barriers and location of smaller C fibers and Aδ fibers in nerves also may influence the rate of local anesthetic action. Different combinations of Na^+ channel subtypes are also expressed in these nerve fibers, but all of these Na^+ channels have similar affinity for block by local anesthetics.

Effect of pH

Local anesthetics tend to be only slightly soluble as unprotonated amines. Therefore, they generally are marketed as water-soluble salts, usually hydrochlorides. Because local anesthetics are weak bases (typical pK_a values range from 8 to 9), their hydrochloride salts are mildly acidic. This property increases the stability of the local anesthetic esters and the catecholamines added as vasoconstrictors. Under usual conditions of administration, the pH of the local anesthetic solution rapidly equilibrates to that of the extracellular fluids.

Although the unprotonated species of the local anesthetic is necessary for diffusion across cellular membranes, it is the cationic species that interacts preferentially with Na^+ channels. The results of experiments on anesthetized mammalian nonmyelinated fibers support this conclusion (Ritchie and Greengard, 1966). In these experiments, conduction could be blocked or unblocked merely by adjusting the pH of the bathing medium to 7.2 or 9.6, respectively, without altering the amount of anesthetic present. The primary role of the cationic form also was demonstrated by Narahashi and Frazier, who perfused the extracellular and axoplasmic surface of the giant squid axon with tertiary and quaternary amine local anesthetics and found that the quaternary amines were active only when perfused intracellularly (Narahashi and Frazier, 1971). However, the unprotonated molecular forms also possess anesthetic activity (Butterworth and Strichartz, 1990).

Prolongation of Action by Vasoconstrictors

The duration of action of a local anesthetic is proportional to the time of contact with nerve. Consequently, maneuvers that keep the drug at the nerve prolong the period of anesthesia. For example, *cocaine* inhibits the neuronal membrane transporters for catecholamines, thereby potentiating the effect of norepinephrine (NE) at α adrenergic receptors in the vasculature, resulting in vasoconstriction and reduced *cocaine* absorption in vascular beds where α adrenergic effects predominate (see Chapters 10 and 14). In clinical practice, a vasoconstrictor, usually *epinephrine*, is often added to local anesthetics. The vasoconstrictor performs a dual service. By decreasing the rate of absorption, it localizes the anesthetic at the desired site and allows the drug's elimination to keep pace with its entry into the systemic circulation, thereby reducing the drug's systemic toxicity. Note, however, that *epinephrine* dilates skeletal muscle vascular beds via actions at β_2 adrenergic receptors and therefore has the potential to increase systemic toxicity of anesthetic deposited in muscle tissue.

Some of the vasoconstrictor agents may be absorbed systemically, occasionally to an extent sufficient to cause untoward reactions (see the next section). There also may be delayed wound healing, tissue edema, or necrosis after local anesthesia. These effects seem to occur partly because sympathomimetic amines increase the O_2 consumption of the tissue; this, together with the vasoconstriction, leads to hypoxia and local tissue damage. Thus, the use of vasoconstrictors in local anesthetic preparations for anatomical regions with limited collateral circulation is avoided.

Undesired Effects of Local Anesthetics

In addition to blocking conduction in nerve axons in the peripheral nervous system, local anesthetics interfere with the function of all organs in which conduction or transmission of impulses occurs. Thus, these agents affect the CNS, autonomic ganglia, neuromuscular junctions, and all forms of muscle (for a review, see Covino, 1987; Garfield and Gugino, 1987; Gintant and Hoffman, 1987). The danger of such adverse reactions is proportional to the concentration of local anesthetic achieved in the circulation. In general, for local anesthetics with chiral centers, the *S*-enantiomer is less toxic than the *R*-enantiomer (McClure, 1996).

CNS

Following absorption, local anesthetics may cause CNS stimulation, producing restlessness and tremor that may progress to clonic convulsions. In general, the more potent the anesthetic, the more readily convulsions may be produced. Alterations of CNS activity are thus predictable from the local anesthetic agent in question and the blood concentration achieved. Central stimulation is followed by depression; death usually is caused by respiratory failure.

The apparent stimulation and subsequent depression produced by applying local anesthetics to the CNS presumably is due solely to depression of neuronal activity; a selective depression of inhibitory neurons likely accounts for the excitatory phase *in vivo*. Rapid systemic administration of local anesthetics may produce death with no or only transient signs of CNS stimulation. Under these conditions, the concentration of the drug probably rises so rapidly that all neurons are depressed simultaneously. Airway control, along with ventilatory and circulatory support, are essential features of treatment in the late stage of intoxication. Intravenously administered benzodiazepines are the drugs of choice for both the prevention and the arrest of convulsions. Neither *propofol* nor a rapidly acting barbiturate is preferred; both are more likely to produce cardiovascular depression than a benzodiazepine (see Chapter 20).

Although drowsiness is the most frequent complaint that results from the CNS actions of local anesthetics, *lidocaine* may produce dysphoria or euphoria and muscle twitching. Moreover, *lidocaine* may produce a loss of consciousness that is preceded only by symptoms of sedation (Covino, 1987). Whereas other local anesthetics also show the effect, *cocaine* has a particularly prominent effect on mood and behavior. These effects of *cocaine* and its potential for abuse are discussed in Chapter 28.

Cardiovascular System

Following systemic absorption, local anesthetics act on the cardiovascular system. The primary site of action is the myocardium, where decreases in electrical excitability, conduction rate, and force of contraction occur. In addition, most local anesthetics cause arteriolar dilation. Untoward cardiovascular effects usually are seen only after high systemic concentrations are attained and CNS symptoms are evident. However, on rare occasions, lower doses of some local anesthetics will cause cardiovascular collapse and death, probably due to either an action on the pacemaker or the sudden onset of ventricular fibrillation. Ventricular tachycardia and fibrillation are relatively uncommon consequences of local anesthetics other than *bupivacaine*. The antiarrhythmic effects of local anesthetics such as *lidocaine* and *procainamide* are discussed in Chapter 34. Finally, it should be stressed that untoward cardiovascular effects of local anesthetic agents may result from their inadvertent intravascular administration, especially if *epinephrine* is also present.

Smooth Muscle

Local anesthetics depress contractions in the intact bowel and in strips of isolated intestine (Zipf and Dittmann, 1971). They also relax vascular and bronchial smooth muscle, although low concentrations initially may produce contraction (Covino, 1987). Spinal and epidural anesthesia, as well as instillation of local anesthetics into the peritoneal cavity, cause sympathetic nervous system paralysis, which can result in increased tone of gastrointestinal musculature (described under Clinical Uses). Local anesthetics may increase the resting tone and decrease the contractions of isolated human uterine muscle; however, uterine contractions are seldom depressed directly during intrapartum regional anesthesia.

Neuromuscular Junction and Ganglia

Local anesthetics also affect transmission at the neuromuscular junction. At concentrations at which the muscle responds normally to direct electrical stimulation, *procaine* can block the response of skeletal muscle to maximal motor-nerve volleys and to acetylcholine (ACh). Similar effects occur at autonomic ganglia. These effects are due to block of nicotinic ACh receptors by high concentrations of the local anesthetic (Charnet et al., 1990; Neher and Steinbach, 1978).

Hypersensitivity

Rare individuals are hypersensitive to local anesthetics. The reaction may manifest itself as an allergic dermatitis or a typical asthmatic attack (Covino, 1987). It is important to distinguish allergic reactions from toxic side effects and from the effects of coadministered vasoconstrictors. Hypersensitivity seems to occur more frequently with local anesthetics of the ester type and frequently extends to chemically related compounds. For example, individuals sensitive to *procaine* also may react to structurally similar compounds (e.g., *tetracaine*) through reaction to a common metabolite. Although allergic responses to agents of the amide type are uncommon, solutions of such agents may contain preservatives such as methylparaben that may provoke an allergic reaction (Covino, 1987). Local anesthetic preparations containing a vasoconstrictor also may elicit allergic responses due to the sulfite added as an antioxidant for the catecholamine/vasoconstrictor.

Metabolism

Local anesthetics of the ester type (e.g., *tetracaine*) are hydrolyzed and inactivated primarily by a plasma esterase, probably plasma cholinesterase. The liver also participates in hydrolysis of local anesthetics. Because spinal fluid contains little or no esterase, anesthesia produced by the intrathecal injection of an anesthetic agent will persist until the local anesthetic agent has been absorbed into the circulation. The amide-linked local anesthetics are, in general, degraded by the hepatic CYPs, with the initial reactions involving *N*-dealkylation and subsequent hydrolysis (Arthur, 1987). However, with *prilocaine*, the initial step is hydrolytic, forming *o*-toluidine metabolites that can cause methemoglobinemia. The extensive use of amide-linked local anesthetics in patients with severe hepatic disease requires caution.

Toxicity

The metabolic fate of local anesthetics is of great practical importance because toxicity can result from an imbalance between their rates of absorption and elimination. The rate of absorption of many local anesthetics into the systemic circulation can be considerably reduced by the incorporation of a vasoconstrictor agent in the anesthetic solution. However, the rate of degradation of local anesthetics varies greatly, and this is a major factor in determining the safety of a particular agent. Because toxicity is related to the concentration of free drug, binding of the anesthetic to proteins in the serum and to tissues reduces toxicity. For example, in intravenous regional anesthesia of an extremity, about half of the original anesthetic dose still is tissue bound 30 min after the restoration of normal blood flow (Arthur, 1987). Reversing the effects of local anesthetic systemic toxicity is a clinical challenge. One developing approach is promising and unusual: intravenous lipid emulsion therapy (Weinberg, 2012). Whether the lipids simply provide a favorable milieu of micelles into which lipophilic drugs can partition or the effect involves more complex biochemical pathways is not yet clear (Ok et al., 2018).

Plasma binding sites serve to moderate local anesthetic levels in blood. The amide-linked local anesthetics bind extensively (55%–95%) to plasma proteins, particularly α_1-acid glycoprotein. Many factors increase (e.g., cancer, surgery, trauma, myocardial infarction, smoking, and uremia) or decrease (e.g., oral contraceptives) the level of this glycoprotein, thereby changing the amount of anesthetic delivered to the liver for metabolism and thus influencing systemic toxicity. Age-related changes in protein binding of local anesthetics also occur. The neonate is relatively deficient in plasma proteins that bind local anesthetics and thereby is more susceptible to toxicity. Plasma proteins are not the sole determinant of local anesthetic availability. Uptake by the lung also may play an important role in the distribution of amide-linked local anesthetics. Finally, reduced cardiac output slows delivery of the amide compounds to the liver, reducing their metabolism and prolonging their plasma half-lives.

Local Anesthetics and Related Agents

Cocaine

Chemistry

Cocaine, an ester of benzoic acid and methylecgonine, occurs in abundance in the leaves of the coca shrub. Egconine is an amino alcohol base closely related to tropine, the amino alcohol in *atropine*. It has the same fundamental structure as the synthetic local anesthetics (see Figure 25–1).

Pharmacological Actions and Preparations

The clinically desired actions of *cocaine* are the blockade of nerve impulses as a consequence of its local anesthetic properties and local vasoconstriction secondary to inhibition of the NE transporter (NET) (see Table 10–5). Toxicity and its potential for abuse have steadily decreased the clinical uses of *cocaine*. Its high toxicity is due to reduced catecholamine uptake in both the central and peripheral nervous systems and the resulting prolongation of transmitter dwell time in the synaptic cleft. *Cocaine*'s euphoric properties are due primarily to inhibition of catecholamine uptake, particularly dopamine, in the CNS. Other local anesthetics do not block the uptake of NE and do not produce the sensitization to catecholamines, vasoconstriction, or mydriasis characteristic of *cocaine*. Currently, *cocaine* is used primarily for topical anesthesia of the upper respiratory tract, where its combination of both vasoconstrictor and local anesthetic properties provides anesthesia and shrinking of the mucosa. *Cocaine hydrochloride* is provided as a 1%, 4%, or 10% solution for topical application. For most applications, the 1% or 4% preparation is preferred to reduce toxicity. Because of its abuse potential, *cocaine* is listed as a schedule II controlled substance by the U.S. Drug Enforcement Administration.

Lidocaine

Lidocaine, an aminoethylamide (Figure 25–1), is the prototypical amide local anesthetic.

Pharmacological Actions and Preparations

Lidocaine produces faster, more intense, longer lasting, and more extensive anesthesia than does an equal concentration of *procaine*. *Lidocaine* is an alternative choice for individuals sensitive to ester-type local anesthetics.

A *lidocaine* transdermal patch is used for relief of pain associated with postherpetic neuralgia. The combination of *lidocaine* (2.5%) and *prilocaine* (2.5%) under an occlusive dressing (EMLA, others) is used as an anesthetic prior to venipuncture, skin graft harvesting, and infiltration of anesthetics into genitalia. *Lidocaine* in combination with *tetracaine* in a formulation that generates a "peel" is approved for topical local analgesia prior to superficial dermatological procedures such as filler injections and laser-based treatments. *Lidocaine* in combination with *tetracaine* is also supplied in a formulation that generates heat on exposure to air, which is used prior to venous access and superficial dermatological procedures such as excision, electrodessication, and shave biopsy of skin lesions. The mild warming is intended to increase skin temperature by up to 5°C for the purpose of enhancing delivery of local anesthetic into the skin.

ADME

Lidocaine is absorbed rapidly after parenteral administration and from the gastrointestinal and respiratory tracts. Although it is effective when used without any vasoconstrictor, *epinephrine* decreases the rate of absorption, thereby decreasing the probability of toxicity and prolonging the duration of action. In addition to preparations for injection, *lidocaine* is formulated for topical, ophthalmic, mucosal, and transdermal use.

Lidocaine is dealkylated in the liver by CYPs to monoethylglycine xylidide and glycine xylidide, which can be metabolized further to monoethylglycine and xylidide. Both monoethylglycine xylidide and glycine xylidide retain local anesthetic activity. In humans, about 75% of the xylidide is excreted in the urine as the further metabolite 4-hydroxy-2,6-dimethylaniline (Arthur, 1987).

Toxicity

The side effects of *lidocaine* seen with increasing dose include drowsiness, tinnitus, dysgeusia, dizziness, and twitching. As the dose increases, seizures, coma, and respiratory depression and arrest will occur. Clinically significant cardiovascular depression usually occurs at serum *lidocaine* levels that produce marked CNS effects. The metabolites monoethylglycine xylidide and glycine xylidide may contribute to some of these side effects.

Clinical Uses

Lidocaine has a wide range of clinical uses as a local anesthetic; it has utility in almost any application where a local anesthetic of intermediate duration is needed. *Lidocaine* also is used as an antiarrhythmic agent (see Chapter 34).

Bupivacaine

Bupivacaine has a wide range of clinical uses as a local anesthetic; it has utility in almost any application where a local anesthetic of long duration is needed.

Pharmacological Actions and Preparations

Bupivacaine is a widely used amide local anesthetic; its structure is similar to that of *lidocaine* except that the amine-containing group is a butyl piperidine (Figure 25–1). *Bupivacaine* is a potent agent capable of producing prolonged anesthesia. Its long duration of action plus its tendency to provide more sensory than motor block has made it a popular drug for providing prolonged analgesia during labor or the postoperative period. By taking advantage of indwelling catheters and continuous infusions, *bupivacaine* can be used to provide several days of effective analgesia. Recently, a liposomal *bupivacaine* preparation has been FDA-approved. While safe and effective, its clinical superiority over conventional *bupivacaine* has not been demonstrated (Hussain et al., 2021).

ADME

Bupivacaine is more slowly absorbed than *lidocaine*, so plasma levels increase more slowly following a *bupivacaine* nerve block or epidural. Conversely, *bupivacaine* levels fall more slowly following cessation of a continuous *bupivacaine* infusion than would be predicted from single-injection pharmacokinetics. *Bupivacaine* is primarily metabolized in the liver by CYP3A4 to pipecolylxylidine, which is then glucuronidated and excreted.

Toxicity

Bupivacaine is more cardiotoxic than equieffective doses of *lidocaine*. Clinically, this is manifested by severe ventricular arrhythmias and myocardial depression after inadvertent intravascular administration. Although *lidocaine* and *bupivacaine* both rapidly block cardiac Na^+ channels during systole, *bupivacaine* dissociates much more slowly than *lidocaine* during diastole, so a significant fraction of Na^+ channels at physiological heart rates remains blocked with *bupivacaine* at the end of diastole (Clarkson and Hondeghem, 1985). Thus, the block by *bupivacaine* is cumulative and substantially more than predicted by its local anesthetic potency. At least a portion of the cardiac toxicity of *bupivacaine* may be mediated centrally; direct injection of small quantities of *bupivacaine* into the medulla can produce malignant ventricular arrhythmias (Thomas et al., 1986). *Bupivacaine*-induced cardiac toxicity can be difficult to treat, and its severity is enhanced by coexisting acidosis, hypercarbia, and hypoxemia, emphasizing the importance of prompt airway control in resuscitation from *bupivacaine* overdose.

Local Anesthetics Suitable for Injection

The number of synthetic local anesthetics is so large that it is impractical to consider them all here. Some local anesthetic agents are too toxic to be given by injection. Their use is restricted to topical application to the eye (see Chapter 74), the mucous membranes, or the skin (see Chapter 75). Many local anesthetics are suitable, however, for infiltration or injection to produce nerve block; some of these also are useful for topical application. The discussion below presents the main categories of local anesthetics; agents are listed alphabetically.

Articaine

Articaine is approved in the U.S. for dental and periodontal procedures. Although it is an amide local anesthetic, it also contains an ester, whose hydrolysis terminates its action. Thus, *articaine* exhibits rapid onset (1–6 min) and duration of action of about 1 h.

Chloroprocaine

Chloroprocaine is a chlorinated derivative of *procaine*. Its major assets are its rapid onset and short duration of action and its reduced acute toxicity due to rapid metabolism (plasma $t_{1/2}$ ~ 25 sec). Enthusiasm for its use has been tempered by reports of prolonged sensory and motor block after epidural or subarachnoid administration of large doses. This toxicity appears to have been a consequence of low pH and the use of sodium metabisulfite as a preservative in earlier formulations. There are no reports of neurotoxicity with newer preparations of *chloroprocaine* that contain calcium ethylenediaminetetraacetic acid (EDTA) as the preservative, although these preparations are not recommended for intrathecal administration. A higher-than-expected incidence of muscular back pain following epidural anesthesia with *2-chloroprocaine* has also been reported (Stevens et al., 1993). This back pain is thought to be due to tetany in the paraspinous muscles, which may be a consequence of Ca^{2+} binding by the EDTA included as a preservative; the incidence of back pain appears to be related to the volume of drug injected and its use for skin infiltration.

Mepivacaine

Mepivacaine is an intermediate-acting amino amide with pharmacological properties resembling those of *lidocaine*. *Mepivacaine*, however, is more toxic to the neonate and thus is not used in obstetrical anesthesia. The increased toxicity of *mepivacaine* in the neonate is related to ion trapping of this agent because of the lower pH of neonatal blood and the pK_a of *mepivacaine*, rather than to its slower metabolism in the neonate. *Mepivacaine* appears to have a slightly higher therapeutic index in adults than does *lidocaine*. Its onset of action is similar to, and its duration slightly longer (~20%) than, that of *lidocaine* in the absence of a coadministered vasoconstrictor. *Mepivacaine* is not effective as a topical anesthetic.

Prilocaine

Prilocaine is an intermediate-acting amino amide. It has a pharmacological profile similar to that of *lidocaine*. The primary differences are that it causes little vasodilation and thus can be used without a vasoconstrictor; its increased volume of distribution reduces its CNS toxicity, making it suitable for intravenous regional blocks (described further in the chapter). The use of *prilocaine* is largely limited to dentistry because the drug is unique among the local anesthetics in its propensity to cause methemoglobinemia. This effect is a consequence of the metabolism of the aromatic ring to *o*-toluidine. Development of methemoglobinemia is dependent on the total dose administered, usually appearing after a dose of 8 mg/kg. If necessary, it can be treated by the intravenous administration of *methylene blue* (1–2 mg/kg).

Ropivacaine

The cardiac toxicity of *bupivacaine* stimulated interest in developing a less-toxic, long-lasting local anesthetic. One result of that search was the development of the amino ethylamide *ropivacaine*; the *S*-enantiomer was chosen because it has a lower toxicity than the *R*-isomer (McClure, 1996). *Ropivacaine* is slightly less potent than *bupivacaine* in producing anesthesia. *Ropivacaine* appears to be suitable for both epidural and regional anesthesia, with a duration of action similar to that of *bupivacaine*. Interestingly, it seems to be even more motor-sparing than *bupivacaine*.

Procaine

Procaine is no longer marketed in the U.S. as a single entity. It is an ingredient of some long-acting intramuscular formulations of *penicillin*.

Tetracaine

Tetracaine is a long-acting amino ester. It is significantly more potent and has a longer duration of action than *procaine*. *Tetracaine* may exhibit increased systemic toxicity because it is more slowly metabolized than the other commonly used ester local anesthetics. Currently, it is widely used in spinal anesthesia when a drug of long duration is needed. *Tetracaine* also is incorporated into several topical anesthetic preparations. With the introduction of *bupivacaine*, *tetracaine* is rarely used in peripheral nerve blocks because of the large doses often necessary, its slow onset, and its potential for toxicity.

Agents Used Primarily to Anesthetize Mucous Membranes and Skin

Some agents are useful as topical anesthetic agents on the skin or mucous membranes, although too irritating or too ineffective to be applied to the eye. These preparations are effective in the symptomatic relief of anal and genital pruritus, poison ivy rashes, and numerous other acute and chronic dermatoses. They sometimes are combined with a glucocorticoid or antihistamine and are available in a number of proprietary formulations.

Dibucaine

Dibucaine is a quinoline derivative. Its toxicity resulted in its removal from the U.S. market as an injectable preparation. It retains wide popularity outside the U.S. as a spinal anesthetic. It currently is available as an over-the-counter ointment for cutaneous use.

Dyclonine

Dyclonine hydrochloride is readily absorbed through the skin and mucous membranes. Its onset is rapid; its duration of action is short. *Dyclonine* is an active ingredient in a number of over-the-counter medications, including sore throat lozenges, a patch for cold sores, and a 0.75% solution.

Pramoxine

Pramoxine hydrochloride is a surface anesthetic agent that is not a benzoate ester. Its distinct chemical structure may help minimize the danger of cross-sensitivity reactions in patients allergic to other local anesthetics. *Pramoxine* produces satisfactory surface anesthesia and is reasonably well tolerated on the skin and mucous membranes. It is too irritating to be used on the eye or in the nose, but an otic solution containing chloroxylenol is marketed. Many preparations, including creams, lotions, sprays, gel, wipes, and foams, usually containing 1% *pramoxine*, are available for topical application.

Anesthetics With Low Aqueous Solubility

Some local anesthetics have low aqueous solubility and consequently are absorbed too slowly to cause classical local anesthetic toxicity. These compounds can be applied directly to wounds and ulcerated surfaces, where they remain localized for long periods of time, producing a sustained anesthetic action. Chemically, they are esters of para-aminobenzoic acid lacking the terminal amino group possessed by the previously described local anesthetics. The most important member of the series is *benzocaine* (ethyl aminobenzoate), which is incorporated into a large number of topical preparations. *Benzocaine* can cause methemoglobinemia (see the discussion of methemoglobinemia in the section on *prilocaine*); consequently, dosing recommendations must be followed carefully.

Agents for Ophthalmic Use

Anesthesia of the cornea and conjunctiva can be obtained readily by topical application of local anesthetics. However, most of the local anesthetics that have been described are too irritating for ophthalmological use. The two compounds used most frequently today are *proparacaine* and *tetracaine*. In addition to being less irritating during administration, *proparacaine* has the advantage of bearing little antigenic similarity to the other benzoate local anesthetics. Thus, it sometimes can be used in individuals sensitive to the amino ester local anesthetics.

For use in ophthalmology, these local anesthetics are instilled a single drop at a time. If anesthesia is incomplete, successive drops are applied until satisfactory conditions are obtained. The duration of anesthesia is determined chiefly by the vascularity of the tissue; thus, it is longest in normal cornea and shortest in inflamed conjunctiva. In the latter case, repeated instillations may be necessary to maintain adequate anesthesia. Long-term administration of topical anesthesia to the eye has been associated with retarded healing, pitting, sloughing of the corneal epithelium, and predisposition of the eye to inadvertent injury. Thus, these drugs should not be prescribed for self-administration. For issues of drug delivery, pharmacokinetics, and toxicity unique to drugs for ophthalmic use, see Chapter 74.

Biological Toxins: Tetrodotoxin and Saxitoxin

The two biological toxins, tetrodotoxin and saxitoxin, block the pore of the Na^+ channel. Tetrodotoxin is found in the gonads and other visceral tissues of some fish of the order Tetraodontiformes (to which the Japanese *fugu*, or puffer fish, belongs); it also occurs in the skin of some newts of the family Salamandridae and of the Costa Rican frog *Atelopus*. *Saxitoxin* is elaborated by the dinoflagellates *Gonyaulax catenella* and *G. tamarensis* and retained in the tissues of clams and other shellfish that eat these organisms. Given the right conditions of temperature and light, the *Gonyaulax* may multiply so rapidly as to discolor the ocean, causing the condition known as *red tide*. Shellfish feeding on *Gonyaulax* at this time become extremely toxic to humans and are responsible for periodic outbreaks of paralytic shellfish poisoning (Sakai and Swanson, 2014; Stommel and Watters, 2004). Although these toxins are chemically distinct, they have similar mechanisms of action. Both toxins, in nanomolar concentrations, specifically block the outer mouth of the pore of Na^+ channels in the *membranes* of excitable cells. As a result, the action potential is blocked. The receptor site for these toxins is formed by amino acid residues in the *P* loop of the Na^+ channel α subunit (Figure 25–2) in all four domains (Catterall, 2000; Shen et al., 2019; Terlau et al., 1991). Not all Na^+ channels are equally sensitive to tetrodotoxin; some Na^+ channels in cardiac myocytes and dorsal root ganglion neurons are resistant, and a tetrodotoxin-resistant Na^+ channel is expressed when skeletal muscle is denervated. Tetrodotoxin and saxitoxin are exceedingly potent; the minimal lethal dose of each in the mouse is about 8 μg/kg. Both toxins have caused fatal poisoning in humans due to paralysis of the respiratory muscles; therefore, the treatment of severe cases of poisoning requires support of respiration. Blockade of vasomotor nerves, together with a relaxation of vascular smooth muscle, seems to be responsible for the hypotension that is characteristic of tetrodotoxin poisoning. Early gastric lavage and pressor support also are indicated. If the patient survives paralytic shellfish poisoning for 24 h, the prognosis is good.

Clinical Uses of Local Anesthetics

Local anesthesia is the loss of sensation in a body part without the loss of consciousness or the impairment of central control of vital functions. It offers two major advantages over general anesthesia. First, physiological perturbations associated with general anesthesia are avoided. Second, neurophysiological responses to pain and stress can be modified beneficially. However, local anesthetics have the potential to produce deleterious side effects. Proper choice of a local anesthetic and care in its use are the primary determinants in avoiding these problems.

There is a poor relationship between the amount of local anesthetic injected and peak plasma levels in adults. Furthermore, peak plasma levels vary widely depending on the area of injection. They are highest with interpleural or intercostal blocks and lowest with subcutaneous infiltration. Thus, recommended maximum doses serve only as general guidelines. This discussion summarizes the pharmacological and physiological consequences of the use of local anesthetics categorized by method of administration. A more comprehensive discussion of their use and administration is presented in textbooks on regional anesthesia (Cousins et al., 2008).

Topical Anesthesia

Anesthesia of mucous membranes of the nose, mouth, throat, tracheobronchial tree, esophagus, and genitourinary tract can be produced by direct application of aqueous solutions of salts of many local anesthetics or by suspension of the poorly soluble local anesthetics. *Tetracaine* (2%), *lidocaine* (2%–10%), and *cocaine* (1%–4%) typically are used. *Cocaine* is used only in the nose, nasopharynx, mouth, throat, and ear, where it uniquely produces vasoconstriction as well as anesthesia. The shrinking of mucous membranes decreases operative bleeding while improving surgical visualization. Comparable vasoconstriction can be achieved with other local anesthetics by the addition of a low concentration of a vasoconstrictor such as *phenylephrine* (0.005%). *Epinephrine*, topically applied, has no significant local effect and does not prolong the duration of action of local anesthetics applied to mucous membranes because of poor penetration. *Maximal safe total dosages* for topical anesthesia in a healthy 70-kg adult are 300 mg for *lidocaine*, 150 mg for *cocaine*, and 50 mg for *tetracaine*.

Peak anesthetic effect following topical application of *cocaine* or *lidocaine* occurs within 2 to 5 min (3–8 min with *tetracaine*), and anesthesia lasts for 30 to 45 min (30–60 min with *tetracaine*). Anesthesia is entirely superficial; it does not extend to submucosal structures. This technique does not alleviate joint pain or discomfort from subdermal inflammation or injury.

Local anesthetics are absorbed rapidly into the circulation following topical application to mucous membranes or denuded skin. Thus, topical anesthesia always carries the risk of systemic toxic reactions. Systemic toxicity has occurred even following the use of local anesthetics to control discomfort associated with severe diaper rash in infants. Absorption is particularly rapid when local anesthetics are applied to the tracheobronchial tree. Concentrations in blood after instillation of local anesthetics into the airway are nearly the same as those following intravenous injection. Surface anesthetics for the skin and cornea have been described earlier in the chapter.

Eutectic mixtures of local anesthetics *lidocaine* (2.5%)/*prilocaine* (2.5%) (EMLA) and *lidocaine* (7%)/*tetracaine* (7%) (Pliaglis) bridge the gap between topical and infiltration anesthesia. The efficacy of each of these combinations lies in the fact that the mixture has a melting point less than that of either compound alone, existing at room temperature as an oil that can penetrate intact skin. These creams produce anesthesia to a maximum depth of 5 mm and are applied as a cream on intact skin under an occlusive dressing in advance (~30–60 min) of any procedure. These mixtures are effective for procedures involving skin and superficial subcutaneous structures (e.g., venipuncture and skin graft harvesting). Beware: the component local anesthetics will be absorbed into the systemic circulation, potentially producing toxic effects. Guidelines are available to calculate the maximum amount of cream that can be applied and area of skin covered. These mixtures must not be used on mucous membranes or abraded skin, as rapid absorption across these surfaces may result in systemic toxicity.

Infiltration Anesthesia

Infiltration anesthesia is the injection of local anesthetic directly into tissue without taking into consideration the course of cutaneous nerves. Infiltration anesthesia can be so superficial as to include only the skin. It also can include deeper structures, including intra-abdominal organs, when these too are infiltrated.

The duration of infiltration anesthesia can be approximately doubled by the addition of *epinephrine* (5 μg/mL) to the injection solution; *epinephrine* also decreases peak concentrations of local anesthetics in blood. *Epinephrine-containing solutions are generally not injected into tissues supplied by end arteries—for example, fingers and toes, ears, the nose, and the penis—because of a concern that the resulting vasoconstriction may cause gangrene.* Similarly, *epinephrine* should be avoided in solutions injected intracutaneously. Because *epinephrine* also is absorbed into the circulation, its use should be avoided in those for whom adrenergic stimulation is undesirable.

The local anesthetics most frequently used for infiltration anesthesia are *lidocaine* (0.5%–1%) and *bupivacaine* (0.125%–0.25%). When used without *epinephrine*, up to 4.5 mg/kg of *lidocaine* or 2 mg/kg of *bupivacaine* can be employed in adults. When *epinephrine* is added, these amounts can be increased by one-third. Tumescent anesthesia is a special case of infiltration anesthesia for which large doses and volumes of *lidocaine* and *epinephrine* are administered (Holt, 2017).

Infiltration anesthesia and other regional anesthetic techniques have the advantage of providing satisfactory anesthesia without disrupting normal bodily functions. The chief disadvantage of infiltration anesthesia is that relatively large amounts of drug must be used to anesthetize relatively small areas. This is no problem with minor surgery. When major surgery is performed, however, the amount of local anesthetic that is required makes systemic toxic reactions likely. The amount of anesthetic required to anesthetize an area can be reduced significantly and the duration of anesthesia increased markedly by specifically blocking the nerves that innervate the area of interest. This can be done at one of several levels: subcutaneously, at major nerves, or at the level of the spinal roots.

Field Block Anesthesia

Field block anesthesia is produced by subcutaneous injection of a solution of local anesthetic to anesthetize the region distal to the injection. For example, subcutaneous infiltration of the proximal portion of the volar surface of the forearm results in an extensive area of cutaneous anesthesia that starts 2 to 3 cm distal to the site of injection. The same principle can be applied with particular benefit to the scalp, the anterior abdominal wall, and the lower extremity.

The drugs, concentrations, and doses recommended are the same as for infiltration anesthesia. The advantage of field block anesthesia is that less drug can be used to provide a greater area of anesthesia than when infiltration anesthesia is used. Knowledge of the relevant neuroanatomy obviously is essential for successful field block anesthesia.

Nerve Block Anesthesia

Injection of a solution of a local anesthetic into or about individual peripheral nerves or nerve plexuses produces even greater areas of anesthesia than do the techniques already described. Blockade of mixed peripheral nerves and nerve plexuses also usually anesthetizes somatic motor nerves, producing skeletal muscle relaxation, which is essential for some surgical procedures. The areas of sensory and motor block usually start several centimeters distal to the site of injection. Brachial plexus blocks are particularly useful for procedures on the upper extremity and shoulder. Intercostal nerve blocks are effective for anesthesia and relaxation of the anterior abdominal wall. Cervical plexus block is appropriate for surgery of the neck. Sciatic and femoral nerve blocks are useful for surgery distal to the knee. Other useful nerve blocks prior to surgical procedures include blocks of individual nerves at the wrist and at the ankle, blocks of individual nerves such as the median or ulnar nerve at the elbow, and blocks of sensory cranial nerves.

There are four major determinants of the onset of sensory anesthesia following injection near a nerve:

1. Proximity of the injection to the nerve
2. Concentration and volume of drug
3. Degree of ionization of the drug
4. Time

Local anesthetic is never intentionally injected into the nerve; this would be painful and could cause nerve damage. Instead, the anesthetic agent is deposited as close to the nerve as possible, a placement that can be assisted by ultrasound visualization of the nerve. Thus, the local anesthetic must diffuse from the site of injection into the nerve on which it acts. The rate of diffusion is determined chiefly by the concentration of the drug, its degree of ionization (ionized local anesthetic diffuses more slowly), its hydrophobicity, and the physical characteristics of the tissue surrounding the nerve. Higher concentrations of local anesthetic will provide a more rapid onset of peripheral nerve block. The utility of higher concentrations, however, is limited by systemic toxicity and by direct neural toxicity of concentrated local anesthetic solutions. For a given concentration, local anesthetics with lower pK_a values tend to have a more rapid onset of action because more drug is uncharged at neutral pH. For example, the onset of action of *lidocaine* occurs in about 3 min; 35% of *lidocaine* is in the basic form at pH 7.4. In contrast, the onset of action of *bupivacaine* requires about 15 min; only 5% to 10% of *bupivacaine* is uncharged at this pH. Increased hydrophobicity might be expected to speed onset by increased penetration into nerve tissue. However, it also will increase binding to tissue lipids. Furthermore, the more hydrophobic local anesthetics also are more potent (and toxic) and thus must be used at lower concentrations, decreasing the concentration gradient for diffusion. Tissue factors also play a role in determining the rate of onset of anesthetic effects. The amount of connective tissue that must be penetrated can be significant in a nerve plexus compared to isolated nerves and can slow or even prevent adequate diffusion of local anesthetic to the nerve fibers.

Duration of nerve block anesthesia depends on the physical characteristics of the local anesthetic used and the presence or absence of vasoconstrictors. Especially important physical characteristics are lipid solubility and protein binding. Local anesthetics can be broadly divided into three categories:

- Those with a short (20- to 45-min) duration of action in mixed peripheral nerves, such as *procaine*
- Those with an intermediate (60- to 120-min) duration of action, such as *lidocaine* and *mepivacaine*
- Those with a long (400- to 450-min) duration of action, such as *bupivacaine*, *ropivacaine*, and *tetracaine*

Block duration of the intermediate-acting local anesthetics such as *lidocaine* can be prolonged by the addition of *epinephrine* (5 μg/mL). The degree of block prolongation in peripheral nerves following the addition of *epinephrine* appears to be related to the intrinsic vasodilating properties of the local anesthetic and thus is most pronounced with *lidocaine*.

The types of nerve fibers that are blocked when a local anesthetic is injected about a mixed peripheral nerve depend on the concentration of drug used, nerve fiber size, internodal distance, and frequency and pattern of nerve impulse transmission (see the previous sections on Frequency and Voltage Dependence and Differential Sensitivity of Nerve Fibers). Anatomical factors are similarly important. A mixed peripheral nerve or nerve trunk consists of individual nerves surrounded by an investing epineurium. The vascular supply usually is centrally located. When a local anesthetic is deposited about a peripheral nerve, it diffuses from the outer surface toward the core along a concentration gradient. Consequently, nerves in the outer mantle of the mixed nerve are blocked first. These fibers usually are distributed to more proximal anatomical structures than are those situated near the core of the mixed nerve and often are motor. If the volume and concentration of local anesthetic solution deposited about the nerve are adequate, the local anesthetic eventually will diffuse inward in amounts adequate to block even the most centrally located fibers. Lesser amounts of drug will block only nerves in the mantle and the smaller and more sensitive central fibers. Furthermore, because removal of local anesthetics occurs primarily in the core of a mixed nerve or nerve trunk, where the vascular supply is located, the duration of blockade of centrally located nerves is shorter than that of more peripherally situated fibers.

The choice of local anesthetic and the amount and concentration administered are determined by the nerves and the types of fibers to be blocked, the required duration of anesthesia, and the size and health of the patient. For blocks of 2 to 4 h, *lidocaine* (1%–1.5%) can be used in the amounts recommended previously (see Infiltration Anesthesia). *Mepivacaine* (up to 7 mg/kg of a 1%–2% solution) provides anesthesia that lasts about as long as that from *lidocaine*. *Bupivacaine* (2–3 mg/kg of a 0.25%–0.375% solution) can be used when a longer duration of action is required. Alternatively, addition of 5 μg/mL *epinephrine* slows systemic absorption and therefore prolongs duration and lowers the plasma concentration of the intermediate-acting local anesthetics.

Peak plasma concentrations of local anesthetics depend on the amount injected, the physical characteristics of the local anesthetic, whether *epinephrine* is used, the rate of blood flow to the site of injection, and the surface area exposed to local anesthetic. This is of particular importance in the safe application of nerve block anesthesia because the potential for systemic reactions is related to peak free serum concentrations. For example, peak concentrations of *lidocaine* in blood following injection of 400 mg without *epinephrine* for intercostal nerve blocks average 7 μg/mL; the same amount of *lidocaine* used for block of the brachial plexus results in peak concentrations in blood of about 3 μg/mL (Covino and Vassallo, 1976). Therefore, the amount of local anesthetic that can be injected must be adjusted according to the anatomical site of the nerve(s) to be blocked to minimize untoward effects. Addition of *epinephrine* can decrease peak plasma concentrations by 20% to 30%. Multiple nerve blocks (e.g., intercostal block) or blocks performed in vascular regions require reduction in the amount of anesthetic that can be given safely because the surface area for absorption or the rate of absorption is increased.

Intravenous Regional Anesthesia (Bier Block)

The Bier block technique relies on using the vasculature to bring the local anesthetic solution to the nerve trunks and endings. In this technique, an extremity is exsanguinated with an Esmarch (elastic) bandage, and a proximally located tourniquet is inflated to 100 to 150 mmHg above the systolic blood pressure. The Esmarch bandage is removed, and the local anesthetic is injected into a previously cannulated vein. Typically, complete anesthesia of the limb ensues within 5 to 10 min. Pain from the tourniquet and the potential for ischemic nerve injury limit tourniquet inflation to 2 h or less. However, the tourniquet should remain inflated for at least 15 to 30 min to prevent toxic amounts of local anesthetic from entering the circulation following deflation. *Lidocaine*, 40 to 50 mL (0.5 mL/kg in children) of a 0.5% solution without *epinephrine*, is the drug of choice for this technique. For intravenous regional anesthesia in adults using a 0.5% solution without *epinephrine*, the dose administered should not exceed 4 mg/kg.

The attractiveness of the Bier block lies in its simplicity. Its primary disadvantages are that it can be used only for a few anatomical regions, sensation (pain) returns quickly after tourniquet deflation, and premature release or failure of the tourniquet can produce toxic levels of local anesthetic (e.g., 50 mL of 0.5% *lidocaine* contains 250 mg of *lidocaine*). For the last reason and because its longer duration of action offers no advantage, the more cardiotoxic agent *bupivacaine* is not recommended for this technique. Intravenous regional anesthesia is used most often for surgery of the forearm and hand but can be adapted for the foot and distal leg.

Spinal Anesthesia

Spinal anesthesia follows the injection of local anesthetic into the cerebrospinal fluid (CSF) in the lumbar space. For a number of reasons, including the ability to produce anesthesia of a considerable fraction of the body with a dose of local anesthetic that produces negligible plasma levels, spinal anesthesia remains one of the most popular forms of anesthesia. In most adults, the spinal cord terminates above the second lumbar vertebra; between that point and the termination of the thecal sac in the sacrum, the lumbar and sacral roots are bathed in CSF. Thus, in this region, there is a relatively large volume of CSF within which to inject drug, thereby minimizing the potential for direct nerve trauma.

The following is a brief discussion of the physiological effects of spinal anesthesia relating to the pharmacology of the local anesthetics. See more specialized texts (Cousins et al., 2008) for additional details.

Physiological Effects of Spinal Anesthesia

Most of the physiological side effects of spinal anesthesia are a consequence of the sympathetic blockade produced by local anesthetic block of the sympathetic fibers in the spinal nerve roots. A thorough understanding of these physiological effects is necessary for the safe and successful application of spinal anesthesia. Although some effects may be deleterious and require treatment, others can be beneficial for the patient or can improve operating conditions.

Most sympathetic fibers leave the spinal cord between T1 and L2 (see Figure 10–1). Although local anesthetic is injected below these levels in the lumbar portion of the dural sac, cephalad spread of the local anesthetic occurs with all but the smallest volumes injected. This cephalad spread is of considerable importance in the practice of spinal anesthesia and potentially is under the control of numerous variables, of which patient position and baricity (density of the drug relative to the density of the CSF) are the most important (Greene, 1983). The degree of sympathetic block is related to the height of sensory anesthesia; often, the level of sympathetic blockade is several spinal segments higher because the preganglionic sympathetic fibers are more sensitive to low concentrations of local anesthetic. The effects of sympathetic blockade involve both the actions (now partially unopposed) of the parasympathetic nervous system and the response of the unblocked portion of the sympathetic nervous system. Thus, as the level of sympathetic block ascends, the actions of the parasympathetic nervous system are increasingly dominant, and the compensatory mechanisms of the few unblocked sympathetic nervous system are diminished. As most sympathetic nerve fibers leave the cord at T1 or below, few additional effects of sympathetic blockade are seen with cervical levels of spinal anesthesia. The consequences of sympathetic blockade will vary among patients as a function of age, physical conditioning, and disease state. Interestingly, sympathetic blockade during spinal anesthesia appears to be minimal in healthy children.

Clinically, the most important effects of sympathetic blockade during spinal anesthesia are on the cardiovascular system. At all but the lowest levels of spinal blockade, some vasodilation will occur. Vasodilation is more marked on the venous than on the arterial side of the circulation, resulting in blood pooling in the venous capacitance vessels. This reduction in circulating blood volume is well tolerated at low levels of spinal anesthesia in healthy patients. With an increasing level of block, this effect becomes more marked, and venous return becomes gravity dependent. If venous return decreases too much, cardiac output and organ perfusion decline precipitously. Venous return can be increased by a modest (10°–15°) head-down tilt or by elevating the legs.

At high levels of spinal blockade, the cardiac accelerator fibers, which exit the spinal cord at T1–T4, will be blocked. This is detrimental in patients dependent on elevated sympathetic tone to maintain cardiac output (e.g., during congestive heart failure or hypovolemia), and it also removes one of the compensatory mechanisms available to maintain organ perfusion during vasodilation. Thus, as the level of spinal block ascends, the rate of cardiovascular compromise can accelerate if not carefully observed and treated. Sudden asystole also can occur, presumably because of loss of sympathetic innervation in the continued presence of parasympathetic activity at the sinoatrial node (Caplan et al., 1988). In the usual clinical situation, blood pressure serves as a surrogate marker for cardiac output and organ perfusion. Treatment of hypotension usually is warranted when the blood pressure decreases to about 30% of *resting* values.

Therapy is aimed at maintaining brain and cardiac perfusion and oxygenation. To achieve these goals, administration of oxygen, fluid infusion, manipulation of patient position, and the administration of vasoactive drugs are all options. In practice, patients typically are administered a bolus (500–1000 mL) of fluid prior to the induction of spinal anesthesia in an attempt to prevent some of the deleterious effects of spinal blockade. Because the usual cause of hypotension is decreased venous return, possibly complicated by decreased heart rate, drugs with preferential venoconstrictive and chronotropic properties are preferred. For this reason, *ephedrine*, 5–10 mg intravenously, often is the drug of choice. In addition to the use of *ephedrine* to treat deleterious effects of sympathetic blockade, direct-acting α_1 adrenergic receptor agonists such as *phenylephrine* (see Chapter 14) can be administered by either bolus or continuous infusion.

A beneficial effect of spinal anesthesia partially mediated by the sympathetic nervous system is on the intestine. Sympathetic fibers originating from T5 to L1 inhibit peristalsis; thus, their blockade produces a small, contracted intestine. This, together with flaccid abdominal musculature, produces excellent operating conditions for some types of bowel surgery. The consequences of spinal anesthesia on the respiratory system are mostly mediated by effects on the skeletal musculature. Paralysis of the intercostal muscles will reduce a patient's ability to cough and clear secretions, which may produce dyspnea in patients with bronchitis or emphysema. Respiratory arrest during spinal anesthesia seldom occurs due to paralysis of the phrenic nerves or to toxic levels of local anesthetic in the CSF of the fourth ventricle; it is much more likely to be due to medullary ischemia secondary to hypotension.

Pharmacology

Currently in the U.S., the drugs most commonly used in spinal anesthesia are *lidocaine*, *tetracaine*, and *bupivacaine*. The choice of local anesthetic is primarily determined by the desired duration of anesthesia. General guidelines are to use *lidocaine* for short procedures, *bupivacaine* for intermediate-to-long procedures, and *tetracaine* for long procedures. As mentioned, the factors contributing to the distribution of local anesthetics in the CSF have received much attention because of their importance in determining the height of block. The most important pharmacological factors include the amount, and possibly the volume, of drug injected and its baricity. The speed of injection of the local anesthesia solution also may affect the height of the block, just as the position of the patient can influence the rate of distribution of the anesthetic agent and the height of blockade achieved (described in the next section). For a given preparation of local anesthetic, administration of increasing amounts leads to a fairly predictable increase in the level of block attained. For example, 100 mg of *lidocaine*, 20 mg of *bupivacaine*, or 12 mg of *tetracaine* usually will result in a T4 sensory block. More complete tables of these relationships can be found in standard anesthesiology texts.

Epinephrine often is added to spinal anesthetics to increase the duration or intensity of block. *Epinephrine*'s effect on duration of block is dependent on the technique used to measure duration. A commonly used measure of block duration is the length of time it takes for the block to recede by two dermatomes from the maximum height of the block, while a second is the duration of block at some specified level, typically L1. In most studies, addition of 200 μg of *epinephrine* to *tetracaine* solutions prolongs the duration of block by both measures. However, addition of *epinephrine* to *lidocaine* or *bupivacaine* does not affect the first measure of duration but does prolong the block at lower levels. In different clinical situations, one or the other measure of anesthesia duration may be more relevant, and this must be kept in mind when deciding whether to add *epinephrine* to spinal local anesthetics.

The mechanism of action of vasoconstrictors in prolonging spinal anesthesia is uncertain. It has been hypothesized that these agents decrease spinal cord blood flow, decreasing clearance of local anesthetic from the CSF, but this has not been convincingly demonstrated. *Epinephrine* and other α adrenergic agonists have been shown to decrease nociceptive transmission in the spinal cord, and studies in genetically modified mice suggested that α_{2A} adrenergic receptors play a principal role in this response (Stone et al., 1997). Such actions may contribute to the beneficial effects of *epinephrine*, *clonidine*, and *dexmedetomidine* when these agents are added to spinal local anesthetics.

Drug Baricity and Patient Position

The baricity of the local anesthetic injected will determine the direction of migration within the dural sac. Hyperbaric solutions will tend to settle in the dependent portions of the sac, while hypobaric solutions will tend to migrate in the opposite direction. Isobaric solutions usually will stay in the vicinity where they were injected, diffusing slowly in all directions. Consideration of the patient position during and after the performance of the block and the choice of a local anesthetic of the appropriate baricity is crucial for a successful block during some surgical procedures. *Lidocaine* and *bupivacaine* are marketed in both isobaric and hyperbaric preparations and, if desired, can be diluted with sterile, preservative-free water to make them hypobaric.

Complications

Persistent neurological deficits following spinal anesthesia are extremely rare. Thorough evaluation of a suspected deficit should be performed in collaboration with a neurologist. Neurological sequelae can be both immediate and late. Possible causes include introduction of foreign substances (such as disinfectants, ultrasound gel, or talc) into the subarachnoid space, infection, hematoma, or direct mechanical trauma. Aside from drainage of an abscess or hematoma, treatment usually is ineffective; thus, avoidance and careful attention to detail while performing spinal anesthesia are necessary.

High concentrations of local anesthetic can cause irreversible block. After administration, local anesthetic solutions are diluted rapidly, quickly reaching nontoxic concentrations. However, there are several reports of transient or longer-lasting neurological deficits following *lidocaine* spinal anesthesia, particularly with 5% *lidocaine HCl* (i.e., ~180 mM) in 7.5% glucose (Forget et al., 2019).

Spinal anesthesia sometimes is regarded as contraindicated in patients with preexisting disease of the spinal cord. No experimental evidence exists to support this hypothesis. Nonetheless, it is prudent to avoid spinal anesthesia in patients with progressive diseases of the spinal cord. However, spinal anesthesia may be useful in patients with a fixed, chronic spinal cord injury.

A more common sequela following any lumbar puncture, including spinal anesthesia, is a postural headache with classic features. The incidence of headache decreases with increasing age of the patient and decreasing needle diameter. Headache following lumbar puncture must be thoroughly evaluated to exclude serious complications such as meningitis. Treatment usually is conservative, with bed rest and analgesics. If this approach fails, an epidural blood patch with the injection of autologous blood can be performed; this procedure is usually successful in alleviating postdural puncture headaches, although a second blood patch may be necessary. If two epidural blood patches are ineffective in relieving the headache, the diagnosis of postdural puncture headache should be reconsidered. Intravenous caffeine (500 mg as the benzoate salt administered over 4 h) also has been advocated for the treatment of postdural puncture headache; however, the efficacy of caffeine is less than that of a blood patch, and relief usually is transient.

Evaluation of Spinal Anesthesia

Spinal anesthesia is a safe and effective technique, especially during surgery involving the lower abdomen, the lower extremities, and the perineum. It often is combined with intravenous medication to provide sedation and amnesia. The physiological perturbations associated with low spinal anesthesia often have less potential harm than those associated with general anesthesia. The same does not apply for high spinal anesthesia. The sympathetic blockade that accompanies levels of spinal anesthesia adequate for mid- or upper abdominal surgery, coupled with the difficulty in achieving visceral analgesia, is such that equally satisfactory and safer operating conditions can be realized by combining the spinal anesthetic with a "light" general anesthetic or by the administration of a general anesthetic and a neuromuscular blocking agent.

Epidural Anesthesia

Epidural anesthesia is administered by injecting local anesthetic into the epidural space—the space bounded by the ligamentum flavum posteriorly, the spinal periosteum laterally, and the dura anteriorly. Epidural anesthesia can be performed in the sacral hiatus (caudal anesthesia) or in the lumbar, thoracic, or cervical regions of the spine. Its current popularity arises from the development of catheters that can be placed into the epidural space, allowing either continuous infusions or repeated bolus administration of local anesthetics. The primary site of action of epidurally administered local anesthetics is on the spinal nerve roots. However, epidurally administered local anesthetics also may act on the spinal cord and on the paravertebral nerves.

The selection of drugs available for epidural anesthesia is similar to that for major nerve blocks. As for spinal anesthesia, the choice of drugs to be used during epidural anesthesia is dictated primarily by the duration of anesthesia desired. However, when an epidural catheter is placed, short-acting drugs can be administered repeatedly, providing more control over the duration of block. *Bupivacaine*, 0.5% to 0.75%, is used when a long duration of surgical block is desired. Due to enhanced cardiotoxicity in pregnant patients, the 0.75% solution is not approved for obstetrical use. Lower concentrations—0.25%, 0.125%, or 0.0625%—of *bupivacaine*, often with 2 μg/mL of *fentanyl* added, frequently are used to provide analgesia during labor. They also are useful preparations for providing postoperative analgesia in certain clinical situations. *Lidocaine* 2% is the most frequently used intermediate-acting epidural local anesthetic. *Chloroprocaine*, 2% or 3%, provides rapid onset and a very short duration of anesthetic action. However, its use in epidural anesthesia has been clouded by controversy regarding its potential ability to cause neurological complications if the drug is accidentally injected into the subarachnoid space (discussed previously). The addition of *epinephrine* frequently prolongs the duration of action and reduces the toxicity of epidurally administered local anesthetics. Addition of *epinephrine* also makes inadvertent intravascular injection easier to detect and modifies the effect of sympathetic blockade during epidural anesthesia.

For each anesthetic agent, a relationship exists between the volume of local anesthetic injected epidurally and the segmental level of anesthesia achieved. For example, in 20- to 40-year-old, healthy, nonpregnant patients, each 1 to 1.5 mL of 2% *lidocaine* will give an additional segment of anesthesia. The amount needed decreases with increasing age and during pregnancy and in children. The concentration of local anesthetic used determines the type of nerve fibers blocked. The highest concentrations are used when sympathetic, somatic sensory, and somatic motor blockade are required. Intermediate concentrations allow somatic sensory anesthesia without muscle relaxation. Low concentrations will block only preganglionic sympathetic fibers. As an example, with *bupivacaine*, these effects might be achieved with concentrations of 0.5%, 0.25%, and 0.0625%, respectively. The total amounts of drug that can be injected with safety at one time are approximately those mentioned in the sections Nerve Block Anesthesia and Infiltration Anesthesia. Performance of epidural anesthesia requires a greater degree of skill than does spinal anesthesia. The technique of epidural anesthesia and the volumes, concentrations, and types of drugs used are described in detail in standard texts on regional anesthesia (Cousins et al., 2008).

A significant difference between epidural and spinal anesthesia is that the dose of local anesthetic used can produce high concentrations in blood following absorption from the epidural space. Peak concentrations of *lidocaine* in blood following injection of 400 mg (without *epinephrine*) into the lumbar epidural space average 3 to 4 μg/mL; peak concentrations of *bupivacaine* in blood average 1 μg/mL after the lumbar epidural injection of 150 mg. Addition of *epinephrine* (5 μg/mL) decreases peak plasma concentrations by about 25%. Peak blood concentrations are a function of the total dose of drug administered rather than the concentration or volume of solution following epidural injection (Covino and Vassallo, 1976). The risk of inadvertent intravascular injection is increased in epidural anesthesia, as the epidural space contains a rich venous plexus.

Another major difference between epidural and spinal anesthesia is that there is no zone of differential sympathetic blockade with epidural anesthesia; thus, the level of sympathetic block is close to the level of sensory block. Because epidural anesthesia does not result in the zones of differential sympathetic blockade observed during spinal anesthesia, cardiovascular responses to epidural anesthesia might be expected to be less prominent. In practice, this is not the case; the potential advantage of epidural anesthesia is offset by the cardiovascular responses to the high concentration of anesthetic in blood that occurs during epidural anesthesia. This is most apparent when *epinephrine* is added to the epidural injection. The resulting concentration of *epinephrine* in blood is sufficient to produce significant β_2 adrenergic receptor–mediated vasodilation. As a consequence, blood pressure decreases, even though cardiac output increases due to the positive inotropic and chronotropic effects of *epinephrine* (see Chapter 14). The result is peripheral hyperperfusion and hypotension. Differences in cardiovascular responses to equal levels of spinal and epidural anesthesia also are observed when a local anesthetic such as *lidocaine* is used without *epinephrine*. This may be a consequence of the direct effects of high concentrations of *lidocaine* on vascular smooth muscle and the heart. The magnitude of the differences in responses to equal sensory levels of spinal and epidural anesthesia varies, however, with the local anesthetic used for the epidural injection (assuming no *epinephrine* is used). For example, local anesthetics such as *bupivacaine*, which are highly lipid soluble, are distributed less into the circulation than are less lipid-soluble agents such as *lidocaine*.

High concentrations of local anesthetics in blood during epidural anesthesia are of particular concern when this technique is used to control pain during labor and delivery. Local anesthetics cross the placenta, enter the fetal circulation, and at high concentrations may cause depression of the neonate. The extent to which they do so is determined by dosage, acid-base status, level of protein binding in both maternal and fetal blood, placental blood flow, and solubility of the agent in fetal tissue. These concerns have been lessened by the trend toward using more dilute solutions of *bupivacaine* for labor analgesia.

Epidural and Intrathecal Opiate Analgesia

Small quantities of opioid injected intrathecally or epidurally produce segmental analgesia (Yaksh and Rudy, 1976). This observation led to the clinical use of spinal and epidural opioids during surgical procedures and for the relief of postoperative and chronic pain (Cousins and Mather, 1984). As with local anesthesia, analgesia is confined to sensory nerves that enter the spinal cord dorsal horn in the vicinity of the injection. Presynaptic opioid receptors inhibit the release of substance P and other neurotransmitters from primary afferents, while postsynaptic opioid receptors decrease the activity of certain dorsal horn neurons in the spinothalamic tracts (Willcockson et al., 1986; see also Chapters 10 and 23). Because conduction in autonomic, sensory, and motor nerves is not affected by the opioids, blood pressure, motor function, and nonnociceptive sensory perception typically are not influenced by spinal opioids. The volume-evoked micturition reflex is inhibited, as manifested by urinary retention. Other side effects include pruritus, nausea, and vomiting in susceptible individuals. Delayed respiratory depression and sedation, presumably from cephalad spread of opioid within the CSF, occur infrequently with the doses of opioids currently used.

Spinally administered opioids by themselves do not provide satisfactory anesthesia for surgical procedures. Thus, opioids have found the greatest use in the treatment of postoperative and chronic pain, providing excellent analgesia following thoracic, abdominal, pelvic, or lower extremity surgery without the side effects associated with high doses of systemically administered opioids. For postoperative analgesia, spinally administered *morphine*, 0.2 to 0.5 mg, usually will provide 8 to 16 h of analgesia. Placement of an epidural catheter and repeated boluses or an infusion of opioid permits an increased duration of analgesia. *Morphine*, 2 to 6 mg every 6 h, commonly is used for bolus injections, while *fentanyl*, 20 to 50 μg/h, often combined with *bupivacaine* at 5 to 20 mg/h, is used for infusions. For cancer pain, repeated doses of epidural opioids can provide analgesia of several months' duration. The dose of epidural *morphine* is far less than the dose of systemically administered *morphine* that would be required to provide similar analgesia, thus reducing the complications that usually accompany the administration of high doses of systemic opioids, particularly sedation and constipation. Unfortunately, as with systemic opioids, tolerance will develop to the analgesic effects of epidural opioids, but this usually can be managed by increasing the dose.

Drug Facts for Your Personal Formulary: *Local Anesthetics*

Drugs	Therapeutic Uses or Duration	Clinical Pharmacology and Tips
Topical Anesthesia		
Lidocaine	• Superficial anesthesia of mucous membranes	• 2%–10% solution • ~30-min duration • Maximal healthy adult dose, ~4 mg/kg
Cocaine	• Superficial anesthesia of mucous membranes of nose, mouth, ear	• 1%–4% solution • ~30-min duration • Maximal healthy adult dose, ~1–3 mg/kg (maximum 400 mg); pediatric dose, <1 mg/kg • Vasoconstriction + anesthesia
Eutectic mixtures, oil, or cream: Lidocaine (2.5%)/prilocaine (2.5%) (EMLA) or Lidocaine (7%)/tetracaine (7%)	• Superficial anesthesia of cutaneous structures	• Effective to ~5-mm depth • Requires 30–60 min of contact to establish effective anesthesia • Should not be used on mucous membranes or abraded skin due to rapid absorption • Consult package insert for maximum dose
Infiltration Anesthesia		
Lidocaine	• Superficial anesthesia of cutaneous structures • Addition of dilute sodium bicarbonate (10:1—lidocaine: 8.4% sodium bicarbonate, ~0.75 mg/mL sodium bicarbonate) can lessen pain on injection	• 0.5%–1.0% solution • Maximal healthy adult dose, ~4 mg/kg • Addition of epinephrine (5 μg/mL) increases duration of action and maximal safe lidocaine dose
Bupivacaine	• Superficial anesthesia of cutaneous structures	• 0.125%–0.25% solution • Maximal healthy adult dose, ~2 mg/kg • Addition of epinephrine (5 μg/mL) increases duration of action and maximal safe bupivacaine dose

Drug Facts for Your Personal Formulary: *Local Anesthetics* (*continued*)

Drugs	Therapeutic Uses or Duration	Clinical Pharmacology and Tips
Nerve Block Anesthesia • Use with epinephrine-containing test dose • Risk of intravenous injection		
Articaine	• 1 h duration	• For dental and periodontal procedures • 4% solution, typically with epinephrine • Contains both an amide and ester, so degraded in both plasma and liver
Lidocaine, mepivacaine	• 1–2 h duration • Addition of epinephrine prolongs duration and increases maximal safe level of drug • Identification of blocked nerves (nerve stimulation or ultrasound) may increase safety and success of block	Safe doses depend on vascularity of tissue, generally: • Lidocaine: 1%–1.5%, maximal healthy adult dose, ~4 mg/kg • Mepivacaine: 1%–2%, maximal healthy adult dose, ~7 mg/kg (maximum 400 mg)
Bupivacaine, ropivacaine	• 6–8 h duration • Longer duration of sensory block with bupivacaine than with ropivacaine • Addition of epinephrine prolongs duration and increases maximal safe level of drug	Safe doses depend on vascularity of tissue, generally: • Bupivacaine: 0.25%–0.375%, maximal healthy adult dose, ~2–3 mg/kg (maximum 400 mg) • Ropivacaine: 0.5%–0.75%, maximal healthy adult dose, ~3–4 mg/kg (maximum 200 mg) • Infusions through a catheter placed adjacent to the nerve can provide sustained analgesia • Identification of blocked nerves (nerve stimulation or ultrasound) may increase safety and success of block
Epidural Anesthesia • Use with epinephrine-containing test dose • Risk of intravenous injection • Spread of block dependent on dose and volume injected • Epidural catheter allows repeated dosing • Consider coagulation status of patient		
Chloroprocaine	• Short duration • Epinephrine prolongs action	• 2%–3% solution • Possible increased incidence of postprocedure back pain
Lidocaine	• Intermediate duration • Epinephrine prolongs action	• 2% solution • Maximal healthy adult dose, ~4 mg/kg
Bupivacaine	• Long duration	• 0.5% solution • Maximal healthy adult dose, ~2–3 mg/kg
Ropivacaine	• Long duration	• 0.5%–1.0% solution • Maximal healthy adult dose, ~2–3 mg/kg • May have less toxicity than equiefficacious dose of bupivacaine
Spinal Anesthesia • Dose and baricity of anesthetic strongly influence spread • Addition of opioids can prolong analgesia • Consider coagulation status of patient		
Lidocaine	• Short duration (60–90 min)	• ~25–50 mg for perineal and lower extremity surgery • Association of spinal lidocaine with transient neurological symptoms
Tetracaine	• Long duration (210–240 min)	• Duration increased by epinephrine • ~5 mg for perineal surgery • ~10 mg for lower extremity surgery
Bupivacaine	• Long duration (210–240 min)	• ~10 mg for perineal and lower extremity surgery • 15–20 mg for abdominal surgery

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Chapter 26

Cannabinoids

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INTRODUCTION

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Introduction

The psychoactive and medicinal properties of *Cannabis* species have been appreciated for millennia. However, only recently have we developed an understanding of the compounds present in cannabis that produce these effects. The primary psychoactive compounds in cannabis are the phytocannabinoids, which are meroterpenoids typically synthesized from olivetolic acid and geranyl pyrophosphate. Δ^9-Tetrahydrocannabinol (THC) is the phytocannabinoid responsible for the characteristic psychoactivity of *Cannabis*. Another major phytocannabinoid, cannabidiol (CBD), may have distinct therapeutic benefits. The effects of other phytocannabinoids are less well understood. THC produces its effects by engaging the endocannabinoid system, an endogenous signaling system comprised of endogenous cannabinoids (endocannabinoids), endocannabinoid receptors, and the enzymes responsible for endocannabinoid synthesis and degradation. The endocannabinoid system is widespread throughout the body and is involved in regulation of stress, pain, reward, metabolism, and inflammation, among other physiological actions.

Endogenous Cannabinoids

The endocannabinoids (eCB) are defined as endogenously produced molecules that bind to cannabinoid receptors. The prototypical eCBs are arachidonate-derived signaling lipids that are produced by cells throughout most organ systems in the body but have been primarily studied within the nervous system (Blankman and Cravatt, 2013; Hillard, 2015, 2018). The lipophilic eCB molecules often associate with protein binding partners, such as fatty acid binding proteins or albumin in the blood, to facilitate transport through aqueous compartments. eCBs exert their actions through a family of receptors as discussed below.

Anandamide

The first eCB to be discovered was *N*-arachidonoylethanolamine (AEA; Figure 26–1) and was given the name *anandamide* in reference to the Sanskrit word *ānanda* for bliss (Blankman and Cravatt, 2013). AEA is an arachidonic acid molecule conjugated to an ethanolamine group. Within the brain, AEA is believed to be synthesized and mobilized in a varying, but continuous, manner and not undergo vesicular storage, a process often referred to as "on-demand" synthesis. AEA is a high-affinity (low nM) but low-efficacy agonist at the cannabinoid receptors, making it a partial agonist (Hillard, 2015), and is also known to act as an agonist at transient receptor potential channel vanilloid 1 (TRPV1) receptors at high concentrations (high nM–low μM range). Bulk levels of AEA exist at the low pmol/g brain weight concentration; microdialysis studies within the brain have found extracellular levels of AEA to be in the low nanomolar range. AEA concentrations within the blood are typically found in the low pmol/mL range; although the tissue depot source from which circulating AEA is derived is not well established, it likely involves a combination of genesis from blood cells and vascular, hepatic, and adipose tissue.

2-Arachidonoyl Glycerol

The second eCB to be discovered was 2-arachidonoyl glycerol (2-AG; Figures 26–1 and 26–2). 2-AG is an arachidonic acid molecule conjugated to a glycerol group. Within the brain, there is evidence for storage of 2-AG in membrane lipid rafts. Bulk 2-AG concentrations quantified from extracted brain tissue indicate that it is present in the low nmol/g range, which is about 1000-fold higher than AEA; however, extracellular levels determined through microdialysis studies indicate that 2-AG concentrations are only about 2- to 5-fold higher than AEA and found in a similar low nanomolar range. Based on this, it is predicted that about 90% of 2-AG in the brain exists as bulk 2-AG. This membrane-associated 2-AG may function as a storage reservoir for arachidonic acid, which is then utilized as a requisite substrate to form neuroinflammatory prostaglandins (Nomura et al., 2011). In contrast to AEA, 2-AG is a full agonist at the cannabinoid receptors, with an affinity in the nanomolar range, lower than that found for AEA (Hillard, 2015, 2018). Within the blood, circulating levels of 2-AG and AEA are in the lower pmol/mL range. As with AEA, the tissue source of circulating 2-AG is not well understood.

The Endocannabinoid-ome: Beyond AEA and 2-AG

While AEA and 2-AG are the most well-established and well-studied eCB molecules, other fatty acid ethanolamides, monoacyl glycerols, and even

Abbreviations

ABHD: α/β hydrolase domain-containing protein
AEA: anandamide
2-AG: 2-arachidonoyl glycerol
ALT: alanine aminotransferase
CBC: cannabichromene
CBCA: cannabichromenic acid
CBD: cannabidiol
CBDA: cannabidiolic acid
CBGA: cannabigerolic acid
CBN: cannabinol
CBNA: cannabinolic acid
COX-2: cyclooxygenase-2
DAG: diacylglycerol
DAGL: diacylglycerol lipase
eCB: endocannabinoid
FAAH: fatty acid amide hydrolase
GIRK: G protein-coupled inwardly rectifying K^+ channel
GPCR: G protein-coupled receptor
LTD: long-term depression
MAGL: monoacylglycerol lipase
MAP kinase: mitogen-activated protein kinase
NAPE-PLD: *N*-acyl phosphatidylethanolamine–specific phospholipase D
NArPE: *N*-arachidonoyl phosphatidylethanolamine
NREM: non–rapid eye movement
7-OH-CBD: 7-hydroxy-cannabidiol
11-OH-THC: 11-hydroxy-tetrahydrocannabinol
PAG: periaqueductal gray
PLC: phospholipase C
PPAR: peroxisome proliferator-activated receptor
REM: rapid eye movement
THC: Δ^9-tetrahydrocannabinol
THCA: Δ^9-tetrahydrocannabinolic acid
THCV: Δ^9-tetrahydrocannabivarin
TRPV1: transient receptor potential channel vanilloid 1
VTA: ventral tegmental area

peptides could represent additional members of the eCB family. Of these, the molecule hemopressin, a derivative of the alpha chain of hemoglobin, has garnered the most attention. Hemopressin has high affinity for cannabinoid receptors but, unlike AEA and 2-AG, appears to function as an inverse agonist (Hillard, 2015). The physiological relevance of hemopressin and the conditions under which it is synthesized and released are not well established. Other molecules, such as virodhamine and noladin ether, have been proposed as additional eCBs, but it is unclear whether these molecules are synthesized and released in a physiological manner *in vivo*. Thus, the discussion of the eCB system is focused exclusively on AEA and 2-AG. The pathways of synthesis and degradation for AEA and 2-AG are detailed in Figure 26–2 and discussed below.

Synthesis

Anandamide

The biosynthesis of AEA appears to involve several redundant pathways that all initiate from a common precursor, *N*-arachidonoyl phosphatidylethanolamine (NarPE). NarPE is formed via a Ca^{2+}-dependent enzymatic process, by an unknown *N*-acyl transferase, which removes arachidonic acid from the *sn-1* position of a membrane phospholipid donor and catalyzes the formation of an amide bond with the ethanolamine from phosphatidylethanolamine (Blankman and Cravatt, 2013). There are three pathways by which NarPE can be converted to AEA. The best established pathway is via hydrolysis by *N*-acyl phosphatidylethanolamine–specific phospholipase D (NAPE-PLD)–mediated liberation of phosphatidic acid from NarPE to produce AEA. Supporting the importance of the NAPE-PLD pathway, NAPE-PLD inhibitors reduce brain AEA levels up to 50% (Mock et al., 2020). NarPE can also be converted to lysoNArPE by α/β hydrolase domain-containing protein (ABHD) 4 or phospholipase A_2. LysoNArPE is deacylated to glycerolphospho-*N*-arachidonoylethanolamine (GP-AEA) and then converted to AEA by glycerophosphodiesterase 1 (GDE1). Finally, NarPE can be converted to a phospho-AEA by a phospholipase C (PLC)–like enzyme and then dephosphorylated to AEA by the tyrosine phosphatase PTPN22. This pathway of AEA biosynthesis may occur primarily in macrophages during inflammatory challenges.

2-Arachidonoyl Glycerol

The biosynthesis of 2-AG occurs via the enzyme diacylglycerol lipase (DAGL) (Gao et al., 2010). The DAGLα isoform is responsible for 2-AG biosynthesis in the CNS, whereas the DAGLβ isoform generates 2-AG in peripheral organs, such as the liver. 2-AG synthesis begins with the hydrolysis of *sn-2* arachidonoyl phosphatidylinositol 4,5-bisphosphate (PIP2) from membrane phospholipids by PLCβ to form diacylglycerol (DAG), which is then converted to 2-AG via DAGL-mediated hydrolysis of the ester bond at the *sn-1* position of DAG. Figure 26–2 also shows an alternative pathway.

Degradation

Anandamide

Anandamide is primarily degraded by fatty acid amide hydrolase (FAAH), which is widely expressed throughout the body. FAAH is a membrane protein associated with intracellular membranes of the endoplasmic reticulum and mitochondria. FAAH-mediated hydrolysis of AEA generates free arachidonic acid and ethanolamine. FAAH is constitutively active; its expression is regulated by reproductive and metabolic hormones; its enzymatic activity is regulated by protein kinase A and by stimulation of receptors linked to the G_s–adenylyl cyclase–cyclic AMP pathway. Within neurons, FAAH tends to be localized within somatodendritic compartments, and the intracellular transit of AEA from lipophilic membrane compartments to FAAH may be mediated by fatty acid binding proteins (Kaczocha et al., 2009). Genetic deletion or pharmacological inhibition of FAAH results in a large increase of AEA tissue content. In humans, a single nucleotide polymorphism, P129T, in the *FAAH* gene increases FAAH protein degradation, resulting in a reduction in FAAH expression and a consequential elevation in constitutive AEA signaling (Hillard, 2015). A second FAAH gene (*FAAH2*) exists in humans that is not present in rodents. Its relevance has yet to be elucidated, although an individual with significant psychiatric and neurological symptoms was identified to have a rare missense mutation in *FAAH2* (Sirrs et al., 2015). In addition to AEA, FAAH hydrolyses other fatty acid amides and *N*-acyl taurines. As a result, FAAH inhibition produces widespread alterations in lipid signaling species. AEA can also be oxidized by cyclooxygenase-2 (COX2), a reaction that generates bioactive prostaglandin ethanolamides.

2-Arachidonoyl Glycerol

2-Arachidonoyl glycerol is degraded by monoacylglycerol lipase (MAGL). Genetic deletion or pharmacological inhibition of MAGL results in a large increase of 2-AG tissue content throughout the whole body. MAGL-mediated hydrolysis generates free arachidonic acid and glycerol. In the brain, approximately 90% of 2-AG exists in membrane compartments where it acts as a reservoir for arachidonic acid. In response to inflammatory processes, MAGL rapidly catabolizes 2-AG to liberate arachidonic acid, which is then oxidized by COX-2 to generate neuroinflammatory prostaglandins (Nomura et al., 2011). Hence, inhibition of MAGL suppressed prostaglandin formation following inflammatory insults in the brain. MAGL is widely expressed throughout the nervous system and peripheral organs. Unlike FAAH, MAGL is localized to presynaptic compartments and not in the somatodendritic portion of neurons. Although MAGL activity can also be detected in the cytosolic fractions of cells, as with FAAH, membrane association of MAGL facilitates 2-AG hydrolysis. MAGL also hydrolyzes 1(3)- and 2-monoacylglycerols; thus, MAGL

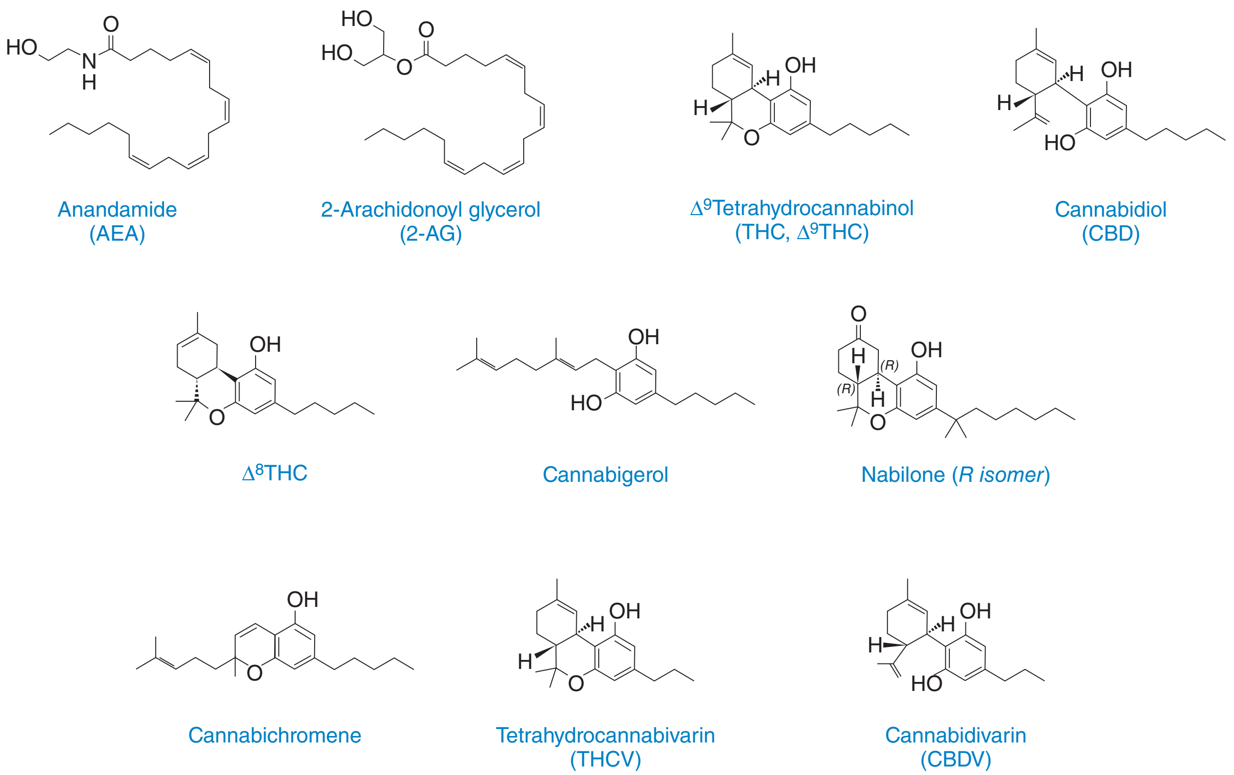
Anandamide
(AEA)
2-Arachidonoyl glycerol
(2-AG)
Δ9Tetrahydrocannabinol
(THC, Δ9THC)
Cannabidiol
(CBD)
Δ8THC
Cannabigerol
Nabilone (R isomer)
Cannabichromene
Tetrahydrocannabivarin
(THCV)
Cannabidivarin
(CBDV)

Figure 26–1 *Structures of selected cannabinoid molecules.* The endocannabinoids anandamide and 2-arachidonoyl glycerol have in common arachidonic acid and differ by being the amide of ethanolamine and ester of glycerol, respectively. The structures of the major phytocannabinoids, Δ^9-tetrahydrocannabinol (Δ^{9}THC) and cannabidiol, differ in the presence of the third ring in THC. The intermediate phytocannabinoid metabolite, cannabigerol, is monocyclic. Tetrahydrocannabivarin and cannabidivarin differ from THC and cannabidiol in having a propyl instead of pentyl side chain on the aromatic ring. Δ^{8}THC, cannabigerol, and cannabichromene are minor cannabinoids.

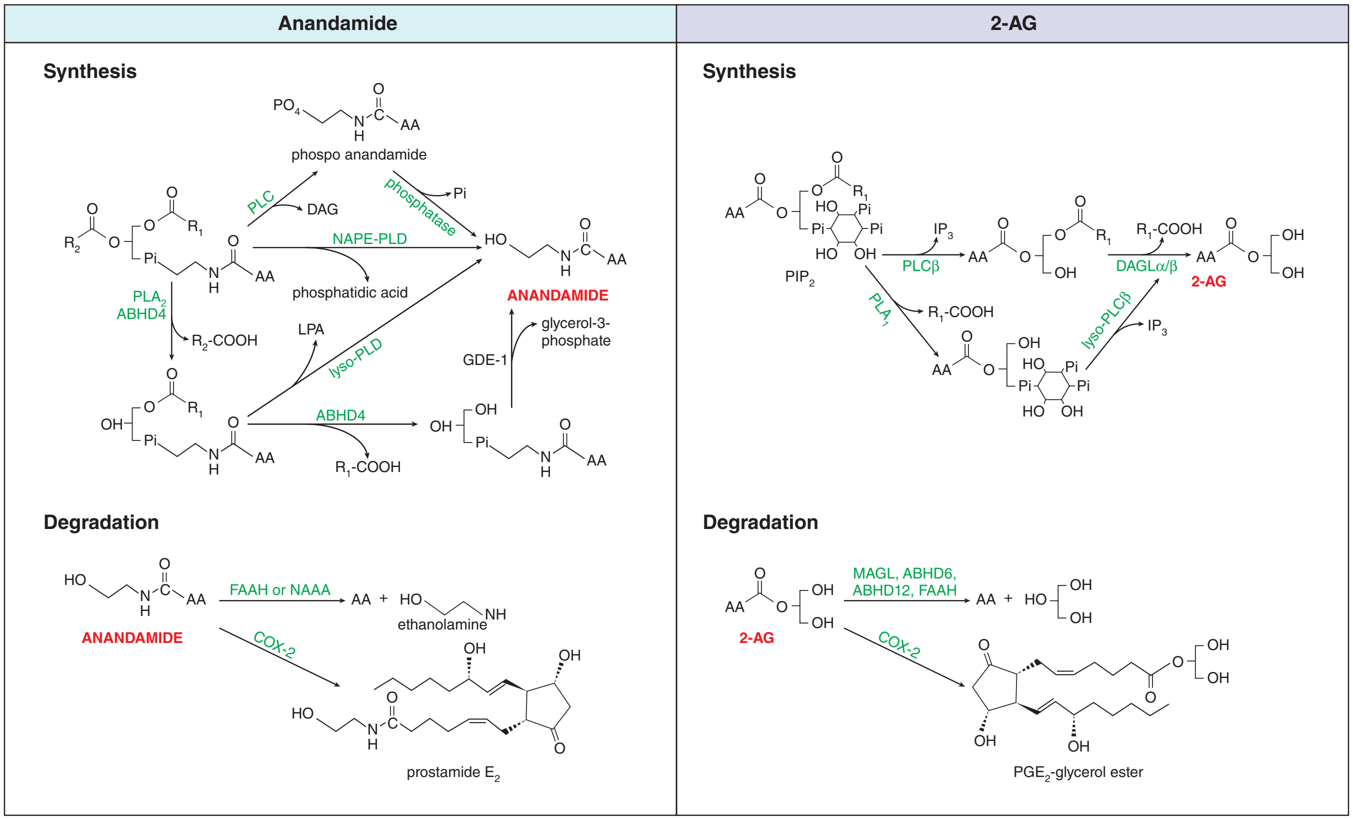
Anandamide
2-AG
Synthesis
phospo anandamide
PLC
DAG
phosphatase
Pi
NAPE-PLD
phosphatidic acid
ANANDAMIDE
PLA2
ABHD4
R2-COOH
LPA
lyso-PLD
GDE-1
glycerol-3-phosphate
ABHD4
R1-COOH
Synthesis
PIP2
IP3
PLCβ
R1-COOH
DAGLα/β
2-AG
PLA1
R1-COOH
lyso-PLCβ
IP3
Degradation
ANANDAMIDE
FAAH or NAAA
AA + HO NH
ethanolamine
COX-2
prostamide E2
Degradation
2-AG
MAGL, ABHD6, ABHD12, FAAH
AA + HO
COX-2
PGE2-glycerol ester

Figure 26–2 *Major synthetic and degradative pathways for anandamide and 2-arachidonoyl glycerol (2-AG).* **Left panel:** Primary synthetic and degradative pathways for anandamide. **Right panel:** Primary synthetic and degradative pathways for 2-AG. Only major pathways are shown. Enzymes are noted in green. AA, arachidonic acid; GDE-1, glycerophosphodiester phosphodiesterase 1; IP$_3$, inositol trisphosphate; LPA, lyso-phosphatidic acid; lyso-PLC, lyso-phospholipid–preferring phospholipase C; NAAA, N-acylethanolamine amidohydrolase; PIP$_2$, phosphatidylinositol bisphosphate (PI[4,5]P$_2$); PLA$_2$, phospholipase A$_2$

inhibition results in widespread elevations of lipid signaling species (Blankman and Cravatt, 2013). In addition to MAGL, hydrolases such as ABHD6 and ABHD12 can also degrade 2-AG. ABHD6 localizes to the somatodendritic compartment of neurons, which may regulate 2-AG hydrolysis prior to release from the cell, as opposed to MAGL, which is localized on presynaptic compartments and catabolizes 2-AG after it has exerted its action at cannabinoid receptors (Cao et al., 2019). ABHD12, on the other hand, has been estimated to be responsible for approximately 9% of 2-AG hydrolysis in the brain, and its gene transcripts have been identified within microglial cells of the brain. Inhibition of either ABHD6 or ABHD12 can impact 2-AG signaling but to a much lesser degree then seen after MAGL inhibition. 2-AG can also be oxygenated by COX2 to form prostaglandin glycerols, for which a physiological role has yet to be established (Hermanson et al., 2014).

Physiological Roles of Endocannabinoids

Mediators of Endocannabinoid Action

The lipophilic nature of THC delayed the identification of its mechanism of action for many years. The first convincing evidence that its actions were mediated by a G protein-coupled receptor (GPCR) came from the pioneering work of Allyn Howlett and colleagues (Howlett et al., 1990; Howlett and Abood, 2017). These studies showed that THC and related synthetic cannabinoids inhibited adenylyl cyclase via a pertussis toxin–sensitive GPCR and that this receptor was highly expressed in many brain regions, consistent with the psychotropic effects of THC. Subsequent autoradiographic mapping using high-affinity synthetic cannabinoids confirmed the widespread and high levels of this cannabinoid receptor across the brain (Herkenham et al., 1991). This receptor (now designated as the CB1 cannabinoid receptor) was cloned and its distribution, actions, and regulation characterized (Howlett et al., 2002). Within a few years, a second cannabinoid receptor, the CB2 receptor, was cloned from an immune cell line. The psychoactivity after consumption of cannabis is likely mediated by CB1 cannabinoid receptors, whereas the immune modulatory effects are likely CB2 cannabinoid receptor mediated (Mackie, 2008).

CB1 Cannabinoid Receptors

CB1 receptors are highly expressed in presynaptic terminals of a subset of cortical GABAergic interneurons (often coexpressing the neuromodulator, CCK) and at lower levels in many other nerve terminals (Hu and Mackie, 2015). The role of these presynaptic as well as somatic CB1 receptors in mediating synaptic plasticity and neuronal excitability is discussed below. CB1 receptor expression changes with age, potentially explaining effects of cannabinoids on the developing CNS (Bara et al., 2021). CB1 receptors are also expressed in nonneuronal cell types such as astrocytes and outside the brain in hepatocytes, adipocytes, skeletal muscle, and endocrine cells (Covelo et al., 2021; Fong and Heymsfield, 2009).

CB2 Cannabinoid Receptors

CB2 receptors are highly expressed in immune cells (including microglia) and are expressed at lower levels in other cell types such as neurons, endothelial cells, pericytes, and keratinocytes. CB2 receptors may mediate immunomodulatory effects of THC and could be important for reducing drug craving and pain.

CB1 and CB2 Receptor Signaling

CB1 and CB2 are GPCRs and usually couple to inhibitory G proteins and arrestins, although coupling to G_s to activate adenylyl cyclase or $G_{q/11}$ to activate PLC has been observed in some experimental conditions. As G_i-coupled receptors, the canonical CB1 and CB2 signaling pathways include inhibition of adenylyl cyclase and voltage-gated Ca^{2+} channels and activation of mitogen-activated protein kinases (MAP kinases) and inwardly rectifying K^+ channels (Howlett et al., 2002; Mackie, 2008). Both CB1 and CB2 receptors show functional selectivity or biased agonism, whereby certain ligands favor activating specific subsets of G proteins and/or arrestin signaling pathways. This functional selectivity needs to be considered when evaluating the behavioral and physiological consequences of structurally diverse cannabinoids acting at cannabinoid receptors, particularly CB2 receptors (Atwood et al., 2012).

Non-CB1/CB2 Targets of Endocannabinoids

Endocannabinoids and some synthetic ligands can engage other targets in addition to CB1 and CB2, including ion channels (discussed below), peroxisome proliferator-activated receptors (PPARs), and synthetic cannabinoid receptor ligands. Among PPARs, PPARα and PPARγ are activated by eCBs and may contribute to the pharmacological effects of cannabinoids (Pistis and O'Sullivan, 2017). Genes targeted by PPARs include those involved in the regulation of metabolism, inflammation, neuroprotection, and cellular differentiation.

eCBs as Retrograde Messengers. Endocannabinoids are major retrograde messengers in the nervous system and mediate several forms of synaptic plasticity (Chevaleyre et al., 2006; Ohno-Shosaku and Kano, 2014). As retrograde messengers, eCBs are synthesized "on demand" by the postsynaptic neuron and travel retrogradely across the synapse to activate presynaptic CB1 receptors, suppressing neurotransmission from CB1-expressing nerve terminals (Figure 26–3). Depending on the duration of eCB production, eCB-mediated synaptic plasticity may be transient or sustained (Figure 26–4). Both forms of plasticity involve stimulation of the postsynaptic neuron (by depolarization and Ca^{2+} influx via voltage-sensitive Ca^{2+} channels and/or activation of a $G_{q/11}$-linked GPCR and release of Ca^{2+} from intracellular stores). This activates diacylglycerol lipase α to produce 2-AG (Figures 26–2 and 26–3). Two well-described transient forms of eCB-mediated synaptic plasticity are *depolarization-stimulated suppression* of excitation (if excitatory transmission is suppressed) or depolarization-stimulated suppression of inhibition (if inhibitory transmission is suppressed) and *metabotropic-stimulated suppression* of excitation (if excitatory transmission is suppressed) or metabotropic-stimulated suppression of inhibition (if inhibitory transmission is suppressed). These transient forms of plasticity start within a second of stimulation of the postsynaptic neurons and can last for tens of seconds (Wilson et al., 2001).

Sustained low-frequency activity of excitatory synapses may lead to a persistent eCB-mediated long-term depression (LTD) (Chevaleyre et al., 2006). Induction of LTD depends on sustained eCB production. However, established LTD is maintained independent of eCBs or CB1 receptors. The network implications of eCB-mediated synaptic plasticity depend on the activity of the CB1-expressing synapse: If the synapse is not active, there will be little effect; it also depends on whether the inhibited synapse is excitatory or inhibitory in nature and the relationship between the inputs driving eCB synthesis and the presynaptic terminals expressing CB1 receptors (Soltesz et al., 2015).

Nonretrograde Effects of eCBs on Neuronal Excitability. In addition to their role as retrograde messengers, eCBs may modify neuronal excitability in diverse ways. The best characterized include:

- Direct modulation of ion channels
- Activation of G protein-coupled inwardly rectifying K^+ channels (GIRKs)
- Enhancement of a hyperpolarization-activated cation channel (I_h)

Endocannabinoids may directly modulate ion channels, including $5HT_3$, TRPV1, $GABA_A$, glycine, and others (Soderstrom et al., 2017). When relating *in vitro* reports to what occurs *in vivo*, it is important to appreciate that some of these reported effects only occur with high eCB concentrations that are unlikely to be reached *in vivo*. Levels of eCBs produced by intense neuronal activity activate somatic CB1 receptors to open GIRK channels to hyperpolarize the neuron (Bacci et al., 2004).

I_h is a cation channel regulating dendritic excitability and playing a central role in synaptic plasticity and learning. Enhancing I_h activity impairs learning, and I_h activation by CB1 receptors is a possible mechanism for THC impairment of learning. CB1 enhancement of I_h involves a signaling cascade consisting of c-Jun-N-terminal kinase 1 (JNK1), guanylyl cyclase, cyclic GMP, and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (Maroso et al., 2016).

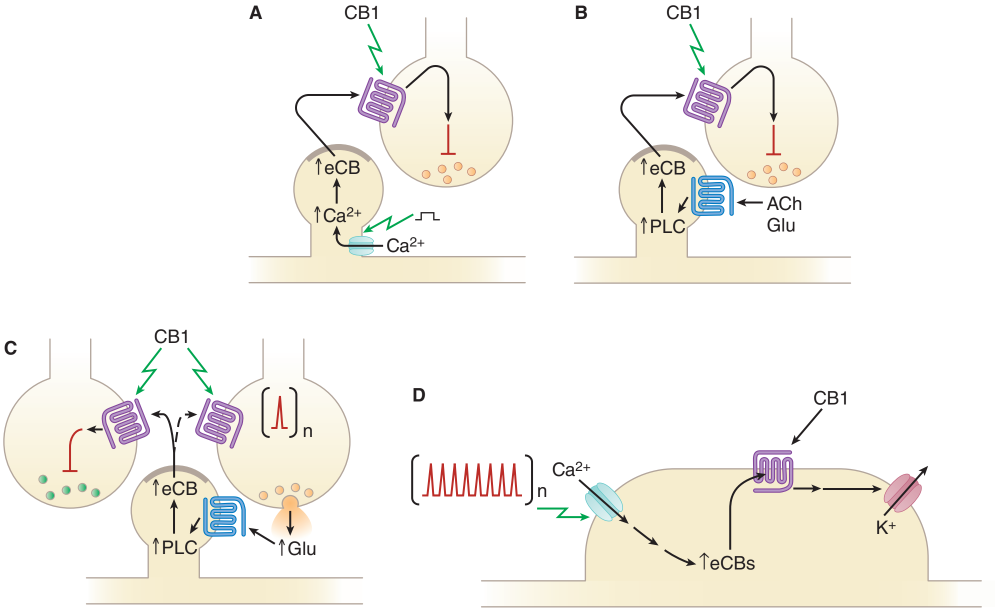

Figure 26–3 *Endocannabinoid-mediated synaptic plasticity and autocrine endocannabinoid signaling.* Unlike classical neurotransmitters, eCBs are "made on demand" following neuronal activity. Often, they are made in the postsynaptic cell and diffuse retrogradely to inhibit neurotransmitter from the presynaptic terminal (eCB-mediated synaptic plasticity; **A–C**). Occasionally, they act in an autocrine fashion (**D**). **A.** *Depolarization-induced suppression of inhibition/excitation (DSI/DSE).* Strong depolarization of the postsynaptic neuron leads to postsynaptic production of endocannabinoids, which diffuse retrogradely across the synapse. The endocannabinoids engage presynaptic CB1 receptors (▥), whose activation decreases synaptic vesicle release and synaptic transmission. If the presynaptic terminal is inhibitory, the phenomenon is denoted as DSI; if the presynaptic terminal is excitatory, the effect is termed DSE. **B.** *Metabotropic-induced suppression of excitation/inhibition (MSI/MSE).* In MSI/MSE, activation of postsynaptic GPCR → PLC pathways leads to the production of 2-AG, which diffuses retrogradely across the synapse to activate presynaptic CB1 receptors, suppressing synaptic transmission. If the presynaptic terminal is inhibitory, the phenomenon is denoted as MSI; if the presynaptic terminal is excitatory, it is referred to as MSE. Concurrent depolarization and GPCR → PLC activation can synergistically increase 2-AG production, resulting in a form of coincidence detection. **C.** *Homosynaptic and heterosynaptic endocannabinoid-mediated long-term depression (eLTD).* In homosynaptic eLTD, repeated stimulation (typically at a low frequency) of an excitatory terminal causes prolonged release of endocannabinoids from the postsynaptic neuron. This prolonged activation of presynaptic CB1 receptors induces a long-term depression of the excitatory terminal that extends long after the presynaptic stimulation has ceased. In heterosynaptic eLTD, LTD occurs at CB1-expressing synapses adjacent to the stimulated synapse. **D.** *Slow self-inhibition (SSI).* Classical SSI occurs when a CB1-expressing neuron is strongly stimulated ($[\wedge\wedge\wedge\wedge\wedge\wedge\wedge\wedge]_n$), producing endocannabinoids that activate somatic CB1 receptors, opening inwardly rectifying K^+ channels and leading to hyperpolarization and a prolonged inhibition of excitability. A paracrine variation on this autocrine theme occurs when the endocannabinoids diffuse away from the cell, activating somatic CB1 receptors on neighboring neurons and hyperpolarizing and suppressing firing in those neurons (Kreitzer et al., 2002).

Physiological Roles of Endocannabinoids

Stress and Its Resolution

Regular users of cannabis cite its ability to reduce stress and anxiety as a primary motivation for its use. Similarly, eCB signaling can reduce stress and anxiety. Research in both humans and rodents has found that exposure to stress results in the mobilization of eCBs, both in the brain and the periphery, and that glucocorticoids are an integral mediator in this response. This release of eCBs following stress is important for the termination of the stress response. As such, increasing eCB function can restrict or limit responses to stress. Within the brain, eCBs gate stress-induced excitation in brain regions such as the amygdala (Figure 26–5), which restricts the development of anxiety and the release of stress hormones (Gray et al., 2015; Morena et al., 2016). CB1 receptors are also localized to sympathetic nerve terminals in the periphery, and activation of these receptors by eCBs following stress exposure is important for restricting the autonomic response to stress. Exposure to chronic stress impairs eCB function. A loss of the ability of eCB signaling to restrict stress responses likely contributes to the development of stress-induced allostatic load (Morena et al., 2016). Human studies have found that the P129T genetic variant in FAAH, which reduces FAAH and elevates AEA signaling, is associated with lower levels of trait anxiety, enhanced top-down emotional control, improved inhibition of fear, and blunted neural and physiological responses to stress (Hariri et al., 2009; Petrie et al., 2021).

Reward

Intoxication with cannabis does produce a state of mild euphoria, and cannabis possesses addiction potential, albeit less than what is seen with drugs such as opiates. Rodents will self-administer cannabis vapor and will exert effort to seek cannabis vapor, both hallmark traits of substances that are reinforcing (Ferland and Hurd, 2020; Freels et al., 2020). The ability of cannabinoids to enhance reward is mediated by their actions in the ventral tegmental area (VTA; see Figure 26–5), a small brain nucleus with a high density of dopaminergic neurons. CB1 receptors in the VTA are primarily localized to the axon terminals of inhibitory GABA neurons that impinge upon VTA dopaminergic neurons. Activation of CB1 in the VTA inhibits GABA release, which disinhibits dopaminergic neurons, promoting the release of dopamine within the nucleus accumbens, a process known to be central for the encoding of rewarding salient events and for the motivation to engage in rewarding actions (Dubreucq et al., 2013; Wenzel and Cheer, 2018). Reinforcing stimuli, including both cocaine and voluntary exercise, require eCB signaling in the VTA to produce their rewarding effects. Disruption of eCB signaling inhibits motivation for rewarding stimuli and reduces engagement in pleasurable activities. In humans, the elevated AEA signaling associated with the P129T variant of FAAH increases neural reactivity of the nucleus accumbens to rewarding cues (Hariri et al., 2009). Pharmacological antagonism of CB1 receptors has been linked to the development of depression with anhedonia, an inability to experience pleasure (Christensen et al., 2007).

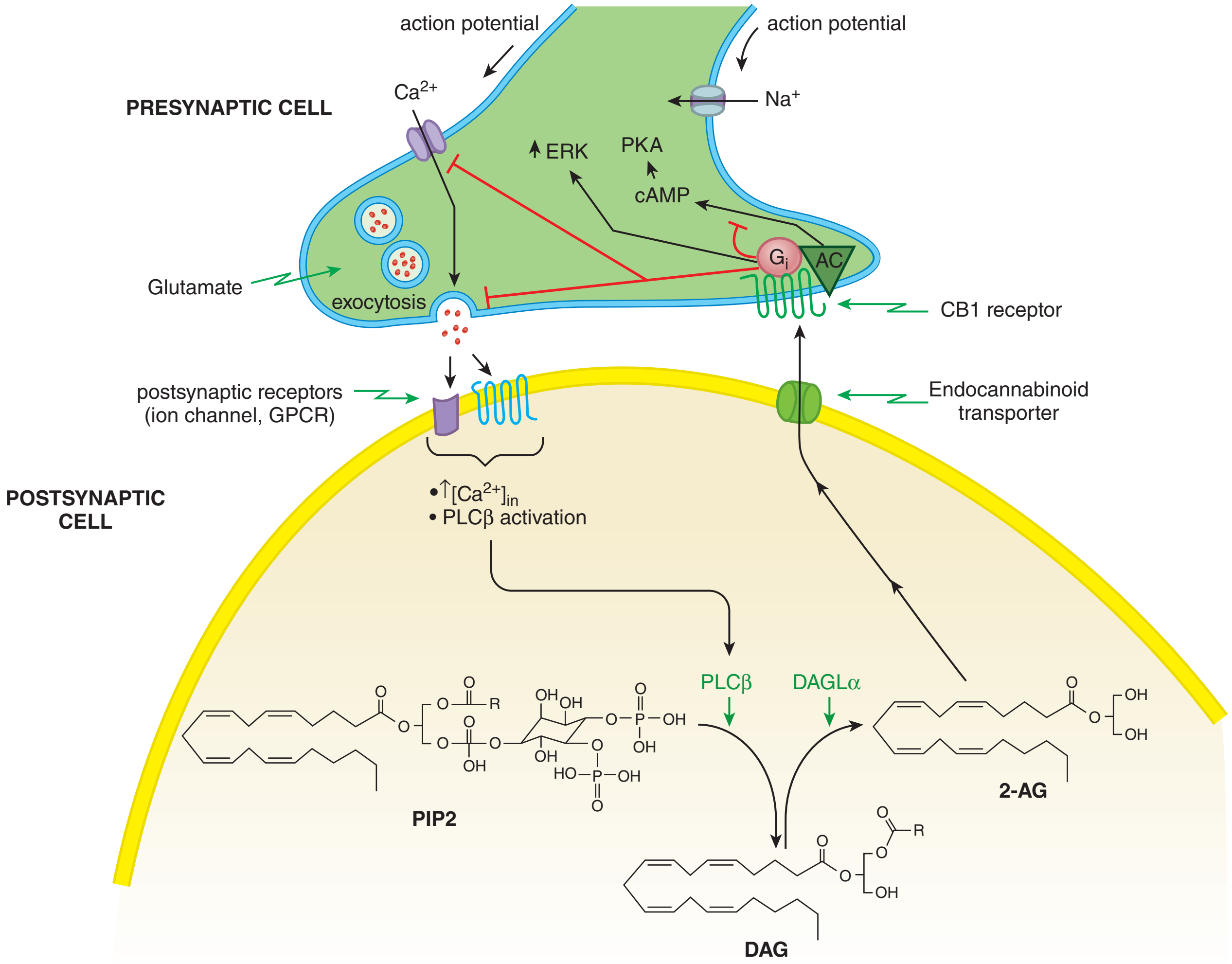

Figure 26–4 *2-Arachidonoyl glycerol (2-AG) synthesis and retrograde signaling in the CNS.* 2-AG acts as a retrograde messenger to limit presynaptic transmitter release. If the transmitter inhibited is excitatory, then feedback is negative. If the transmitter is inhibitory, then the feedback is positive. PIP2, phosphatidyl 4,5-bisphosphate.

Appetite and Metabolism

An increase in the consumption of sweet, palatable food, often referred to as "the munchies," is one of the prototypical effects of cannabis consumption in humans. eCB signaling is prominent within feeding circuits in the brain (see Figure 26–5). Within the hypothalamus, eCB levels fluctuate in response to nutritional status, where fasting elevates eCB levels and subsequent feeding and satiety decrease these levels (Lau et al., 2017). eCB signaling regulates feeding through regulation of the excitability of neurons within the arcuate nucleus of the hypothalamus, which are known to drive food intake (the AgRP/NPY neurons) and inhibit food intake (the POMC/MCH4 neurons). Thus, eCB signaling can rapidly increase or suppress food-seeking behavior and consumption. eCB signaling is also embedded into canonical hormonal cascades involved in regulating feeding. Leptin, a potent anorectic peptide produced by adipose tissue, rapidly suppresses hypothalamic eCB signaling to inhibit feeding. Hunger-stimulating hormones, such as ghrelin or glucocorticoids, promote food intake via recruitment of eCB signaling (Balsevich et al., 2018; Lau et al., 2017).

Peripheral eCB signaling also influences food intake and metabolic processes (Maccarrone et al., 2015; Ruiz de Azua and Lutz, 2019). Stimulation of cannabinoid receptors on vagal afferents and sympathetic nerve terminals can enhance food intake. Excess eCB activity in peripheral organs can have adverse effects on metabolic processes. Activation of hepatic CB1 receptors promotes the development of fatty liver and hepatic steatosis. Adipose tissue CB1 receptor activation can augment adipogenesis and fat accumulation. Elevated levels of endocannabinoids have been noted in rodent models of obesity and in human obese populations. Elevated eCB activity can promote the development of obesity and metabolic disorders; conversely, blockade of CB1 receptors produces anorectic effects, weight loss, and the prevention of metabolic consequences of obesity, such as insulin resistance and the development of type 2 diabetes in multiple species including humans (Lau et al., 2017; Ruiz de Azua and Lutz, 2019). Curiously, however, cannabis use in humans is generally not associated with obesity. Several large-scale population studies report that cannabis users have a lower body mass index and lower rates of obesity than noncannabis users. Because THC is a partial agonist at CB1 receptors, THC may occlude CB1 activation by 2-AG, a full agonist and thus limit some of the negative metabolic effects driven by elevated eCB function (Le Foll et al., 2013; Sidney, 2016).

Pain

Management of chronic pain and chemotherapy-induced nausea are the most common and most scientifically established therapeutic uses of cannabis in humans (Committee on the Health Effects of Marijuana, 2017; see also Chapter 54). CB1 receptors are distributed throughout multiple levels of pain circuits, including cortical, midbrain, spinal, and peripheral sites of action. CB1 receptors are synthesized within many dorsal root ganglion neurons and transported to peripheral afferent fibre nerve terminals. Peripheral eCB signaling can suppress pain initiation directly through activation of these receptors (Piomelli et al., 2014). eCBs also can act on CB1 receptors, and in some situations at TRPV1 receptors, within

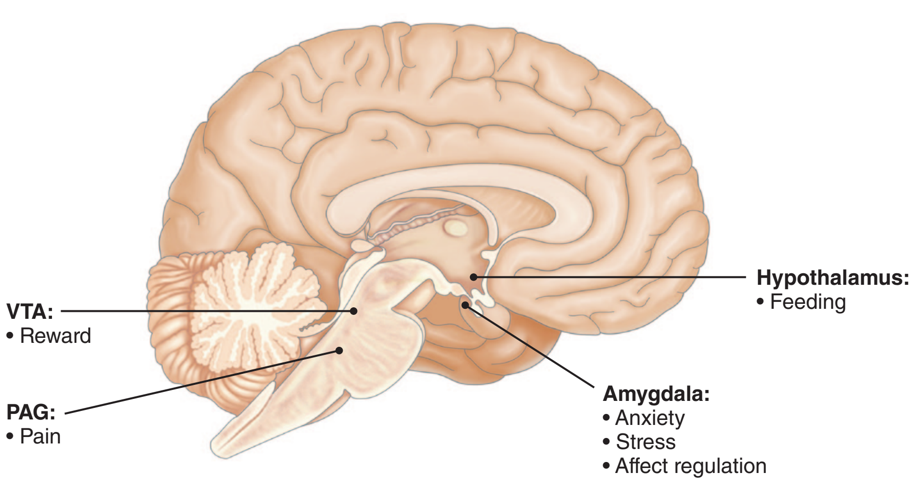

Figure 26–5 *Impact of endo- and phytocannabinoids on physiological processes in specific brain regions.* Cannabinoid signaling in the amygdala is the primary hub whereby endocannabinoids and THC influence anxiety states, stress responses, and affective processes. The capacity of cannabinoids to enhance rewarding stimuli has been localized to actions in the VTA. Cannabinoids increase feeding behavior through localized actions within hypothalamic nuclei. The analgesic effects of cannabinoids are driven through multiple pathways, including spinal and peripheral nerve pathways, but in the brain, the primary site of action is within the PAG.

spinal networks to influence pain processing (Woodhams et al., 2017). Cannabinoids can produce analgesia through activation of CB1 receptors within the periaqueductal gray (PAG; see Figure 26–5) and rostral ventromedial medulla of the descending pain circuit. eCBs act in higher-order brain circuits, primarily the cortical-amygdalar pathway, to influence pain processing, likely by influencing the affective component of pain. Exposure to noxious stimuli can enhance release of eCBs both in the periphery and within this distributed supraspinal pain circuit to act as endogenous regulators of pain initiation and sensitivity (Piomelli et al., 2014; Woodhams et al., 2017). Acute stress exposure produces transient analgesia via local release of eCBs within the PAG (Hohmann et al., 2005). A Scottish woman was discovered to possess both the P129T FAAH mutation as well as an upstream deletion in an FAAH pseudogene, which collectively resulted in robust elevations in AEA. These mutations were associated with a phenotype of pain insensitivity and accelerated healing (Habib et al., 2019).

Inflammation

CB2 receptors are primarily localized to immune cells and tissue, both in the periphery and in the brain. Most immune cells express CB2 receptors at varying levels, including T cells, monocytes, natural killer cells, and neutrophils, as well as microglia within the CNS. Activation of CB2 receptors on immune cells acts to reduce inflammation, primarily via the suppression of the release of proinflammatory cytokines, as well as by inhibiting cell proliferation and migration. Within the brain, CB2 receptors on microglia are rapidly induced by inflammation or damage and act to suppress the release of inflammatory cytokines and promote the release of anti-inflammatory cytokines. Within the periphery, CB2 receptors on T cells gate migration into tissues, such as the CNS, by reducing expression of adhesion factors. Deficits in T-cell CB2 receptors are associated with their enhanced infiltration into the CNS in pathological conditions such as multiple sclerosis (Malfitano et al., 2014). CB2-mediated activation of MAP kinases is integral to its ability to promote the release of anti-inflammatory cytokines and engage in reparative functions (Eljaschewitsch et al., 2006). CB1 receptors are also localized on some immune cells, but CB2 receptors appear to be the primary mechanism for most of the anti-inflammatory actions of eCBs.

eCB molecules can also regulate inflammation in the brain independently of cannabinoid receptors. 2-AG in the brain is predominately sequestered to cell membrane domains where it acts as a reservoir for arachidonic acid. In response to inflammatory stimuli, MAGL activity rapidly increases, metabolizing this membrane-associated 2-AG and liberating arachidonic acid, which is then converted to inflammatory prostaglandins via COX-2 (Nomura et al., 2011). MAGL localized within astrocytes (but not neurons) in the brain mediates the generation of neuroinflammatory molecules from 2-AG catabolism (Viader et al., 2015).

Sleep

The self-reported prosomnogenic effects of cannabis are often cited as a primary reason for continued consumption among recreational and medical cannabis users (Kesner and Lovinger, 2020). Cannabis can reduce the latency to onset of sleep and nighttime awakenings as well as promote non–rapid eye movement (NREM) sleep, while reducing the percentage of time spent in rapid eye movement (REM) sleep. eCB levels fluctuate in the brain and in the circulation in a circadian manner, and this diurnal cycle is disrupted following sleep. Elevating 2-AG signaling via MAGL inhibition similarly increases time spent sleeping and enhances NREM sleep while suppressing REM sleep (Kesner and Lovinger, 2020). Administration of a CB1 receptor antagonist promotes wakefulness and arousal and reduces NREM sleep. In humans, there have been multiple reports of sleep disruption following administration of the CB1 receptor antagonist *rimonabant*. eCB signaling may contribute to normative sleep-wake cycles.

Phytocannabinoids

Synthesis of THC and CBD by Cannabis

Our understanding of the synthetic pathways of the phytocannabinoids has expanded rapidly in the last decade. Details can be found in recent reviews (e.g., Gulck and Moller, 2020; Tahir et al., 2021) (Figure 26–6). Δ^9THC and its isomer, CBD (Figure 26–1), are synthesized in the trichomes of the cannabis plant, with the highest levels synthesized in the flowers of the female plant and lower levels produced in other aerial components and the male plant. Classic phytocannabinoid synthesis involves initial steps leading to the production of geranyl pyrophosphate and olivetolic acid. These are then joined by an aromatic prenyltransferase to form the initial phytocannabinoid, cannabigerolic acid (CBGA). CBGA then serves primarily as a substrate for Δ^9-tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) synthases, which catalyze the formation of THCA and CBDA. THCA and CBDA have little psychoactivity on their own but are important precursors. THCA and CBDA undergo decarboxylation (enhanced by gentle heating or light) to form biologically active THC and CBD, respectively. THCA and THC can also undergo spontaneous oxidation to form cannabinolic acid (CBNA) and cannabinol (CBN), with the latter lacking both the classic psychoactivity of THC and the biological activity of CBD. Interestingly, several other species of plants can also synthesize phytocannabinoids and closely related molecules with cannabimimetic effects (Gulck and Moller, 2020).

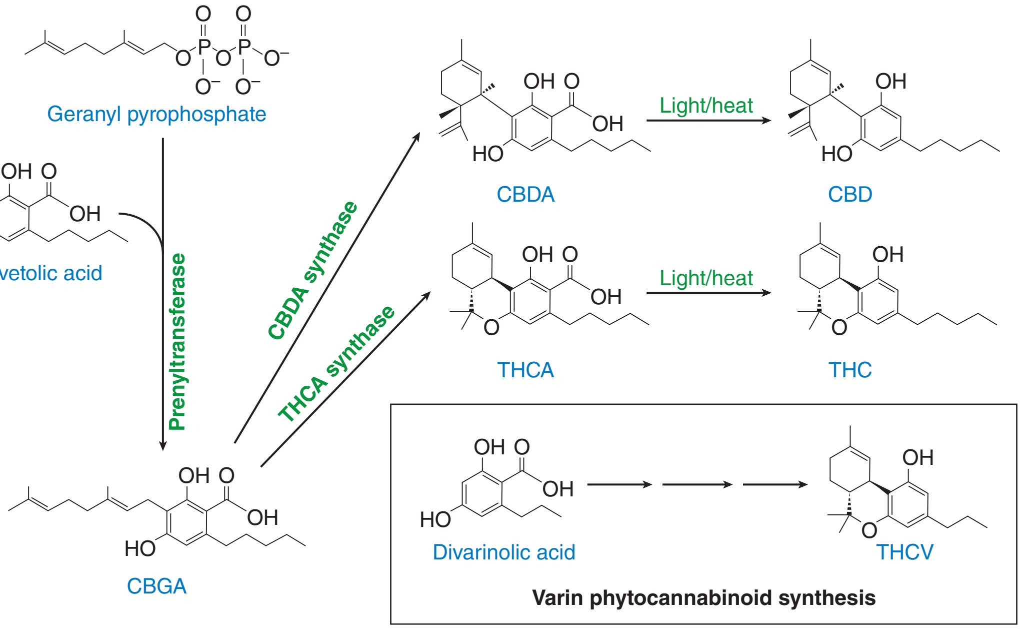

Figure 26–6 *Phytocannabinoid synthesis.* In the first committed step of phytocannabinoid synthesis, cannabigerolic acid synthase (CBGAS) prenylates olivetolic acid with geranyl pyrophosphate to produce cannabigerolic acid (CBGA). CBGA is then a substrate for THC synthase, CBD synthase, or CBC synthase, whose levels in experimental models can be varied by directed breeding programs. The synthases produce the acid forms of the phytocannabinoids (THCA, CBDA, and CBCA), which undergo nonenzymatic decarboxylation by light exposure or mild heating, yielding the more familiar THC, CBD, and CBC. *In the box:* Under growth conditions where butyl coenzyme A (CoA) is higher than hexanoyl CoA, divarinolic acid is produced (rather than olivetolic acid). Divarinolic acid is then synthesized to the varin cannabinoids, using the same enzymatic pathways that synthesize THC and CBD.

In addition to THC, CBD, and CBN, several "minor" cannabinoids may be synthesized in the plant under various growth conditions. Cannabichromenic acid (CBCA) is formed from CBGA by an incompletely characterized CBCA synthase and is subsequently decarboxylated to cannabichromene (CBC) (Gulck and Moller, 2020). Δ^8THC is a positional isomer of Δ^9THC and is likely a degradation product of Δ^9THC. Δ^8THC retains Δ^9THC psychoactivity.

A particularly interesting group of minor phytocannabinoids with a pharmacology distinct from Δ^9THC and CBD are the so-called varin cannabinoids, distinguished by their propyl side chain (see Figure 26–6). THC and CBD both contain a pentyl side chain derived from olivetolic acid. If the primary resorcinol available for the prenyltransferase is varinolic acid instead of olivetolic acid, then phytocannabinoids with propyl side chains result (tetrahydrocannabivarin acid and cannabidivarin acid, decarboxylated to Δ^9-tetrahydrocannabivarin [THCV] and cannabidivarin, respectively; see Figure 26–1). These varin phytocannabinoids have a pharmacology that distinguishes them from the pentyl phytocannabinoids, with THCV being a particularly potent CB1 cannabinoid receptor antagonist and a low-efficacy CB2 cannabinoid receptor agonist (McPartland et al., 2015).

Generally, the levels and activity of THCA and CBDA synthase in cannabis cultivars vary in a reciprocal fashion. Thus, plants that have been bred to have high THCA synthase activity will produce high levels of THC and low levels of CBD, while plants with high CBDA synthase activity will produce little THC. Selective breeding can result in a total THC content of dried plant material exceeding 30% (Swift et al., 2013). Importantly, the product specificities of THCA and CBDA synthases are not absolute. Therefore, plants producing high amounts CBD can also produce detectable levels of THC. This has important legal implications as cannabis plants with greater than 0.3% THC (w/w) are considered THC-producing in the U.S. and under current U.S. federal law are subject to U.S. Drug Enforcement Administration confiscation. Of note, CBD can be converted to THC under mildly acidic conditions, as might occur in the stomach, but whether this occurs in the human body under normal physiological conditions is not unequivocally established (Golombek et al., 2020).

In addition to phytocannabinoids, cannabis plants synthesize a range of other molecules, including terpenes and flavonoids. Terpenes give cannabis cultivars their distinctive aromas. Terpenes, built from isoprene units (2-methyl-1,3-butadiene), occur in myriad structures. The extent to which terpenes in cannabis contribute to or modify its psychoactivity or underlie its possible therapeutic benefits remains unknown (Booth et al., 2020).

Synthetic Cannabinoids

Considerable effort has been expended on synthesizing novel CB1 cannabinoid receptor agonists for therapeutic applications, such as analgesia. This has resulted in a rich medicinal chemistry of cannabinoid agonists of diverse structural classes. In parallel, widespread drug testing for THC (and its metabolites), coupled with the very long elimination half-life of THC metabolites, has motivated the recreational use of synthetic cannabinoids for individuals for whom a positive drug test may result in loss of their job. Because the structures of synthetic cannabinoids are often substantially different from THC, these compounds escape detection by common urine drug screens. This has led to the widespread recreational use of a wide variety of synthetic cannabinoids, known generically as "spice." In almost all cases, synthetic cannabinoids are high-efficacy CB1 agonists. In addition, their "off-target" activities have not been characterized, so they may engage an unpredictable repertoire of additional receptors, resulting in unpredictable psychoactivity. Finally, synthetic cannabinoids are typically prepared in illicit labs, with little quality control, so they may also be contaminated with unwanted molecular species.

Interactions Between eCBs, THC, and Synthetic Cannabinoids

The varying intrinsic efficacies of 2-AG, AEA, THC, and the synthetic cannabinoids used recreationally (the "spice" compounds) give rise to several possible interactions. For example, THC is a potent, low-efficacy agonist at CB1 receptors, whereas 2-AG is a less potent but highly efficacious agonist. Thus, under conditions where either CB1 receptor density or postreceptor coupling is limited, THC may antagonize endogenous 2-AG signaling. Conversely, under circumstances of high CB1 receptor density and ample postreceptor coupling, THC and 2-AG could produce similar cellular effects. THC/2-AG interactions under the former condition may explain human behavioral data where even very high doses of the CB1 antagonist *rimonabant* weakly antagonize the subjective effects of THC, while *rimonabant* strongly attenuates THC-induced tachycardia (Huestis et al., 2001). Whether THC antagonizes, mimics, or augments eCB action likely varies across synapses. However, "spice" compounds are typically highly efficacious agonists, fully and indiscriminately activating CB1 receptors. With this property, "spice" compounds likely counter AEA signaling (AEA is a low-efficacy agonist). The very high efficacy of "spice" compounds (together with these compounds potentially engaging targets beyond CB1 and the presence of adulterants in the various "spice" formulations purchased by consumers) may explain their propensity to produce more frequent and more severe adverse effects than THC (Atwood et al., 2010; Deng et al., 2018; Huestis et al., 2001; Laaris et al., 2010; Straiker and Mackie, 2005).

Approved and Promising Pharmacological Applications

Dronabinol

Dronabinol is chemically synthesized THC formulated in sesame oil in a capsule and is also available as an oral solution.

Mechanism of Action

Dronabinol is a low-efficacy agonist of CB1 and CB2 cannabinoid receptors.

ADME

Dronabinol absorption from the gastrointestinal tract is high, as is first-pass metabolism, resulting in a bioavailability of only 10% to 20%. Peak plasma THC concentrations typically occur 1 to 3 h after oral administration. Concurrent food consumption slows time to peak THC plasma levels and increases exposure but does not affect peak plasma levels. THC undergoes successively oxidation at C11, leading to 11-hydroxy-tetrahydrocannabinol (11-OH-THC), followed by 11-nor-carboxy-THC. Notably, 11-OH-THC retains high activity at CB1 cannabinoid receptors. Thus, after oral consumption of *dronabinol*, levels of 11-OH-THC may be greater than those of THC, and most biological effects may be due to this metabolite. Excretion is primarily via the bile, with lesser amounts in the urine. THC metabolism is prolonged, with metabolites being detected in the feces and urine more than 5 weeks after a single dose. Most pharmacokinetic parameters for capsule and oral suspension forms of *dronabinol* are similar but not identical.

While inhalation is not an approved route of administration, it is important to note that the pharmacokinetics of THC vary dramatically if consumption occurs via the pulmonary route as opposed to oral ingestion in capsule form. For example, the bioavailability of inhaled (smoked) THC is 60% to 70%. For comparison, orally ingested THC produces blood levels in the range of 2 to 20 ng/mL, which peak around 60 to 90 min after ingestion and produce intoxication for up to 8 to 12 h (Huestis, 2005). Inhaled THC produces blood levels of THC that are dramatically higher, around 60 to 200 ng/mL, but peak quickly, around 15 min after inhalation, and rapidly return to baseline. However, frank intoxication persists for 90 to 240 min (Huestis, 2005).

Therapeutic Uses

Dronabinol is approved in the U.S. for the treatment of nausea and vomiting in patients receiving cancer chemotherapy who have not responded to conventional antiemetics as well as for anorexia associated with weight loss in patients with AIDS. See Chapter 54 for additional information on the use of *dronabinol* as an antinauseant.

Adverse Effects and Drug Interactions

The most common adverse effects reported with *dronabinol* involve the CNS and the cardiovascular system. CNS adverse effects include exacerbation of mania, depression, and schizophrenia as well as dizziness, fatigue, and cognitive impairment. Cardiovascular adverse effects include tachycardia and hypo- or hypertension. Abdominal pain and *increased* nausea and vomiting are occasionally reported with *dronabinol* use, which may be manifestations of cannabinoid hyperemesis syndrome. Care should be used in prescribing *dronabinol* to individuals who may be at greater risk for any of these adverse effects. Treatment includes holding or reducing the *dronabinol* dose or administration before sleep, depending on the nature and severity of the adverse effect. Phase 1 metabolism of *dronabinol* appears to be chiefly by CYP2C9 and CYP3A4, with the former having a greater contribution. Interactions with other drugs that may induce or inhibit these CYPs is likely. Conversely, high doses of THC may impair metabolism of drugs metabolized by these CYPs. CYP2C9 is highly polymorphic. Individuals who are homozygous for CYP2C9*3 (i.e., *3/*3) are classified as poor metabolizers and may be unusually susceptible to adverse drug responses when CYP2C9 is involved (see Chapter 7).

Pediatric and Geriatric Indications

Dronabinol is not approved for pediatric use. While there has been some interest in using *dronabinol* in treating agitation in dementia, it is not approved for this use.

Clinical Use

To treat nausea and vomiting associated with cancer chemotherapy after traditional pharmacological regimens are unsuccessful, *dronabinol* is begun at 5 mg/m^2 given 1 to 3 h before chemotherapy and then every 2 to 4 h after chemotherapy up to four to six doses per day. The first dose should be given at least 30 min prior to eating. Later doses can be given without regard to fed status; however, dosing relative to meals should be kept constant to facilitate titration. If the antiemetic response is inadequate, the dose can be increased in increments of 2.5 mg/m^2 to a maximum dose of 15 mg/m^2. CNS-related adverse effects increase significantly at higher doses, so patients must be carefully monitored for these and the dose decreased if necessary.

To treat anorexia associated with weight loss in AIDS, *dronabinol* is started at 2.5 mg twice a day, before lunch and before sleep. If the response is inadequate, the dose can be increased slowly to 5 mg twice daily, before lunch and in the evening. The maximum recommended dose is 10 mg twice daily. If CNS adverse effects are troublesome, the prelunch dose can be reduced or eliminated, keeping the evening dose. In Chapter 54, the section on Antinauseants and Antiemetics also presents information on the clinical use of *dronabinol* and *nabilone* (see below for discussion of *nabilone*).

Nabiximols

Nabiximols is a standardized extract from cannabis consisting of approximately equal amounts of THC and CBD and lesser amounts of minor cannabinoids, terpenes, and triglycerides. It is formulated as an ethanol-containing, peppermint-flavored, metered-dose oromucosal spray. It is not FDA-approved in the U.S. but is available in other countries.

Mechanism of Action

The THC in *nabiximols* is a low-efficacy agonist of CB1 and CB2 cannabinoid receptors. The CBD in *nabiximols* has the potential to interact with multiple targets (see section on CBD). The role of the minor constituents of *nabiximols* in its therapeutic efficacy is not known. There is the potential for a pharmacokinetic interaction between THC and CBD in *nabiximols* because of the overlapping CYPs involved in their metabolism.

ADME

Nabiximols pharmacokinetics are notably variable between patients, with mixed oromucosal and intestinal absorption contributing to this variability. Peak plasma THC concentrations typically occur 1 h after oromucosal administration. Peak plasma levels of THC and CBD can vary 10-fold between subjects receiving the same dose, and exposure may vary 3- to 4-fold. Co-consumption of food with *nabiximols* increases peak plasma levels and exposure for both THC (~2-fold) and CBD (~4-fold). The pharmacokinetic variability emphasizes that patients receiving *nabiximols* should be consistent in taking the drug relative to their food consumption. Importantly, because of the oromucosal route of administration of *nabiximols*, peak levels of THC and CBD are achieved more slowly and are much lower than seen with inhaled cannabis or vaporized cannabinoid preparations. Metabolism of *nabiximols* is dominated by the metabolism of its two primary active components, THC and CBD. Details of their metabolism can be found in the sections on *dronabinol* and CBD, respectively. *Nabiximols* have been shown to inhibit CYP3A4 in a time-dependent fashion, even at clinically used doses. Thus, there is the potential for *nabiximols* to increase levels of other drugs metabolized by CYP3A4. Conversely, CYP3A4 inhibitors (e.g., *ketoconazole* and congeners) can substantially increase exposure to THC and CBD, and inducers of CYP3A4 (e.g., *rifampin*, St. John's wort) can decrease THC and CBD exposure. If the doses of drugs in these classes are increased or decreased when a patient is receiving *nabiximols*, their dosing should be reviewed.

Therapeutic Uses

Nabiximols is approved in several countries (but not the U.S.) for the treatment of moderate to severe spasticity and associated pain in multiple sclerosis. There is also interest in using *nabiximols* for treating chronic pain, although the clinical data on this indication are mixed.

Pediatric and Geriatric Indications

Nabiximols is not approved for pediatric use.

Adverse Effects and Drug Interactions

The most common adverse effects reported with *nabiximols* include dizziness, drowsiness, fatigue, and problems with memory/concentration; these effects likely arise from THC acting at CB1 cannabinoid receptors. Treatment for adverse effects involves holding or reducing the *nabiximols* dose, depending on the severity of the adverse effect. In clinical practice, these adverse effects are generally mild in intensity and diminish with continued use, suggesting that tolerance develops.

Clinical Use

Each dose (100-μL spray) of *nabiximols* contains 2.7 mg of THC and 2.5 mg of CBD. Typically, to minimize adverse effects, therapy is initiated with a single evening dose, with an additional evening dose every 2 days and morning doses, similarly titrated upward, added on the fifth day of treatment, to a maximum of 12 doses per day. Titration is stopped when adequate symptom relief is obtained or bothersome adverse effects occur. If adequate relief is not obtained after 4 weeks of treatment, treatment is generally stopped.

Cannabidiol

An oral solution of cannabis-derived CBD is FDA-approved for use in certain seizure disorders, as detailed below and in Chapter 20.

Mechanism of Action

The mechanism of action of CBD in reducing seizures remains unknown. Anxiolysis mediated by CBD appears to involve activation of serotonin $5HT_{1A}$ receptors. Anti-inflammatory actions of CBD seem to be mediated by a potentiation of adenosine signaling.

ADME

Peak plasma concentrations typically occur 3 to 5 hours after oral administration. Bioavailability of orally administered CBD is typically low and variable but is enhanced by concomitant fatty food consumption (3- to 15-fold increase in exposure). CBD half-life is long and variable (14–30+ h) and increases with chronic dosing.

Cannabidiol is primarily metabolized by hydroxylation of the C7 methyl group by CYP2C19, followed by oxidation to the C7 carboxylic acid by CYP3A4. The C7 hydroxyl cannabidiol metabolite (7-hydroxycannabidiol [7-OH-CBD]) is analogous to 11-OH-THC and appears to retain antiepileptic activity (Huestis, 2005). CBD metabolites are subject to phase II glucuronidation, primarily at the phenolic oxygen. CBD metabolites are excreted mostly via the feces with small amounts via urine.

Therapeutic Uses

Cannabidiol has been approved to treat medication-resistant seizures in Dravet and Lennox-Gastaut syndromes and tuberous sclerosis complex. Additional clinical trials are ongoing to determine its efficacy in other medication-resistant epilepsies. While there is evidence that acute administration of high doses of CBD reduces social anxiety disorder in certain settings, its efficacy for broadly treating anxiety disorders is not established (Wright et al., 2020). CBD has attracted interest as an antipsychotic agent, but its efficacy remains to be defined (Schoevers et al., 2020).

Adverse Effects and Drug Interactions

The most common adverse effects of CBD at the doses used to treat epilepsies include somnolence, fatigue, decreased appetite, diarrhea, and elevated hepatic transaminases. Liver transaminase elevations are dose-related, and alanine aminotransferase (ALT) elevations of greater than 3 times the upper limit of normal were observed in more than 10% of patients taking CBD at doses of 10 to 25 mg/kg per day. Approximately one-third of ALT elevations resolved despite continued administration of CBD. The risk of ALT elevation is almost doubled in patients receiving *valproate* (with or without *clobazam*) with CBD. Thus, in patients receiving CBD, serum transaminases and bilirubin should be monitored, particularly early in therapy or after a change in dose.

Due to the high doses of CBD administered as an antiepileptic, the likelihood that patients receiving CBD are also receiving multiple other drugs (which may induce or inhibit CYPs), and CBD interactions with several CYPs, CBD has a high potential to cause significant bidirectional drug-drug interactions. The best described is with the antiepileptic *clobazam*, where plasma levels of *clobazam* and its active metabolite, nor-clobazam, often increase dramatically following initiation of CBD therapy. This is consistent with CBD's inhibition of CYP3A4 and CYP2C19, which are responsible for metabolizing *clobazam* and nor-clobazam, respectively. Conversely, coadministration of CBD with *clobazam* increases 7-OH-CBD. *Clobazam* levels should be monitored when CBD is administered with *clobazam*. Coadministration of *valproate* with CBD does not increase *valproate* exposure but, as mentioned above, does increase the chance of liver transaminase elevation.

Pediatric and Geriatric Indications

In the U.S., *cannabidiol* is approved by the FDA for the treatment of seizures associated with Lennox-Gastaut syndrome, Dravet syndrome, or tuberous sclerosis complex in patients aged 1 year and older. In the E.U., the E.M.A. has granted orphan drug status for CBD to be used as adjunctive therapy with *clobazam* to treat seizures associated with Lennox-Gastaut or Dravet syndrome and as adjunctive therapy to treat seizures associated with tuberous sclerosis complex.

Clinical Use

For the treatment of seizures associated with Lennox-Gastaut or Dravet syndrome, CBD is typically started at 2.5 mg/kg twice daily. This is increased, as necessary, to 5 mg/kg twice daily after the initial week of treatment. If indicated, this dose can be increased to 10 mg/kg twice daily in weekly increments of 2.5 mg/kg per dose.

For the treatment of seizures associated with tuberous sclerosis complex, CBD is started at 2.5 mg/kg twice daily and increased in weekly increments of 2.5 mg/kg per dose to 12.5 mg/kg twice daily as tolerated.

It is important to note that the doses of CBD necessary to effectively treat seizures are much higher than the doses of CBD typically consumed from over-the-counter preparations available in the U.S.

Cannabidiol has also been examined for utility in other disease states. Several randomized clinical trials examined CBD as treatment for schizophrenia; results were mixed. In one study, 300 mg/day, but not 600 mg/day, of CBD improved cognitive impairment in schizophrenia (Hallak et al., 2010). Another study found that 800 mg/day of CBD was as effective as *amisulpride* but that CBD produced fewer negative side effects (e.g., extrapyramidal symptoms) than *amisulpride* (Leweke et al., 2012). The third trial found that 1000 mg/day of CBD improved positive symptoms but had no impact on negative symptoms (McGuire et al., 2018), while the fourth trial, which studied a dose of 600 mg/day, found no effect on cognitive outcomes (Boggs et al., 2018). In persons with social anxiety disorder, one trial reported that a single dose of 600 mg of CBD did produce improvements in stress-induced anxiety and cognitive impairment (Bergamaschi et al., 2011). Another trial found that 3 consecutive days of administration of either 400 or 800 mg of CBD reduced craving and anxiety to drug cues in abstinent individuals with heroin use disorder (Hurd et al., 2019).

Note that in all clinical trials where efficacy of CBD has been reported, the doses are upward of 200 mg/day, with upper ranges reaching 1600 mg/day. This dose range is much higher than the doses that are typically used off-label by the general public, which tend to fall closer to 5 to 25 mg/day. Given the poor bioavailability of most oral CBD preparations, the lack of any identified pharmacological targets for CBD at the very low nanomolar concentrations that would be achieved by this dosing, and the failure to see clinical benefit in any trials using oral CBD at this low dose range, it seems highly unlikely that doses of CBD taken in this range are producing any biological impact on the individuals taking them. There is strong reason to believe that expectancy bias is having a robust impact on individuals claiming benefit from dosing of CBD at this level and that these effects are likely placebo effects.

Nabilone

Nabilone is a synthetic form of THC that is similar to, but structurally distinct from, THC itself (see Figure 26–1). *Nabilone* was previously FDA-approved and marketed in capsule form but has been discontinued in the U.S. since 2019 due to reports of serious hypersensitivity reactions.

Mechanism of Action

Nabilone has similar pharmacology to THC. It is a low-efficacy agonist of CB1 and CB2 cannabinoid receptors but is somewhat more efficacious than THC at activating both CB receptors.

ADME

Nabilone is taken orally and has high absorption from the gut (~96% absorption into bloodstream), with peak plasma levels occurring at around 2 h. As with THC, nabilone is subject to high first-pass metabolism, but over a dose range of 1 to 4 mg, the pharmacokinetics remain relatively linear. The distribution and metabolism of *nabilone* have not been well defined. *Nabilone* metabolism is primarily hepatic and likely involves several CYPs and hydroxylation sites on the dimethyl-heptyl side chain and formation of carboxylic analogues, similar to THC. There is also evidence that metabolites of *nabilone* are generated from the reduction of the ketone group on C9, producing several isomeric alcohols, which are primarily excreted in feces.

Therapeutic Uses

Nabilone is used in Canada, the United Kingdom, and Mexico for the treatment of chemotherapy-induced nausea and vomiting in patients who have not responded to conventional antiemetics. *Nabilone* has also been used off-label for the management of nightmares in individuals with posttraumatic stress disorder, for chronic neuropathic pain, cannabis withdrawal in hospitalized patients, and in the management of agitation in early dementia.

Adverse Effects and Drug Interactions

The most common adverse effects reported with *nabilone* involve the CNS and the cardiovascular system. CNS adverse side effects manifest as increased anxiety, nervousness, and panic and, in extreme rare cases, hallucinations and delusions. Sedation, dizziness, and impairments in normative cognitive function have also been seen, particularly at higher doses (≥5 mg). Cardiovascular adverse effects include mild tachycardia and postural hypotension at doses of 2.5 to 5 mg and above. Many of these adverse effects, particularly postural hypotension, may occur more frequently in elderly populations.

The metabolism of *nabilone* is not well established but likely involves the same CYP enzymes as those that metabolize THC. As such, there is a potential for drug interactions, although no major interactions have been identified. There are potential functional interactions with other substances that produce sedation or dizziness, such as benzodiazepines, opiates, some antidepressants (such as *quetiapine*), and alcohol.

Pediatric and Geriatric Indications

Nabilone is not approved for pediatric use. While there has been some interest in using *nabilone* in treating agitation in dementia, it is not approved for this use.

Clinical Use

To treat nausea and vomiting associated with cancer chemotherapy after traditional pharmacological regimens are unsuccessful, *nabilone* is begun at 1 to 2 mg given 1 to 3 h before chemotherapy. An initial dose may also be given the night prior to chemotherapy administration to offset nausea, and dosing may continue every 8 to 12 h for 24 h following chemotherapy (up to 48 h following chemotherapy if needed), without exceeding a total of 6 mg within 24 h. CNS-related adverse effects increase significantly at higher doses, so initial dosing is best at 1 mg to limit adverse side effects, with the dose increased up to 2 mg if not effective.

Off-label use of *nabilone* for control of nightmares in posttraumatic stress disorder consists of taking *nabilone* 1 h prior to bed, starting at a dose of 0.5 mg per night and titrating up to a maximal dose of 4 mg per night depending on efficacy and side effects. Similar dose ranges are used for off-label management of pain and for agitation in dementia.

Cannabinoid Drug Development

Peripherally Restricted CB1 Antagonists

Motivated by preclinical data showing that peripheral CB1 antagonism could be metabolically beneficial (Nogueiras et al., 2008) and on the assumption that the CB1 inhibitor *rimonabant* (see Box 26–1) induced anxiety and depression by a central mechanism, efforts have been directed toward the development and testing of *peripherally restricted* CB1 antagonists/inverse agonists. Several peripherally restricted CB1 antagonists are being evaluated in clinical trials to test whether they are effective therapy for obesity and the metabolic dysregulation accompanying obesity.

BOX 26–1 ■ Lessons From Clinical Trials and Postmarketing Surveillance

Cannabis consumption often causes an urge to consume calorically dense foods, a phenomenon colloquially known as “the munchies.” Soon after the identification of CB1 cannabinoid receptors, several pharmaceutical companies initiated drug discovery programs to identify CB1 receptor antagonists, based on the reasoning that blocking CB1 receptors might decrease the consumption of highly palatable foods and be a novel pharmacological treatment for obesity. Among these companies, Sanofi-Aventis was the first to identify such an antagonist (specifically, an inverse agonist), designated as *rimonabant*.

In clinical trials, *rimonabant* produced a modest but consistent weight loss across diverse populations of obese subjects (Scheen, 2008). Interestingly, *rimonabant* improved metabolic parameters to a greater extent than predicted from the modest weight loss (Van Gaal et al., 2008). Further preclinical and clinical investigations supported the notion that *rimonabant*-induced weight loss was not a result of decreased food intake, which was only transient following initiation of the drug. Rather, weight loss was due to a sustained increase in energy expenditure brought about by antagonism by *rimonabant* of CB1 receptors on adipocytes, hepatocytes, and skeletal muscle (Addy et al., 2008; Kunos and Tam, 2011). Based on the favorable weight loss data, *rimonabant* was approved for use in the European Union as a treatment for obesity. However, concerns emerged that patients taking *rimonabant* were at a higher risk for anxiety, depression, and suicide. Because of these concerns, *rimonabant* failed to gain FDA approval and was withdrawn from the market in the European Union a few months later.

Endocannabinoid Catabolic Enzyme Inhibitors: FAAH and MAGL Inhibitors

A major approach within the cannabinoid field is the development of specific inhibitors that target the catabolism of eCB molecules: FAAH inhibition to potentiate AEA signaling and MAGL inhibition to potentiate 2-AG signaling (Blankman and Cravatt, 2013). MAGL inhibitors have been tested clinically by Pfizer and Abide/Lundbeck. The Pfizer compound did not go past phase I testing of brain penetrance. The Abide/Lundbeck MAGL inhibitor Lu AG06466 (formerly ABX1431) is currently in clinical trials but was ineffective in Tourette syndrome (Muller-Vahl et al., 2021). Given the ability of MAGL inhibition to modulate the generation of central inflammatory molecules (Nomura et al., 2011), ongoing clinical studies are focused on neurodegenerative diseases.

Compared to MAGL inhibitors, FAAH inhibitors have been more extensively tested. The first FAAH inhibitor entering clinical development, PF-04457845, was developed by Pfizer. It is a covalent, irreversible inhibitor of FAAH that produces up to 10-fold elevations in circulating AEA and related *N*-acyl ethanolamines for 24 h (Li et al., 2012). No serious adverse side effects were detected, and the compound had good CNS penetration and saturation of FAAH in the brain. The initial clinical study on osteoarthritic pain failed (Huggins et al., 2012). In 2019, a phase IIa trial with PF-04457845 showed safety and efficacy in treating cannabis withdrawal and dependence in men, with notable improvements in mood, anxiety, and sleep changes associated with withdrawal (D'Souza et al., 2019). This indication is currently being tested in a multisite trial (ClinicalTrials.gov identifier: NCT03386487). In 2020, an experimental trial in healthy humans showed that PF-04457845 reduced affective, autonomic, and physiological responses to stress and improved fear extinction (Mayo et al., 2020). Ongoing studies are examining the efficacy of FAAH inhibition in posttraumatic stress disorder.

The Janssen FAAH inhibitor, JNJ-42165279, is a reversible inhibitor of FAAH (Postnov et al., 2018). JNJ-42165279 was ineffective in individuals with anxious depression (ClinicalTrials.gov identifier: NCT02498392) but showed some benefit in patients with social anxiety disorder (Schmidt et al., 2021). Another FAAH inhibitor, SSR-411298, was tested for geriatric depression (ClinicalTrials.gov identifier: NCT00822744) and found to have no benefit.

Whether eCB-based drugs may have clinical utility remains to be determined. MAGL inhibitors hold promise for treating neuroinflammatory and neurodegenerative diseases, while FAAH inhibitors could be useful for the treatment of stress-related psychiatric disorders, such as social anxiety disorder and posttraumatic stress disorder, and for the management of cannabis use disorder.

Drug Facts for Your Personal Formulary: *Cannabinoids*

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Cannabinoid receptor agonists		
Nabiximols (cannabidiol and THC)	• Spasticity associated with multiple sclerosis	• Oromucosal spray • Slow upward dose titration
Dronabinol	• Nausea/vomiting associated with chemotherapy • Appetite and weight loss in HIV infection	• Diverse CNS and cardiovascular adverse effects possible • Consider evening dosing to decrease adverse effects
Nabilone	• Nausea/vomiting associated with chemotherapy	• CNS and cardiovascular adverse effects and their treatment, similar to dronabinol
Cannabidiol		
Cannabidiol	• Decrease seizures associated with Lennox-Gastaut syndrome, Dravet syndrome, and tuberous sclerosis complex	• May elevate liver enzymes • Significant drug-drug interactions, particularly with clobazam • Bioavailability affected by food • Usual dose range, 10–20 mg/kg

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Chapter 27

Ethanol

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HUMAN CONSUMPTION OF ETHANOL: A BRIEF HISTORY AND CURRENT PERSPECTIVE

BLOOD ETHANOL CONCENTRATION

METABOLISM OF ETHANOL

- Absorption and Gastric Metabolism
- Liver Metabolism

METHANOL

EFFECTS OF ETHANOL ON PHYSIOLOGICAL SYSTEMS

- The Wisdom of Shakespeare
- Central Nervous System
- Endocrine System
- Sexual and Reproductive Function
- Bone
- Body Temperature
- Kidneys
- Cardiovascular System
- Lungs
- Skeletal Muscle
- Digestive System
- Hematological Effects
- Immune System
- Neuroimmune System
- Cancers

TERATOGENIC EFFECTS: FETAL ALCOHOL SPECTRUM DISORDERS

CLINICAL USES OF ETHANOL

DRUG INTERACTIONS

COMORBIDITY OF ALCOHOL USE DISORDER WITH OTHER PSYCHIATRIC DISORDERS

ALCOHOL USE DISORDER AND GENETICS

PHARMACOTHERAPIES FOR ALCOHOL USE DISORDER

- Disulfiram
- Naltrexone
- Acamprosate
- Other Drugs With Repurposing Potential
- Treatment Outlook

Ethanol (CH_3CH_2OH), or beverage alcohol, is a two-carbon alcohol that is rapidly distributed in the body and brain. Ethanol alters many neurochemical systems and has rewarding and addictive properties. It is the oldest recreational drug and likely contributes to more morbidity, mortality, and public health costs than all illicit drugs combined. The 5th edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5) integrates alcohol abuse and alcohol dependence into a single disorder called *alcohol use disorder* (AUD), with mild, moderate, and severe subclassifications (American Psychiatric Association, 2013). In the DSM-5, all types of substance abuse and dependence have been combined into a single substance use disorder (SUD) on a continuum from mild to severe. A diagnosis of AUD requires that at least two of the 11 DSM-5 behaviors be present within a 12-month period (*mild AUD*: 2–3 criteria; *moderate AUD*: 4–5 criteria; *severe AUD*: 6–11 criteria). The four main behavioral effects of AUD are impaired control over drinking, negative social consequences, risky use, and altered physiological effects (tolerance, withdrawal). This chapter presents an overview of the prevalence and harmful consequences of AUD in the U.S., the systemic nature of the disease, neurocircuitry and stages of AUD, comorbidities, fetal alcohol spectrum disorders, genetic risk factors, and pharmacotherapies for AUD.

Human Consumption of Ethanol: A Brief History and Current Perspective

The use of alcoholic beverages is documented as far back as 10,000 BC. By about 3000 BC, the Greeks, Romans, and inhabitants of Babylon were incorporating ethanol into religious festivals, while also using it for pleasure and in medicinal practice. Over the last 2000 years, alcoholic beverages have been identified in most cultures, including pre-Columbian America about AD 200 and the Islamic world in the 8th century.

The dangers of heavy consumption of alcohol have long been recognized by almost all cultures. The increase in ethanol consumption in the 1800s, along with industrialization and the need for a dependable workforce, contributed to widespread organized efforts to discourage drunkenness, including a constitutional ban on the sale of alcoholic beverages in the U.S. from 1920 to 1933.

Today, AUD is one of the most prevalent psychiatric disorders worldwide. In the U.S., among adults 18 years and older, AUD is highly comorbid with other substance use and psychiatric disorders. In 2019, about 14.5 million people in the U.S. had AUD (Substance Abuse and Mental Health Services Administration, 2019). Roughly one-third of men (36%) and one-quarter of women (23%) will meet the criteria for a mild, moderate, or severe AUD in their lifetimes (Grant et al., 2015). Among ethnic groups, Native Americans have the highest prevalence of AUD. According to the Centers for Disease Control and Prevention (CDC), alcohol-related deaths are the third leading preventable cause of death in the U.S. (the first is tobacco and the second is poor diet and physical inactivity). Alcohol-impaired driving accounts for almost one-third of the overall driving fatalities. The cost of excessive drinking in the U.S. reached about $249 billion in 2010 (Sacks et al., 2015).

Binge drinking to a blood ethanol concentration (BEC) of 0.08% or above is the most common, costly, and deadly pattern of excessive drinking. This pattern of intake is defined as four or more drinks on the same occasion (within ~2 h) for females and five or more drinks for males and accounts for 77% of the total cost of excessive alcohol use in the U.S. (Sacks et al., 2015). In the past, AUD was more prevalent in men than

Abbreviations

ADH: alcohol dehydrogenase
ALDH: aldehyde dehydrogenase
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
APA: American Psychiatric Association
ARBD: alcohol-related birth defect
ARDS: acute respiratory distress syndrome
ARND: alcohol-related neurodevelopmental disorder
AUD: alcohol use disorder
BEC: blood ethanol concentration
CDC: U.S. Centers for Disease Control and Prevention
CYP: cytochrome P450
DSM-5: *Diagnostic and Statistical Manual of Mental Disorders* (5th edition)
FAS: fetal alcohol syndrome
GABA: γ-aminobutyric acid
GHB: γ-hydroxybutyric acid
GI: gastrointestinal
5HT: 5-hydroxytryptamine, serotonin
LPS: lipopolysaccharide
NMDA: *N*-methyl-D-aspartate
PTSD: posttraumatic stress disorder
SSRI: selective serotonin reuptake inhibitor
SUD: substance use disorder

women, but in the last decade, the difference between the sexes has narrowed for both AUD and binge drinking. In the 2019 National Survey on Drug Use and Health, more adults ages 18 and older reported binge drinking (~26%) in the past month than reported heavy alcohol use (~6%). Despite the high prevalence, morbidity, mortality, and socioeconomic costs, AUD often goes undiagnosed and untreated.

Blood Ethanol Concentration

Compared with other drugs, surprisingly large amounts of ethanol are required for physiological effects. Ethanol is consumed in gram quantities. In contrast, most other drugs with affinities for specific proteins are taken in milligram or microgram doses. The alcohol content of beverages typically ranges from 4% to 6% (volume/volume) for beer, 10% to 15% for wine, and 40% and higher for distilled spirits. The proof of an alcoholic beverage is twice its percentage of alcohol (e.g., 40% alcohol is 80 proof). A 12-oz bottle of beer (355 mL), a 5-oz glass of wine (148 mL), and a 1.5-oz "shot" of 40% liquor (44 mL) each contain about 14 g ethanol (the density of ethanol is 0.79 g/mL at 25°C) and constitute what is defined as a "standard drink" in the U.S.

Because the ratio of ethanol in end-expiratory alveolar air and blood is relatively consistent, BECs in humans are readily estimated by the measurement of ethanol levels in expired air; the partition coefficient for ethanol between blood and alveolar air is about 2100:1. In all states except Utah (0.05% w/v), the legally allowed BEC for operating a motor vehicle is 80 mg% (80 mg ethanol per 100 mL blood; 0.08% w/v), which is equivalent to a concentration of 17 mM ethanol in the blood. The consumption of one standard drink by a 70-kg person would produce a BEC of about 30 mg%. However, it is important to note that this is an estimation because the BEC is determined by multiple factors (e.g., rate of drinking, sex, body weight and water percentage, rates of metabolism and stomach emptying).

Metabolism of Ethanol

Absorption and Gastric Metabolism

After oral administration, ethanol is absorbed rapidly into the bloodstream from the stomach and small intestine and distributes into total body water (~0.65 L/kg body weight). Due to high surface area, absorption occurs more rapidly from the small intestine than from the stomach; delays in gastric emptying (e.g., due to the presence of food) slow ethanol absorption. Peak blood levels occur about 30 min after ingestion of ethanol if the stomach is empty. Because of first-pass metabolism by gastric and liver alcohol dehydrogenase (ADH), oral ingestion of ethanol leads to lower blood ethanol concentrations (BECs) than would be obtained if the same dose were administered intravenously. Compared with males, females have very little gastric ADH and consequently absorb more alcohol into their bloodstreams. In addition, the activities of liver ADH isozymes are lower in females. Thus, females generally metabolize ethanol more slowly than males. In addition, women are generally smaller and have lower body water percentages than men; this results in females reaching higher BECs than men after consuming the same amounts of alcohol.

Liver Metabolism

Only small amounts of ethanol are excreted in urine, sweat, and breath. The main enzymes involved in ethanol metabolism are ADH, catalase, and CYP2E1. CYPs 1A2 and 3A4 may also participate. Ethanol is metabolized primarily by sequential hepatic oxidation, first to acetaldehyde by ADH and then to acetic acid by aldehyde dehydrogenase (ALDH) (Figure 27–1). Each metabolic step requires NAD$^+$; thus, oxidation of 1 mol ethanol (46 g) to 1 mol acetic acid requires 2 mol NAD$^+$ (~1.3 kg). This greatly exceeds the supply of NAD$^+$ in the liver; thus, the bioavailability of NAD$^+$ limits ethanol metabolism to about 8 g/h (10 mL/h, 170 mmol/h) in a 70-kg adult. Ethanol metabolism proceeds via zero-order kinetics at BECs greater than 10 mg% and by first-order kinetics at BECs less than 10 mg%. Genetic variants of ADH and ALDH influence risk for developing AUD and are discussed later in the section on genetics.

In addition to limiting the rate of ethanol metabolism, the large increase in the hepatic NADH:NAD$^+$ ratio resulting from ethanol oxidation has other profound consequences. The function of NAD$^+$-requiring enzymes is impaired, resulting in accumulation of lactate, reduced activity of the tricarboxylic acid cycle, and accumulation of acetyl-CoA (which is produced from ethanol-derived acetic acid; Figure 27–1). The combination of increased NADH and elevated acetyl-CoA supports fatty acid synthesis and the storage and accumulation of triacylglycerides; ketone bodies then accrue, exacerbating lactic acidosis.

Although ADH is responsible for the majority of ethanol metabolism, CYP2E1 accounts for about 10%. This constituent of the microsomal ethanol-oxidizing system can be altered by acute or chronic ethanol consumption. Competition between ethanol and other drugs (e.g., *phenytoin* and *warfarin*) that are metabolized by CYP2E1 is observed after acute consumption of ethanol. CYP2E1 is also induced by chronic consumption of ethanol, and this is the primary mechanism for the development of pharmacokinetic tolerance to alcohol. This increased CYP2E1 activity also increases the clearance of other CYP2E1 substrates, leading to a requirement for increased dosing. The increased activity also increases susceptibility to certain toxins (e.g., CCl_4, which CYP2E1 metabolizes and thereby activates to the highly reactive trichloromethyl radical). Ethanol metabolism by the CYP2E1 pathway elevates NADP$^+$ and limits the availability of NADPH for the regeneration of reduced glutathione, thereby enhancing oxidative stress.

The mechanisms underlying hepatic disease resulting from heavy ethanol use probably reflect a complex combination of these metabolic factors, CYP2E1 induction (and enhanced activation of toxins and production of H_2O_2 and oxygen radicals), and possibly enhanced release of endotoxin as a consequence of ethanol's effect on gram-negative flora in the gastrointestinal (GI) tract. The often poor nutritional status of

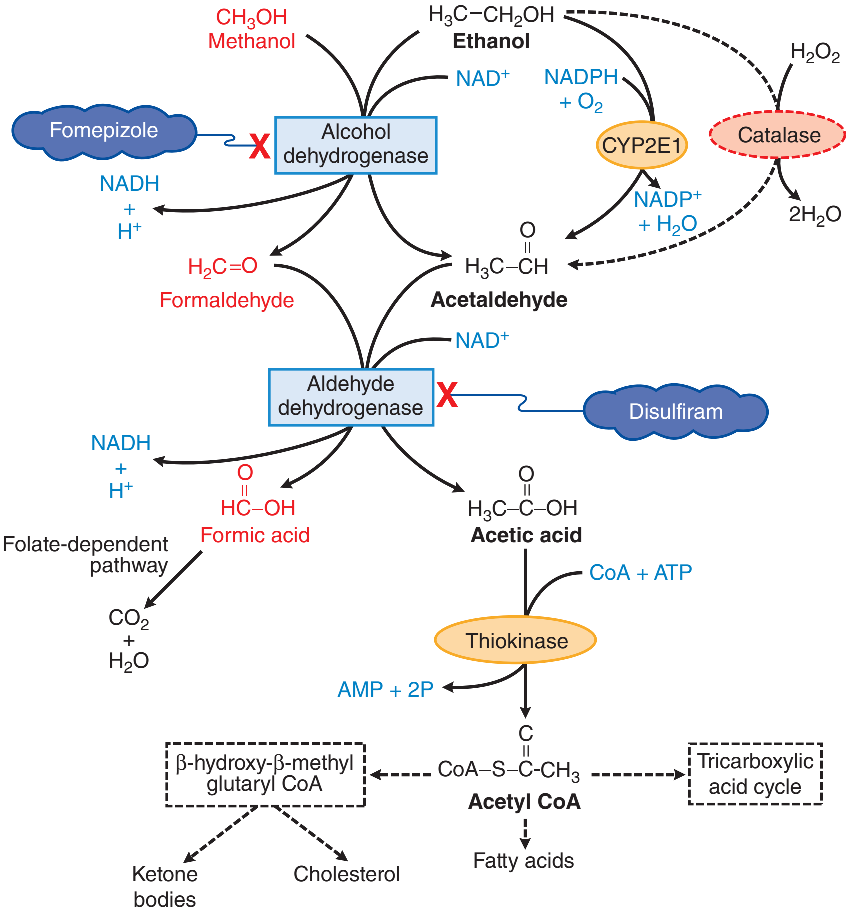

CH3OH
Methanol
H3C–CH2OH
Ethanol
NAD+
NADPH
+ O2
H2O2
Fomepizole
Alcohol dehydrogenase
CYP2E1
Catalase
NADH
+
H+
NADP+
+ H2O
2H2O
H2C=O
Formaldehyde
O
H3C–CH
Acetaldehyde
NAD+
Aldehyde dehydrogenase
Disulfiram
NADH
+
H+
O
HC–OH
Formic acid
O
H3C–C–OH
Acetic acid
Folate-dependent pathway
CO2
+
H2O
CoA + ATP
Thiokinase
AMP + 2P
C
CoA–S–C–CH3
Acetyl CoA
β-hydroxy-β-methyl glutaryl CoA
Tricarboxylic acid cycle
Fatty acids
Ketone bodies
Cholesterol

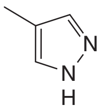
Figure 27–1 *Metabolism of ethanol and methanol.*

alcoholics (malabsorption and lack of vitamins A, D, and thiamine), suppressed of immune function, and a variety of other generalized effects likely compound the more direct adverse effects of excessive ethanol consumption. An overview of ethanol metabolism and how it can lead to tissue injury is found in the work of Molina et al. (2014).

Methanol

Methanol (CH_3OH), also known as methyl or wood alcohol, is an important industrial reagent and solvent found in products such as paint removers, shellac, and antifreeze. Methanol is added to industrial-use ethanol to make it unsafe for human consumption. Ingestion of as little as 8 g (10 mL) of methanol produces toxicity ranging from blindness to death. The toxic effects take about 12 or more hours to manifest themselves and are dependent on methanol metabolism to formaldehyde and formic acid (see Figure 27–1). Methanol poisoning consists of headache, GI distress, and pain (partially related to pancreatic necrosis), difficulty breathing, restlessness, and blurred vision. The visual disturbances occur from injury to ganglion cells of the retina and the optic nerve by formic acid, which produces inflammation, atrophy, and potential bilateral blindness. Severe metabolic acidosis can develop due to the accumulation of formic acid, and respiratory depression may be severe, resulting in coma or death.

Methanol is rapidly absorbed via the eyes, oral administration, inhalation, and through the skin, with the last two routes most relevant in industrial settings. Absorption of methanol taken orally typically occurs within 30 to 60 min. Methanol is metabolized by ADH to formaldehyde, which is then metabolized to formic acid by ALDH. Competition between methanol and ethanol for ADH is the basis for using ethanol to treat methanol poisoning because ethanol slows the rate of formic acid production, lessening the toxicity associated with methanol consumption.



FOMEPIZOLE

Fomepizole (4-methylpyrazole), an ADH inhibitor (Figure 27–1) that is administered alone, or in combination with hemodialysis, is also used to treat methanol or ethylene glycol poisoning. Plasma levels of 0.8 mg/L are effective in inhibiting ADH. *Fomepizole* should not be used with ethanol because it prolongs the half-life of ethanol. Treatment of methanol poisoning also consists of using sodium bicarbonate to combat acidosis.

Effects of Ethanol on Physiological Systems

The Wisdom of Shakespeare

William Shakespeare described the acute pharmacological effects of imbibing ethanol in the Porter scene (act 2, scene 3) of *Macbeth*. The Porter, awakened from an alcohol-induced sleep by Macduff, explains three effects of alcohol and then wrestles with a fourth effect that combines the contradictory aspects of soaring overconfidence with physical impairment:

> **Porter:** … and drink, sir, is a great provoker of three things.
>
> **Macduff:** What three things does drink especially provoke?
>
> **Porter:** Marry, sir, nose-painting [*cutaneous vasodilation*], sleep [*CNS depression*], and urine [*inhibition of antidiuretic hormone (vasopressin) secretion, exacerbated by volume loading*]. Lechery, sir, it provokes and

> unprovokes: it provokes the desire, but it takes away the performance; therefore, much drink may be said to be an equivocator with lechery: it makes him, and it mars him; it sets him on, and it takes him off; it persuades him, and disheartens him; makes him stand to, and not stand to [*the imagination desires what the corpus cavernosum cannot deliver*]; in conclusion, equivocates him in a sleep, and, giving him the lie, leaves him.

Research findings have since provided the physiological correlates for Shakespeare's enumeration (see the bracketed additions to the Porter's words in the preceding paragraph). The most noticeable consequences of the recreational use of ethanol still are well summarized by the gregarious and garrulous Porter, whose delighted and devilish demeanor demonstrates a frequently observed influence of modest concentrations of ethanol on the CNS.

Central Nervous System

Ethanol is primarily a CNS depressant. Ingestion of low to moderate amounts of ethanol, like that of other sedative/hypnotics such as barbiturates and benzodiazepines, can have anxiolytic actions and produce behavioral disinhibition. Individual signs of intoxication vary from expansive and vivacious effects to uncontrolled mood swings and emotional outbursts that may have violent components. With more severe intoxication, CNS function becomes progressively more impaired, ultimately to the point of general anesthesia. Due to respiratory depression, there is little margin between the concentrations yielding the anesthetic and lethal effects of ethanol.

Acute Intoxication and Treatment

Many factors influence the BEC, including body weight, body composition, and the rate of absorption from the GI tract. In women, with a generally lower volume of distribution for ethanol, BECs may be about 30% to 50% higher than in men for the same quantity consumed.

Signs of intoxication typical of CNS depression are observed in most people after two or three drinks, with the most prominent effects seen at times of peak BEC, about 30 to 60 min following consumption on an empty stomach. These symptoms include an initial stimulatory effect (perhaps due to inhibition of CNS inhibitory systems), giddiness, muscle relaxation, and impaired judgment. Higher blood levels (~80 mg/dL or ~17 mM) are associated with slurred speech, incoordination, unsteady gait, and impaired attention; levels between 80 and 200 mg/dL are associated with more intense mood lability and greater cognitive deficits, potentially accompanied by aggressiveness and anterograde amnesia (an "alcoholic blackout," i.e., loss of memory of events that transpired while intoxicated). BECs greater than 200 mg/dL can produce nystagmus and sedation, while levels of 300 mg/dL and higher produce failing vital signs, coma, and death. Heavy drinkers, who have developed significant pharmacodynamic tolerance, will display markedly less impairment at these BECs. All of these symptoms are likely to be exacerbated and occur at a lower BEC if ethanol is taken with other CNS depressants (e.g., benzodiazepines or barbiturates) or with any drug or medication that promotes sedation and incoordination (e.g., antihistamines). Also see the section on Drug Interactions later in the chapter.

The treatment of acute ethanol intoxication is based on the severity of respiratory and CNS depression. If respiratory depression is not severe, careful observation is the primary treatment. Patients with evidence of respiratory depression should be intubated to protect the airway and to provide ventilatory assistance; stomach lavage can also be considered if absorption is not yet complete. Because it is freely miscible with water, ethanol can be removed from the blood by hemodialysis. The usual protocol involves observing the patient in the emergency room for 4 to 6 h while the ingested ethanol is metabolized. The symptoms associated with diabetic coma, drug intoxication, cardiovascular accidents, and skull fractures are similar and may be confused with profound alcohol intoxication. Testing for breath odor in a case of suspected intoxication can be misleading because there can be other causes of similar breath odor (e.g., diabetic ketoacidosis or other metabolic acidosis). Determining blood ethanol levels is necessary to confirm the absence or presence of alcohol intoxication, and diabetes or other underlying conditions should also be considered in patients with positive BECs. There is no safe pharmacological treatment to increase the rate of ethanol metabolism.

Chronic Effects and Treatment

The transient effects of heavy ethanol consumption that produce a "hangover"—the next-morning syndrome of headache, thirst, nausea, hyperexcitability, and cognitive impairment—may reflect ethanol withdrawal, dehydration, or mild acidosis. Insomnia is a common and persistent problem in AUD, even after weeks of abstinence (Brower, 2015), and should be treated because it may contribute to relapse. Ethanol affects respiration and muscle relaxation, and heavy drinking can produce sleep apnea, especially in older dependent individuals. Treatment of insomnia must take into account possible pharmacodynamic interactions of ethanol with another CNS depressant.

Ethanol-induced cognitive impairment may be related to changes in synaptic plasticity (e.g., long-term potentiation and long-term depression), like those reported in preclinical studies of the hippocampus, prefrontal cortex, striatum, and nucleus accumbens (Abrahao et al., 2017). AUD causes shrinkage of the brain due to loss of both white and gray matter, and chronic heavy drinking increases the risk of developing *alcoholic dementia*. The cognitive deficits and brain atrophy observed after a heavy drinking period partially reverse over the weeks to months following abstinence. Chronic alcohol abuse also reduces overall brain metabolism, which reverses during detoxification.

Wernicke-Korsakoff syndrome is a degenerative neurological disorder that encompasses Wernicke encephalopathy and Korsakoff syndrome. These two closely related disorders often occur together, with most individuals developing encephalopathy first. Some researchers believe these are different stages of the same disorder. The disorder is caused by thiamine deficiency and is most commonly seen in those with AUD. It may also result independently of AUD, from malnutrition or conditions that cause nutritional deficiencies. The classic triad of symptoms are confusion, ataxia, and eye abnormalities (double vision, nystagmus, ophthalmoplegia); however, it is underdiagnosed because of a wide range of symptoms that overlap with other conditions (Chandrakumar et al., 2018). In its early stages, symptoms of Wernicke encephalopathy are usually reversible with high doses of thiamine, but the mental impairments in Korsakoff syndrome respond more slowly and incompletely. Untreated Wernicke encephalopathy leads to death in up to 20% of cases, and about 75% of those who survive the encephalopathy go on to develop Korsakoff psychosis, characterized by memory impairment, apathy, anterograde and retrograde amnesia, and confabulation (Chandrakumar et al., 2018; Thomson et al., 2012). Wernicke-Korsakoff syndrome is a medical emergency, and early treatment with intravenous thiamine (followed by oral maintenance treatment) is essential to reverse the Wernicke symptoms and prevent progression or reduce the severity of the Korsakoff state.

Molecular Targets: Neurotransmitter and Neuromodulator Systems

Ethanol alters the release of neurotransmitters and neuropeptides and modulates pre-, post-, and extrasynaptic activity through diverse molecular targets (reviewed in Abrahao et al., 2017). The dopaminergic system and the endogenous opioid system are sensitive to low concentrations of ethanol and play key roles in mediating its rewarding effects, although ethanol is not believed to directly act at either opioid or dopamine receptors. Ethanol perturbs the balance between excitatory and inhibitory transmission, for example, by enhancing the function of inhibitory cys-loop ligand-gated ion channels (e.g., $GABA_A$ and glycine) or inhibiting the function of excitatory ionotropic glutamate receptors (e.g., *N*-methyl-D-aspartate [NMDA], α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA], kainate) (Table 27–1). GIRK, $5HT_3$(serotonin) receptor-gated, and L-type Ca^{2+} channels are also sensitive to ethanol at low concentrations. Table 27–1 shows the overall effects of acute ethanol on some of the neurotransmitter and neuromodulator systems that are involved in the neurocircuitry of addiction.

TABLE 27–1 ■ MOLECULAR SITES OF ETHANOL ACTION

LIGAND- AND VOLTAGE-GATED ION CHANNELS	EFFECTS OF ACUTE ETHANOL
GABA_A receptor-operated channels	Enhancement
Glycine receptor-operated channels	Enhancement
Glutamate receptor-operated channels (e.g., NMDA, AMPA, kainate)	Inhibition
Nicotinic acetylcholine receptor-operated channels	Subunit-specific
5HT_3 receptor-operated channels	Enhancement
G protein–coupled inwardly rectifying K^+ (GIRK) channels	Enhancement
Large conductance, Ca^{2+}-activated K^+ channels (BK)	Enhancement
L-type Ca^{2+} channels	Inhibition

The enhancement or inhibition of channel function recorded here represents an overall consensus of acute ethanol effects. Sensitivity and overall effects may depend on ethanol concentration, time of exposure, channel subunit composition, brain region, cell type, posttranslational modifications, and other factors.

Putative ethanol binding sites in many of the channel proteins in Table 27–1 have been identified through molecular modeling of high-resolution crystal structures (reviewed in Cui and Koob, 2017). Unlike other drugs, ethanol has a relatively weak affinity for its molecular targets; however, the ease with which ethanol is distributed and diffuses through membranes greatly broadens its targets and long-term neurobiological effects. Considering this, it is not surprising that the molecular mechanisms responsible for alcohol intoxication, reward, and dependence remain poorly understood.

Neurocircuitry and Stages of AUD

AUD is the chronically relapsing and compulsive use of alcohol, comprising three interacting stages that progressively worsen over time: binge-intoxication, withdrawal-negative affect, and preoccupation-anticipation (“craving”). The neurocircuitry of the basal ganglia is thought to mediate the neurobiological basis of the binge-intoxication stage, including the facilitation of incentive salience, a form of motivational salience associated with reward. The basal ganglia are associated with key functions, including voluntary motor control and procedural learning related to routine behaviors or habits. Release of dopamine and opioid peptides in the ventral striatum (nucleus accumbens) is associated with the reinforcing actions of alcohol (Volkow et al., 2007). Endocannabinoid signaling may also contribute to the motivational and reinforcing properties of ethanol, and ethanol drinking alters endocannabinoid levels and cannabinoid receptor 1 expression in brain nuclei in addiction pathways (Pava and Woodward, 2012). Alcohol use facilitates incentive salience by imparting motivational properties to previously neutral stimuli. Activation of the ventral striatum leads to recruitment of basal ganglia–globus pallidus–thalamic–cortical loops that engage the dorsal striatum habit formation, hypothesized to be the beginning of compulsive-like responding for drugs.

Tolerance rapidly develops to the rewarding effects of alcohol and is defined as a reduced behavioral or physiological response to the same dose of drug, or the requirement of a higher dose to obtain the same effect (see Chapter 28); this may be pharmacokinetic and/or pharmacodynamic in nature. The major forms of pharmacodynamic (functional) tolerance are *acute* and *chronic*. Acute functional tolerance, also known as the Mellanby effect, occurs within hours of alcohol administration and is due to CNS adaptations to its effects. This is demonstrated by comparing behavioral impairment at the same BECs on the ascending limb of the absorption phase of the BEC–time curve and on the descending limb of the curve, as metabolism reduces the BEC. Behavioral impairment and subjective feelings of intoxication are much greater at a given BEC on the ascending than on the descending limb. Chronic tolerance also develops in the long-term heavy drinker. In contrast to acute tolerance, chronic tolerance has both pharmacodynamic and pharmacokinetic elements, the latter due to induction of alcohol-metabolizing enzymes, primarily CYP2E1. In general, the maximum pharmacokinetic tolerance attained would be a doubling of the normal metabolic rate.

Dependence is defined by a withdrawal syndrome observed several hours to days after alcohol consumption is terminated. The symptoms and severity are determined by the amount and duration of drinking and include major motivational changes, sleep disruption, autonomic nervous system (sympathetic) activation, tremors, and, in severe cases, seizures. In addition, 2 or more days after withdrawal, some individuals experience *delirium tremens*, characterized by hallucinations, delirium, tachycardia, and a potentially fatal fever or cardiac arrhythmia. Individuals with AUD also show evidence of negative emotional states during acute withdrawal that persist into protracted abstinence; such states include symptoms related to anxiety, dysphoria, and depression. Persistent depression/anxiety-like symptoms are important treatment considerations. Delirium tremens require management as a medical emergency in an intensive care unit or another inpatient setting. Patients should be kept in a quiet and comfortable environment, and their vital signs routinely monitored. Electrolyte, fluid, and nutritional deficiencies must be corrected. Long-acting benzodiazepines such as *diazepam* or *chlordiazepoxide* are the treatments of choice, but *phenobarbital*, *dexmedetomidine*, or *propofol* may be used in benzodiazepine-refractory patients. Comorbidities are common in delirium tremens patients, especially liver dysfunction, and this may affect benzodiazepine dosing regimens. Lastly, clinical management of delirium tremens would be incomplete if it is not done in the context of anticipated treatment of the patient’s underlying AUD. Two processes are thought to form the neurobiological basis for the withdrawal–negative affect stage: decreased functioning in reward systems in the ventral striatum and recruitment of the stress systems in the extended amygdala. As dependence develops, brain stress systems, involving corticotropin-releasing factor, norepinephrine, and dynorphin, are recruited (Koob, 2014), producing a powerful motivation for drug seeking.

The preoccupation-anticipation (“craving”) stage involves dysregulation of prefrontal cortex circuits, causing loss of executive control. The completion of complex tasks in the AUD brain may involve two opposing systems. A “go” system (the anterior cingulate cortex and dorsolateral prefrontal cortex) engages habits via the basal ganglia, while the “stop” system (the ventrolateral prefrontal cortex and orbitofrontal cortex) inhibits the basal ganglia incentive salience and the extended amygdala stress system (Koob, 2015). Individuals with AUD show impairments in decision making, spatial information, and behavioral inhibition, all of which drive craving. Craving can be divided into “reward” craving (drug seeking induced by drugs or stimuli linked to drugs) and “relief” craving (drug seeking induced by an acute stressor or a state of stress) (Heinz et al., 2003). Thus, deficits in prefrontal cortical control of basal ganglia and extended amygdala function may explain individual differences in the predisposition to and perpetuation of addiction. Residual dysregulation of the neurocircuits mediating incentive salience and stress responsivity help perpetuate compulsive drug taking and relapse.

Endocrine System

The endocrine system comprises glands that produce and secrete hormones into the blood, affecting almost every cell and organ, and regulating many processes, including metabolism, energy, electrolyte balance, stress response, growth, development, and reproduction. The endocrine system also controls communication between the immune and nervous systems and is essential for maintaining homeostasis. Acute and chronic ethanol differentially alters endocrine function via the hypothalamic-pituitary-adrenal axis, the hypothalamic-pituitary-gonadal axis, the hypothalamic-pituitary-thyroid axis, the hypothalamic-pituitary-growth hormone/insulin-like growth factor-1 axis, and the hypothalamic-posterior pituitary axis (Rachdaoui and Sarkar, 2017). Chronic ethanol intake disrupts hormones from the endocrine portions of the pancreas and adipose tissue and the pituitary hormone prolactin, causing hyperprolactinemia. The hormonal disturbances contribute to many conditions, such as thyroid and cardiovascular disease, immune and reproductive dysfunction, cancer, diabetes, and psychological disorders.

Sexual and Reproductive Function

Many drugs of abuse, including ethanol, have disinhibiting effects that may initially increase libido. Despite the notion that ethanol enhances sexual function, the opposite effect generally prevails, as Shakespeare's Porter noted. Sexual dysfunction in AUD includes decreased sexual arousal, increased ejaculatory latency, and decreased orgasmic pleasure. Hyperprolactinemia can decrease fertility in men and women with AUD by leading to hypogonadism, reduced sperm production, impotence, and menstrual cycle irregularities. Ethanol's adverse effects on the hypothalamic-pituitary-gonadal axis also contribute to decreased libido and fertility, gonadal atrophy, and menstrual irregularities.

Bone

Maintaining healthy bone requires a balanced bone remodeling process, where old bone is removed by osteoclasts and new bone is formed by osteoblasts. Chronic heavy drinking disrupts this balance by inhibiting bone formation and interfering with bone resorption (Maurel et al., 2012). Binge or heavy drinking is particularly concerning during adolescence and in young adults when it can dramatically compromise bone health. As bone mass and bone mineral density decrease, the risk of osteoporosis and fractures increase. In addition to bone loss from the direct effects of alcohol on both osteoblasts and osteoclasts, there are indirect mechanisms, such as poor vitamin D metabolism and Ca^{2+} absorption. Hormonal deficiencies in men and women with AUD are also contributing factors. Testosterone, an anabolic hormone important for production of osteoblasts, is decreased, and reduced estrogen levels increase bone fragility. Elevated levels of cortisol and parathyroid hormone in AUD also decrease bone formation.

Body Temperature

Ingestion of ethanol causes an initial feeling of warmth due to enhanced cutaneous vasodilatation. Heat is transferred from the body core to the periphery, and the core temperature falls due to ethanol's effect on the central temperature-regulating mechanism in the hypothalamus. Intake of high quantities of ethanol may lead to pronounced decreases in body temperature, especially in cold ambient temperatures. Alcohol is a major risk factor contributing to deaths from hypothermia.

Kidneys

Ethanol inhibits the release of vasopressin (antidiuretic hormone) from the posterior pituitary gland, resulting in enhanced diuresis. This dehydrating effect can lead to headaches and nausea. Alcohol-dependent individuals in withdrawal exhibit increased vasopressin release and a consequential retention of water, as well as dilutional hyponatremia.

Cardiovascular System

There is a complex relationship between ethanol consumption and cardiovascular disease, a leading cause of death and disability. Heavy alcohol use greatly increases risk for cardiovascular disease. Although observational studies have suggested lower risk in light to modest drinkers, the perceived benefit may be the product of healthier lifestyles in this group. Drinking recommendations from clinicians should remain guarded given that risk depends on dose and many other factors (e.g., sex, age, drinking patterns, lifestyle factors, and genetic risk), that there is the potential for other ethanol-related problems, and that there is a lack of randomized controlled trials on ethanol's long-term effects on cardiovascular function.

Serum Lipoproteins

A common risk factor for cardiovascular disease is the composition of lipids in the blood. Low alcohol consumption may have positive effects on lipid profiles by increasing high-density lipoprotein cholesterol (see Chapter 37), which transports cholesterol to the liver for elimination, and by decreasing total cholesterol, triglycerides, and low-density lipoprotein cholesterol, which causes cholesterol to accumulate in the arteries. The overall decrease in cholesterol accumulation in arterial walls decreases cardiovascular disease.

Hypertension and Stroke

In general, alcohol-risk relationships for hypertension tend to be J-shaped in women, with decreased risk found at low doses (Piano, 2017). However, based on a meta-analysis, women drinking more than 20 g ethanol/day and men drinking more than 30 g/day showed increased risk of hypertension. Even small increases in blood pressure can increase mortality from coronary artery disease and from stroke. In men and women, approximately one to two drinks per day may have no effect on or slightly reduce stroke events; however, greater daily alcohol consumption increases stroke risk (Piano, 2017).

Cardiac Arrhythmias and Cardiomyopathy

Arrhythmias like atrial fibrillation are a serious consequence of consuming large amounts of alcohol, particularly during binge drinking. Atrial fibrillation is one of the most common arrhythmias and is strongly associated with cardiovascular events such as stroke. Alcoholic cardiomyopathy is an acquired form of dilated cardiomyopathy associated with long-term, heavy alcohol consumption (Piano, 2017). It is characterized by a dilated left ventricle and increased mass, normal or reduced left ventricular wall thickness, and reduced ejection fraction in advanced stages. Cardiomyopathy may be caused by oxidative stress, cell death, impaired mitochondrial function, altered fatty acid metabolism and transport, and increased protein breakdown.

Lungs

The lungs are often overlooked as a site of ethanol action. However, individuals with AUD are more likely to develop pneumonia, tuberculosis, respiratory syncytial virus infection, and acute respiratory distress syndrome (ARDS) (Simet and Sisson, 2015). ARDS is quite deadly in a healthy person, and someone with AUD is at even greater risk. ARDS can occur in young AUD patients, whereas cirrhosis or other alcohol-related diseases take much longer to develop. The increased susceptibility to pulmonary infections, injury, and inflammation is caused by impaired immune responses. For example, alcohol impairs neutrophil production and release, lymphocyte function (e.g., T-cell function), ciliary function in airways, and the function of alveolar macrophages. Thus, the "alcoholic lung" is immunocompromised and under oxidative stress. The lungs then become depleted of the vital antioxidant glutathione.

Skeletal Muscle

Skeletal muscle dysfunction (myopathy) is a very common clinical manifestation in AUD, but it often goes unrecognized (Simon et al., 2017). Approximately 50% of chronic drinkers may develop alcoholic myopathy, which is five times more common than the incidence of liver cirrhosis. Cirrhosis may also influence the development of myopathy, and myopathy worsens the clinical outcome. The decreased muscle mass in alcohol-induced myopathy results from an imbalance in protein synthesis and degradation. In particular, there is decreased protein synthesis of myofibrillar and sarcoplasmic proteins. Some of the specific anabolic and catabolic mechanisms that are implicated in muscle wasting in AUD may involve decreased mammalian target of rapamycin–mediated protein synthesis and excessive protein degradation via the ubiquitin proteosome pathway and the autophagic-lysosomal system (Simon et al., 2017). Functional improvements in muscle strength occur over time following abstinence or reduced drinking. Optimizing nutritional status can also improve muscle function.

Digestive System

The body priorities metabolizing alcohol over other functions. As ethanol is absorbed and metabolized, it can damage the organs of the digestive system and increase the risk of several cancers (discussed in a later section). Heavy drinking damages the mucosa of the esophagus, stomach, and intestines, disrupts gastric and intestinal barriers, impairs nutrient and protein absorption, impairs immune responses, and causes gastritis, pancreatitis, and liver diseases. The gut dysbiosis caused by ethanol has widespread inflammatory consequences.

Esophagus and Stomach

Ethanol directly damages the mucosa of the esophagus and stomach. It lowers esophageal sphincter pressure, impairs motility, and alters gastric acid secretion. AUD patients may develop gastroesophageal reflux disease, ulcers, or acute and chronic gastritis. Heavy drinking is also a predisposing factor for Mallory-Weiss syndrome, which is characterized by mucosal tears at the gastroesophageal junction and upper GI bleeding. Proton pump inhibitors and histamine H_2 antagonists are used to decrease gastric acidity.

Intestines

Ethanol damages the intestinal mucosa directly and indirectly by altering the microbiota and impairing mucosal immune function. Many individuals with AUD have chronic diarrhea caused by malabsorption in the small intestine. The rectal fissures and pruritus ani that are associated with heavy drinking are likely related to chronic diarrhea. Diarrhea is caused by structural and functional changes in the small intestine; for example, the intestinal mucosa has flattened villi, and digestive enzyme levels often are decreased. These changes are usually reversible after a period of abstinence.

Pancreas

Heavy alcohol use is the most common cause of both acute and chronic pancreatitis in the U.S. Acute alcoholic pancreatitis is characterized by the abrupt onset of abdominal pain, nausea, vomiting, and increased levels of serum or urine pancreatic enzymes. Treatment usually consists of intravenous fluid replacement (often with nasogastric suction) and opioid pain medication. Similar to alcoholic cirrhosis, chronic pancreatitis results from progressive cellular destruction and fibrosis and develops after recurrent episodes of acute pancreatitis. Ethanol metabolites and by-products like reactive oxygen species directly injure pancreatic acinar cells, causing stellate cells to produce extracellular matrix, which leads to the atrophy-fibrosis sequelae characteristic of chronic pancreatitis. Compared with the liver, the ability to oxidize ethanol in the pancreas is low. However, during chronic alcohol abuse, the pancreas has a much greater capacity than the liver for nonoxidative metabolism of ethanol to fatty acid ethyl esters (Rasineni et al., 2020). Inhibition of hepatic ADH during chronic drinking and the resulting increased production of fatty acid ethyl esters in the pancreas may be a major mechanism in the pathogenesis of chronic pancreatitis. The clinical symptoms are pain, malnutrition, and diabetes mellitus. Insulin may be needed to control the hyperglycemia (see Chapter 51), and pancreatic enzyme capsules containing lipase, amylase, and proteases can treat malabsorption (see Chapter 54).

Liver

As the main organ involved in ethanol metabolism, the liver is a well-known target for the pathological effects of chronic ethanol. According to the CDC in 2018, about 43% of deaths due to liver disease among individuals ages 12 and older involved alcohol. Over time, the dose-dependent effects progress from fat accumulation (steatosis) to fatty liver accompanied by inflammation (steatohepatitis), to collagen deposition (fibrosis), to fibrous scarring and loss of liver cells (cirrhosis). However, heavy drinkers may develop cirrhosis without first developing hepatitis.

Ethanol perturbs most aspects of hepatic lipid metabolism (You and Arteel, 2019), and the accumulation of fat in the liver is an early event that can develop over a few days. The generation of excess NADH, via metabolism of ethanol and acetaldehyde by ADH and ALDH, inhibits the tricarboxylic acid cycle and the oxidation of fat, leading to steatosis (see Figure 27–1). Fibrosis, resulting from tissue necrosis and chronic inflammation, is the underlying cause of alcoholic cirrhosis. Fatty liver disease is common in those with AUD, while a minority will go on to develop cirrhosis.

Patients with alcohol-related liver disease show changes in the composition of their gut microbiomes, increased intestinal permeability, and increased circulating levels of the gut-derived microbial endotoxin LPS (lipopolysaccharide). LPS is an important trigger for steatosis, inflammation, and fibrosis by stimulating the generation of reactive oxygen species and a cascade of events that culminate in the transcription of proinflammatory cytokines.

Fatty liver disease and acute alcoholic hepatitis are usually reversible with abstinence. In severe cases of alcoholic hepatitis or cirrhosis, abstinence may stop or delay disease progression, but cirrhosis is usually terminal if the patient does not receive a liver transplant.

Hematological Effects

While nutritional deficiencies can play a role in alcohol-related anemias, alcohol also directly interferes with red blood cell production. Heavy drinking is associated with different anemias (e.g., macrocytic and sideroblastic anemias). Thrombocytopenia is common in heavy drinkers and can complicate alcohol withdrawal syndrome (Silczuk and Habrat, 2020). Leukopenia is also commonly found in AUD and increases susceptibility to infections.

Immune System

Ethanol disrupts innate and adaptive immune signaling, further increasing the risk of infection and causing systemic inflammation that can damage organs and increase cancer risk. Immune responses are central to the pathogenesis of alcoholic liver disease. In addition to effects on granulocytes (leukopenia), ethanol alters lymphocyte composition, decreases T-cell mitogenesis, and changes immunoglobulin production. Individuals with AUD have weakened resistance to infections (e.g., to *Klebsiella* pneumonia and tuberculosis). The function of alveolar macrophages and neutrophils in the airways is impaired, causing damage that may not be evident until the patient encounters a secondary respiratory infection. As a consequence of ethanol's harmful effects on the gut microbiome and the integrity of the GI tract, gut microbes escape into the bloodstream where they go on to trigger or exacerbate immune and proinflammatory responses in different tissues and organs. The immune dysfunction is not confined to peripheral injury or illness. It is not clear whether peripheral responses are required to induce neuroinflammation or if ethanol also has direct proinflammatory effects in the brain.

Neuroimmune System

The impact of immune signaling in the brain on the neurobiological and behavioral changes in AUD or other CNS diseases has been an area of focus over the past decade (Erickson et al., 2019). Chronic ethanol consumption increases the levels of circulating innate immune signaling molecules (e.g., proinflammatory cytokines) that are capable of reaching the brain, where they elicit long-lasting neuroimmune responses through the transcription of immune-related genes and the activation of astrocytes and microglia. These cells are the principal immune mediators in the brain, responding to and releasing molecules like cytokines and chemokines. Neurons also mediate immune responses and express immune-related genes. Although microglia are the resident macrophages in the brain, there is new appreciation for how they directly communicate with neurons. The transition of microglia to reactive states is a diverse process with different functional states that may dictate healthy CNS function or trigger neuroinflammatory processes in psychiatric and neurodegenerative diseases. Drugs that inhibit neuroimmune/proinflammatory signaling have opened new avenues for therapeutics and are currently being studied for their potential to reduce alcohol drinking.

Cancers

Ethanol is a procarcinogen and increases the risk of several types of cancers (National Cancer Institute, 2020). People who drink 50 g (~3.5 drinks) or more of alcohol daily are at two to three times greater risk of cancers of the oral cavity, pharynx, and larynx; tobacco use further escalates these risks. Compared with non- or occasional drinkers, this level of drinking is associated with a 1.5 times greater risk of developing colorectal cancer. AUD is a major risk factor for esophageal squamous cell carcinoma, as is genetic deficiency of ALDH2. AUD is a primary cause of liver cancer. Epidemiological studies show a higher risk of breast cancer in women who are heavy drinkers. A notable complication in the treatment of cancer patients with AUD is that ethanol can interfere in the metabolism of some chemotherapeutic agents. The effects of acetaldehyde, a demonstrated carcinogen in animal models, and oxidative stress are widely cited mechanisms for the increased rate of carcinogenesis among individuals with AUD. Evidence also points to a role for aberrant DNA methylation patterns and other epigenetic modifications that control genome activity as mechanisms in alcohol-induced cancer development and progression. Epigenetics refers to processes that affect gene expression without changes in DNA sequence.

Teratogenic Effects: Fetal Alcohol Spectrum Disorders

Ethanol is the most common teratogen in humans. Ethanol from a mother's blood passes freely to her baby through the umbilical cord, and it can be assumed that embryo or fetal BECs will be similar to maternal levels. Children born to mothers who are heavy drinkers display mental deficits and a common pattern of distinct dysmorphology known as FAS (fetal alcohol syndrome). The diagnosis of FAS is typically based on the observance of a triad of abnormalities associated with a history of prenatal ethanol exposure (Dorrie et al., 2014):

- A cluster of craniofacial abnormalities
- CNS dysfunction (structural or functional)
- Pre- or postnatal growth deficiencies (weight or height)

Fetal alcohol spectrum disorder is not a diagnostic term used by clinicians but rather an umbrella term that encompasses all of the disabilities caused by prenatal alcohol exposure. For example, children who do not meet all the criteria for a diagnosis of FAS may show physical or mental deficits consistent with partial phenotypes, including *partial FAS, alcohol-related neurodevelopmental disorder (ARND), and alcohol-related birth defect (ARBD)* (Dorrie et al., 2014). The DSM-5 characterizes *neurobehavioral disorder associated with prenatal alcohol exposure* as a condition associated with problems in thinking, behavior, and life skills (Hagan et al., 2016). The incidence of FAS is about 0.5 to 2 per 1000 live births in the general U.S. population, while the incidence of FAS, ARND, and ARBD combined is at least 1%. Early diagnosis and treatment intervention are important for these children. Higher rates of FAS occur in children born to African and Native American women. Children of binge-drinking mothers show severe mental and behavioral deficits, likely due to the high peak BECs (Dorrie et al., 2014).

The FAS craniofacial abnormalities associated with maternal drinking in the first trimester consist of microcephaly, shortened palpebral fissures, thin upper lip, smooth philtrum, and epicanthal folds. Magnetic resonance imaging studies demonstrate decreased volumes in the basal ganglia, corpus callosum, cerebrum, and cerebellum that correlate with the facial abnormalities. CNS dysfunction attributed to *in utero* ethanol exposure consists of hyperactivity; attention and mental deficits; learning disabilities; language, memory, and motor disorders; and psychiatric conditions. FAS is the number one preventable cause of cognitive and attention deficits in the Western world, with afflicted children consistently scoring lower than their peers on a variety of IQ tests. Although the evidence is not conclusive, it has been suggested that even moderate alcohol consumption (28 g/day) in the second trimester of pregnancy is correlated with impaired academic performance of children at age 6. Maternal age also may be a factor: Pregnant women over age 30 who drink alcohol create greater risks to their children than do younger women who consume similar amounts of alcohol. In addition, the intake of high amounts of alcohol, particularly during the first trimester, greatly increases the chances of spontaneous abortion. Alcohol use is risky throughout pregnancy, and there is no known safe amount. Current recommendations are to drink no alcohol during pregnancy.

Clinical Uses of Ethanol

For the treatment of poisoning by methanol or ethylene glycol, *fomepizole* is the first-line treatment, but systemically administered ethanol is also used; it acts at ADH to competitively slow the metabolism of methanol to formaldehyde and thence formic acid (see Figure 27–1). Dehydrated alcohol injected in close proximity to nerves or sympathetic ganglia is used to relieve long-lasting pain related to trigeminal neuralgia, inoperable carcinoma, and other conditions. Epidural, subarachnoid, and lumbar paravertebral injections of ethanol are also administered for inoperable pain. For example, lumbar paravertebral ethanol injections destroy sympathetic ganglia and thereby cause vasodilation, provide pain relief, and promote healing of lesions in patients with vascular disease of the lower extremities.

Drug Interactions

There are hundreds of over-the-counter and prescription medications that can interact adversely with ethanol. Ethanol increases the risk of liver damage or internal bleeding associated with *aspirin* or other nonsteroidal anti-inflammatory drug use. Due to synergistic effects in the CNS, caution must be taken when administering sedatives in patients who have ingested heavy doses of ethanol or other CNS depressants. Acute ethanol intoxication decreases general anesthetic requirements, and elective surgery should be postponed in intoxicated patients. In contrast, chronic ethanol drinking increases anesthetic requirements largely due to pharmacodynamic cross-tolerance. An additional complication is the use of neuromuscular blockers and sedative/anesthetic agents in patients with AUD presenting with compromised liver function. This is particularly true for patients administered *succinylcholine* and benzodiazepines.

Pharmacokinetic interactions between ethanol and other drugs must also be considered. *Acute administration* of ethanol inhibits the function of enzymes responsible for metabolizing a variety of different drugs, including *codeine*, *morphine*, *phenytoin*, some benzodiazepines, *tolbutamide*, and *warfarin*, among others. One in five prescription opioid deaths involves alcohol, and mixing these drugs increases risk of overdose and death. Because ethanol is a substrate for CYP2E1, any drug being metabolized by this CYP isozyme will be metabolized at a slower rate in the presence of ethanol. In contrast, the *chronic administration* of ethanol acts as an enzyme inducer, particularly of CYP2E1, increasing the rate of metabolism of *phenytoin*, *warfarin*, *propranolol*, and benzodiazepines. Activation of CYP2E1 in heavy drinkers can change *acetaminophen* into a toxic chemical and exacerbate hepatoxicity (see Figure 9–4).

Comorbidity of Alcohol Use Disorder With Other Psychiatric Disorders

Patients diagnosed with a mood or anxiety disorder are about twice as likely to suffer from a drug abuse disorder and vice versa. AUD is comorbid with other SUDs, major depressive and bipolar disorders, certain phobias, and antisocial and borderline personality disorders. AUD or other SUDs also co-occur with schizophrenia. Interestingly, there is a common neuroimmune link among AUD and some comorbid psychiatric disorders, suggesting a rationale for the development of therapeutics that target the key neuroimmune pathways. Although selective serotonin reuptake inhibitors (SSRIs) have not been shown to be effective treatments for AUD in those without a mental health disorder, SSRIs and other antidepressants may decrease alcohol intake when AUD and depression co-occur. If alcohol use occurs as a consequence of depression, treating the underlying problem can decrease drinking.

Exposure to traumatic events, and the debilitating, long-term anxiogenic symptoms that often result, are a cornerstone of posttraumatic stress disorder (PTSD). Classic symptoms include:

- Intrusion or reexperiencing the event
- Avoidance of internal and external reminder

- Negative changes in mood and cognition
- Hyperarousal and reactivity

PTSD increases the risk of developing AUD or other SUDs. There is a high prevalence of comorbid AUD and PTSD in the U.S., and, not surprisingly, a dual diagnosis exacerbates the symptoms of each disorder and worsens the prognosis for recovery. AUD and PTSD share considerable overlap in neural substrates and neuropathologies (Gilpin and Weiner, 2017). The SSRIs *sertraline* and *paroxetine* are FDA-approved for PTSD, but few studies have examined their efficacy in patients with PTSD and AUD. In one clinical trial, *sertraline* modestly improved PTSD symptoms and drinking measures but only in a subset of patients with early-onset PTSD and less severe AUD (Gilpin and Weiner, 2017). Verplaetse et al. (2018) have reviewed different treatment interventions for comorbid PTSD and AUD. For example, *naltrexone* (FDA-approved for AUD and discussed later) has modest effects on alcohol outcomes but does not significantly improve PTSD symptoms. Randomized clinical trials are needed to study potential drug combinations in these comorbid diseases.

Alcohol Use Disorder and Genetics

As with other complex trait disorders, the development and progression of AUD are influenced by multiple genetic and environmental factors, including stressors such as emotional, physical, or sexual abuse and drinking patterns within one's culture and peer group. The heritability of AUD is estimated to be 50% to 60%, as judged by family and twin studies.

Among the genetic variants identified, the most significant are in the ethanol-metabolizing enzymes. Linkage analyses show that genes clustered in the *ADH* region affect risk for alcohol dependence (Edenberg et al., 2006). For example, the *ADH1B*2* variant is found in high frequencies in Asian populations and may protect against AUD. It results in faster metabolism of ethanol and a transient higher blood level of acetaldehyde, which is associated with a lower risk for heavy drinking, but a higher risk for esophageal, head, and neck cancers in those who drink. The *ADH1B*3* single nucleotide polymorphism found in individuals of African descent is also associated with lower incidence of AUD.

ALDH2 is the most efficient isozyme in humans for the metabolism of ethanol-derived acetaldehyde. Low levels of acetaldehyde are rewarding and stimulating, while high blood levels produce toxic reactions such as vomiting, diarrhea, and unstable blood pressure; thus, variation in ALDH2 activity could affect the rewarding or aversive properties of ethanol. The genetic variant *ALDH2*2* encodes for an enzyme that cannot fully convert acetaldehyde to acetate; and in those who drink, acetaldehyde (a group 1 carcinogen) can accumulate to toxic levels, increasing risk of esophageal, head, and neck cancers. Approximately 10% of East Asians are homozygous for *ALDH2*2* and develop severe adverse reactions after the consumption of one drink or less. Similar effects occur if ethanol is consumed with the ALDH inhibitor *disulfiram* (the first pharmacotherapy for AUD, discussed in the next section of this chapter). Almost 40% of East Asians are heterozygous for *ALDH2*2* and experience facial flushing and enhanced sensitivity to alcohol but do not necessarily report adverse responses. Because ALDH2 deficiency poses increased risk of carcinogenesis, those individuals who drink should not dismiss mild responses to alcohol as inconsequential.

As gene discovery efforts continue to progress, findings from a large sample size show the polygenicity of alcohol use phenotypes and enrichment of candidate genes in tissues from cortical and subcortical regions known to be involved in AUD or SUDs (Liu et al., 2019). Many classical neurotransmitter and neuromodulator risk genes for AUD have been identified in genome-wide association studies, as well as robust changes in genes involved in brain stress and immune signaling. A genome-wide meta-analysis in individuals of European ancestry identified new risk loci for problematic alcohol use and also found genetic correlations with other substance use and psychiatric traits, particularly major depressive disorder (Zhou et al., 2020).

TABLE 27–2 ■ FDA-APPROVED MEDICATIONS FOR TREATING ALCOHOL USE DISORDER

MEDICATION	USUAL DOSE	MECHANISM/EFFECT
Disulfiram	250 mg/d (range of 125–500 mg/d)	• Inhibits ALDH with resulting ↑ acetaldehyde in the presence of ethanol. Abstinence is reinforced to avoid the adverse reaction.
Naltrexone (oral)	50 mg/d	• Opioid receptor antagonist; may ↓ drinking by ↓ the rewarding and reinforcing properties of alcohol.
Naltrexone (IM)	380 mg/4 weeks	• Same mechanism and effect as oral naltrexone. Long-acting depot formulation may improve bioavailability and patient compliance.
Acamprosate	666 mg three times daily	• May modulate glutamate and GABA neurotransmission; may work best in patients who are abstinent at treatment initiation.

Pharmacotherapies for Alcohol Use Disorder

In the U.S., the lifetime prevalence rate for AUD is estimated to be 29% (Grant et al., 2015). Despite evidence-based treatment options, however, AUD often goes undiagnosed and untreated. There are still only a few FDA-approved drugs for AUD: *disulfiram*, *naltrexone* (oral and long-acting injectable), and *acamprosate* (Table 27–2). *Disulfiram* inhibits ALDH and thereby alters the pharmacokinetics of ethanol; the other agents have neurobiological mechanisms. They have reasonable efficacy, with effect sizes similar to those of antidepressant drugs. However, they are prescribed in less than 9% of patients (Kranzler and Soyka, 2018).

Benzodiazepines are the treatment of choice for management of acute alcohol withdrawal (see Table 27–2) and to prevent the progression from minor withdrawal symptoms to major ones, such as seizures and delirium tremens (see Chapter 28).

Disulfiram

Disulfiram (tetraethylthiuram disulfide) was the first drug approved to treat alcohol abuse but is not considered a first-line treatment today. It inhibits ALDH and rapidly increases the blood acetaldehyde concentration by 5 to 10 times the level measured when ethanol is administered alone. *Disulfiram* irreversibly inactivates cytosolic and mitochondrial forms of ALDH to varying degrees. It is unlikely that *disulfiram*, itself, is responsible for ALDH inactivation *in vivo*. Several active metabolites, especially diethylthiomethylcarbamate, behave as suicide-substrate inhibitors of ALDH *in vitro*; these metabolites reach significant concentrations in plasma following the administration of *disulfiram*.

Alcohol consumption by individuals previously treated with *disulfiram* gives rise to marked signs and symptoms of acetaldehyde poisoning. At BECs of 5 to 10 mg%, mild effects are noted, increasing markedly in severity as the BEC reaches 50 mg%. If the patient attains a BEC of 125 to 150 mg%, loss of consciousness may occur. Within 5 to 10 min, the face feels hot and soon becomes flushed and scarlet in appearance. As the vasodilation spreads over the whole body, intense throbbing is felt in the head and neck, and a pulsating headache may develop. Respiratory difficulties, nausea, vomiting, sweating, thirst, chest pain, hypotension, orthostatic syncope, weakness, vertigo, blurred vision, and confusion are observed. The facial flush is then replaced by pallor, and blood pressure may fall to levels seen in shock. Thus, the use of *disulfiram* requires careful medical supervision and patient education and should only be attempted in motivated patients committed to maintaining abstinence. Patients must learn to avoid disguised forms of alcohol that may be present in sauces, fermented vinegar, cough syrups, and even aftershave lotions. *Disulfiram* does not reduce craving; its effectiveness is based on the fear of adverse effects in the presence of ethanol, resulting in poor patient compliance.

Disulfiram should not be administered until the patient has abstained from alcohol for at least 12 h. The FDA-approved dosage is 250 to 500 mg/day. Unless sedation (the most common side effect) is prominent, the daily dose should be taken in the morning, the time when the resolve not to drink may be strongest. Sensitization to alcohol may last as long as 14 days after the last dose because of the slow rate of restoration of ALDH. It should be used with caution in patients with liver disease and is contraindicated in those with cardiovascular disease.

Disulfiram or its metabolites can inhibit many enzymes with sulfhydryl groups, producing a wide spectrum of biological effects. Hepatic CYPs are inhibited, thereby interfering with the metabolism of *phenytoin*, *chlordiazepoxide*, barbiturates, *warfarin*, and other drugs.

Naltrexone

Naltrexone, an opioid receptor antagonist, is approved for AUD in both oral and extended-release injectable forms. *Naltrexone* is chemically related to *naloxone* but has higher bioavailability and a longer duration of action when administered orally. It is also approved for treatment of opioid dependence (see Chapters 23 and 28). *Naltrexone* reduces endogenous opioid activity in mesolimbic reward pathways and may reduce the reinforcing effects of alcohol by blunting opioid-mediated dopamine transmission. *Naltrexone* is typically prescribed after no opioids have been taken for 7 to 10 days at a dose of 50 mg/day (see Table 27–2). The extended-release intramuscular formulation (380 mg) may increase bioavailability and overcome problems with medication adherence. It is approved for monthly injection in those who can abstain from alcohol in an outpatient setting before starting *naltrexone*. *Naltrexone* is underutilized in clinical practice, although current American Psychiatric Association (APA) guidelines recommend it as a first-line drug.

A meta-analysis shows that oral *naltrexone* reduces risk of relapse to any drinking and to binge drinking (reviewed in Kranzler and Soyka, 2018). Both oral (50 mg/day) and injectable (380 mg, single injection) *naltrexone* increased the likelihood of no binge drinking in a 1-month pilot study of male veterans (Busch et al., 2017).

Naltrexone is well tolerated, the most common adverse effect being nausea. Other side effects are headache, dizziness, anxiety, and insomnia. Patients should also be monitored for development of depression. Hepatotoxicity is associated with oral doses exceeding 300 mg; the drug is contraindicated in patients with liver failure or acute hepatitis and should be used with caution in patients with active liver disease. One potential concern with intramuscular *naltrexone* is injection-site reactions. *Naltrexone* blocks the effects of opioid analgesics and can cause withdrawal in an opioid-dependent patient. It can be used following detoxification to prevent relapse in patients with opioid use disorder. If opioids are needed for pain management, higher doses may be required and should be administered under medical supervision.

Acamprosate

Acamprosate (*N*-acetylhomotaurine) is FDA-approved for AUD and may have a better outcome in patients who are abstinent at treatment initiation. The mechanism of action of *acamprosate* has not yet been elucidated. It is generally well tolerated, with diarrhea being the main side effect. A meta-analysis of randomized clinical trials showed that *acamprosate* is associated with reduced risk of drinking in abstinent patients but does not reduce the likelihood of binge drinking (Jonas et al., 2014). The FDA-approved dose is 1998 mg/day, divided into three doses, which may limit patient compliance (see Table 27–2). *Acamprosate* is contraindicated in patients with renal failure.

Other Drugs With Repurposing Potential

The following drugs are not FDA-approved for AUD but are approved in other countries or are being used off-label, or there is emerging evidence of their possible clinical utility.

Baclofen, a $GABA_B$ receptor agonist, is a skeletal muscle relaxant used to reduce spasticity. It is approved to treat AUD in France and is indicated for use in patients not responsive to other treatments. There is increased risk of sedation when combining *baclofen* with alcohol. In a meta-analysis of randomized clinical trials, *baclofen* was associated with a delay in return to drinking and a greater likelihood of abstinence, especially in those with higher daily alcohol use at baseline (reviewed in Kranzler and Soyka, 2018). Although some results have been promising, evidence overall is uncertain regarding its use, particularly as a first-line treatment. For a review of *baclofen* in treating AUD, see de Beaurepaire et al., 2019.

Nalmefene (an analogue of *naltrexone*) is a mu- and delta-opioid receptor antagonist with partial agonist activity at kappa receptors. Compared with *naltrexone*, *nalmefene* has a longer half-life, greater bioavailability, and decreased risk of liver toxicity. The European Medicines Agency has recommended *nalmefene* for as-needed use (18 mg) to reduce heavy drinking. Such targeted use may be beneficial in high-risk situations or in problem drinkers who are not medication compliant or may otherwise not seek treatment. In a meta-analysis of randomized clinical trials, *nalmefene* was associated with a slight reduction in the number of binge-drinking days and in total alcohol consumption at 6 months (reviewed in Kranzler and Soyka, 2018).

Gabapentin is an anticonvulsant and GABA analogue that inhibits the $\alpha 2\delta$-1 subunit of voltage-gated Ca^{2+} channels. It is primarily used to treat neuropathic pain, restless leg syndrome, and as an adjunctive treatment for partial-onset seizures. It is prescribed off-label for anxiety and other psychiatric disorders. APA guidelines suggest using it to treat AUD after first trying *naltrexone* or *acamprosate*. In one clinical trial, *gabapentin* (particularly at 1800 mg/day) increased the rate of abstinence and prevented binge drinking (reviewed in Kranzler and Soyka, 2018). *Gabapentin* is also associated with decreased alcohol craving and is used to treat mild to moderate alcohol withdrawal (Ahmed et al., 2019). For additional information on *gabapentin*, see Chapter 20.

Topiramate is an anticonvulsant that modulates voltage-gated sodium channels and GABA and glutamate activity in the CNS. It is FDA-approved for seizure disorders and migraine prevention and is used off-label as an adjunct therapy for chronic weight management. As with *gabapentin*, current APA guidelines suggest using *topiramate* as a second-line treatment for AUD, typically after trying *naltrexone* or *acamprosate*. A meta-analysis of randomized clinical trials showed *topiramate* is associated with a greater number of abstinent days and reduced number of binge-drinking days (reviewed in Kranzler and Soyka, 2018). Paresthesia and dysgeusia are the most common side effects.

Sodium oxybate, the sodium salt of γ-hydroxybutyric acid (GHB), is FDA-approved to treat cataplexy and excessive daytime sleepiness in narcolepsy. *Sodium oxybate* is approved in Italy and Austria to prevent relapse and to treat alcohol withdrawal (Caputo et al., 2016; van den Brink et al., 2018). It is structurally similar to GABA and thought to act by mimicking the effects of ethanol at $GABA_A$ and $GABA_B$ receptors, increasing dopamine levels in the mesocorticolimbic circuitry, and reducing craving. Because GHB is a recreational ("street") drug, there has been concern regarding its abuse potential and pharmacodynamic interactions with alcohol; however, this has not been an issue in clinical settings in patients without polydrug use and psychiatric comorbidities.

There are a number of other drugs being studied for their potential to treat AUD. ***Varenicline***, which is approved for smoking cessation, also reduces craving and alcohol consumption in some studies. *Varenicline* is a partial agonist at $\alpha 3\beta 4$, $\alpha 4\beta 2$, and $\alpha 6\beta 2$ subtypes of nicotinic acetylcholine receptors (nAChRs) and acts as a high-efficacy agonist at $\alpha 7$ nAChRs. For more information on *varenicline*, consult Chapter 13. Other compounds in under investigation for the treatment of AUD in at least some subgroups of patients include the dopamine receptor partial agonist *aripiprazole*, the NMDA receptor antagonist *ifenprodil*, and the α_1 adrenergic receptor antagonists *prazosin* and *doxazosin*. Drugs that target immune and inflammatory pathways are also being studied for their potential to treat AUD. For example, *apremilast* (an inhibitor of phosphodiesterase type 4 and an anti-inflammatory drug approved to treat psoriasis) decreased alcohol drinking in a human laboratory study. Lastly, *suvorexant*, an antagonist of OX_1 and OX_2 orexin receptors that is used to treat insomnia, may reduce relapse drinking by treating the sleep disruptions commonly found in AUD.

Treatment Outlook

Compared with other psychiatric disorders, there is a significant gap in pharmacotherapies for AUD. In the U.S., fewer than 1 in 10 individuals with a 12-month diagnosis of AUD receive any treatment. This is particularly concerning because, as described throughout this chapter, AUD is a systemic disease targeting the central and peripheral nervous systems, the digestive tract, the heart and vascular system, the lungs, bone and skeletal muscle, and the endocrine and immune systems. AUD is one of the leading causes of preventable morbidity and mortality.

Despite the overall modest effect sizes of available drugs, they do benefit some patients beyond what psychosocial treatment alone can accomplish. Clinicians are urged to address this public health burden by increasing screening measures and discussing evidence-based treatments (pharmacological and psychosocial) for patients who meet the criteria for AUD. Screening for other comorbid psychiatric illnesses in AUD individuals is also critical. As with any polygenic disease, the goal should be to initiate treatment earlier, before there is prolonged history of abuse with neurobiological or inflammatory insults. Although abstinence has traditionally been the main goal, reducing risky drinking behavior may be a valid treatment outcome for some. Evidence for this comes from patients in clinical trials who reported that alcohol-related problems increase with their number of days of binge drinking. New tools are being used in the search for new treatments, such as combining transcriptomic and computational approaches to find existing drugs that will target the dysregulated genes and biological pathways in AUD (Ferguson et al., 2019). Identifying improved pharmacotherapies and broadening access to treatment are major goals in the fight against this systemic disease.

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Drug Facts for Your Personal Formulary: *Drugs Used to Treat Alcohol Use Disorder or Methanol Poisoning*

FDA-Approved Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Disulfiram 250–500 mg/day	• AUD (for patients committed to sustained abstinence)	• ALDH inhibitor. • Causes adverse effects from increased acetaldehyde when taken with alcohol. • Requires medical supervision and patient education. • Poor patient compliance.
Naltrexone Oral: 50 mg/day (50–100 mg) IM: 380 mg every 4 weeks (150–400 mg)	• AUD • Opioid use disorder (after opioid detoxification)	• Opioid receptor antagonist. • Available in oral and extended-release injectable formulations. • Contraindicated in patients with liver disease or taking opioids concurrently. • 2018 APA guidelines recommend use as a first-line treatment.
Acamprosate 1998 mg/day	• AUD	• May restore glutamate and GABA imbalances in the brain. • Safer for patients with hepatic disease; contraindicated in patients with severe cardiovascular disease. • 2018 APA guidelines recommend use as a first-line treatment.
Benzodiazepines	• Management of alcohol withdrawal • Anxiety/panic/seizure disorders • Insomnia • Anesthetic premedication	• ↑ GABA binding at $GABA_A$ receptors. • Chlordiazepoxide, lorazepam, diazepam, oxazepam, midazolam, and clorazepate are used in the U.S. to manage alcohol withdrawal symptoms.
Fomepizole	• Methanol or ethylene glycol poisoning	• ADH inhibitor.
Drugs Not FDA-Approved for AUD but Approved Elsewhere or Found Clinically Useful		
Gabapentin	• AUD (off-label) • Anticonvulsant • Neuropathic pain • Restless leg syndrome	• Blocks voltage-gated Ca^{2+} channels. • 2018 APA guidelines suggest use in AUD, typically after first trying naltrexone or acamprosate.
Topiramate	• AUD (off-label) • Anticonvulsant • Migraine prevention	• Acts on voltage-gated sodium channels and $GABA_A$ and glutamate receptors. • 2018 APA guidelines suggest use in AUD, typically after first trying naltrexone or acamprosate.
Nalmefene	• Approved for AUD in European Union (18 mg/day, as needed) • Opioid overdose/dependence	• Opioid receptor antagonist. • Less liver toxicity and longer acting than naltrexone. • Once approved for opioid overdose but now discontinued in the U.S.
Baclofen	• Approved for AUD in France • Spasticity	• $GABA_B$ receptor agonist, skeletal muscle relaxant, and antispasmodic agent.
Sodium oxybate	• Approved for AUD in Italy and Austria • Cataplexy • Narcolepsy	• GABA analogue and form of GHB. • Acts on $GABA_B$ and extrasynaptic $GABA_A$ receptors.

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Chapter 28

Drug Use Disorders and Addiction

Christine Konradi and Yasmin L. Hurd

A BRIEF HISTORY OF THE USE OF PSYCHOACTIVE AND ADDICTIVE SUBSTANCES

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A Brief History of the Use of Psychoactive and Addictive Substances

Addictive and hallucinogenic substances have been used throughout mankind’s cultural history. Traditionally, these substances have been used by healers for medicinal purposes and by priests in religious ceremonies. Hallucinogenic plants and mushrooms were used in pre-Columbian Mesoamerican cultures, opium has been known to man since prehistoric times, and alcoholic substances have been widely socially accepted for thousands of years. The advent of chemistry and pharmaceutical companies in the 19th century allowed for the analysis of psychoactive substances in coca leaves and poppy seeds, leading to the extraction and identification of the active ingredients and the synthesis of new compounds that were modeled after the botanical material. The hope was to find substances that could cure medical conditions and relieve pain without some of the addictive or adverse properties of the botanical extracts. Cocaine, morphine, and heroin were synthesized, and, although it seems absurd from today’s vantage point, were marketed as less addictive and more beneficial for addiction treatment. Morphine was a blessing during the American Civil War, as it was 100 years later during the U.S. involvement in Vietnam, and in both wars, many soldiers developed dependencies.

Regulatory Responses in the U.S. to Issues of Purity, Use, and Misuse of Drugs

Initial steps to regulate pharmaceuticals were introduced with the Pure Food and Drugs Act in 1906. That act stipulated that active ingredients be listed on the label of a drug’s packaging and that drugs could not fall below purity levels established by the U.S. Pharmacopeia (National Formulary). However, addictive drugs continued to be legally available without prescriptions as long as they were properly labeled. In 1908, with opium dens in most major cities, the first Opium Commissioner was appointed, and in 1914, the Harrison Narcotics Act was enacted. It regulated and taxed the production, importation, and distribution of heroin and cocaine. For the first time, doctors and pharmacists had to keep records of prescriptions.

The temperance movement and Prohibition, solidified by the 18th Amendment, had the unintended consequences of encouraging organized crime, bootlegging, and poisonings with methanol-laced industrial alcohol. With the termination of Prohibition by the passage of the 21st Amendment, organized crime turned to trafficking in other addictive substances such as heroin, which was legally grown in Turkey for medicinal purposes. The Controlled Substances Act (CSA), enacted in 1970, was a response to the Counterculture Generation and the return of

Abbreviations

Δ9-THC: Δ-9-tetrahydrocannabinol
ADHD: attention-deficit/hyperactivity disorder
AIDS: acquired immune deficiency syndrome
AUD: alcohol use disorder
CBD: cannabidiol
CDC: U.S. Centers for Disease Control and Prevention
CRF: corticotropin-releasing factor
CSA: Controlled Substances Act
DA: dopamine
DAT: dopamine transporter
DEA: U.S. Drug Enforcement Administration
DMT: dimethyltryptamine
DSM-5: *Diagnostic and Statistical Manual of Mental Disorders* (fifth edition)
GABA: γ-aminobutyric acid
GPCR: G protein-coupled receptor
5HT: serotonin (5-hydroxytryptamine)
KOR: κ (kappa) opioid receptor
LSD: d-lysergic acid diethylamide
MDA: methylenedioxy-amphetamine
MDMA: 3,4-methylenedioxy-methamphetamine
MOR: μ (mu) opioid receptor
MPH: methylphenidate
NDEWS: National Drug Early Warning System
NDTA: National Drug Threat Assessment
NE: norepinephrine
NIAAA: National Institute on Alcohol Abuse and Alcoholism
NIDA: National Institute on Drug Abuse
NMDA: *N*-methyl-D-aspartate
OUD: opioid use disorder
PCP: phencyclidine
psilocybin: 4-phosphoryloxy-N,N-dimethyltryptamine
PTSD: posttraumatic stress disorder
SAMSA: Substance Use and Mental Health Services Administration
SUD: substance use disorder

heroin-addicted soldiers from Vietnam. In its wake, the Drug Enforcement Administration (DEA) and the CSA were established. The CSA established federal drug policy under which the manufacture, importation, possession, use, and distribution of certain substances are regulated. Further, the CSA established classifications of drugs into five schedules, based on their accepted medical use and their likelihood to cause dependence (Table 28–1). The CSA also serves as the national implementing legislation for the Single Convention on Narcotic Drugs, an international treaty to prohibit production and supply of specific drugs. The DEA and the U.S. Food and Drug Administration (FDA) determine which substances are added to or removed from the various schedules.

TABLE 28–1 ■ DRUG SCHEDULING OF CONTROLLED SUBSTANCES

SCHEDULE	DEFINITION	EXAMPLES
I	Drugs with no currently accepted medical use and a high potential for abuse	Heroin, LSD[a], marijuana[a], MDMA, peyote, methaqualone
II	Drugs with a high potential for abuse, with use potentially leading to severe psychological or physical dependence	Combination products with <15 mg of hydrocodone per dosage unit (Vicodin), cocaine, methamphetamine, PCP, methadone, hydromorphone (Dilaudid), meperidine (Demerol), oxycodone (OxyContin), fentanyl, Dexedrine, Adderall, Ritalin
III	Drugs with a moderate to low potential for physical and psychological dependence	Products containing <90 mg of codeine per dosage unit (Tylenol with codeine), ketamine, anabolic steroids, testosterone
IV	Drugs with a low potential for abuse and low risk of dependence	Xanax, Soma, Darvon, Darvocet, Valium, Ativan, Talwin, Ambien, Tramadol
V	Drugs with lower potential for abuse than Schedule IV and consist of preparations containing limited quantities of certain narcotics. Schedule V drugs are generally used as antidiarrheals, antitussives, and analgesics.	Cough preparations with <200 mg of codeine or per 100 mL (Robitussin AC), Lomotil, Motofen, Lyrica, Parepectolin

MDMA, 3,4-methylenedioxy-methamphetamine.
[a] The absence of therapeutic properties is debated.

In 1995, the slow-releasing opioid drug *OxyContin* was introduced by Purdue Pharma, and in 2001, The Joint Commission rolled out new pain management standards that consider pain as a fifth vital sign. The resultant exuberance of prescribing of opioids contributed to a new addiction epidemic, with the death toll from opioids rising to 75,000 annually (Centers for Disease Control and Prevention [CDC], 2021). Clearly addiction is a national problem in the U.S.

Neurobiological Mechanisms of Addiction

The interaction of a ligand with a receptor on a neuron activates an intracellular signal transduction pathway that leads to, among other things, a change in gene expression patterns and a modification of how that neuron communicates in the future. This type of response to neurotransmitters, hormones, and neurotrophic factors is the basis of memory formation. Without this level of neural plasticity, memory would not be possible (Figure 28–1).

Drugs are exogenous ligands that tap into the same process. Psychoactive drugs (i.e., drugs that have target sites in the brain and lead to alterations in perception, mood, consciousness, cognition, or behavior) can produce lasting neuroplastic changes. In the case of prescription drugs that treat neuropsychiatric disorders, the response is a desired adaptation. In the case of drugs of abuse, this neuroplasticity is a form of maladaptive memory. A characteristic of substance use disorders (SUDs) is the further change in brain circuits that may persist beyond detoxification and cause intense drug craving and relapse.

Addiction begins with the exposure to substances (e.g., cocaine) or presentation of behaviors (e.g., gambling) that directly and intensely affect brain function and perception. Many drugs of abuse activate the reward pathway in the brain, which involves a dopaminergic connection from the ventral tegmental area to the *nucleus accumbens*. Drugs can activate the reward system through a direct action on the dopaminergic neurons, as is the case with stimulants, or indirectly through neuronal circuits that regulate those neurons, as is the case with opioids. The mechanism of action of alcohol, sedatives, and hypnotics is less clear-cut and includes neurotransmitter systems such as γ-aminobutyric acid (GABA), glutamate, 5HT (5-hydroxytryptamine, serotonin), and others.

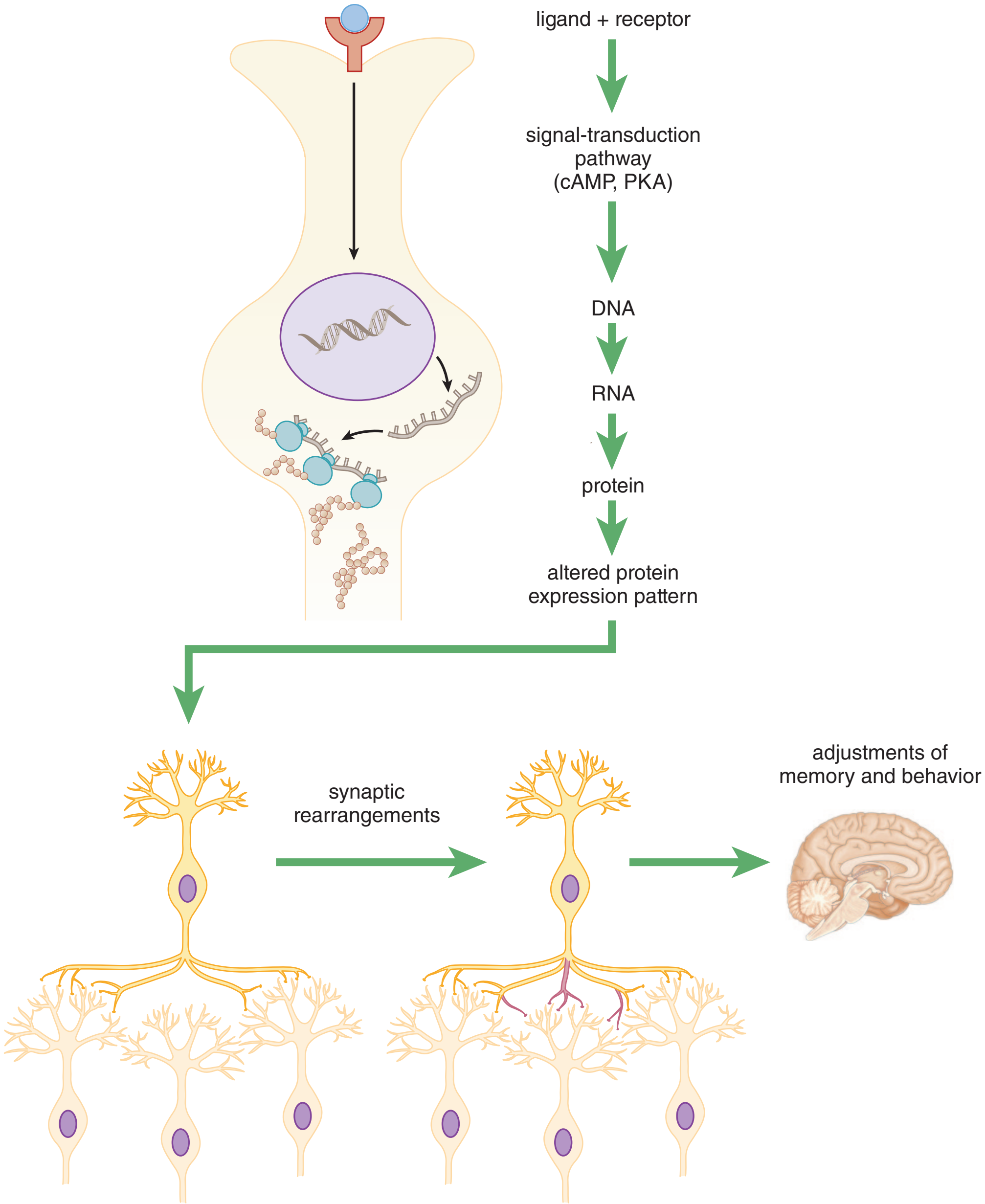

ligand + receptor
signal-transduction pathway (cAMP, PKA)
DNA
RNA
protein
altered protein expression pattern
synaptic rearrangements
adjustments of memory and behavior

Figure 28–1 *From ligand-receptor interaction to long-term physical and behavioral changes in the CNS.* Drugs of abuse can alter the release of an endogenous ligand (i.e., release of DA, 5HT, NE) or serve as ligands themselves (e.g., THC isomers, opioids, nicotine, etc.). Interaction with a receptor initiates a cascade of signaling in the neuron that can involve ion channels, kinases, phosphatases, and other enzymes. Strong signals can be propagated to the nucleus where transcription factors are initiating mRNA synthesis to temporarily alter protein expression patterns. The newly synthesized proteins may change the physiology, structure, and connectivity of neurons. Some of this hard wiring of information generated by drugs might be irreversible and cannot be extinguished.

The reward system motivates normal behavior, and most humans simply enjoy the experience without an uncontrollable urge to repeat it. However, some individuals become drawn into compulsive repetition of the experience and develop SUDs or addictive behaviors, focusing on the immediate pleasure despite negative long-term consequences and neglecting important social, familial, and occupational responsibilities. The National Survey on Drug Use and Health reported that about 7% of Americans met the criteria for SUD in 2019 (Substance Abuse and Mental Health Services Administration, 2020) (Table 28–2), while about 10% have had SUDs at some time in their lives (Grant et al., 2016). This is lower than the approximately 17% of people above 18 years of age that used illicit drugs in 2019 or the approximately 25% of binge alcohol users in the month prior to the survey, underscoring that not all exposure to drugs leads to SUDs (Substance Abuse and Mental Health Services Administration, 2020).

Definitions and Pharmacological Phenomena

Using appropriate terminology is important to convey accurate patient information among healthcare professionals. Here are some of the most prevalent terms used in addiction research and treatment and their associated pharmacological phenomena.

Substance Use Disorders

The fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5) bases the diagnosis of SUD on a pathological pattern of behaviors related to the use of a chemical substance (American Psychiatric Association, 2013). The individual has impaired control and may take the substance in larger amounts or over a longer period of time than was originally intended. An individual might report multiple

TABLE 28–2 ■ SUBSTANCE USE DISORDER FOR SPECIFIC SUBSTANCES IN 2019 (AS A PERCENTAGE OF THOSE AGE ≥12 YEARS IN U.S. POPULATION WHO MET CRITERIA FOR SUBSTANCE USE DISORDER)

Illicit Drugs	3.0
Marijuana	1.8
Cocaine	0.4
Heroin	0.2
Hallucinogens	0.1
Inhalants	0.0
Methamphetamine	0.4
Misuse of psychotherapeutics	0.8
Pain relievers	0.5
Stimulants	0.2
Tranquilizers or sedatives	0.2
Tranquilizers	0.2
Sedatives	0.1
Opioids	0.6
Illicit drugs other than marijuana	1.5
Alcohol	5.3
Both Illicit Drugs and Alcohol	0.9
Illicit Drugs or Alcohol	7.4
Illicit Drugs Only	2.1
Alcohol Only	4.4

unsuccessful efforts to decrease or discontinue use and spend a great deal of time obtaining the substance, using the substance, or recovering from its effects. Craving, defined as an intense desire or urge for the drug, is manifested particularly in an environment where the drug was previously obtained or used. Negative consequences associated with SUDs can be failure to fulfill obligations at work, school, or home; persistent or recurrent social or interpersonal problems; and withdrawal from family, recreational activities, and hobbies, which are replaced with use of the substance. Other hallmarks are risky overall behavior and risky use of the substance. Pharmacological criteria include the development of tolerance and withdrawal symptoms when levels of the substance decline (see below).

Addiction and Addictive Behaviors

Certain excessive behaviors can fit the same pathological criteria described in SUDs. These behavioral addictions include gambling, sex addiction, and shopping addiction. While SUD is a form of addiction, it is more appropriately labeled SUD in healthcare settings, which is considered a more neutral term. The American Society of Addiction Medicine defines addiction as a treatable, chronic medical disease involving complex interactions among brain circuits, genetics, the environment, and an individual's life experiences. People with addiction use substances or engage in behaviors that become compulsive and often continue despite harmful consequences.

Treatment outcomes for addiction and SUDs are generally as successful as those for other chronic diseases, such as diabetes or cardiovascular disease. While relapses are not uncommon, they fit into similar patterns as other chronic diseases that require healthy behavioral adaptations.

Tolerance

According to DSM-5, tolerance refers to the need of increased doses of a substance to achieve the desired effect or a reduction in effect even though the usual dose is consumed. Consider a stylized drug dose-response curve (Figure 28–2). As the dose of the drug increases, the observed effect of the drug increases. However, with tolerance, the curve shifts to the right due to the repeated use of the drug; that is, a given dose of the drug produces less effect.

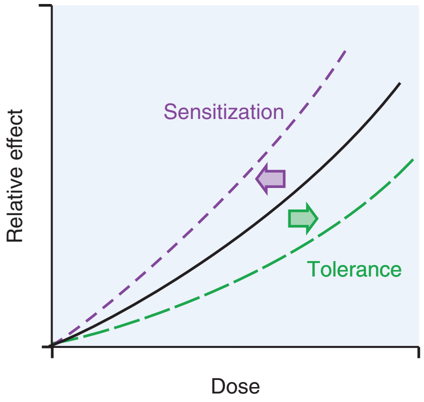


Figure 28–2 *Shifts in a dose-response curve with tolerance and sensitization.* The solid black curve describes the dose-response relationship to initial doses (the "control" curve). With tolerance, there is a shift of the curve to the right such that higher doses are required to achieve equivalent effects. With sensitization, the dose-response shifts leftward, and a given dose produces a greater effect than in the control case.

Tolerance can be life threatening in the case of opioids, for example, where the tolerance to respiratory depression does not develop at the same rate as the tolerance to sedating or euphoric effects. The discrepancy between tolerance to euphorigenic effects (rapid) and tolerance to effects on vital functions such as respiration and blood pressure (slow) can lead to potentially fatal overdoses as the user seeks the euphoria.

Pharmacologists define multiple aspects of tolerance:

- *Innate tolerance* refers to genetically determined lack of sensitivity to a drug the first time it is experienced.
- *Acquired tolerance* can be divided into three major types—*pharmacokinetic, pharmacodynamic*, and *learned tolerance*—and includes acute, reverse, and cross-tolerance.

Consider the following examples:

1. *Pharmacokinetic* or *dispositional tolerance* refers to changes in the distribution or metabolism of a drug after repeated administrations, such that a given dose produces a lower blood concentration than the same dose did on initial exposure. The most common mechanism is an increase in the rate of metabolism of the drug. For example, chronic administration of *phenobarbital* induces hepatic cytochrome P450 isoforms (CYPs) 1A2, 2C9, 2C19, and 3A4, thereby enhancing the metabolism of drugs that are substrates for these enzymes, including the barbiturate itself.
2. *Pharmacodynamic tolerance* refers to adaptive changes that have taken place within systems affected by the drug so that response to a given concentration of the drug is altered (usually reduced). Examples include drug-induced changes in receptor density or efficiency of receptor coupling to signal transduction pathways, such as upregulation of β adrenergic receptors during treatment of a hypertensive patient with a β receptor antagonist.
3. *Learned tolerance* refers to a reduction in the effects of a drug due to compensatory mechanisms that are acquired by past experiences.

- *Behavioral tolerance* is a type of learned tolerance. A common example is learning to walk a straight line despite the motor impairment produced by alcohol intoxication. At higher levels of intoxication, behavioral tolerance is overcome, and the behavioral deficits are obvious.

- *Conditioned tolerance* (situation-specific tolerance) develops when environmental cues or situations consistently are paired with the administration of a drug. When a drug affects homeostatic balance by producing sedation and changes in blood pressure, pulse rate, gut activity, and so on, there is usually a reflexive counteraction or adaptation in the direction of maintaining the status quo. If a drug is always taken in the presence of specific environmental cues (e.g., smell of drug preparation and sight of syringe), these cues begin to predict the effects of the drug, and the adaptations begin to occur, which will prevent the full manifestation of the drug's effects (i.e., cause tolerance). This mechanism follows classical (Pavlovian) principles of learning and results in drug tolerance under circumstances where the drug is "expected."

- *Acute tolerance* refers to rapid tolerance developing with repeated use on a single occasion, such as in a "binge." For example, repeated doses of cocaine over several hours produce a decrease in response to subsequent doses of cocaine during the binge. This is the opposite of *sensitization*, observed with an intermittent-dosing schedule.

- *Sensitization* or *reverse tolerance* refers to an increase in response with repetition of the same dose of the drug. Sensitization results in a shift to the left of the dose-response curve (see Figure 28–2). Sensitization, in contrast to acute tolerance during a binge, requires a longer interval between doses, usually about 1 day. Sensitization often occurs with stimulants such as cocaine or amphetamine, but also occurs with other drugs.

- *Cross-tolerance* occurs when repeated use of a drug in a given category confers tolerance not only to that drug but also to other drugs in the same pharmacological category, such as opioid receptor agonists. Understanding cross-tolerance is important in the medical management of persons dependent on a drug. For example, a patient on methadone maintenance therapy might not experience analgesia elicited by morphine.

- *Detoxification* is a medically supervised withdrawal from the drug of abuse. It is often the first phase of treatment of drug dependence that involves the gradual decrease of the dose of the drug to reduce withdrawal symptoms, thereby weaning the patient from the drug. Detoxification can be accomplished with any medication in the same category as the initial drug of dependence. For example, users of heroin show cross-tolerance to other opioids. Thus, the detoxification of heroin-dependent patients can be accomplished with any medication that is an agonist at μ opioid receptors (MORs), such as *buprenorphine*.

Degrees of tolerance depend on the type of opioid, its half-life, and the route of administration. An addicted individual who is craving the "high" may risk overdose by not understanding the intricacies of the pharmacological profile of related drugs. This is especially dangerous when tolerance contributes to the individual progressing to more dangerous routes of administration (e.g., intravenous injection) or to highly potent opioids such as *fentanyl*. Accidental overdose has reached epidemic proportions and become so common in the U.S. that death in this manner now exceeds the toll for traffic accidents in young people (Rudd et al., 2016).

Allostasis and Physical Dependence

Physical dependence is a state that develops as a result of the adaptation (tolerance) produced by a resetting of homeostatic mechanisms in response to repeated drug use. The organism maintains "stability through change" by a process known as *allostasis* (McEwen, 1998). In the case of continued drug exposure, a person becomes physically dependent and requires continued administration of the drug to maintain normal function. If administration of the drug is stopped abruptly, there is a new imbalance, and the affected systems must readjust to a new equilibrium without the drug. An example is the rapid physical and emotional adaptation to opioid exposure that requires increasing doses of opioids to maintain the effect desired by the user (Figure 28–3).

Withdrawal Syndrome

DSM-5 describes withdrawal as a syndrome that occurs when blood or tissue concentrations of a substance decline in an individual who had maintained prolonged heavy use of that substance. The appearance of a withdrawal syndrome when administration of the drug is terminated is the only actual evidence of physical dependence. The types of withdrawal symptoms depend on the pharmacological category of the drug. For example, withdrawal of a stimulant causes sedation during withdrawal. Withdrawal of an opioid produces craving for the opioid and physical symptoms, such as nausea, vomiting, and diarrhea. Withdrawal associated with cannabis use disorder also includes somatic symptoms such as sweating, headache, and abdominal pain. Dependence and withdrawal have both physical and emotional presentations. Removal of a drug from the system often causes negative emotional states such as dysphoria, anxiety, and irritability. Significant

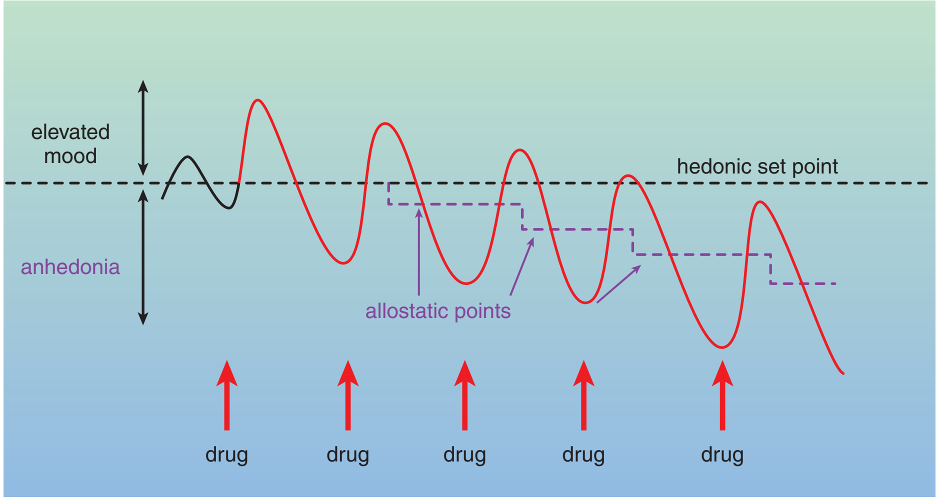


Figure 28–3 *Shifts in the hedonic set point during prolonged periods of opioid exposure.* Within the neuronal network, a balance is maintained between the multitude of receptors and their ligands. To protect the functional integrity, biological mechanisms are employed to counterbalance overactivity of any one system. Emotional experiences are rooted within this biological framework. The recalibration of interdependent elements to maintain a stable equilibrium explains why people with opioid use disorder (OUD) need to increase their drug intake over time to reach a similar emotional state to previous exposure experiences. With every exposure to opioids and the induced shift toward a state of elevated mood, the system counteracts downward toward equilibrium. When opioid levels fall, the counterbalancing measures are revealed as a drop in the hedonic setpoint and a reduced ability to feel pleasure (anhedonia). If drugs are taken in rapid succession, new allostatic points are established and the network is calibrated toward the presence of drugs. Eventually, drug use needs to be maintained to avoid the intensely unpleasant emotional and physical consequences of withdrawal. (Figure adapted from Koob, 2015.)

withdrawal has not been documented in humans after repeated use of phencyclidine (PCP), other hallucinogens, and inhalants, which are not drugs that are generally used on a chronic daily basis.

Pharmacokinetic variables are of considerable importance to the amplitude and duration of the withdrawal syndrome. Short-acting substances tend to have a higher potential for the development of withdrawal symptoms, while longer-acting substances tend to have longer withdrawal duration. The longer the half-life of a substance, the longer is the time between cessation of drug taking and the onset of withdrawal symptoms, and the longer the withdrawal duration.

Tolerance, physical dependence, and withdrawal are all biological phenomena. They are the natural consequences of repeated, chronic drug use and can be reproduced in experimental animals. However, these symptoms in themselves do not imply that the individual is involved in drug misuse or addiction. *Patients who take medicines for appropriate medical indications and in correct dosages still may show tolerance, physical dependence, and withdrawal symptoms* if the drug is stopped abruptly rather than gradually. A physician prescribing a medication that normally produces tolerance must understand the difference between dependence and SUD and be mindful of withdrawal symptoms if the dose is reduced.

Reinforcement

The reinforcing properties of a drug can be propagated through positive reinforcement, negative reinforcement, or both.

- *Positive reinforcement* in the context of addiction refers to the capacity of drugs to produce positive effects that increase the likelihood that the individual will take the drug again. The more strongly reinforcing a drug is, the greater is the likelihood that the drug will be abused. The reinforcing properties of drugs are often associated with their capacity to increase neuronal activity in the brain mesolimbic reward pathway. Cocaine, amphetamine, ethanol, opiates, cannabinoids, and nicotine increase extracellular dopamine (DA) levels in the reward pathway, which consists of neurons arising in the ventral tegmental area that terminate in the ventral striatum, specifically the *nucleus accumbens* region. In contrast, drugs that block DA receptors generally produce bad feelings (i.e., *dysphoric effects*). Despite strong correlative findings, a precise causal relationship between DA and euphoria/dysphoria has not been established, and other findings emphasize additional roles of 5HT, glutamate, norepinephrine (NE), endogenous opioids, and GABA in mediating the reinforcing effects of drugs.
- *Negative reinforcement* is the process by which removal of an aversive stimulus increases the probability of a response. In the case of addiction, the aversive stimulus is the negative emotional state induced by drug withdrawal that reinforces drug taking. Various neurotransmitter systems have been implicated in negative reinforcement, such as the drug-induced increase in dynorphin, activation of κ opioid receptors (KOR), and corticotropin-releasing factor (CRF) hyperactivation during drug withdrawal (Koob, 2015; Zhou et al., 2019).

Relapse and Reinstatement

SUDs and addictions are chronic diseases. According to the National Institute on Drug Abuse (NIDA), more than 85% of people with addictions who stop using a drug reinstate drug use within a year. This is not due to physical symptoms, but to emotional craving. People in recovery are vulnerable to relapse when presented with cues associated with drug taking, in times of heightened stress, and when re-exposed to drugs after abstinence. While addiction is never considered "healed," lifelong remission and recovery are possible.

Substance-Induced Disorders

Substance-induced disorders are different from SUDs. They are defined by the development of a substance-specific syndrome due to the recent ingestion of a substance (i.e., intoxication). Clinically significant manifestations develop during or shortly after use of the substance. Substance intoxication is reversible and is common among those with SUDs but also occurs in individuals without SUD. In contrast, the development of substance-induced mental disorders is potentially severe and may in some cases leave persistent CNS syndromes.

An example is alcohol, which may produce depressive symptoms during intoxication, and anxiety syndromes during the acute withdrawal period. Stimulant substances and synthetic cannabinoids (manufactured drugs that activate cannabinoid receptors) can cause psychotic disorders or anxiety disorders, and depressive episodes during withdrawal.

Factors That Affect the Liability to Become Addicted

Most individuals who initiate use of a drug with addictive potential do not develop an SUD, although about 7% of persons aged 12 years or older met the criteria for SUD or alcohol use disorder (AUD) in 2019 (see Table 28–2). This trend increased slightly over the past 5 years due to an increased availability of, and addiction to, cannabis (Figure 28–4).

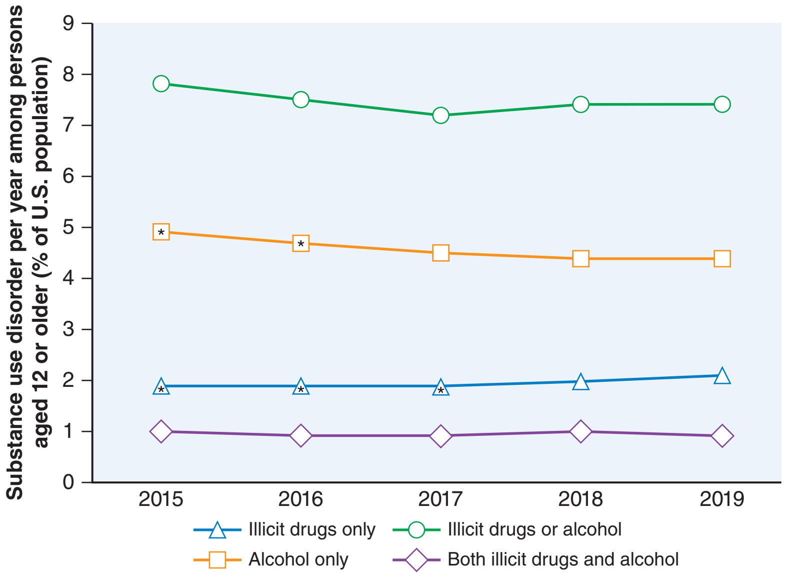


Figure 28–4 *Percentage of persons aged 12 years or older who met criteria for SUDs from 2015 to 2019 in the U.S.* Asterisk denotes the difference to 2019 at $p \le 0.05$. (Data from Substance Abuse and Mental Health Services Administration, 2020.)

TABLE 28–3 ■ MULTIPLE SIMULTANEOUS VARIABLES AFFECTING ONSET AND CONTINUATION OF DRUG ABUSE AND ADDICTION

Agent (drug)

- Availability
- Purity/potency
- Mode of administration
  - Chewing (absorption via oral mucous membranes)
  - Gastrointestinal
  - Intranasal
  - Subcutaneous and intramuscular
  - Intravenous
  - Inhalation
  - Speed of onset and termination of effects (pharmacokinetics: combination of agent and host)

Host (user)

- Age at first exposure
- Heredity (genetic vulnerability vs. genetic resilience)
  - Innate tolerance
  - Speed of developing tolerance
  - Likelihood of experiencing intoxication as pleasure
- Absorption, distribution, metabolism, and elimination
- Psychiatric symptoms
- Prior experiences/expectations
- Propensity for risk-taking behavior

Environment

- Access to enforcers
- Affordability of enforcers
- Social setting
- Community attitudes
  - Peer influence, role models
- Employment or educational opportunities
- Stressful environment
- Conditioned stimuli: environmental cues become associated with drugs after repeated use in the same environment

TABLE 28–4 ■ USE, ADDICTION, AND RISK AMONG USERS OF TOBACCO, ETHANOL, AND ILLICIT DRUGS IN THE U.S., 1992–1994

AGENT	EVER USED[a] (%)	ADDICTION (%)	RISK OF ADDICTION (%)
Tobacco	75.6	24.1	31.9
Alcohol	91.5	14.1	15.4
Illicit drugs	51.0	7.5	14.7
Cannabis	46.3	4.2	9.1
Cocaine	16.2	2.7	16.7
Stimulants	15.3	1.7	11.2
Anxiolytics	12.7	1.2	9.2
Analgesics	9.7	0.7	7.5
Psychedelics	10.6	0.5	4.9
Heroin	1.5	0.4	23.1
Inhalants	6.8	0.3	3.7

[a] The ever-used and addiction percentages are those of the general population. The risk of addiction is specific to the drug indicated and refers to the percentage who met criteria for addiction among those who reported having used the agent at least once (i.e., each value in the rightmost column was obtained by expressing the number in the Addiction column as a percentage of the number in the Ever Used column, subject to errors of rounding).
Data *source*: Anthony et al., 1994. This study was repeated in 2001–2003: see Degenhardt et al., 2007.

Many variables operate simultaneously to influence the likelihood that a person initiating drug use will lose control and develop an addiction. These variables can be organized into three categories: agent (drug), host (user), and environment (Table 28–3).

Drug Variables

Pharmacokinetics in this instance refers to the onset, duration, and intensity of drug effects. It is influenced by properties related to the drug and by factors related to the user. *The abuse liability of a drug is correlated with the rate at which drug levels peak in the body*. Routes of administration that produce more rapid and efficient absorption into the bloodstream and brain—injecting, smoking, or “snorting”—tend to result in a more intense intoxication and are more reinforcing than oral administration. When coca leaves are chewed, cocaine is absorbed slowly; this produces low cocaine levels in the blood and few, if any, behavioral problems. Crack cocaine, on the other hand, is alkaloidal cocaine (free base) that can be readily vaporized by heating. Simply inhaling the vapors produces blood levels comparable to those resulting from intravenous cocaine owing to the large pulmonary surface area for absorption into the circulation following inhalation. Thus, inhalation of crack cocaine is much more addictive than chewing, drinking, or sniffing cocaine. The risk for developing addiction among those who try nicotine is about twice that for those who try cocaine (Table 28–4). This does not imply that the pharmacological addiction liability of nicotine is twice that of cocaine. Rather, the combination of variables listed in Table 28–3 in the categories of the Agent (e.g., mode of administration), Host, and Environment influence the development of addiction. For example, not only is nicotine smoked, which provides a rapid brain uptake, but nicotine is more accessible in society and can be administered more frequently than cocaine, thus increasing its addiction potential.

The influence of pharmacokinetics is fittingly illustrated by methylphenidate (MPH), the stimulant drug prescribed in attention-deficit/hyperactivity disorder (ADHD). Intravenous administration of MPH increases extracellular DA in the brain at a higher rate than cocaine and has similar reinforcing effects (i.e., self-report of feeling “high”) as cocaine. The intravenous peak plasma concentration occurs almost immediately after dosing. In contrast, oral administration of MPH medication reaches peak plasma levels at a much lower speed and is rarely perceived as reinforcing, even though peak levels are eventually as high as after intravenous administration (Volkow et al., 2003). The recent availability of slow-release formulations of amphetamine compounds for the treatment of ADHD are another approach to prevent misuse of this group of drugs.

Host (User) Variables

Effects of drugs vary among individuals. Differences in genes that encode enzymes involved in drug transport, metabolism, elimination, and receptor-mediated responses may contribute to different effects of the drug across the addiction cycle (e.g., euphoria, reinforcement). For example, innate tolerance to alcohol due to enhanced metabolism may represent a biological trait that contributes to the development of alcoholism. While innate tolerance increases vulnerability to alcoholism, impaired metabolism may *protect* against it. Similarly, individuals who inherit a gene associated with slow nicotine metabolism may experience unpleasant effects when beginning to smoke and reportedly have a lower probability of becoming nicotine dependent (Thorgeirsson et al., 2010).

Psychiatric disorders constitute another category of host variables. People with anxiety, depression, insomnia, or even shyness may find that certain drugs give them relief (see Drug Summary Table). Alcohol and cannabis use are examples of self-medication. However, the apparent beneficial effects can be transient, and misuse of a drug may lead to tolerance and eventually compulsive, uncontrolled drug use. While psychiatric symptoms are seen commonly in drug abusers presenting for treatment, many of these symptoms begin *after* the person starts abusing drugs. Thus, drugs of abuse can produce more psychiatric symptoms than they relieve.

Age at first exposure is another critical factor. The majority of adults who end up with AUD or SUD had their first exposure to alcohol or drugs during adolescence. While the early initiation of drug taking could be part of an innate adolescent behavioral pattern such as risk-taking, the effect of drug exposure on the still-developing brain can facilitate use disorders and psychiatric disorders.

Drug use and vulnerability can differ between the sexes. Men are more likely than women to abuse illicit drugs and to overdose on drugs. However, females are as likely as males to develop SUDs and may be more susceptible to craving and relapse.

Environmental Variables

Initiating and continuing illegal drug use is influenced significantly by societal norms and peer pressure. A permissive environment, easy access to drugs, and community attitudes influence substance abuse. Drug policies such as marijuana legalization have the potential to increase SUDs, a viewpoint expressed in the theory of "gateway" drugs. However, while cannabis use in the states that legalized recreational marijuana did increase, there is no proven connection with other substance use trends (Figure 28–5).

People with adverse childhood experiences appear to account for a half to two-thirds of serious problems with drug use (Dube et al., 2003). Recent stressful situations can also lead to the initiation of drug taking, as well as cause drug reinstatement in abstinent persons with former SUDs. Similarly, environmental cues related to drug taking or re-exposure to small amounts of drugs or alcohol can lead to relapse.

Government Agencies Involved in Data Collection, Substance Use Disorder Research, and Drug Regulations

Much of what we know about drug use in the U.S. is collected by federal agencies or by projects funded by these agencies. The latest data are found on their websites, which provide access to current trends on a daily, weekly, and annual basis, including information on changing trends and novel illicit drugs that enter the U.S. market.

Synthetic cannabinoids and synthetic cathinones (e.g., ethylone) are the most common classes of new psychoactive substances, although opioids, phenethylamines, tryptamines, benzodiazepines, and piperazines are also of concern. With the influx of novel drugs and changes in the political landscape, trends are constantly changing (DEA Strategic Intelligence Section, 2021). For example, among people aged 12 or older, the percentage who used marijuana in the past year increased from 11.0% in 2002 to 17.5% in 2019. Over the same period, the percentage who used cocaine decreased from 2.5% to 2.0% (Substance Abuse and Mental Health Services Administration, 2020). According to the CDC, 70,630 drug overdose deaths occurred in the U.S. in 2019, accounting for 21.6 deaths per 100,000 persons. This number rose to approximately 85,000 drug overdose deaths in 2020, during the COVID-19 pandemic (Ahmad et al., 2021) (Figure 28–6). Drug overdose deaths are particularly driven by opioids and synthetic opioids. These data do not include deaths from excessive alcohol use, which results in around 29 deaths per 100,000. There can be no doubt that SUDs are a significant public health problem in the U.S. and that healthcare professionals and pharmacologists need timely information to make informed decisions about treatment and prevention of SUDs.

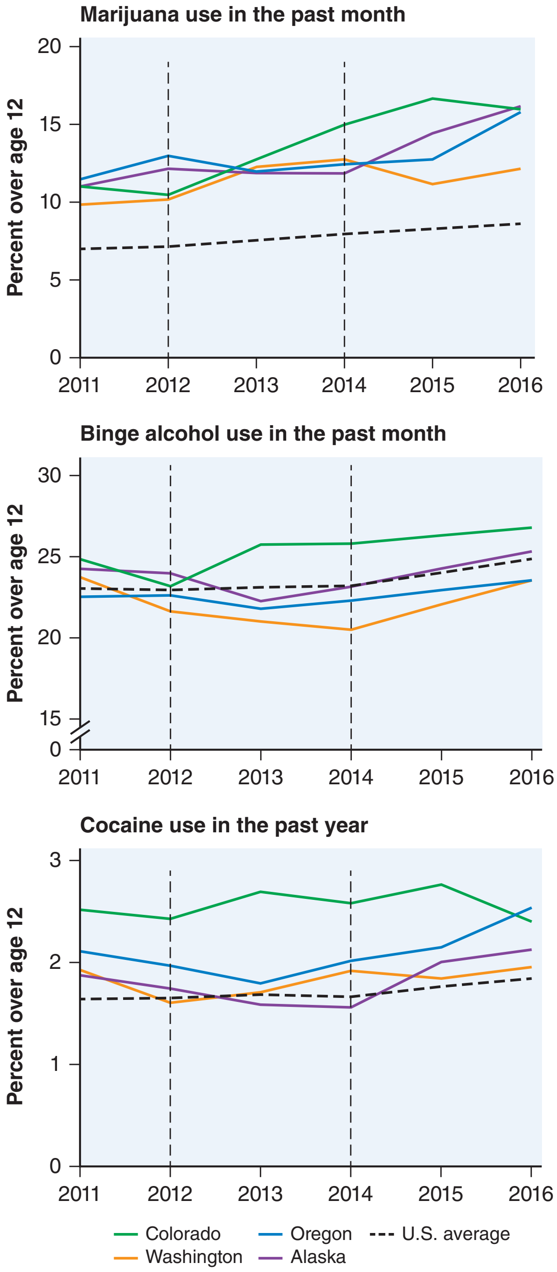


Figure 28–5 *No remarkable effects of marijuana legalization in four states on cocaine use and binge alcohol drinking.* Marijuana use was legalized in Colorado and Washington in 2012 and in Oregon and Alaska in 2014. Population age 12 and older was surveyed. Marijuana use and binge alcohol use were surveyed for 1 month of the year indicated, and cocaine use was surveyed for the entire year. Marijuana use was above the U.S. average in states that legalized recreational use and increased further after legalization. Binge alcohol use stayed within the U.S. national average, while cocaine use did not significantly change. (Data from Substance Abuse and Mental Health Services Administration, 2020.)

National Institute on Drug Abuse (NIDA) and National Institute on Alcohol Abuse and Alcoholism (NIAAA)

The National Institute on Drug Abuse (NIDA) supports research on drug use and its consequences (https://www.drugabuse.gov/). This includes understanding how drugs work in the brain and throughout the body, developing and testing new SUD treatment and prevention approaches, and tracking emerging drug use trends. NIDA funds the National Drug Early Warning System (NDEWS), a source with the most up-to-date

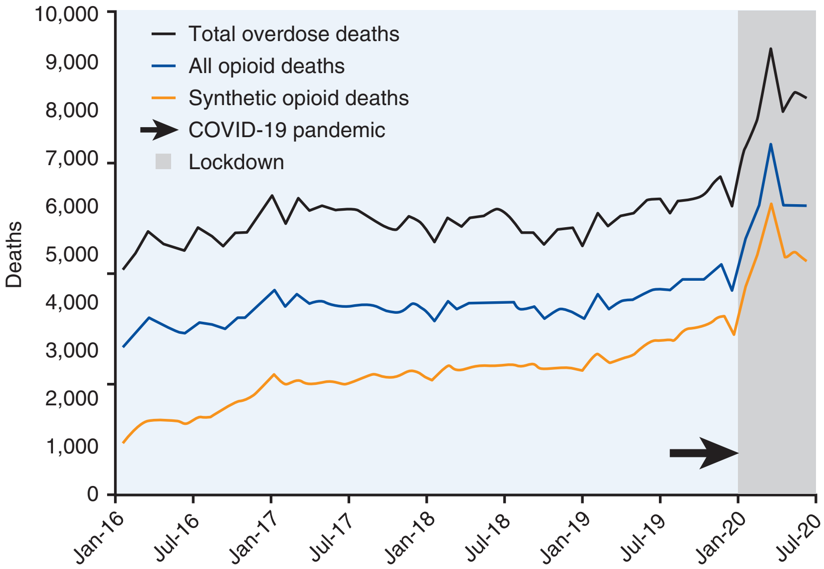


Figure 28–6 *The impact of the COVID-19 pandemic on overdose deaths in the U.S.* Numbers are fatalities per time unit. (Data from Ahmad et al., 2021.)

information on emerging substance use trends (Cottler et al., 2020). On the NDEWS website, pharmacologists can find information about novel psychoactive substances emerging in communities throughout the U.S. AUDs, though arguably among SUDs, are handled through a different entity, the National Institute on Alcohol Abuse and Alcoholism (NIAAA; https://www.niaaa.nih.gov/). AUD-relevant information is found on the NIAAA website, including the National Epidemiologic Survey on Alcohol and Related Conditions.

Substance Use and Mental Health Services Administration (SAMHSA)

SAMHSA, an agency within the U.S. Department of Health and Human Services, has the mission of reducing the impact of substance abuse and mental illness on U.S. communities and to improve SUD prevention, treatment, and recovery services (https://www.samhsa.gov/). Through the National Survey on Drug Use and Health, SAMHSA provides up-to-date information on tobacco, alcohol, and drug use (SAMHSA, 2020). Data collected in 2019 showed that 13.0% of people aged 12 or older (35.8 million people) used an illicit drug in the past month.

U.S. Drug Enforcement Administration (DEA), Controlled Substances Act, and Drug Schedules

The DEA, a branch of the Department of Justice, is charged with enforcing the controlled substances laws (https://www.dea.gov/). Drugs, other substances, and certain chemicals used to make drugs are classified into five distinct categories or schedules (see Table 28–1). Classification takes into consideration a drug's approved medical use and its abuse potential; new substances are added on a regular basis. The DEA publishes the annual National Drug Threat Assessment (NDTA), a comprehensive assessment of the threat posed to the U.S. by illicit drugs (DEA Strategic Intelligence Section, 2021). The NDTA also combines SUD-relevant information from the CDC, such as overdose deaths by specific drugs. Moreover, in its annual update, it lists new psychoactive substances that have entered the U.S.

Clinical and Pharmacological Topics: CNS Depressants

Alcohol is the most commonly used CNS depressant. Persons with SUDs frequently use multiple drugs in combination, most often in combination with ethanol, leading to combined SUD and AUD (see Figure 28–4). When confronted with a patient exhibiting signs of overdose or withdrawal, these possible combinations will require different and specific treatments.

Alcohol (Ethanol) and Alcohol Use Disorder

More than 90% of American adults report experience with ethanol ("alcohol"), and over 50% report alcohol intake in the past month (Figure 28–7) (SAMHSA, 2020). While SAMHSA's annual numbers of AUD in persons 12 years and older are between 5% and 6%, studies in persons 18 years and older find even higher numbers and a lifetime prevalence of 29.1% (Grant et al., 2015). Alcohol use continues to be one of the deadliest substances, with liver disease and alcohol-related accidents causing approximately 95,000 deaths every year.

Ethanol is classified as a depressant because it produces sedation and sleep. However, the initial effects of alcohol, particularly at lower doses, are often perceived as stimulating owing to a suppression of inhibitory systems. Heavy use of ethanol causes the development of tolerance and physical dependence sufficient to produce an alcohol withdrawal syndrome when intake is stopped (Table 28–5).

Tolerance, Physical Dependence, and Withdrawal

The symptoms of mild intoxication by alcohol vary among individuals. Some experience motor incoordination and sleepiness. Others initially become stimulated. As the blood level increases, the sedating effects increase, with eventual coma and death occurring at high blood alcohol levels. The innate tolerance to alcohol varies greatly among individuals and is often traced to a family history of alcoholism (Hu et al., 2005). Experience with alcohol can produce greater tolerance (acquired tolerance) such that extremely high blood levels (300–400 mg/dL) may occur in alcoholics who do not appear grossly sedated. In these cases, the lethal dose does not increase proportionately to the sedating dose; thus, the margin of safety is decreased.

The development of tolerance and dependence by heavy drinkers often leads to drinking in the morning to restore blood alcohol levels diminished during the night. The alcohol withdrawal syndrome generally depends on the amount of the average daily dose and usually is "self-medicated" by resumption of alcohol ingestion. Withdrawal symptoms are experienced frequently but usually are not severe or life threatening unless they occur in conjunction with other problems, such as infection, trauma, malnutrition, or electrolyte imbalance. In the context of such complications, the syndrome of *delirium tremens* becomes likely, especially after a long period of heavy drinking in people with an AUD, which under the most severe situation can lead to seizures and death.

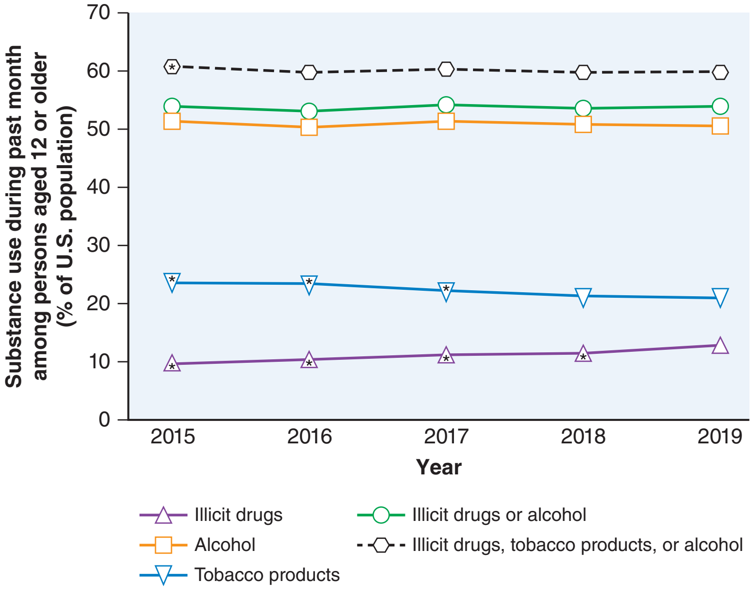


Figure 28–7 *Experience with substance use during 1 month annually among persons aged 12 or older in the U.S. from 2015 to 2019.* Asterisk denotes the difference to 2019 at $p \leq 0.05$. (Data from Substance Abuse and Mental Health Services Administration, 2020; data retrieved June 4, 2021.)

AUD produces cross-tolerance to other sedatives, such as benzodiazepines. This tolerance is present in abstinent alcoholics, but while drinking, it can enhance the properties of other sedatives. This is particularly true for benzodiazepines, which are relatively safe when given alone but are potentially lethal in combination with alcohol. The chronic use of alcohol and other sedatives is associated with the development of depression and the risk of suicide. Cognitive deficits have been reported in individuals with AUD even when tested while sober. These deficits usually improve with abstinence. More severe memory impairments are caused by neurological disorders due to nutritional deficiencies (e.g., thiamine deficiency) common in those with excessive alcohol consumption. Medical complications of alcohol abuse and dependence include liver disease, cardiovascular disease, endocrine and gastrointestinal effects, and malnutrition, in addition to the CNS dysfunctions noted above. Ethanol readily crosses the placental barrier, producing fetal alcohol syndrome that can lead to intellectual disability in the offspring.

TABLE 28–5 ■ ALCOHOL WITHDRAWAL SYNDROME

Alcohol craving
Tremor, irritability
Nausea
Sleep disturbance
Tachycardia
Hypertension
Sweating
Perceptual distortion
Seizures (6–48 h after last drink)
Visual (and occasionally auditory or tactile) hallucinations (12–48 h after last drink)
Delirium tremens (48–96 h after last drink; rare in uncomplicated withdrawal)
- *Severe agitation, confusion*
- *Fever, profuse sweating*
- *Tachycardia, dilated pupils*
- *Nausea, diarrhea*

Data from Substance Abuse and Mental Health Services Administration, 2020.

Pharmacological Interventions

For treating AUD, the American Psychiatric Association recommends that treatment be initiated after a thorough clinical evaluation for co-occurring conditions and supplemented with behavioral therapies.

Detoxification. Although most mild cases of alcohol withdrawal never come to medical attention, severe cases require general evaluation; attention to hydration and electrolytes; vitamins, especially high-dose thiamine; and a sedating medication that has cross-tolerance with alcohol. To block or diminish the symptoms described in Table 28–5, a short-acting benzodiazepine such as *oxazepam* can be used at a dose of 15 to 30 mg every 6 to 8 h according to the stage and severity of withdrawal. However, benzodiazepines should be avoided in AUD beyond treatment of acute withdrawal (Reus et al., 2018). Anticonvulsants such as *carbamazepine* also have been shown to be effective in alcohol withdrawal.

Pharmacotherapy. Detoxification is the immediate goal, and doing so in a medically controlled setting will reduce negative effects of the withdrawal syndrome. Complete abstinence is the objective of long-term treatment, and this is best accomplished by a combination of relapse prevention, anticraving medication, and cognitive behavioral therapy. Various pharmacotherapies are available for treating AUD after detoxification. Of these, three are FDA-approved for AUD: *disulfiram*, *naltrexone*, and *acamprosate*.

Disulfiram has been useful in some programs that focus behavioral efforts to promote ingestion of the medication. *Disulfiram* blocks aldehyde dehydrogenase (see Chapter 27), resulting in the accumulation of acetaldehyde, which produces an unpleasant flushing reaction and nausea when alcohol is ingested. Knowledge that this unpleasant reaction will ensue may help the patient to resist the urge to resume drinking alcohol. However, *disulfiram* has not proven to be effective in controlled clinical trials because so many patients choose to stop taking the medication rather than the alcohol.

Naltrexone, an opioid receptor antagonist, blocks the endorphin activation properties of alcohol. *Naltrexone* is available in both PO (25, 50, or 100 mg) and extended-release IM formulations (e.g., Vivitrol; 380 mg IM injected every 4 weeks). Chronic administration of *naltrexone* decreases the rate of relapse to heavy drinking in randomized clinical trials. The effects varied from strong to weak, but overall, reduction in heavy drinking is a consistent finding (de Laat et al., 2021). *Naltrexone* works best in combination with behavioral treatment programs that encourage

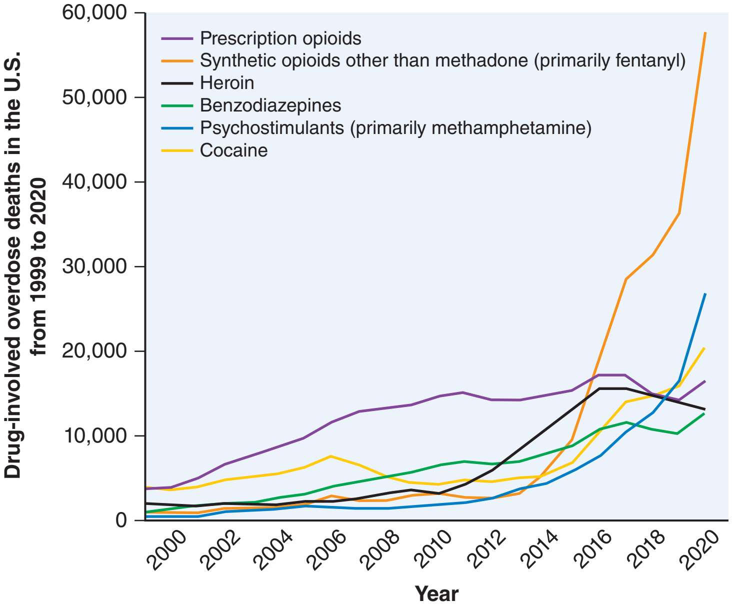


Figure 28–8 *Annual drug-involved overdose deaths in the U.S. between 1999 and 2020.* (Data retrieved from Centers for Disease Control and Prevention, National Center for Health Statistics. Multiple Cause of Death 1999–2020 on CDC WONDER Online Database, released in 2021. Data are from the Multiple Cause of Death Files, 1999–2020, as compiled from data provided by the 57 vital statistics jurisdictions through the Vital Statistics Cooperative Program. Accessed at http://wonder.cdc.gov/mcd-icd10.html on December 23, 2021.)

adherence to medication and abstinence from alcohol. *Naltrexone* is also approved for the treatment of opioid use disorder (OUD; see below).

Acamprosate, a mixed-acting antagonist at NMDA (*N*-methyl-D-aspartate) receptors and a positive allosteric modulator of $GABA_A$ receptors, appears to normalize the dysregulated neurotransmission associated with chronic ethanol intake, thereby attenuating one of the mechanisms that lead to relapse. *Acamprosate* is administered PO in delayed-release formulation (two 333-mg tablets three times a day). *Acamprosate* may also have neuroprotective actions.

Gabapentin has been studied off-label as an aid in the transition to the abstinent state, possibly by improvement in sleep and mood disturbance (PO 900–1800 mg/day). *Gabapentin,* also used as an anticonvulsant, indirectly reduces NMDA glutamate receptor function by disrupting voltage-dependent Ca^{2+} channels (see Chapters 16 and 20).

Topiramate, an anticonvulsant that inhibits carbonic anhydrase, can inhibit the reinforcing effect of alcohol. Like other therapeutic agents used in AUD, *topiramate* facilitates $GABA_A$ activity and suppresses glutamatergic activity.

Opioids and Opioid Use Disorder

Opioid agonists activate the MOR in pain pathways and are strong analgesics (see Chapter 23). They also stimulate the reward pathway, which makes them liable for misuse. Because of the high plasticity within the opioid system and the rapid adaptation to opioid exposure, these drugs have a high allostatic load (see Figure 28–3). Importantly, opioids inhibit the respiratory center in the brain, which explains the high risk of lethal overdose associated with their use.

Opioid Overdose

Nearly 70% of all drug overdose deaths in the U.S. in 2018 involved an opioid (DEA Strategic Intelligence Section, 2021; Wilson et al., 2020). The purity of street heroin in the U.S. has increased over the past decade from about 4% (4 mg heroin per 100-mg bag; range 0–8 mg/100 mg; the rest was nonopioid filler such as quinine) to a purity of 45% to 75% (45–75 mg heroin per 100-mg bag), with some samples testing as high as 90%. This increase in purity has led to increased levels of physical dependence among heroin addicts. Users who interrupt regular dosing now develop more severe withdrawal symptoms. The more potent supplies can be smoked or administered nasally (snorted), making heroin use accessible to people who would not insert a needle into their veins.

While deaths involving heroin have decreased by 4%, deaths due to other synthetic opioids such as *fentanyl* and *fentanyl analogues* are on the rise (Wilde et al., 2019) (Figure 28–8). *Fentanyl* is extremely powerful, 50 to 100 times more potent than *morphine* and 10 times more potent than heroin. *Fentanyl* is sometimes added to illicit drugs such as cocaine and to counterfeit "pharmaceutical" pills. Because of its extreme potency, *fentanyl* is a significant contributor to the increasing rates of overdose deaths in the U.S.

The most troubling issue with opioid dependence was initiated by changes in medical practice. At the turn of the 21st century, increasing interest in minimizing pain combined with intense marketing by the pharmaceutical industry changed prescription opioid practices. In some cases, clear overprescribing, especially of extended-release formulations of *oxycodone*, led to an epidemic of abuse, addiction, and overdose deaths (see Figure 28–8). Sales of *oxycodone* and *hydrocodone* peaked in 2012 (Figure 28–9), and opioid overdoses became a common cause of death in many communities. In the absence of FDA action, the CDC stepped in with prescribing guidelines (Dowell et al., 2016). States followed with prescription drug monitoring programs, electronic databases that track controlled substance prescriptions in each state.

With the stringent control of opioid prescriptions, heroin and other illegal synthetic opioids became the drugs of choice. Although there is no legal supply of heroin in the U.S., the drugs are brought into the country by transnational criminal organizations. Use of illegal opioids has increased significantly over the past 20 years, and a concomitant increase in overdose deaths has been observed. This public health crisis created by a combination of good intentions, financial opportunities, inadequate critical evaluation, and insufficient regulatory oversight serves as a cautionary tale how to approach and evaluate the opportunities and downsides of pharmacological therapies and treatments.

Opioid Tolerance, Dependence, and Withdrawal

Injection of a potent opioid produces a high and intense pleasure ("rush") often compared with sexual orgasm. There are some differences among the opioids in their acute effects; for instance, *morphine* and *codeine* produce a more prominent histamine-releasing effect (causing itching), whereas *meperidine* is notable for producing excitation or confusion.

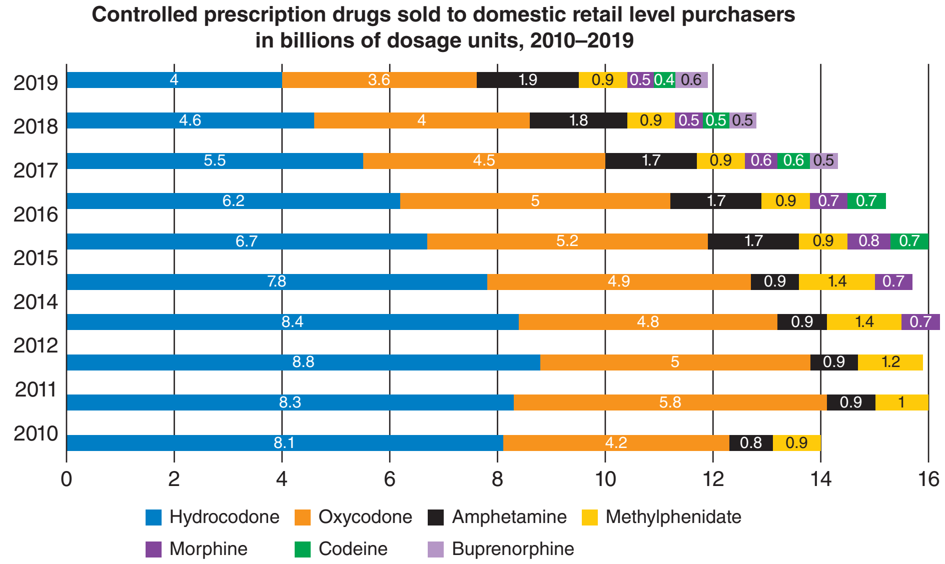


Figure 28–9 *Controlled prescription drugs sold to domestic retail level purchasers in billions of dosage units.* (Data from U.S. Drug Enforcement Administration, 2020 National Drug Threat Assessment, https://www.dea.gov/documents/2021/03/02/2020-national-drug-threat-assessment)

Even experienced opioid addicts, however, cannot distinguish between intravenously administered heroin and the common opioid *hydromorphone*, often used for pain management in hospitalized patients.

Heroin has high lipid solubility, crosses the blood-brain barrier quickly, and is deacetylated to the active metabolites 6-monoacetyl morphine and morphine. After the intense euphoria, which lasts from 45 sec to several minutes, there is a period of sedation and tranquility ("on the nod") lasting up to an hour. The effects of heroin wear off in 3 to 5 h, depending on the dose. Experienced users may inject two to four times daily. Thus, the heroin-addicted individual is constantly oscillating between being "high" and feeling the sickness of early withdrawal (as depicted in Figure 28–10). This produces many problems in the homeostatic systems regulated at least in part by endogenous opioids.

Tolerance develops early to the euphoria-producing effects of heroin and other opioids (Figure 28–3). While there is tolerance to the respiratory depressant, analgesic, sedative, and emetic properties, the levels of tolerance in the different systems vary. Thus, while increasing doses are needed to maintain a level of euphoria, reduced adaptations of the respiratory system to increasing doses can lead to death. Overdose is likely to occur when potency of the street sample is unexpectedly high or when the heroin is mixed with a more potent synthetic opioid such as *fentanyl*.

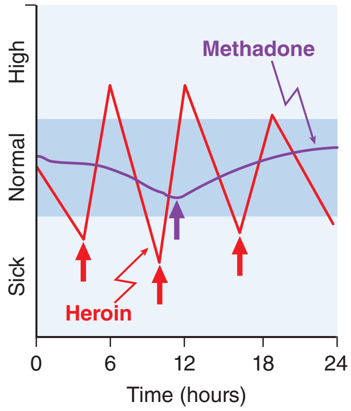


Figure 28–10 *Comparative time courses of response to heroin and methadone.* A person who injects heroin (↑) several times per day oscillates between being sick and being high (red line). In contrast, a *methadone* patient (purple line) remains in the "normal" range (blue band) with little fluctuation after dosing once per day. Ordinate values represent the subject's mental and physical state, not plasma levels of the drug.

Addiction to heroin or other short-acting opioids produces behavioral disruptions and usually becomes incompatible with a productive life. Chronic heroin use is associated with pulmonary infections (especially tuberculosis), and when injected, it is associated with bacterial infections, skin abscesses, endocarditis, and viral infections such as hepatitis C and AIDS (acquired immunodeficiency syndrome).

Opioids are frequently used in combination with other drugs, such as heroin and cocaine ("speedball"). Users report improved euphoria with that combination, and there is evidence of reducing negative side effects of the other substance. For example, cocaine can reduce the signs of opiate withdrawal, and heroin may reduce the irritability seen in chronic users of cocaine.

Pharmacological Interventions for Opioid Use Disorders

Withdrawal and Detoxification. The first stage of treatment generally addresses physical dependence and detoxification. The opioid withdrawal syndrome (Table 28–6), though unpleasant, is not life threatening. It begins within 6 to 12 h after the last dose of a short-acting opioid and as long as 72 to 84 h after a long-acting opioid medication. The duration and

TABLE 28–6 ■ CHARACTERISTICS OF OPIOID WITHDRAWAL

SYMPTOMS	SIGNS
Regular withdrawal	Pupillary dilation
Craving for opioids	Sweating
Restlessness, irritability	Piloerection ("gooseflesh")
Increased sensitivity to pain	Tachycardia
Nausea, cramps	Vomiting, diarrhea
Muscle aches	Increased blood pressure
Dysphoric mood	Yawning
Insomnia, anxiety	Fever
Protracted withdrawal	Cyclic changes in weight, pupil size, respiratory center sensitivity
Anxiety	
Insomnia	
Drug craving	

Data from Ahmad et al., 2021.

intensity of the syndrome relate to the $t_{1/2}$ of the agent. Thus, heroin withdrawal is brief (5–10 days) and intense, whereas *methadone* withdrawal is slower in onset and longer-lasting.

Opioid withdrawal signs and symptoms are normally treated pharmacologically. The most common approach consists of the transfer to a medically managed prescription opioid medication and then gradual dose reduction. Addiction to short-acting opioids such as heroin is most frequently treated with a long-acting opioid such as *methadone*, a full MOR agonist. The initial dose of *methadone* for the purposes of withdrawal is typically 20 to 30 mg. The first day's total dose can be determined by the response and then reduced by 20% per day during the course of detoxification. Although the duration of the *methadone* detoxification regimen is not correlated to better long-term outcomes, long-term maintenance of *methadone* treatment to prevent relapse and allow restoration of social connections is associated with better outcomes. *Buprenorphine*, a long-acting partial agonist with high affinity to the MOR, is also used to induce detoxification. Initial *buprenorphine* sublingual dose is typically 2 mg, and if well tolerated, subsequent 2-mg doses are administered every 1 to 2 h, titrating up to a total dose of 8 mg with gradual tapering during the course of detoxification.

A second approach during detoxification involves the use of oral *clonidine*, an α_2 adrenergic agonist that decreases adrenergic neurotransmission from the locus coeruleus. This medication is approved for the treatment of hypertension but is commonly used off-label to reduce symptoms of opioid withdrawal (0.1–0.3 mg PO every hour up to four doses). Many of the autonomic symptoms of opioid withdrawal, such as rhinorrhea and lacrimation, result from the loss of opioid suppression of the locus coeruleus system during the abstinence syndrome. *Clonidine* can alleviate many of these symptoms, although not the generalized aches and opioid craving. *Lofexidine*, a similar medication, is FDA-approved for use as an opioid withdrawal suppressant and administered PO at 3.2 mg/day split into four doses. With *clonidine* and *lofexidine*, the dose must be titrated according to the stage and severity of withdrawal; postural hypotension is a common side effect.

A third strategy for treating opioid withdrawal involves activation of the endogenous opioid system without medication. The techniques proposed include acupuncture and several methods of CNS activation such as transcranial magnetic stimulation, transcranial direct current stimulation, and auricular vagus nerve stimulation. These approaches may have therapeutic benefits by directly or indirectly modulating the neurocircuitry affected in OUD. While attractive theoretically, these strategies are not yet validated or currently implemented in the clinic.

Long-Term Management of OUDs. While in-patient detoxification is an important first start for treating OUDs, the probability of a quick return to compulsive opioid use is high if patients are discharged from the hospital after withdrawal from opioids without follow-up care. Numerous factors influence relapse. The withdrawal syndrome does not end in 5 to 7 days; a *protracted withdrawal syndrome* (see Table 28–6) can persist for up to 6 months. Physiological measures tend to oscillate, and the allostatic changes leave an extended mark of anxiety, insomnia, and drug craving (see Figure 28–3); during this phase, outpatient drug-free treatment has a very low probability of success, even when the patient has received intensive prior treatment while protected from relapse in a residential program.

Three medications have been approved for long-term OUD management by the FDA: *methadone*, *buprenorphine*, and extended-release *naltrexone*.

Opioid Agonist Treatment. Stabilization and maintenance on *methadone* is considered the most successful treatment of heroin addiction. *Methadone* is available as an oral solution, injectable solution, and tablet. Oral *methadone* has the strongest evidence for effectiveness and has a $t_{1/2}$ of 22 to 24 h. It is typically given as a liquid, with a starting dose of 20 mg/day and titrated up in increments to 60 to 80 mg/day. Patients who relapse repeatedly during drug-free treatment can be transferred directly to *methadone* without requiring detoxification. While treatment with *methadone* has the risk of diversion and misuse of medication, it is a safer alternative to short-acting potent opioids.

The introduction of *buprenorphine* represented a major change in the treatment of OUD. This medication produces minimal withdrawal symptoms when discontinued, has a low potential for overdose, and has a long duration of action. As a partial agonist, *buprenorphine* has ceiling effects on respiratory depression and thus is considered safer than *methadone* for agonist substitution treatment. However, it can precipitate withdrawal and patients need to be abstinent from other opioids before treatment is initiated. The period of abstinence is determined by the $t_{1/2}$ of the opioid the patient has been using. *Buprenorphine* is available as transmucosal tablets or film strips (4–16 mg/day). This is the first OUD treatment that can be prescribed or dispensed in a qualified physician's private office (upon obtaining a special federal waiver) rather than in a specialized clinic, as required for *methadone*. Although *buprenorphine* has a mild addictive potential, it can still be misused if it is snorted as a powder or dissolved and injected. As a solution to this problem, a combination of *buprenorphine* with *naloxone*, a short-acting MOR antagonist, is available. The oral bioavailability of *naloxone* is low (1%–3%), and thus, when *naloxone* is taken orally (sublingually), it is not effective; however, if the patient misuses the medication by injecting a solution of it, the *naloxone* component will block or diminish the subjective high that could be produced by *buprenorphine* alone. An alternative to daily *buprenorphine* is extended-release depot *buprenorphine*, which has shown greater patient satisfaction (Lintzeris et al., 2021).

Interestingly, supervised injectable opioid treatment (e.g., prescribed pharmaceutical heroin) is available in Canada and some European countries but not in the U.S. (Bell et al., 2020).

Opioid Antagonist Treatment. *Naltrexone* is an antagonist with a high affinity for MOR; it will competitively block the effects of heroin and other MOR agonists and is approved for both opioid dependence and alcohol dependence (see section on AUD). *Naltrexone* will not satisfy craving or relieve protracted withdrawal symptoms but will reduce opioid intake and overdose. An extended-release injectable depot version of *naltrexone* is available, since oral *naltrexone* has had limited effectiveness (Lott, 2018). As an MOR antagonist, *naltrexone* will precipitate withdrawal, so individuals who are being treated with *naltrexone* must abstain from all opioids for approximately 7 days before the treatment is initiated.

Naloxone, a full MOR antagonist, has a rapid onset of action that is useful as an emergency measure to acutely treat opioid overdoses. It is available as an injectable (0.4 and 1 mg/mL) and as a nasal spray (4 or 8 mg of *naloxone*) that can be administered even by persons not medically trained. *Naloxone* precipitates opioid withdrawal, which is unpleasant (Table 28–6) but lifesaving by reversing respiratory depression. Persons in opioid-induced respiratory distress must receive medical help since multiple doses of *naloxone* might be required over 24 h until opioid levels in the system decline. The *naloxone* will be competing against an unknown quantity of an MOR agonist; the $t_{1/2}$ of *naloxone* is 60 to 90 min, shorter than the half-lives of the effects of the abused opiates (see Chapter 23). This consideration is particularly critical with overdoses of highly potent opioid agonists such as *fentanyl*. Once a patient in overdose arrives at a medical center, the recommended dosing schedule with *naloxone* is 0.4 or 1 mg IV; dosing might require multiple administrations or continuous infusion (2.5 μg/kg/h) to prevent the occurrence of respiratory depression.

Benzodiazepines

Benzodiazepines, commonly used as sedatives and hypnotics (see Chapters 16 and 22), are, under some circumstances, also used acutely for alcohol detoxification. These agents are widely prescribed, and their abuse is not uncommon. Addiction to benzodiazepines is considered a "hypnotic, sedative, or anxiolytic use disorder." The proportion of patients who become tolerant and physically dependent on benzodiazepines increases after several months of use, and abrupt reduction of the dose or stopping the medication produces withdrawal symptoms (Table 28–7). Drug poisoning deaths by benzodiazepines are on the

TABLE 28–7 ■ BENZODIAZEPINE WITHDRAWAL SYMPTOMS

Following moderate-dose usage

- Anxiety, agitation
- Increased sensitivity to light and sound
- Paresthesias, strange sensations
- Muscle cramps
- Myoclonic jerks
- Sleep disturbance
- Dizziness

Following high-dose usage

- Seizures
- Delirium

decline (see Figure 28–7), but the number of deaths involving the combination of benzodiazepines and opioids has increased, likely due to the fact that both types of drugs suppress respiratory drive.

It can be difficult to distinguish benzodiazepine withdrawal symptoms from the reappearance of the anxiety symptoms for which the benzodiazepine was originally prescribed. Some patients may increase their dose over time as tolerance develops to the sedative effects. Antianxiety benefits, however, seem to continue long after tolerance to the sedating effects has developed. Other patients take the medication for years according to medical directions and function effectively because they take the medication. Patients with a history of alcohol or other drug abuse problems have an increased risk for the development of benzodiazepine abuse and should rarely, if ever, be treated with benzodiazepines on a chronic basis.

Pharmacological Interventions

Patients on long-term prescribed benzodiazepine treatments who wish to stop their medication are able to do so in an outpatient setting, but the process may take months of gradual dose reduction. Withdrawal symptoms may occur, but in most cases, the symptoms are mild. Patients who have been on low doses of benzodiazepines for years usually have no adverse effects. If symptoms of anxiety return, a nonbenzodiazepine such as *buspirone* may be prescribed. Some authorities recommend transferring the patient to a benzodiazepine with a long $t_{1/2}$ during detoxification; others recommend the use of anticonvulsants *carbamazepine* and *phenobarbital*. Intravenous administration of the selective $GABA_A$ receptor antagonist *flumazenil* is useful as an antidote in the treatment of benzodiazepine overdose and in reversing the postsurgical effects of long-acting benzodiazepines used as anesthetics.

Abusers of high doses of benzodiazepines usually require inpatient detoxification. Frequently, benzodiazepine abuse is part of a combined dependence involving alcohol, opioids, and cocaine. Detoxification can be a complex clinical pharmacological challenge requiring knowledge of the pharmacokinetics of each drug. One approach to complex detoxification is to focus on the nonopioid components while temporarily holding the opioid component constant with a low dose of *methadone* or *buprenorphine*. A long-acting benzodiazepine such as *diazepam* or *clorazepate* or a long-acting barbiturate such as *phenobarbital* can be used to block the sedative withdrawal symptoms. After detoxification, the prevention of relapse requires long-term outpatient rehabilitation similar to the treatment of alcoholism. No specific medications have been found to be useful in the rehabilitation of sedative abusers; specific psychiatric disorders such as depression or schizophrenia, if present, require appropriate medications.

Barbiturates

Barbiturates have sedative-hypnotic, anticonvulsant, anesthetic, and respiratory depressant effects. Barbiturates facilitate the activity of $GABA_A$ receptors at allosteric sites that are distinct from the benzodiazepine binding site (see Figure 16–11). Because of their higher abuse and overdose potential, barbiturates are less commonly prescribed than benzodiazepines. Abuse problems with barbiturates resemble those seen with benzodiazepines in many ways, and treatment of abuse and addiction to barbiturates should be handled similarly to interventions for the abuse of alcohol and benzodiazepines. Because drugs in this category frequently are prescribed as hypnotics for patients complaining of insomnia, physicians should be aware of the problems that can develop when the hypnotic agent is withdrawn and of possible causes for insomnia that are treatable by other means. Insomnia is often a symptom of an underlying chronic problem, such as depression or respiratory dysfunction. Long-term prescription of sedative medications can change the physiology of sleep and should be avoided. When the sedative is stopped, there is a rebound effect with worsened insomnia. Whether from prescribed hypnotic or self-administered alcohol, medication-induced rebound insomnia requires detoxification by gradual dose reduction. Patients should be dissuaded from a bedtime drink of alcohol to relieve insomnia since alcohol can result in disordered sleep.

Nicotine

Nicotine and agents for smoking cessation are discussed in depth in Chapter 13. Because nicotine is the source of reinforcement of cigarette smoking, the most common cause of preventable death and disease in the U.S., it is arguably the most dangerous dependence-producing drug. Although more than 80% of smokers express a desire to quit, only 35% try to stop each year, and fewer than 5% are successful in unaided attempts to quit.

Tobacco (nicotine) use disorder is influenced by multiple variables. Nicotine itself produces reinforcement; users compare nicotine to stimulants such as cocaine or amphetamine, although its effects are of lower euphorigenic magnitude. To avoid the addictive effects of nicotine, one would need to smoke no more than 5 cigarettes daily. Many individuals who smoke cigarettes do not constrain themselves to such a small number. In 2019, approximately 11 million people smoked 16 or more cigarettes per day. Nicotine is absorbed readily through the skin, mucous membranes, and lungs. The pulmonary route produces discernible CNS effects in as little as 7 sec. Thus, each puff produces discrete positive reinforcement. With 10 puffs per cigarette, the 1-pack-per-day smoker reinforces the habit 200 times daily.

In dependent smokers, the urge to smoke correlates with a low blood nicotine level, as though smoking were a means to achieve a certain physiological nicotine level to avoid nicotine withdrawal symptoms (Table 28–8). As with other drugs of abuse, nicotine withdrawal involves both physical and mood-related symptoms such as craving, anxiety, depression, insomnia, constipation, or diarrhea. Like with many SUDs, depressed mood (dysthymic disorder, affective disorder) is associated with tobacco use disorder, but it is not known whether depression can predispose one to begin smoking or whether depression develops secondarily during the course of nicotine dependence.

Pharmacological Interventions

The nicotine withdrawal syndrome can be alleviated by nicotine replacement therapy (e.g., nicotine inhaler and nasal spray, nicotine gum or lozenge, or nicotine transdermal patch). Different methods of nicotine delivery provide different blood nicotine levels over varying time courses

TABLE 28–8 ■ NICOTINE WITHDRAWAL SYMPTOMS

- Irritability, impatience, hostility
- Anxiety
- Dysphoric or depressed mood
- Difficulty in concentrating
- Restlessness
- Decreased heart rate
- Increased appetite or weight gain

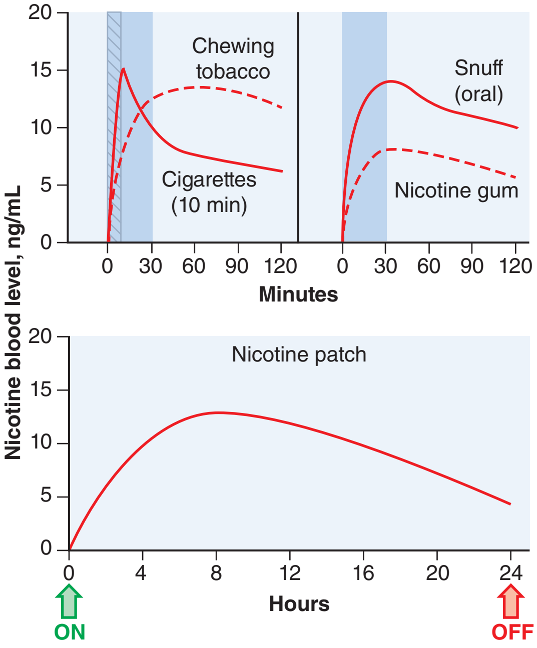


Figure 28–11 *Blood levels of nicotine resulting from different delivery systems.* In the upper panels, the shaded areas indicate the periods of nicotine delivery (30 min except for cigarettes, 10 min). In the lower panel, the arrows indicate the times of application and removal of a nicotine patch. These idealized curves are based on the findings of experiments by Benowitz et al. (1988) and Srivastava et al. (1991).

(Figure 28–11). These methods suppress the symptoms of nicotine withdrawal. Although these treatments result in more smokers achieving abstinence, most resume smoking over the ensuing weeks or months. A sustained-release preparation of the antidepressant *bupropion* (see Chapter 18) improves abstinence rates among smokers and remains a useful option. The cannabinoid CB_1 receptor inverse agonist *rimonabant* shows modest improvement of abstinence rates and reduces weight gain seen frequently in ex-smokers; unfortunately, *rimonabant* was linked to significant adverse depressive and neurological symptoms and is not approved for use in the U.S.

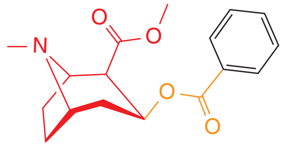
Varenicline, a partial agonist at the $\alpha_2\beta_4$ subtype of the nicotinic acetylcholine receptor, reduces cigarette craving and improves long-term abstinence rates. It has a high receptor affinity and blocks nicotine's access to the receptor. If the treated smoker relapses, there is little reward, and abstinence is more likely to be maintained. A systematic review and multiple treatment meta-analysis of various nicotine cessation replacement therapies showed *varenicline* to be the most effective over time (Mills et al., 2012). See Chapter 13 for more on the pharmacology of *varenicline*.

Clinical and Pharmacological Topics: Psychostimulants

Cocaine

In the U.S., the number of people aged 12 years or older who used cocaine in 2019 was 5.5 million (SAMHSA, 2020). Not all users, however, become addicted. Twenty percent of people who used cocaine met the criteria for SUD defined by the DSM-5 (American Psychiatric Association, 2013). A key factor in addiction is the widespread availability of relatively inexpensive cocaine in the alkaloidal form (free base, "crack"), suitable for smoking, and in the hydrochloride powder form, suitable for nasal or intravenous use.

The reinforcing effects of cocaine and its analogues correlate best with their effectiveness in inhibiting the dopamine transporter (DAT), which takes up DA from the synapse back into cells (see Figure 15–9). This leads to increased DA concentrations at brain sites mediating reward. However, cocaine also blocks both NE and 5HT reuptake, and chronic use of cocaine leads to changes in these neurotransmitter systems as well. Cocaine produces a dose-dependent increase in heart rate and blood pressure accompanied by increased arousal, improved performance on tasks of vigilance and alertness, and a sense of self-confidence and well-being. Higher doses produce euphoria, which has a brief duration and often is followed by a desire for more drug. Repeated doses of cocaine may lead to involuntary motor activity, stereotyped behavior, and paranoia. Irritability and increased risk of violence are found among heavy chronic users. The $t_{1/2}$ of cocaine in plasma is about 50 min, but inhalant (crack) users typically desire more cocaine after 10 to 30 min.



COCAINE

The major route for cocaine metabolism involves hydrolysis of its two ester groups. Tissue esterases and spontaneous hydrolysis remove the methyl ester to produce benzoylecgonine (30%–40%); removal of the benzoyl moiety by butyrylcholinesterase yields ecgonine methyl ester (~50%). Benzoylecgonine, produced on loss of the methyl group, is the major urinary metabolite and can be found in the urine for 2 to 5 days after a binge. As a result, the *benzoylecgonine test* is a valid method for verifying cocaine use; the metabolite can remain detectable in the urine of heavy users for up to 10 days.

Ethanol is frequently abused with cocaine, as it reduces the irritability induced by cocaine. Dual addiction to alcohol and cocaine is common. When cocaine and alcohol are taken concurrently, cocaine may be transesterified to cocaethylene, which is equipotent to cocaine in blocking DAT.

Addiction is the most common complication of cocaine abuse. In general, stimulants tend to be abused more irregularly than opioids, nicotine, and alcohol. Binge use is common, and a binge may last hours to days, terminating only when supplies of the drug are exhausted.

Toxicity

Risks of cocaine, beyond the potential for addiction, include cardiac arrhythmias, myocardial ischemia, myocarditis, aortic dissection, cerebral vasoconstriction, and seizures. Death from traumatic injuries is also associated with cocaine use. Cocaine may induce premature labor and *abruptio placentae*. Cocaine has been reported to produce a prolonged and intense orgasm if taken prior to intercourse, and users often indulge in compulsive and promiscuous sexual activity. However, chronic cocaine use reduces sexual drive. Chronic use is also associated with psychiatric disorders, including anxiety, depression, and psychosis.

Tolerance, Dependence, and Withdrawal

In intermittent users, the euphoric effect of cocaine is typically not subject to sensitization. In contrast, most experienced users become desensitized and, over time, require more cocaine to obtain euphoria (i.e., tolerance develops). Because cocaine typically is used intermittently, even heavy users go through frequent periods of withdrawal or "crash." The symptoms of withdrawal seen in users admitted to hospitals are listed in Table 28–9. Careful studies of cocaine users during withdrawal showed gradual diminution of these symptoms over 1 to 3 weeks. Residual depression, often seen after cocaine withdrawal, should be treated with antidepressant agents if it persists (see Chapter 18).

Pharmacological Interventions

The physiological aspects of cocaine withdrawal are generally mild. The major problem in treatment is not detoxification but helping the patient to resist the urge to resume compulsive cocaine use. At present, there are no medications approved by the FDA to treat cocaine addiction.

TABLE 28–9 ■ COCAINE WITHDRAWAL SYMPTOMS AND SIGNS

Dysphoria, depression
Sleepiness, fatigue
Cocaine craving
Bradycardia

Data from U.S. Drug Enforcement Administration, 2020 National Drug Threat Assessment, https://www.dea.gov/documents/2021/03/02/2020-national-drug-threat-assessment

Behavioral therapy is the treatment of choice, with medication indicated for specific coexisting disorders such as depression. However, researchers are exploring a variety of neuropharmacological targets. Animal models suggest that enhancing GABAergic inhibition can reduce reinstatement of cocaine self-administration, and a controlled clinical trial of *topiramate*, a medication approved to treat epilepsy and prevent migraines, showed a significant reduction in cocaine use or craving. *Topiramate* also reduced the relapse rate in individuals with AUD, prompting current studies in patients dually dependent on cocaine and alcohol (Johnson et al., 2013). *Baclofen*, a $GABA_B$ agonist, was found in a single-site trial to reduce relapse in cocaine addicts but was not effective in a multisite trial. *Modafinil*, a mild stimulant approved for treating narcolepsy and thought to be a weak inhibitor of DAT, has, in several clinical trials for use in cocaine withdrawal, reduced the euphoria produced by cocaine and relieved cocaine withdrawal symptoms (Morgan et al., 2016). *Modafinil* is also currently being tested in clinical trials as a treatment for methamphetamine, alcohol, and other SUDs.

A novel approach being considered for cocaine addiction employs a vaccine that produces anticocaine antibodies capable of preventing cocaine from reaching the brain. Conjugates of cocaine, cocaine metabolites, and cocaine analogues are rendered antigenic by conjugating them to proteins that elicit immune responses that will remove cocaine from the bloodstream. Preclinical trials and phase I trials are currently ongoing (Havlicek et al., 2020).

Amphetamine and Related Agents

Subjective effects similar to those of cocaine are produced by *amphetamine*, *dextroamphetamine*, *methamphetamine*, *phenmetrazine*, *methylphenidate*, and *diethylpropion*. Amphetamines increase synaptic DA, NE, and 5HT primarily by stimulating presynaptic release of stored neurotransmitter (see Chapter 10). Amphetamine-related agents are most often used to treat ADHD, although they do not seem to function as gateway drugs. Intravenous or smoked methamphetamine produces an abuse/dependence syndrome similar to that of cocaine, although clinical deterioration may progress more rapidly. Methamphetamine addiction has become a major public health problem in the U.S. Behavioral and medical treatments for methamphetamine addiction are similar to those used for cocaine, with behavioral therapies as the most effective treatment.

MDMA ("Ecstasy") and MDA

MDMA (3,4-methylenedioxy-methamphetamine) and MDA (methylenedioxy-amphetamine) are synthetic phenylethylamines that have stimulant as well as psychedelic effects. Acute effects are dose dependent and include feelings of energy, altered sense of time, and pleasant sensory experiences with enhanced perception. Negative effects include tachycardia, dry mouth, jaw clenching, and muscle aches. At higher doses, visual hallucinations, agitation, hyperthermia, and panic attacks have been reported. A typical oral dose is one or two 100-mg tablets, producing effects lasting 3 to 6 h, although dosage and potency of street samples are variable (~100 mg of active drug per tablet). Some people do report withdrawal symptoms that include fatigue, loss of appetite, depression, and trouble concentrating.

Caffeine

Caffeine, a mild stimulant, is the most widely used psychoactive drug in the world. It is present in soft drinks, coffee, tea, cocoa, chocolate, and numerous prescription and over-the-counter drugs.

Caffeine is an antagonist at adenosine A_{2A} receptors. It also inhibits cyclic nucleotide phosphodiesterases, mildly increases NE and DA release, and enhances neural activity in numerous brain areas. Caffeine is absorbed from the digestive tract, is distributed rapidly throughout all tissues, and easily crosses the placental barrier. Caffeine is metabolized largely by CYP1A2, with a mean biological $t_{1/2}$ of approximately 5 h, a number that can vary widely. For instance, tobacco smoking reduces the $t_{1/2}$ by approximately 40%; oral contraceptives double it; *fluvoxamine* increases caffeine's half-life 10-fold. Many of caffeine's effects are believed to be due to its competitive antagonism at adenosine A_{2A} receptors. Adenosine is a neuromodulator (see Chapter 16) that caffeine resembles structurally. The mild sedating effects that occur when adenosine activates adenosine receptor subtypes can be antagonized by caffeine. Tolerance to the stimulating effects of caffeine occurs rapidly. Thus, a mild withdrawal syndrome can be produced by abruptly discontinuing the intake of as little as one to two cups of coffee per day. Caffeine withdrawal consists of feelings of fatigue and sedation. With higher doses, headaches and nausea have been reported during withdrawal; vomiting is rare.

Clinical and Pharmacological Topics: Hallucinogens

Hallucinogens are a class of drugs that cause a distortion of reality in the user. While these chemicals are found in certain plants and mushrooms, with historical evidence of their use dating back thousands of years, many are also produced synthetically today. Hallucinogens are divided into two categories: classic hallucinogens (psychedelics such as d-lysergic acid diethylamide [LSD], peyote, psilocybin, ayahuasca, dimethyltryptamine [DMT], and 251-NBOMe) and dissociative drugs (e.g., ketamine, PCP, *Salvia divinorum*). Dissociative drugs do not produce the same psychedelic experience ("trip") as psychedelics but tend to conjure up feelings of detachment. Pharmacologically, many classical hallucinogens act on the serotonergic system, while dissociative drugs act predominantly on the glutamatergic and GABA system. Salvinorin A, the main compound in *S. divinorum*, is different and has a profound effect on KORs.

While hallucinogens are rarely addictive, they seem to play a role in triggering mental disorders such as schizophrenia, yet they are also used to treat disorders such as depression and posttraumatic stress disorder (PTSD) (Galvao-Coelho et al., 2021; Gonzalez-Maeso et al., 2009). Claims about the potential of psychedelic drugs to enhance psychotherapy and to treat addictions are currently not supported by controlled studies.

Classic Hallucinogens

LSD (d-Lysergic Acid Diethylamide)

LSD is one of the most potent hallucinogenic drugs and is greater than 3000 times more potent than mescaline. LSD is sold on the illicit market in a variety of forms. A popular contemporary system involves postage stamp–size papers impregnated with varying doses of LSD (50–300 μg or more). The drug in these papers is absorbed through the oral epithelium.

Effects of hallucinogenic drugs are variable, even in the same individual on different occasions. LSD is absorbed rapidly after oral administration, with effects beginning at 40 to 60 min, peaking at 2 to 4 h, and gradually returning to baseline over 6 to 8 h. At a dose of 100 μg, LSD produces perceptual distortions and sometimes hallucinations; mood changes, including elation, paranoia, or depression; intense arousal; and sometimes a feeling of panic. Signs of LSD ingestion include pupillary dilation, increased blood pressure and pulse, flushing, salivation, lacrimation, and hyperreflexia. Visual effects are prominent. Colors seem more intense, and shapes may appear altered. The user may focus attention on unusual items, such as the pattern of hairs on the back of the hand. A "bad trip" usually consists of severe anxiety, although it also can be marked by periods of intense depression and suicidal thoughts. Visual disturbances usually are prominent. There are no documented toxic fatalities from LSD use, but fatal accidents and suicides have occurred during or shortly after intoxication.

Peyote (Mescaline)

Peyote is a small, spineless cactus with mescaline as its main ingredient. Mescaline can also be produced synthetically. Peyote has been used by natives in the southern part of North America as a part of religious ceremonies, dating back to approximately 4000 BCE. The top of the peyote cactus has disk-shaped buttons that are cut out, dried, and chewed or soaked in water to produce an intoxicating liquid. The DEA exempts peyote use from the CSA in connection with religious ceremonies of the Native American Church.

Psilocybin (4-Phosphoryloxy-N,N-Dimethyltryptamine)

Psilocybin is extracted from certain mushrooms found in tropical and subtropical regions of South America, Mexico, and the U.S. Similar to some other hallucinogenic drugs, psilocybin was ingested during religious ceremonies by indigenous cultures from Mexico and Central America, with historical evidence possibly dating back 6000 years. Psilocybin is consumed raw, mixed with food, or brewed into a tea and produces similar effects to those of LSD. Therapeutic use of psilocybin has been legalized in Oregon.

DMT (N,N-dimethyltryptamine)

DMT is a hallucinogenic chemical found naturally in several Amazonian plant species. It can also be synthesized in the laboratory. Synthetic DMT usually takes the form of a white crystalline powder and is typically vaporized or smoked in a pipe. *Ayahuasca* is a hallucinogenic brew made from Amazonian plants containing DMT along with a vine containing a monoamine oxidase inhibitor that prevents the normal breakdown of DMT in the digestive tract. Ayahuasca tea has traditionally been used for healing and religious purposes in indigenous South American cultures, mainly in the Amazon region, with evidence of its use dating back 1000 years.

251-NBOMe

251-NBOMe is a synthetic, highly potent $5HT_{2A}$ receptor agonist, with a common dose of the hydrochloride salt being 600 to 1200 μg. The drug was discovered in the early 21st century and has become a recreational drug. Routes of administration are similar to those for LSD. 251-NBOMe can cause tachycardia, hypertension, agitation, aggression, hallucinations, and seizures and has been linked to overdose deaths and injury-related deaths.

Tolerance, Physical Dependence, and Withdrawal of Classical Hallucinogens

Frequent, repeated use of psychedelic drugs is unusual; thus, tolerance is not commonly seen. Tolerance does develop to the behavioral effects of LSD after three or four daily doses, but no withdrawal syndrome has been observed.

Pharmacological Interventions

There are no FDA-approved medications that treat addiction to hallucinogens. However, because of the unpredictability of psychedelic drug effects, any use carries some risk. Users may require medical attention because of bad trips. Severe agitation may respond to *diazepam* (20 mg orally). "Talking down" by reassurance also is effective and is the management of first choice. Antipsychotic medications (see Chapter 19) may intensify the experience and thus are contraindicated. A particularly troubling aftereffect of LSD and similar drugs is the occasional occurrence of episodic visual disturbances. These originally were called "flashbacks" and resembled the experiences of prior LSD trips. Flashbacks belong to an official diagnostic category called the *hallucinogen persisting perception disorder*. The symptoms include false fleeting perceptions in the peripheral fields, flashes of color, geometric pseudohallucinations, and positive afterimages. The visual disorder appears stable in half the cases and represents an apparently permanent alteration of the visual system. Precipitants include stress, fatigue, emergence into a dark environment, marijuana, antipsychotic agents, and anxiety states.

Prolonged psychotic reactions lasting 2 days or more may occur after the ingestion of a hallucinogen. Schizophrenic episodes may be precipitated in susceptible individuals, and there is some evidence that chronic use of these drugs is associated with the development of persistent psychotic disorders.

Dissociative Drugs

PCP (Phencyclidine)

PCP, an NMDA receptor antagonist, was developed originally as a general anesthetic in the 1950s and later was abandoned because of a high frequency of postoperative delirium with hallucinations. It was classified as a dissociative anesthetic because, in the anesthetized state, the patient remains conscious with staring gaze, flat facies, and rigid muscles. PCP became a drug of abuse in the 1970s, first in an oral form and then in a smoked version enabling a better regulation of the dose.

As little as 50 μg/kg produces emotional withdrawal, concrete thinking, and bizarre responses to projective testing. Catatonic posturing can resemble that of schizophrenia. A meta-analysis showed that hallucinogen-induced psychosis transitioned to a schizophrenia diagnosis in 26% of cases (Murrie et al., 2020). Abusers taking higher doses may appear to be reacting to hallucinations and may exhibit hostile or assaultive behavior. Anesthetic effects increase with dosage; stupor or coma may occur with muscular rigidity, rhabdomyolysis, and hyperthermia. Intoxicated patients in the emergency room may progress from aggressive behavior to coma, with elevated blood pressure and enlarged, nonreactive pupils. PCP binds with high affinity to sites throughout the brain including the cortex and limbic structures, thus blocking NMDA-type glutamate receptors in regions mediating cognition and emotion (see Table 16–2 and Figure 16–9). Evidence suggests that NMDA receptors are involved in ischemic neuronal death caused by high levels of excitatory amino acids; as a result, PCP analogues that block NMDA receptors but with fewer psychoactive effects are of therapeutic interest.

Medical Intervention. Overdose must be treated by life support; there is no antagonist of PCP effects and no proven way to enhance excretion from the body, although acidification of the urine has been proposed. PCP-induced coma may last 7 to 10 days. The agitated or psychotic state produced by PCP can be treated with *diazepam*. Prolonged psychotic behavior requires antipsychotic medication. Because of the anticholinergic activity of PCP, antipsychotic agents with significant anticholinergic effects such as *chlorpromazine* should be avoided.

Ketamine

Ketamine, another NMDA receptor antagonist, appears to have higher potency for NMDA receptor subunits expressed on GABA interneurons (see Figure 18–2). It is a dissociative drug that is used in surgical anesthesia in children and animals and, more recently, as an antidepressant in adults. It is not used as an anesthetic in adults, due to its psychiatric side effects at higher doses, which led to its being investigated experimentally to understand the neurobiology of schizophrenia. Much of the ketamine sold on the street has been diverted from veterinary offices. Although it is manufactured as an injectable liquid, ketamine is generally evaporated to form a powder that is snorted or compressed into pills for illicit use. Because ketamine is odorless and tasteless and has amnesia-inducing properties, it is sometimes added to drinks to facilitate sexual assault.

Esketamine, the *S*-enantiomer of ketamine, is an FDA-approved rapid-acting antidepressant administered as a nasal spray for treatment-resistant depression. The effect is transient and might require maintenance therapy if other antidepressants are ineffective. Since ketamine is known to cause dependence in some persons, caution is still warranted to prevent potential addiction. For this reason, esketamine must be administered under the direct supervision of a healthcare provider.

Salvia divinorum

Salvia divinorum is a plant species related to the common sage. A number of terpenoids have been extracted from the plant, including *salvinorin A*, a potent naturally occurring hallucinogen. Salvinorin A is a KOR agonist and also has activity at the dopamine receptor. The KOR actions are somewhat mimic in the KOR agonists such as dynorphin are dysphoric and involved in negative reinforcement. Not surprisingly, the experience of salvinorin A is often considered dysphoric by the user but offset by the desired hallucinogenic effects.

Tolerance, Physical Dependence, and Withdrawal of Dissociative Drugs

Of the dissociatives, only PCP is documented to have the potential to be addictive. While behavioral treatments can be successful for a variety of SUDs, it is not clear if they are effective to treat addiction to hallucinogens.

Pharmacological Interventions Pharmacological interventions are used to treat various symptoms associated with use of dissociative drugs, but no approved medications are on the market to treat addiction to these drugs.

Clinical and Pharmacological Topics: Cannabinoids

Cannabis plants have been cultivated for centuries for presumed medicinal and psychoactive properties. Cannabis contains over 113 cannabinoids with varying degrees of psychopharmacological properties. Of these, Δ-9-tetrahydrocannabinol (Δ9-THC) is the main psychoactive cannabinoid that produces most of the characteristic euphorigenic effects of marijuana. In the U.S., marijuana use remains prohibited by federal law, but as of 2021, more than half of all states have various degrees of legalization, ranging from medical use only, to decriminalization, to full legalization. The greater availability of cannabinoid products has led to increased marijuana use and a greater number of marijuana-associated auto accidents. The issues of whether and how to control the use of marijuana have not been resolved, and there is a likelihood that marijuana will be decriminalized on the federal level. The potencies of available botanical forms have generally not been standardized, and the dangers inherent in inhaling a smoke replete with organic molecules have not been defined for marijuana.

Δ9-THC

Cannabinoids such as Δ9-THC target the endogenous cannabinoid system that consists of ligand/receptor/signaling networks described in Chapter 26. The pharmacological effects of Δ9-THC vary with the dose, route of administration, experience of the user, vulnerability to psychoactive effects, and setting of use. Intoxication with marijuana produces changes in mood, perception, and motivation, but the effects most frequently sought are a "high" and a "mellowing out." Effects vary with dose, but typically last about 2 h. During the high, cognitive functions, perception, reaction time, learning, and memory are impaired. Coordination and tracking behavior may be impaired for several hours beyond the perception of the high. Marijuana also produces complex behavioral changes such as giddiness and increased hunger. Unpleasant reactions such as panic or hallucinations and even acute psychosis may occur. These reactions are seen commonly with higher doses and with oral ingestion, since the delayed impact of ingested Δ9-THC (15–60 min) entices people to keep consuming edibles in the belief that they are ineffective. Numerous clinical reports suggest that marijuana use may precipitate a recurrence of psychosis in people with a history of schizophrenia. Cannabis has a high conversion rate from drug-induced psychosis to schizophrenia, at 34% (Murrie et al., 2020). One of the most controversial putative effects of marijuana is the production of an "amotivational syndrome." This syndrome is not an official diagnosis but has been used to describe young people who drop out of social activities and show little interest in school, work, or other goal-directed activity. At the cellular level, there is no evidence that marijuana damages brain cells or produces any permanent functional changes. There is evidence that cannabinoids and the CB_1 receptor, the most abundant GPCR in the mammalian brain, have neuroprotective potential (Antonazzo et al., 2019).

Marijuana has medicinal effects, including antiemetic properties that relieve side effects of chemotherapy. It also has muscle-relaxing effects, anticonvulsant properties, and the capacity to reduce the elevated intraocular pressure of glaucoma. These medical benefits can come at the cost of the psychoactive effects that potentially impair normal activities. *Dronabinol* is an approved synthetic formulation of Δ9-THC (see Chapters 26 and 54).

TABLE 28–10 ■ MARIJUANA WITHDRAWAL SYNDROME

Restlessness
Irritability
Mild agitation
Insomnia
Sleep electroencephalogram disturbance
Nausea, cramping

Tolerance, Dependence, and Withdrawal

Tolerance to most of the effects of marijuana can develop rapidly after only a few doses but also disappears rapidly. Withdrawal symptoms are observed in people with cannabis use disorder (Table 28–10), who use marijuana on a daily basis and then suddenly stop. Negative affective states associated with cannabis withdrawal symptoms include irritability, anxiety, decreased appetite, and disrupted sleep. Presently no FDA-approved medications are available for cannabis use disorder. Several pharmacological interventions under investigation are substitution therapies with exogenous cannabinoid agonists that target the CB_1 receptor, including *dronabinol* and *nabilone*. Heavy users may suffer from accompanying depression and thus may respond to antidepressant medication.

Cannabidiol

Cannabidiol (CBD) is another prominent cannabinoid in cannabis plants. As of 2021, CBD derived from cannabis remains a schedule I drug (see Table 28–1). However, CBD from hemp extract (which contains <0.3% THC) is not regulated and is widely available throughout the U.S. In contrast to Δ9-THC, CBD is not psychoactive and is not an agonist at cannabinoid receptors (Laprairie et al, 2015; Zou and Kumar, 2018). The mechanisms of action of CBD are not well understood but are multifold and include negative allosteric modulation of the cannabinoid receptor, competitive antagonism at the GPR-55 receptor (initially considered an atypical cannabinoid receptor), partial agonism at $5HT_{1A}$ receptors, and agonism at transient receptor potential cation channel subfamily V member 1 (TRPV1). Studies about potential medicinal uses in pain, epilepsy, anxiety, mood disorders, neurodegenerative disorders, and other conditions are currently ongoing, including CBD as a potential treatment for OUD (Hurd et al., 2019). Chapter 26 presents more details about cannabinoids.

Drug Summary Table—Chapter 28

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Drugs of Abuse		
Amphetamine	Used to treat: • ADHD • Obesity • Narcolepsy • Improving cognition and working memory	• High doses can cause psychosis • Increases monoamines (particularly DA) in the neuronal synapse
Barbiturates	• Anxiolytics • Anticonvulsants • Hypnotics • Anti-migraine drugs • Anesthetics in surgical settings	• GABA receptor modulators • Inferior to benzodiazepines • Can cause physical dependance • Danger of overdose death • Increased fatality when combined with other CNS depressants
Benzodiazepines	• Anxiolytics • Anticonvulsants • Sedatives • Hypnotics	• GABA receptor modulators • Are relatively safe when taken alone but can be fatal when combined with other CNS depressants • Can cause physical dependance
Cannabinoids (Δ9-THC)	• Antiemetic (particularly during chemotherapy)	• Δ9-THC partial agonist at the CB_1 cannabinoid receptor • Δ9-THC has psychoactive effects; may cause anxiety at higher doses, and reduce anxiety at lower doses • Associated with transition to schizophrenia • No known lethal effects
Cocaine	• Topical use as local anesthetic in otolaryncology and tear duct surgery	• Can cause strokes and heart attacks • Associated with high mortality rates • Cobalt thiocyanate test used for quick detection of cocaine has a high false-positivity rate • Increases monoamines (particularly DA) in neuronal synapses
Ethanol (alcohol)	• External antiseptic • Antidote to ethylene glycol (antifreeze) and methanol	• Creates toxic metabolites • Common mutation in aldehyde dehydrogenase in East Asia causes a toxic alcohol flush reaction • Leading cause of preventable death
Heroin	• No approved medical use	• Metabolized in the brain into 6-monoacetylmorphine and morphine • Extremely addictive • Together with fentanyl a leading cause of SUD deaths • Rapid neurobiological adaptations require increased doses to avoid withdrawal symptoms • Causes respiratory depression • Increased fatalities when combined with CNS depressants • Metabolites activate MORs
MDMA	• No approved medical use	• "Party-drug," used in raves • High doses can cause psychosis • increases monoamines (particularly 5HT) in the synapse
Methaqualone	• Hypnotic • Sedative • Anxiolytic	• GABA agonist • Similar to barbiturates • Widespread use as recreational drug between 1960 and 1980 • Increases fatality when combined with other CNS depressants
Methylphenidate	Used to treat: • ADHD • Narcolepsy • Improves cognition and working memory	• Similar to amphetamine • Increases monoamines (particularly DA and NE) in the synapse
Nicotine	• Might have benefits in Parkinson's disease, schizophrenia, dementia, depression, and ADHD • Used in nicotine batches to help people quit smoking	• Binds to nicotinic acetylcholine receptors • Highly addictive • Leading cause of preventable death

Drug Summary Table—Chapter 28 (*continued*)

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Drugs used in the treatment of addiction		
Acamprosate	• Used to treat AUD	• Mechanism of action not fully understood but may act as a NMDA receptor antagonist/GABA receptor modulator • Should not be administered to persons with impaired kidneys • May cause depression, anxiety and GI symptoms
Baclofen	• May be used to treat AUD, but evidence is not conclusive • Used to treat spasticity	• $GABA_B$ receptor agonist • Discontinuation can lead to a withdrawal syndrome
Benzodiazepines	• Used to treat alcohol withdrawal syndrome during detoxification	• Increased fatality when combined with alcohol or other CNS depressants! • Not used for prolonged AUD treatment • See under "Drugs of Abuse"
Buprenorphine	• OUD maintenance treatment • OUD induction of treatment • Analgesic • Administered PO, IV, IM, transmucosal, transdermal, or implant	• Partial agonist at MOR, antagonist at KOR • Is combined with naloxone in maintenance treatment to prevent intravenous abuse • Side effects include respiratory depression, somnolence • Concomitant use with CNS depressants amplifies side effects and can be lethal
Bupropion	• Improves abstinence rate in smokers • Atypical antidepressant	• May cause insomnia
Carbamazepine	• Might be used during detoxification • Anticonvulsant	• Sodium channel blocker • Should not be combined with alcohol use
Cannabidiol	• FDA-approved as an anticonvulsant • Being considered as a potential treatment of OUD	• Negative allosteric modulator at the CB_1 receptor • Currently, Schedule I if derived from cannabis plant, but unscheduled if obtained from hemp plant (>0.3% THC).
Clonidine	• Used off label to reduce symptoms of opioid and alcohol withdrawal • Treats hypertension • Treats ADHD	• α_2 adrenergic receptor agonist • May cause insomnia
Disulfiram	• Used to treat AUD	• Inhibits aldehyde dehydrogenases (see also "Ethanol" and toxic flush reaction) • Very unpleasant when alcohol is consumed • Poor compliance
Flumazenil	• Antidote in the treatment of benzodiazepine overdose • Reversing the post-surgical effects of long-acting benzodiazepines used as anesthetics	• $GABA_A$ receptor antagonist
Gabapentin	• Alcohol detoxification • Treats neuropathic pain • Anticonvulsant	
Lofexidine	• Reduces symptoms of opioid withdrawal • Treats hypertension	• α_2 adrenergic receptor agonist
Methadone	• Used in OUD detoxification and in opioid maintenance therapy • May be used as analgesic	• Opioid receptor agonist • Schedule II drug • On WHO list of essential medicines • Side effects similar to other opioids
Modafinil	• Off-label use in cocaine, alcohol, methamphetamine and amphetamine withdrawal • May increase rate of abstinence • Used to treat ADHD • Used to treat Narcolepsy	• Schedule IV controlled substance in the US • Increases wakefulness

Drug Summary Table—Chapter 28 (*continued*)

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Drugs used in the treatment of addiction (cont.)		
Naloxone	• Used for the acute treatment of opioid overdose • Available as nasal spray • Combined with buprenorphine in buprenorphine maintenance therapy	• Rapid-acting competitive opioid receptor antagonist • Precipitates opioid withdrawal • May have to be administered repeatedly over 24 hours • Side effects may include agitation, nausea, vomiting, and tachycardia
Naltrexone	• Used in the management of OUD and AUD	• Opioid receptor antagonist • May precipitate opioid withdrawal and should not be used before opioid detoxification • Side effects may include nausea, anxiety, and insomnia
Oxazepam	• AUD detoxification • Anxiolytic	• Short-acting benzodiazepine
Phenobarbital	• Might be used during drug detoxification • Anticonvulsant	• Barbiturate • On WHO list of essential medicines
Rimonabant	• Some improvement in nicotine abstinence rate • Not approved in the U.S.!	• Cannabinoid CB_1 receptor inverse agonist • Serious adverse effects
Topiramate	• May reduce craving for cocaine and alcohol • Used to treat AUD • Anticonvulsant • Prevention of migraines	• Carbonic anhydrase inhibitor • Increased risk of kidney stones
Varenicline	• Treatment of tobacco use disorder	• Selective partial agonist at $\alpha_4\beta_2$ nicotinic acetylcholine receptors • May cause nausea
Opioid medications used in pain treatment		
Buprenorphine	• OUD treatment • Analgesic • Administered PO, IV, IM, transmucosal, transdermal, or implant	• Partial agonist at MOR, antagonist at KOR • Side effects include respiratory depression, somnolence • Concomitant use with CNS depressants amplify side effects and can be lethal
Fentanyl	• Analgesic • Administered PO, IV, IM, transmucosal, sublingual, transdermal	• Leading cause of drug-related deaths in the U.S. • Respiratory depressant • Side effects include nausea, vomiting, sedation and constipation • On WHO list of essential medicines in cancer pain • 100 times more potent than morphine • Analogues such as carfentanil 10,000 times more potent than morphine
Hydrocodone	• Analgesic • Antitussive • Administered PO	• Similar side effects to oxycodone • Histamine release and itching • Full MOR agonist • Should not be combined with CNS depressants
Morphine	• Analgesic • Administered PO, IV, IM • Is also smoked	• Side effects include respiratory depression, nausea, vomiting, hypotension
Oxycodone	• Analgesic • can be administered PO, IV, IM, or intranasally	• A slow-release formulation, Oxycontin, has been subjected to criticism as a significant contributor to the opioid epidemic • Side effects include euphoria, constipation, nausea, drowsiness, hypotension, and respiratory depression • Should not be combined with CNS depressants

Drug Summary Table—Chapter 28 (*continued*)

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Hallucinogens		
251-NBOMe	• No approved medical use	
Ayahuasca	• No approved medical use	• The most prevalent teas are brewed from *banisteriopsis caapi* and *psychotria viridis* • DMT and an MAOI are the main ingredients • Can cause severe vomiting
DMT	• No approved medical use	
Ketamine	• Anesthetic • Antidepressant	• Esketamine prescribed for treatment resistant depression under medical supervision • Widely used by veterinarians for surgical anesthesia • Can cause psychosis
LSD	• No approved medical use	• Can impair mental function • 1/10th as harmful as alcohol • May trigger psychosis and panic attacks
PCP	• No approved medical use	• May trigger hallucinogen-induced psychosis and transition to schizophrenia
Peyote (Mescaline)	• No approved medical use	• Regularly used and legal in the Native American Church
Psilocybin	• No approved medical use	• Misidentification of poisonous mushrooms resembling psilocybin could lead to unintentional, potentially fatal poisoning
Salvia divinorum	• No approved medical use	• As of 2021, not regulated under the U.S. Controlled Substances Act, though might be regulated in individual states

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Section III

Modulation of Pulmonary, Renal, and Cardiovascular

Chapter 29. Drugs Affecting Renal Excretory Function / 557
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Chapter 29

Drugs Affecting Renal Excretory Function

Edwin K. Jackson

PART I: RENAL PHYSIOLOGY AND DIURETIC DRUG ACTION

- Renal Anatomy and Physiology
- Principles of Diuretic Action
- Inhibitors of Carbonic Anhydrase
- Osmotic Diuretics
- Inhibitors of Na$^+$-K$^+$-2Cl$^-$ Symport: Loop Diuretics, High-Ceiling Diuretics
- Inhibitors of Na$^+$-Cl$^-$ Symport: Thiazide-Type and Thiazide-Like Diuretics
- Inhibitors of Renal Epithelial Na$^+$ Channels: K$^+$-Sparing Diuretics
- Antagonists of Mineralocorticoid Receptors: Aldosterone Antagonists, K$^+$-Sparing Diuretics
- Inhibitors of Sodium-Glucose Symport: SGLT2 Inhibitors, Gliflozins
- Inhibitors of the Nonspecific Cation Channel: Natriuretic Peptides
- Adenosine Receptor Antagonists
- Emerging Diuretics
- Clinical Use of Diuretics

PART II: WATER HOMEOSTASIS AND THE VASOPRESSIN SYSTEM

- Vasopressin Physiology
- Vasopressin Receptor Agonists
- Diseases Affecting the Vasopressin System
- Clinical Use of Vasopressin Agonists
- Clinical Use of Vasopressin Antagonists

The kidney filters the extracellular fluid volume across the renal glomeruli an average of 12 times a day, and the renal nephrons precisely regulate the fluid volume of the body and its electrolyte content via processes of secretion and reabsorption. Disease states such as hypertension, heart failure, renal failure, nephrotic syndrome, and cirrhosis may disrupt this balance. Diuretics increase the rate of urine flow and Na$^+$ excretion and are used to adjust the volume or composition of body fluids in these disorders. Precise regulation of body fluid osmolality is also essential. It is controlled by a finely tuned homeostatic mechanism that operates by adjusting both the rate of water intake and the rate of solute-free water excretion by the kidneys—that is, water balance. Abnormalities in this homeostatic system can result from genetic diseases, acquired diseases, or drugs and may cause serious and potentially life-threatening deviations in plasma osmolality.

Part I of this chapter first describes renal physiology, then introduces diuretics with regard to mechanism and site of action, effects on urinary composition, and effects on renal hemodynamics, and then integrates diuretic pharmacology with a discussion of mechanisms of edema formation and the role of diuretics in clinical medicine. Specific therapeutic applications of diuretics are presented in Chapters 32 (hypertension) and 33 (heart failure). *Part II* of this chapter describes the vasopressin system that regulates water homeostasis and plasma osmolality and factors that perturb those mechanisms and examines pharmacological approaches for treating disorders of water balance.

Part I: Renal Physiology and Diuretic Drug Action

Renal Anatomy and Physiology

The basic urine-forming unit of the kidney is the nephron. The initial part of the nephron, the renal (Malpighian) corpuscle, consists of a capsule (Bowman's capsule) and a tuft of capillaries (the glomerulus) residing within the capsule. The glomerulus receives blood from an afferent arteriole, and blood exits the glomerulus via an efferent arteriole. Ultrafiltrate produced by the glomerulus collects in the space between the glomerulus and capsule (Bowman's space) and enters a long tubular portion of the nephron, where the ultrafiltrate is reabsorbed and conditioned. Each human kidney is composed of about 1 million nephrons. Figure 29–1 illustrates subdivisions of the nephron.

Glomerular Filtration

In the glomerular capillaries, a portion of plasma water is forced through a filter that has three basic components: the fenestrated capillary endothelial cells, a basement membrane lying just beneath the endothelial cells, and the filtration slit diaphragms formed by epithelial cells that cover the basement membrane on its urinary space side. Solutes of small size flow with filtered water (solvent drag) into Bowman's space, whereas formed elements and macromolecules are retained by the filtration barrier.

Overview of Nephron Function

The kidney filters large quantities of plasma, reabsorbs substances that the body must conserve, and leaves behind or secretes substances that must be eliminated. The changing architecture and cellular differentiation along the length of a nephron are crucial to these functions (Figure 29–1). Together, the two kidneys in humans produce about 120 mL of ultrafiltrate/min, yet only 1 mL of urine/min; more than 99% of the glomerular ultrafiltrate is reabsorbed at a high energy cost. The kidneys consume 7% of total-body O$_2$ intake despite comprising only 0.5% of body weight.

The proximal tubule is contiguous with Bowman's capsule and takes a winding path until finally forming a straight portion that dives into the renal medulla. Normally, about 65% of filtered Na$^+$ is reabsorbed in the proximal tubule. This part of the tubule is highly permeable to water, and thus, reabsorption is essentially isotonic. Between the outer and inner strips of the outer medulla, the tubule abruptly changes morphology to become the descending thin limb (DTL), which penetrates the inner medulla, makes a hairpin turn, and then forms the ascending thin limb (ATL). At the juncture between the inner and outer medulla, the tubule once again changes morphology and becomes the thick ascending limb (TAL). Together, the proximal straight tubule, DTL, ATL, and TAL segments are known as the *loop of Henle*.

The DTL is highly permeable to water, yet its permeabilities to NaCl and urea are low. In contrast, the ATL is permeable to NaCl and urea but is impermeable to water. The TAL actively reabsorbs NaCl but is impermeable to water and urea. Approximately 25% of filtered Na$^+$ is reabsorbed in the loop of Henle, mostly in the TAL, which has a large reabsorptive capacity. The TAL passes between the afferent and efferent arterioles and makes contact with the afferent arteriole by means of a cluster of specialized columnar epithelial cells known as the *macula densa*. The macula

Abbreviations

ACTH: corticotropin (previously adrenocorticotropic hormone)
ADH: antidiuretic hormone
AIP: aldosterone-induced protein
Ang: angiotensin (e.g., AngII and AngIII)
ANP: atrial natriuretic peptide
ATL: ascending thin limb
AVP: arginine vasopressin
BNP: brain natriuretic peptide
cGMP: cyclic guanosine monophosphate
CHF: congestive heart failure
CKD: chronic kidney disease
CNGC: cyclic nucleotide-gated cation channel
CNP: C-type natriuretic peptide
CNT: connecting tubule
COX: cyclooxygenase
DAG: diacylglycerol
DCT: distal convoluted tubule
DDAVP: 1-deamino-8-D-AVP (desmopressin)
DI: diabetes insipidus
DTL: descending thin limb
ECFV: extracellular fluid volume
ENaC: epithelial Na^+ channel
ENCC1 or TSC: the absorptive Na^+-Cl^- symporter
ENCC2, NKCC2, or BSC1: the absorptive Na^+-K^+-$2Cl^-$ symporter
ENCC3, NKCC1, or BSC2: the secretory Na^+-K^+-$2Cl^-$ symporter
GFR: glomerular filtration rate
GPCR: G protein-coupled receptor
IMCD: inner medullary collecting duct
IP_3: inositol trisphosphate
LOX: lipoxygenase
MR: mineralocorticoid receptor
NP: natriuretic peptide
NPR: natriuretic peptide receptor (e.g., NPRA, B, or C)
NSAID: nonsteroidal anti-inflammatory drug
OAT: organic anion transporter
PG: prostaglandin
PK: protein kinase (e.g., PKA, PKB, PKG)
PL: phospholipase (e.g., PLC, PLD)
PTH: parathyroid hormone
PVN: paraventricular nucleus
RAS: renin-angiotensin system
RBF: renal blood flow
SGLT2: sodium-glucose cotransporter type 2
SIADH: syndrome of inappropriate secretion of ADH
SON: supraoptic nucleus
TAL: thick ascending limb
TGF: tubuloglomerular feedback
VP: vasopressin
VRUT: vasopressin-regulated urea transporter
vWD: von Willebrand disease

densa is strategically located to sense concentrations of NaCl leaving the loop of Henle. If the concentration of NaCl is too high, the macula densa sends a chemical signal (perhaps adenosine or ATP) to the afferent arteriole of the same nephron, causing it to constrict, thereby reducing the glomerular filtration rate (GFR). This homeostatic mechanism, known as *tubuloglomerular feedback (TGF)*, protects the organism from salt and volume wasting. The macula densa also regulates renin release from the adjacent juxtaglomerular cells in the wall of the afferent arteriole.

Approximately 0.2 mm past the macula densa, the tubule changes morphology once again to become the DCT. Like the TAL, the DCT actively transports NaCl and is impermeable to water. Because these characteristics impart the capacity to produce dilute urine, the TAL and the DCT are collectively called the *diluting segment of the nephron*, and the tubular fluid in the DCT is hypotonic regardless of hydration status. However, unlike the TAL, the DCT does not contribute to the countercurrent-induced hypertonicity of the medullary interstitium (described in material that follows).

The collecting duct system (segments 10–14 in Figure 29–1) is an area of fine control of ultrafiltrate composition and volume. It is here that final adjustments in electrolyte composition are made, via the adrenal steroid *aldosterone*. Vasopressin (also called ADH) modulates water permeability in this part of the nephron as well. The more distal portions of the collecting duct pass through the renal medulla, where the interstitial fluid is markedly hypertonic. In the absence of ADH, the collecting duct system is impermeable to water, and dilute urine is excreted. In the presence of ADH, the collecting duct system is permeable to water, and water is reabsorbed. The movement of water out of the tubule is driven by the steep concentration gradient that exists between tubular fluid and medullary interstitium.

The hypertonicity of the medullary interstitium plays a vital role in the capacity of mammals and birds to concentrate urine, which is accomplished by the unique topography of the loop of Henle and the specialized permeabilities of the loop's subsegments. The "passive countercurrent multiplier hypothesis" proposes that active transport in the TAL concentrates NaCl in the interstitium of the outer medulla. Because this segment of the nephron is impermeable to water, active transport in the ascending limb dilutes the tubular fluid. As the dilute fluid passes into the collecting duct system, water is extracted if, and only if, ADH is present. Because the cortical and outer medullary collecting ducts have low permeability to urea, urea is concentrated in the tubular fluid. The IMCD, however, is permeable to urea, so urea diffuses into the inner medulla, where it is trapped by countercurrent exchange in the vasa recta (medullary capillaries that run parallel to the loop of Henle). Because the DTL is impermeable to salt and urea, the high urea concentration in the inner medulla extracts water from the DTL and concentrates NaCl in the tubular fluid of the DTL. As the tubular fluid enters the ATL, NaCl diffuses out of the salt-permeable ATL, thus contributing to the hypertonicity of the medullary interstitium.

General Mechanism of Renal Epithelial Transport

There are multiple mechanisms by which solutes may cross cell membranes (see also Chapter 4). The kinds of transport achieved in a nephron segment depend mainly on which transporters are present and whether they are embedded in the luminal or basolateral membrane. Figure 29–2 presents a general model of renal tubular transport that can be summarized as follows:

1. Na^+, K^+-ATPase (sodium pump) in the basolateral membrane transports Na^+ into the intercellular and interstitial spaces and K^+ into the cell, establishing an electrochemical gradient for Na^+ across the cell membrane directed inward.
2. Na^+ can diffuse down this Na^+ gradient across the luminal membrane via Na^+ channels and via membrane symporters that use the energy stored in the Na^+ gradient to transport solutes out of the tubular lumen and into the cell (e.g., Na^+-glucose, Na^+-$H_2PO_4^-$, and Na^+-amino acid) and antiporters (e.g., Na^+-H^+) that move solutes into the lumen as Na^+ moves down its gradient and into the cell.
3. Na^+ exits the basolateral membrane into intercellular and interstitial spaces mainly via the Na^+ pump.
4. The action of Na^+-linked symporters in the luminal membrane causes the concentration of substrates for these symporters to rise in the epithelial cell. These substrate/solute gradients then permit simple diffusion or mediated transport (e.g., symporters, antiporters, uniporters, and channels) of solutes into the intercellular and interstitial spaces.
5. Accumulation of Na^+ and other solutes in the intercellular space creates a small osmotic pressure differential across the epithelial cell.

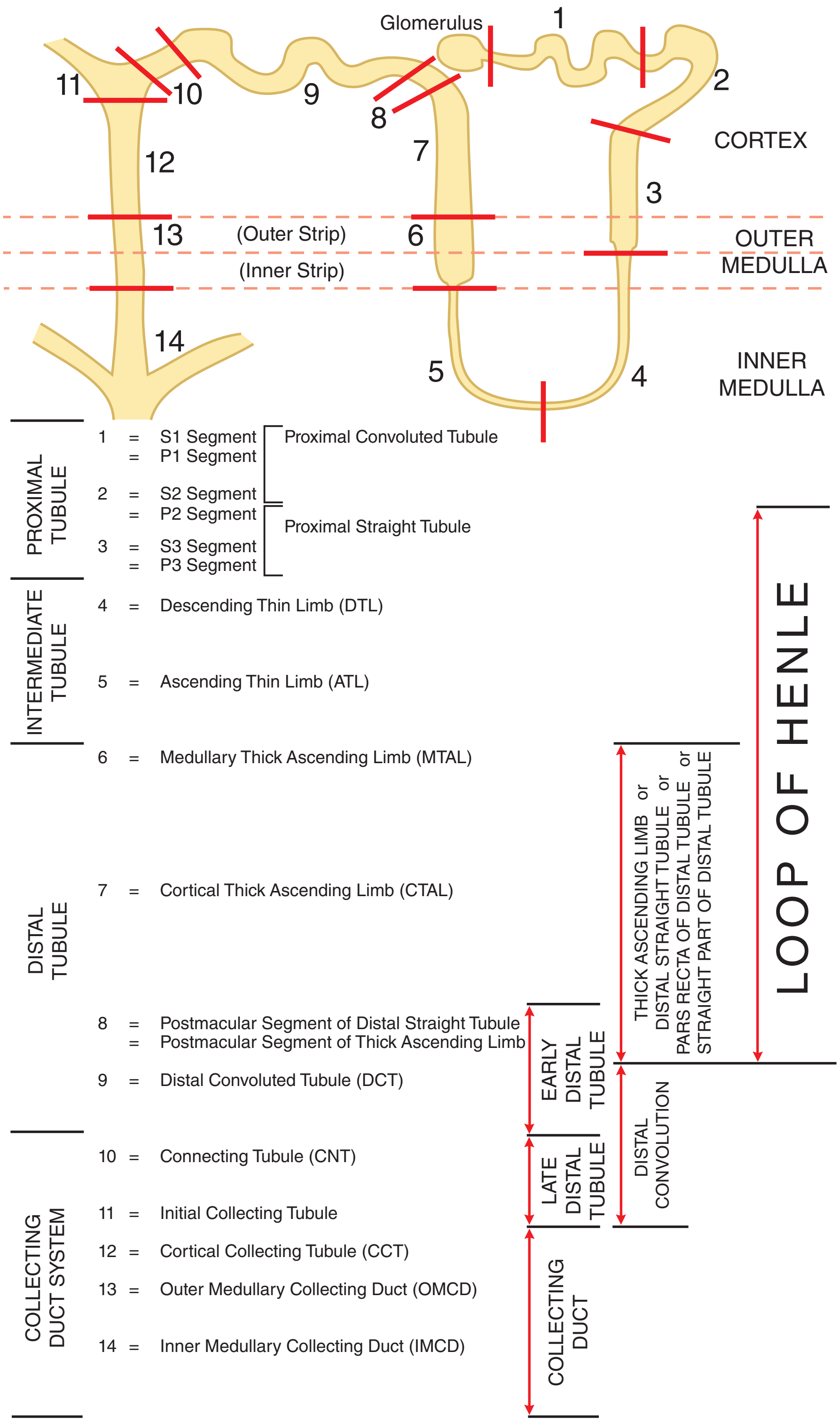

Glomerulus
1
2
3
4
5
6
7
8
9
10
11
12
13
14
CORTEX
OUTER MEDULLA
INNER MEDULLA
(Outer Strip)
(Inner Strip)
PROXIMAL TUBULE
INTERMEDIATE TUBULE
DISTAL TUBULE
COLLECTING DUCT SYSTEM
1 = S1 Segment = P1 Segment
2 = S2 Segment = P2 Segment
3 = S3 Segment = P3 Segment
Proximal Convoluted Tubule
Proximal Straight Tubule
4 = Descending Thin Limb (DTL)
5 = Ascending Thin Limb (ATL)
6 = Medullary Thick Ascending Limb (MTAL)
7 = Cortical Thick Ascending Limb (CTAL)
8 = Postmacular Segment of Distal Straight Tubule = Postmacular Segment of Thick Ascending Limb
9 = Distal Convoluted Tubule (DCT)
10 = Connecting Tubule (CNT)
11 = Initial Collecting Tubule
12 = Cortical Collecting Tubule (CCT)
13 = Outer Medullary Collecting Duct (OMCD)
14 = Inner Medullary Collecting Duct (IMCD)
EARLY DISTAL TUBULE
LATE DISTAL TUBULE
COLLECTING DUCT
THICK ASCENDING LIMB or DISTAL STRAIGHT TUBULE or PARS RECTA OF DISTAL TUBULE or STRAIGHT PART OF DISTAL TUBULE
DISTAL CONVOLUTION
LOOP OF HENLE

Figure 29–1 *Anatomy and nomenclature of the nephron.*

In water-permeable epithelium, water moves into the intercellular spaces driven by the osmotic pressure differential. Water moves through aqueous pores in both the luminal and the basolateral cell membranes, as well as through tight junctions (paracellular pathway). Bulk water flow carries some solutes into the intercellular space by solvent drag.

6. Movement of water into the intercellular space concentrates other solutes in the tubular fluid, resulting in an electrochemical gradient for these substances across the epithelium. Membrane-permeable solutes then move down their electrochemical gradients into the intercellular space by both the transcellular (e.g., simple diffusion, symporters, antiporters, uniporters, and channels) and paracellular pathways. Membrane-impermeable solutes remain in the tubular lumen and are excreted in the urine with an obligatory amount of water.
7. As water and solutes accumulate in the intercellular space, hydrostatic pressure increases, thus providing a driving force for bulk water flow. Bulk water flow carries solute out of the intercellular space into the interstitial space and, finally, into the peritubular capillaries.

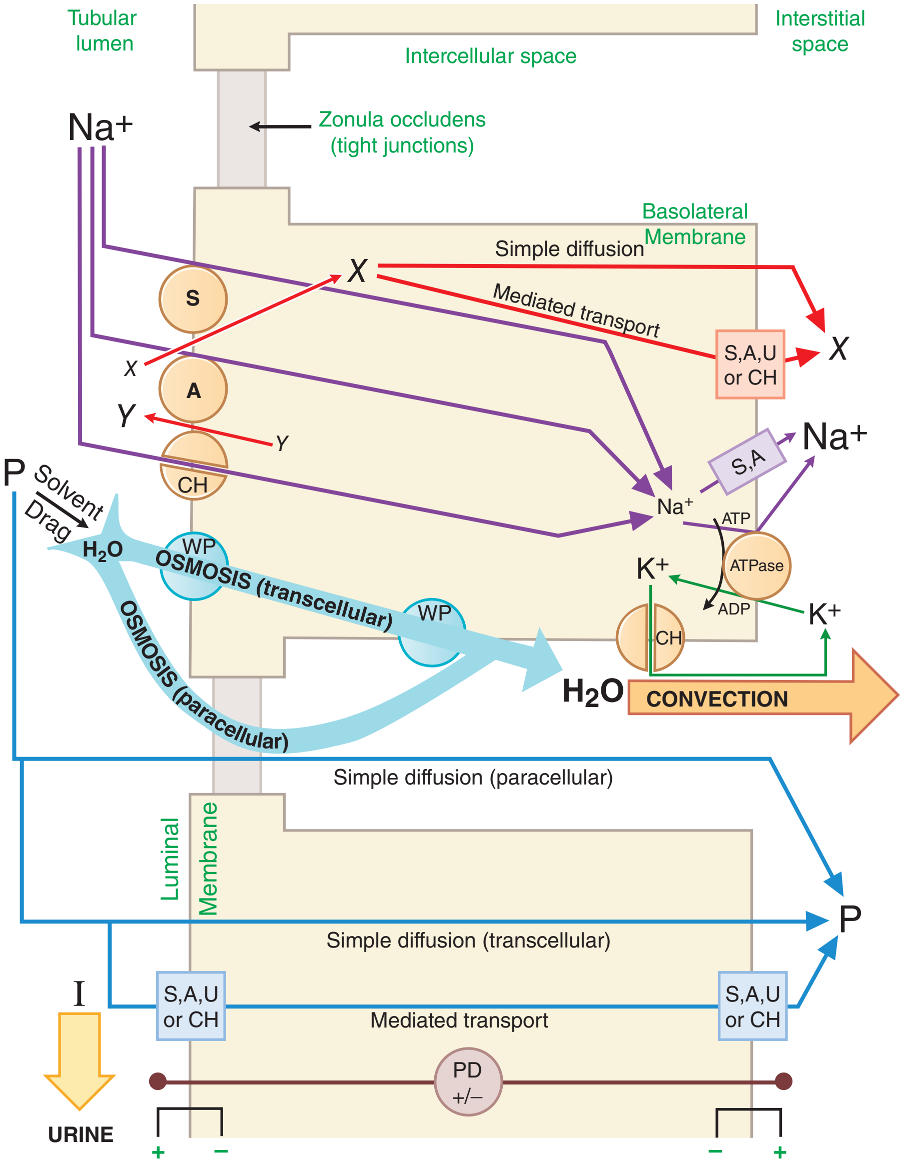

Tubular lumen
Intercellular space
Interstitial space
Na+
Zonula occludens (tight junctions)
Basolateral Membrane
Simple diffusion
X
Mediated transport
S
X
A
Y
Y
CH
S,A,U or CH
X
S,A
Na+
Na+
ATP
ATPase
K+
ADP
K+
CH
P
Solvent Drag
H2O
WP
OSMOSIS (transcellular)
WP
OSMOSIS (paracellular)
H2O
CONVECTION
Simple diffusion (paracellular)
Luminal Membrane
P
Simple diffusion (transcellular)
I
S,A,U or CH
Mediated transport
S,A,U or CH
PD +/–
URINE
+ –
– +

Figure 29–2 *Generic mechanism of renal epithelial cell transport* (see text for details). A, antiporter; ATPase, Na$^+$, K$^+$-ATPase (sodium pump); CH, ion channel; *I*, membrane-impermeable solutes; *P*, membrane-permeable solutes; PD, potential difference across indicated membrane or cell; S, symporter; U, uniporter; WP, water pore; *X* and *Y*, transported solutes.

Organic Acid and Organic Base Secretion

The kidney is a major organ involved in the elimination of organic chemicals from the body. Organic molecules may enter the renal tubules by glomerular filtration or may be actively secreted directly into tubules. The proximal tubule has a highly efficient transport system for organic acids and an equally efficient but separate transport system for organic bases. Current models for these secretory systems are illustrated in Figure 29–3. Both systems are powered by the sodium pump in the basolateral membrane, involve secondary and tertiary active transport, and use a facilitated diffusion step. There are many organic acid and organic base transporters (see Chapter 4). A family of organic anion transporters (OATs) links countertransport of organic anions with dicarboxylates (Figure 29–3A).

Renal Handling of Specific Anions and Cations

Reabsorption of Cl$^-$ generally follows reabsorption of Na$^+$. In segments of the tubule with low-resistance tight junctions (i.e., "leaky" epithelium), such as the proximal tubule and TAL, Cl$^-$ movement can occur paracellularly. Cl$^-$ crosses the luminal membrane by antiport with formate and oxalate (proximal tubule), symport with Na$^+$/K$^+$ (TAL), symport with Na$^+$ (DCT), and antiport with HCO_3^- (collecting duct system). Cl$^-$ crosses the basolateral membrane via symport with K$^+$ (proximal tubule and TAL), antiport with Na$^+$/HCO_3^- (proximal tubule), and Cl$^-$ channels (TAL, DCT, collecting duct system).

Of filtered K$^+$, 80% to 90% is reabsorbed in the proximal tubule (diffusion and solvent drag) and TAL (diffusion), largely through the paracellular pathway. The DCT and collecting duct system secrete variable amounts of K$^+$ by a channel-mediated pathway. Modulation of the rate of K$^+$ secretion in the collecting duct system, particularly by aldosterone, allows urinary K$^+$ excretion to be matched with dietary intake. The transepithelial potential difference V_T, lumen positive in the TAL and lumen negative in the collecting duct system, drives K$^+$ reabsorption and secretion, respectively.

Most of the filtered Ca^{2+} (~70%) is reabsorbed by the proximal tubule by passive diffusion through a paracellular route. Another 25% of filtered Ca^{2+} is reabsorbed by the TAL in part by a paracellular route driven by the lumen-positive V_T and in part by active transcellular Ca^{2+} reabsorption modulated by parathyroid hormone (PTH) (see Chapter 52). Most of

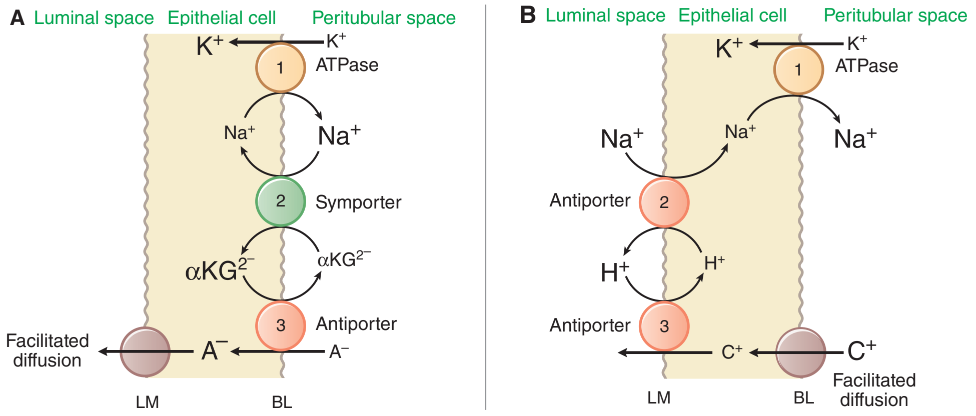


Figure 29–3 *Mechanisms of organic acid* (**A**) *and organic base* (**B**) *secretion in the proximal tubule.* The numbers 1, 2, and 3 refer to primary, secondary, and tertiary active transport, respectively. A^-, organic acid (anion); C^+, organic base (cation); αKG^{2-}, α-ketoglutarate but also other dicarboxylates. BL and LM indicate basolateral and luminal membranes, respectively.

the remaining Ca^{2+} is reabsorbed in DCT by a transcellular pathway. The transcellular pathway in the TAL and DCT involves passive Ca^{2+} influx across the luminal membrane through Ca^{2+} channels (TRPV5, transient receptor potential cation channel V5), followed by Ca^{2+} extrusion across the basolateral membrane by a Ca^{2+}-ATPase. Also, in DCT and CNT, Ca^{2+} crosses the basolateral membrane by Na^+-Ca^{2+} exchanger (antiport). P_i is largely reabsorbed (80% of filtered load) by the proximal tubule. The Na^+-P_i symporter uses the free energy of the Na^+ electrochemical gradient to transport P_i into the cell. The Na^+-P_i symporter is inhibited by PTH.

The renal tubules reabsorb HCO_3^- and secrete protons (tubular acidification), thereby participating in acid-base balance. These processes are described in the section on carbonic anhydrase inhibitors.

Principles of Diuretic Action

Diuretics are drugs that increase the rate of urine flow; clinically useful diuretics also increase the rate of Na^+ excretion (natriuresis) and of an accompanying anion, usually Cl^-. Most clinical applications of diuretics are directed toward reducing extracellular fluid volume by decreasing total-body NaCl content.

Although continued diuretic administration causes a sustained net deficit in total-body Na^+, the time course of natriuresis is finite because renal compensatory mechanisms bring Na^+ excretion in line with Na^+ intake, a phenomenon known as *diuretic braking*. These compensatory mechanisms include activation of the sympathetic nervous system, activation of the renin-angiotensin-aldosterone axis, decreased arterial blood pressure (which reduces pressure natriuresis), renal epithelial cell hypertrophy, increased renal epithelial transporter expression, and perhaps alterations in natriuretic hormones such as atrial natriuretic peptide (ANP). The net effects on extracellular volume and body weight are shown in Figure 29–4.

Diuretics may modify renal handling of other cations (e.g., K^+, H^+, Ca^{2+}, and Mg^{2+}), anions (e.g., Cl^-, HCO_3^-, and $H_2PO_4^-$), and uric acid. In addition, diuretics may alter renal hemodynamics indirectly. Table 29–1 compares the general effects of the major diuretic classes.

Inhibitors of Carbonic Anhydrase

There are three orally administered carbonic anhydrase inhibitors—*acetazolamide, dichlorphenamide,* and *methazolamide* (Table 29–2).

Mechanism and Site of Action

Proximal tubular epithelial cells are richly endowed with the zinc metalloenzyme carbonic anhydrase, which is found in the luminal and basolateral membranes (type IV carbonic anhydrase), as well as in the cytoplasm (type II carbonic anhydrase) (Figure 29–5). Carbonic anhydrase plays a role in $NaHCO_3$ reabsorption and acid secretion.

In the proximal tubule, the free energy in the Na^+ gradient established by the basolateral Na^+ pump is used by a Na^+-H^+ antiporter (Na^+-H^+ exchanger type 3) in the luminal membrane to transport H^+ into the tubular lumen in exchange for Na^+. In the lumen, H^+ reacts with filtered HCO_3^- to form H_2CO_3, which decomposes rapidly to CO_2 and water in the presence of carbonic anhydrase in the brush border. Carbonic anhydrase reversibly accelerates this reaction several thousand–fold. CO_2 is lipophilic and rapidly diffuses across the luminal membrane into the

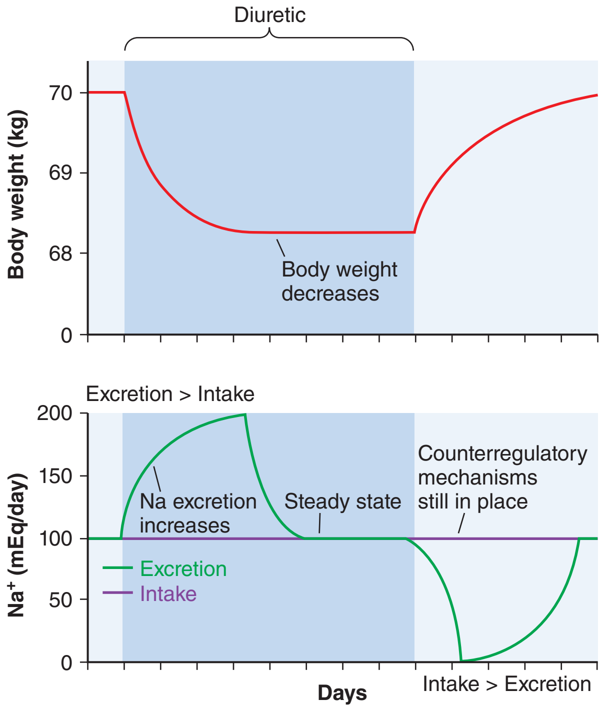


Figure 29–4 *Changes in extracellular fluid volume (ECFV) and weight with diuretic therapy.* The period of diuretic administration is shown in the shaded box along with its effects on body weight in the upper part of the figure and Na^+ excretion in the lower half of the figure. Initially, when Na^+ excretion exceeds intake, body weight and ECFV decrease. Subsequently, a new steady state is achieved where Na^+ intake and excretion are equal but at a lower ECFV and body weight. This results from activation of the RAAS and SNS, "the braking phenomenon." When the diuretic is discontinued, body weight and ECFV rise during a period when Na^+ intake exceeds excretion. A new steady state is then reached as stimulation of the RAAS and SNS wanes. RAAS, renin-angiotensin-aldosterone system; SNS, sympathetic nervous system.

TABLE 29–1 ■ EXCRETORY AND RENAL HEMODYNAMIC EFFECTS OF DIURETICS[a]

DIURETIC MECHANISM (Primary site of action)	CATIONS					ANIONS			URIC ACID		RENAL HEMODYNAMICS			
	Na^+	K^+	H^{+b}	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	$H_2PO_4^-$	*ACUTE*	*CHRONIC*	RBF	GFR	FF	TGF
Inhibitors of CA (proximal tubule)	+	++	–	NC	V	(+)	++	++	I	–	–	–	NC	+
Osmotic diuretics (loop of Henle)	++	+	I	+	++	+	+	+	+	I	+	NC	–	I
Inhibitors of Na^+-K^+-$2Cl^-$ symport (thick ascending limb)	++	++	+	++	++	++	$+^c$	$+^c$	+	–	V(+)	NC	V(–)	–
Inhibitors of Na^+-Cl^- symport (distal convoluted tubule)	+	++	+	V(–)	V(+)	+	$+^c$	$+^c$	+	–	NC	V(–)	V(–)	NC
Inhibitors of renal epithelial Na^+ channels (late distal tubule, collecting duct)	+	–	–	–	–	+	(+)	NC	I	–	NC	NC	NC	NC
Antagonists of mineralocorticoid receptors (late distal tubule, collecting duct)	+	–	–	I	–	+	(+)	I	I	–	NC	NC	NC	NC

[a] Except for uric acid, changes are for acute effects of diuretics in the absence of significant volume depletion, which would trigger complex physiological adjustments.

[b] H^+ includes titratable acid and NH_4^+.

[c] In general, these effects are restricted to those individual agents that inhibit carbonic anhydrase. However, there are notable exceptions in which symport inhibitors increase bicarbonate and phosphate (e.g., metolazone, bumetanide). ++, +, (+), –, NC, V, V(+), V(–), and I indicate marked increase, mild-to-moderate increase, slight increase, decrease, no change, variable effect, variable increase, variable decrease, and insufficient data, respectively. For cations and anions, the indicated effects refer to absolute changes in fractional excretion. CA, carbonic anhydrase; FF, filtration fraction; GFR, glomerular filtration rate; RBF, renal blood flow; TGF, tubuloglomerular feedback.

TABLE 29–2 ■ INHIBITORS OF CARBONIC ANHYDRASE

DRUG	RELATIVE POTENCY	ORAL AVAILABILITY	$t_{1/2}$ (hours)	ROUTE OF ELIMINATION
Acetazolamide	1	~100%	6–9	R
Dichlorphenamide	30	ID	ID	ID
Methazolamide	>1; <10	~100%	~14	~25% R, ~75% M

ID, insufficient data; M, metabolism; R, renal excretion of intact drug.

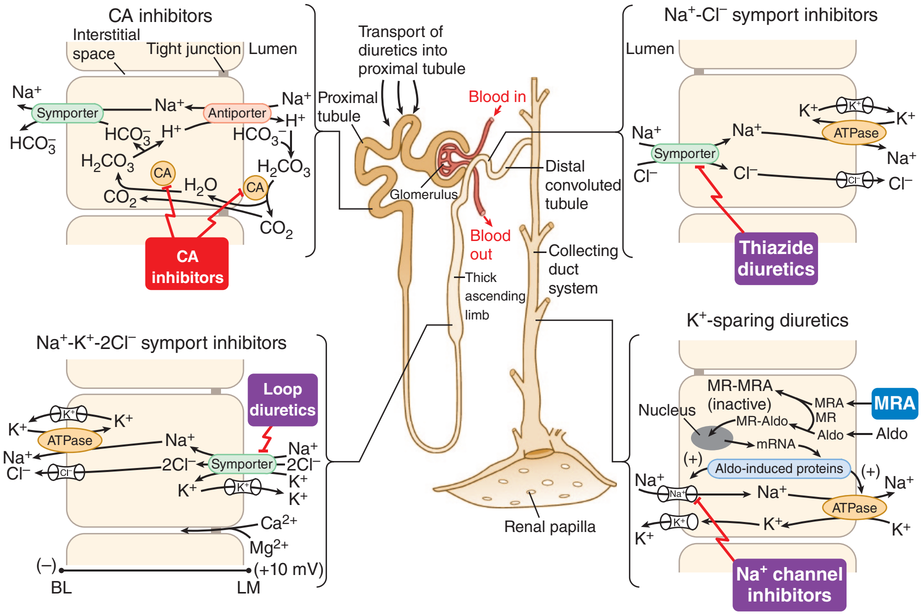

CA inhibitors
Interstitial space
Tight junction
Lumen
Na+
Symporter
HCO3−
Na+
Antiporter
Na+
H+
HCO3−
H+
HCO3−
H2CO3
CA
H2O
H2CO3
CA
CO2
CO2
CA inhibitors
Transport of diuretics into proximal tubule
Proximal tubule
Blood in
Glomerulus
Distal convoluted tubule
Blood out
Collecting duct system
Thick ascending limb
Renal papilla
Na+-Cl− symport inhibitors
Lumen
K+
K+
Na+
ATPase
Na+
Symporter
Na+
Cl−
Cl−
Cl−
Thiazide diuretics
Na+-K+-2Cl− symport inhibitors
Loop diuretics
K+
K+
ATPase
Na+
Na+
Na+
Cl−
2Cl−
Symporter
2Cl−
K+
K+
K+
Ca2+
Mg2+
(−)
(+10 mV)
BL
LM
K+-sparing diuretics
MR-MRA (inactive)
MRA
MR
MRA
Nucleus
MR-Aldo
Aldo
Aldo
mRNA
(+)
Aldo-induced proteins
(+)
Na+
Na+
Na+
ATPase
K+
K+
K+
Na+ channel inhibitors

Figure 29–5 *Sites and mechanisms of action of diuretics.* Three important features are noteworthy: (1) Transport of solute across epithelial cells in all nephron segments involves highly specialized proteins, which for the most part are apical and basolateral membrane integral proteins. (2) Diuretics target and block the action of epithelial proteins involved in solute transport. (3) The site and mechanism of action of a given class of diuretics are determined by the specific protein inhibited by the diuretic. BL, basolateral membrane; CA, carbonic anhydrase; LM, luminal membrane; MR, mineralocorticoid receptor; MRA, mineralocorticoid receptor antagonist.

epithelial cell, where it reacts with water to form H_2CO_3, a reaction catalyzed by cytoplasmic carbonic anhydrase. Continued operation of the Na^+-H^+ antiporter maintains a low proton concentration in the cell, so H_2CO_3 ionizes spontaneously to form H^+ and HCO_3^-, creating an electrochemical gradient for HCO_3^- across the basolateral membrane. The electrochemical gradient for HCO_3^- is used by a Na^+-HCO_3^- symporter (also known as the Na^+-HCO_3^- cotransporter) in the basolateral membrane to transport $NaHCO_3$ into the interstitial space. The net effect of this process is transport of $NaHCO_3$ from the tubular lumen to the interstitial space, followed by movement of water (isotonic reabsorption). Removal of water concentrates Cl^- in the tubular lumen, and consequently, Cl^- diffuses down its concentration gradient into the interstitium by the paracellular pathway.

Carbonic anhydrase inhibitors potently inhibit both the membrane-bound and cytoplasmic forms of carbonic anhydrase and can cause nearly complete elimination of $NaHCO_3$ reabsorption in the proximal tubule. However, due to the large excess of carbonic anhydrase activity in proximal tubules, a large fraction of the enzyme activity must be inhibited before an effect on electrolyte excretion is observed. Although the proximal tubule is the major site of action of carbonic anhydrase inhibitors, carbonic anhydrase is also involved in secretion of titratable acid in the collecting duct system, which is a secondary site of action for this class of drugs.

Effects on Urinary Excretion

Inhibition of carbonic anhydrase is associated with a rapid rise in urinary HCO_3^- excretion to about 35% of filtered load. This, along with inhibition of titratable acid and NH_4^+ secretion in the collecting duct system, results in an increase in urinary pH to about 8 and development of metabolic acidosis. However, even with a high degree of inhibition of carbonic anhydrase, 65% of HCO_3^- is rescued from excretion. The loop of Henle has large reabsorptive capacity and captures most of the Cl^- and a portion of the Na^+. Thus, only a small increase in Cl^- excretion occurs, with HCO_3^- being the major anion excreted along with the cations Na^+ and K^+. The fractional excretion of Na^+ may be as much as 5%, and the fractional excretion of K^+ can be as much as 70%. The increased excretion of K^+ is in part secondary to increased delivery of Na^+ to the distal nephron, as described in the section on inhibitors of Na^+ channels. The effects of carbonic anhydrase inhibitors on renal excretion are self-limiting, probably because the resulting metabolic acidosis decreases the filtered load of HCO_3^- to the point that the uncatalyzed reaction between CO_2 and water is sufficient to achieve HCO_3^- reabsorption.

Effects on Renal Hemodynamics

By inhibiting proximal reabsorption, carbonic anhydrase inhibitors increase delivery of solutes to the macula densa. This triggers TGF, which increases afferent arteriolar resistance and reduces renal blood flow (RBF) and GFR.

Other Actions

Carbonic anhydrase inhibitors have extrarenal sites of action. Carbonic anhydrase in the ciliary processes of the eye mediates formation of HCO_3^- in aqueous humor. Inhibition of carbonic anhydrase decreases the rate of formation of aqueous humor and consequently reduces intraocular pressure. *Acetazolamide* frequently causes paresthesias and somnolence, suggesting an action of carbonic anhydrase inhibitors in the CNS. The efficacy of *acetazolamide* in epilepsy is due in part to the production of metabolic acidosis; however, direct actions of *acetazolamide* in the CNS also contribute to its anticonvulsant action. Due to interference with carbonic anhydrase activity in erythrocytes, carbonic anhydrase inhibitors increase CO_2 levels in peripheral tissues and decrease CO_2 levels in expired gas. *Acetazolamide* causes vasodilation by opening vascular Ca^{2+}-activated K^+ channels; however, the clinical significance of this effect is unclear.

ADME

See Table 29–2 for pharmacokinetic data.

Therapeutic Uses

The efficacy of carbonic anhydrase inhibitors as single agents is low. The combination of *acetazolamide* with diuretics that block Na^+ reabsorption at more distal sites in the nephron causes a marked natriuretic response in patients with low basal fractional excretion of Na^+ (<0.2%) who are resistant to diuretic monotherapy. Even so, the long-term usefulness of carbonic anhydrase inhibitors often is compromised by the development of metabolic acidosis. The major indication for carbonic anhydrase inhibitors is open-angle glaucoma (Scozzafava and Supuran, 2014). Two products developed specifically for this use are *dorzolamide* and *brinzolamide*, which are available only as ophthalmic drops. Carbonic anhydrase inhibitors also may be employed for secondary glaucoma and preoperatively in acute-angle closure glaucoma to lower intraocular pressure before surgery (see Chapter 74). Orally administered *acetazolamide* is also used for the treatment of glaucoma (see Chapter 74) and absence seizures (see Chapter 20). *Acetazolamide* can provide symptomatic relief in patients with *high-altitude illness* or *mountain sickness* (Ritchie et al., 2012). Carbonic anhydrase inhibitors are also useful in patients with familial periodic paralysis. *Dichlorphenamide* is now approved for treating this syndrome. The mechanism for the beneficial effects of carbonic anhydrase inhibitors in altitude sickness and familial periodic paralysis may relate to the induction of a metabolic acidosis. Carbonic anhydrase inhibitors can be useful for correcting metabolic alkalosis, especially one caused by diuretic-induced increases in H^+ excretion. Other off-label uses include normal-pressure hydrocephalus, idiopathic intracranial hypertension, prevention of cystine renal calculi, and respiratory stimulant in stable hypercapnic chronic obstructive pulmonary disease.

Toxicity, Adverse Effects, Contraindications, Drug Interactions

Serious toxic reactions to carbonic anhydrase inhibitors are infrequent; however, these drugs are sulfonamide derivatives and, like other sulfonamides, may cause bone marrow depression, skin toxicity, sulfonamide-like renal lesions, and allergic reactions. With large doses, many patients exhibit drowsiness and paresthesias. Most adverse effects, contraindications, and drug interactions are secondary to urinary alkalinization or metabolic acidosis, including (1) diversion of ammonia of renal origin from urine into the systemic circulation, a process that may induce or worsen hepatic encephalopathy (the drugs are contraindicated in patients with hepatic cirrhosis); (2) calculus formation and ureteral colic due to precipitation of calcium phosphate salts in alkaline urine; (3) worsening of metabolic or respiratory acidosis (the drugs are contraindicated in patients with hyperchloremic acidosis or severe chronic obstructive pulmonary disease); and (4) reduction of the urinary excretion rate of weak organic bases. As such, caution is advised for patients receiving concomitant high-dose salicylates.

Osmotic Diuretics

Osmotic diuretics (Table 29–3) are freely filtered at the glomerulus, undergo limited reabsorption by the renal tubule, and are relatively inert

TABLE 29–3 ■ OSMOTIC DIURETICS

DRUG	ORAL AVAILABILITY	$t_{1/2}$ (hours)	ROUTE OF ELIMINATION
Glycerin	Orally active	0.5–0.75	~80% M, ~20% U
Isosorbide[a]	Orally active	5–9.5	R
Mannitol	Negligible	0.25–1.7[b]	~80% R, ~20% M + B
Urea[a]	Negligible	1.2	R

B, excretion of intact drug into bile; M, metabolism; R, renal excretion of intact drug; U, unknown pathway of elimination.

[a] Not available in the U.S.

[b] In renal failure, 6–36 h.

pharmacologically. Osmotic diuretics are administered in doses large enough to significantly increase the osmolality of plasma and tubular fluid. Of the osmotic diuretics listed, only *glycerin* and *mannitol* are currently available in the U.S.

Mechanism and Site of Action

Osmotic diuretics act in both the proximal tubule and the loop of Henle, with the latter the primary site of action. In the proximal tubule, osmotic diuretics act as nonreabsorbable solutes that limit the osmosis of water into the interstitial space and thereby reduce the luminal Na^+ concentration to the point that net Na^+ reabsorption ceases. By extracting water from intracellular compartments, osmotic diuretics expand extracellular fluid volume, decrease blood viscosity, and inhibit renin release. These effects increase RBF, and the increase in renal medullary blood flow removes NaCl and urea from the renal medulla, thus reducing medullary tonicity. A reduction in medullary tonicity causes a decrease in the extraction of water from the DTL, which in turn limits the concentration of NaCl in the tubular fluid entering the ATL. This latter effect diminishes the passive reabsorption of NaCl in the ATL. In addition, osmotic diuretics inhibit Mg^{2+} reabsorption in the TAL.

Effects on Urinary Excretion

Osmotic diuretics increase urinary excretion of nearly all electrolytes, including Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, and phosphate.

Effects on Renal Hemodynamics

Osmotic diuretics increase RBF by a variety of mechanisms, but total GFR is not significantly altered.

ADME

Pharmacokinetic data on the osmotic diuretics are gathered in Table 29–3. Where available, *glycerin* and *isosorbide* can be given orally, whereas *mannitol* and *urea* must be administered intravenously (with the exception that *mannitol* powder is used by inhalation for diagnosis of bronchial hyperreactivity).

Therapeutic Uses

One use for *mannitol* is in the treatment of dialysis disequilibrium syndrome. Excessively removing solutes from the extracellular fluid by hemodialysis results in a reduction in the osmolality of extracellular fluid. Consequently, water moves from the extracellular compartment into the intracellular compartment, causing hypotension and CNS symptoms (headache, nausea, muscle cramps, restlessness, CNS depression, and convulsions). Osmotic diuretics increase the osmolality of the extracellular fluid compartment and thereby shift water back into the extracellular compartment. By increasing the osmotic pressure of plasma, osmotic diuretics extract water from the eye and brain. Osmotic diuretics are used to control intraocular pressure during acute attacks of glaucoma and for short-term reductions in intraocular pressure both preoperatively and postoperatively in patients who require ocular surgery. Also, *mannitol* is used to reduce cerebral edema and brain mass before and after neurosurgery and to control intracranial pressure in patients with traumatic brain injury (Wakai et al., 2013). A spray-dried form of *mannitol* is FDA approved for managing cystic fibrosis in adults; by hydrating airways, *mannitol* improves mucus clearance. *Mannitol* is also FDA approved for the diagnosis of bronchial hyperreactivity (by oral inhalation) and for antihemolytic urologic irrigation during transurethral procedures. *Mannitol* is often used to treat or prevent acute kidney injury; however, it is questionable whether *mannitol* improves renal outcomes (Nigwekar and Waikar, 2011).

Toxicity, Adverse Effects, Contraindications, Drug Interactions

Osmotic diuretics are distributed in the extracellular fluid and contribute to the extracellular osmolality. Thus, water is extracted from intracellular compartments, and the extracellular fluid volume becomes expanded. In patients with heart failure or pulmonary congestion, this may cause frank pulmonary edema. Extraction of water also causes hyponatremia, which may explain the common adverse effects, including headache, nausea, and vomiting. Conversely, loss of water more than electrolytes can cause hypernatremia and dehydration. Osmotic diuretics are contraindicated in patients who are anuric due to severe renal disease. *Urea* may cause thrombosis or pain if extravasation occurs, and it should not be administered to patients with impaired liver function because of the risk of elevation of blood ammonia levels. Both *mannitol* and *urea* are contraindicated in patients with active cranial bleeding. Glycerin is metabolized and can cause hyperglycemia.

Inhibitors of Na^+-K^+-$2Cl^-$ Symport: Loop Diuretics, High-Ceiling Diuretics

The loop diuretics inhibit activity of the Na^+-K^+-$2Cl^-$ symporter in the TAL of the loop of Henle, hence the moniker *loop diuretics*. Although the proximal tubule reabsorbs about 65% of filtered Na^+, diuretics acting only in the proximal tubule have limited efficacy because the TAL has the capacity to reabsorb most of the unabsorbed material from the proximal tubule. In contrast, inhibitors of Na^+-K^+-$2Cl^-$ symport in the TAL, sometimes called *high-ceiling diuretics*, are highly efficacious because (1) about 25% of the filtered Na^+ load normally is reabsorbed by the TAL, and (2) nephron segments past the TAL do not possess the resorptive capacity to rescue the flood of unabsorbed material exiting the TAL.

Of the inhibitors of Na^+-K^+-$2Cl^-$ symport (Table 29–4), only *furosemide*, *bumetanide*, *ethacrynic acid*, and *torsemide* are available in the U.S. *Furosemide* and *bumetanide* contain a sulfonamide moiety. *Ethacrynic acid* is a phenoxyacetic acid derivative; *torsemide* is a sulfonylurea. *Furosemide* and *bumetanide* are available as oral and injectable formulations. *Torsemide* is available as an oral formulation; *ethacrynate sodium* is available as an injectable solution and *ethacrynic acid* as an oral tablet.

Mechanism and Site of Action

These agents act primarily in the TAL, where the flux of Na^+, K^+, and Cl^- from the lumen into epithelial cells is mediated by a Na^+-K^+-$2Cl^-$ symporter

TABLE 29–4 ■ INHIBITORS OF Na^+-K^+-$2Cl^-$ SYMPORT (LOOP DIURETICS, HIGH-CEILING DIURETICS)

DRUG	RELATIVE POTENCY	ORAL AVAILABILITY	$t_{1/2}$ (hours)	ROUTE OF ELIMINATION
Furosemide	1	~60%	~1.5	~65% R, ~35% M[a]
Bumetanide	40	~80%	~0.8	~62% R, ~38% M
Ethacrynic acid	0.7	~100%	~1	~67% R, ~33% M
Torsemide	3	~80%	~3.5	~20% R, ~80% M
Azosemide[b]	1	~12%	~2.5	~27% R, ~63% M
Piretanide[b]	3	~80%	0.6–1.5	~50% R, ~50% M

M, metabolism; R, renal excretion of intact drug.

[a] Metabolism of furosemide occurs predominantly in the kidney.

[b] Not available in the U.S.

(Figure 29–5). Inhibitors of this symporter block its function (Bernstein and Ellison, 2011; Wile, 2012), bringing salt transport in this segment of the nephron to a virtual standstill. There is evidence that these drugs attach to the Cl^- binding site located in the symporter's transmembrane domain; however, more recent studies challenge this view. Inhibitors of Na^+-K^+-$2Cl^-$ symport also inhibit Ca^{2+} and Mg^{2+} reabsorption in the TAL by abolishing the transepithelial potential difference that is the dominant driving force for reabsorption of these cations.

Na^+-K^+-$2Cl^-$ symporters are found in many secretory and absorbing epithelia. There are two varieties of Na^+-K^+-$2Cl^-$ symporters. The "absorptive" symporter (called *ENCC2, NKCC2*, or *BSC1*) is expressed only in the kidney, is localized to the apical membrane and subapical intracellular vesicles of the TAL, and is regulated by the cyclic AMP/PKA pathway. The "secretory" symporter (called *ENCC3, NKCC1*, or *BSC2*) is a "housekeeping" protein that is expressed widely and, in epithelial cells, is localized to the basolateral membrane. The affinity of loop diuretics for the secretory symporter is somewhat less than for the absorptive symporter (e.g., 4-fold difference for *bumetanide*). Mutations in the Na^+-K^+-$2Cl^-$ symporter cause a form of inherited hypokalemic alkalosis called Bartter syndrome.

Effects on Urinary Excretion

Loop diuretics increase urinary Na^+ and Cl^- excretion profoundly (i.e., up to 25% of the filtered Na^+ load) and markedly increase Ca^{2+} and Mg^{2+} excretion. *Furosemide* has weak carbonic anhydrase–inhibiting activity and thus increases urinary excretion of HCO_3^- and phosphate. All inhibitors of Na^+-K^+-$2Cl^-$ symport increase urinary K^+ and titratable acid excretion. This effect is due in part to increased Na^+ delivery to the distal tubule (the mechanism by which increased distal Na^+ delivery enhances K^+ and H^+ excretion is discussed in the section on Na^+ channel inhibitors). Other mechanisms contributing to enhanced K^+ and H^+ excretion include flow-dependent enhancement of ion secretion by the collecting duct, nonosmotic vasopressin release, and activation of the renin-angiotensin system (RAS) axis.

Acutely, loop diuretics increase uric acid excretion; their chronic administration results in reduced uric acid excretion. Chronic effects of loop diuretics on uric acid excretion may be due to enhanced proximal tubule transport or secondary to volume depletion or to competition between diuretic and uric acid for the organic acid secretory mechanism in the proximal tubule. Asymptomatic hyperuricemia is a common consequence of loop diuretics, but painful episodes of gout are rarely reported (Bruderer et al., 2014). By blocking active NaCl reabsorption in the TAL, inhibitors of Na^+-K^+-$2Cl^-$ symport interfere with a critical step in the mechanism that produces a hypertonic medullary interstitium. Therefore, loop diuretics block the kidney's ability to concentrate urine. Also, because the TAL is part of the diluting segment, inhibitors of Na^+-K^+-$2Cl^-$ symport markedly impair the kidney's ability to excrete a dilute urine during water diuresis.

Effects on Renal Hemodynamics

If volume depletion is prevented by replacing fluid losses, inhibitors of Na^+-K^+-$2Cl^-$ symport generally increase total RBF and redistribute RBF to the midcortex. The mechanism of the increase in RBF is not known but may involve PGs: nonsteroidal anti-inflammatory drugs (NSAIDs) attenuate the diuretic response to loop diuretics in part by preventing prostaglandin (PG)-mediated increases in RBF. Loop diuretics block TGF by inhibiting salt transport into the macula densa so that the macula densa no longer detects NaCl concentrations in the tubular fluid. Therefore, unlike carbonic anhydrase inhibitors, loop diuretics do not decrease the GFR by activating TGF. Loop diuretics are powerful stimulators of renin release. This effect is due to interference with NaCl transport by the macula densa and, if volume depletion occurs, to reflex activation of the sympathetic nervous system and stimulation of the intrarenal baroreceptor mechanism.

Other Actions

Loop diuretics, particularly *furosemide*, acutely increase systemic venous capacitance and thereby decrease left ventricular filling pressure. This effect, which may be mediated by PGs and requires intact kidneys, benefits patients with pulmonary edema even before diuresis ensues. High doses of inhibitors of Na^+-K^+-$2Cl^-$ symport can inhibit electrolyte transport in many tissues. This effect is clinically important in the inner ear and can result in ototoxicity, particularly in patients with preexisting hearing impairment.

ADME

Table 29–4 presents some pharmacokinetic properties of the agents. Because these drugs are bound extensively to plasma proteins, delivery of these drugs to tubules by filtration is limited. However, they are secreted efficiently by the organic acid transport system in the proximal tubule and thereby gain access to the Na^+-K^+-$2Cl^-$ symporter in the luminal membrane of the TAL. For loop diuretics, this involves OAT1 and OAT3 on the basolateral membrane and multidrug resistance-associated protein 4 (MRP-4) on the luminal membrane (Ellison, 2019). Approximately 65% of *furosemide* is excreted unchanged in urine, and the remainder is conjugated to glucuronic acid in the kidney. Thus, in patients with renal disease, the elimination $t_{1/2}$ of *furosemide* is prolonged. *Bumetanide* and *torsemide* have significant hepatic metabolism, so liver disease can prolong the elimination $t_{1/2}$ of these loop diuretics. Oral bioavailability of *furosemide* varies (10%–100%). In contrast, oral availabilities of *bumetanide* and *torsemide* are reliably high.

As a class, loop diuretics have short elimination half-lives; prolonged-release preparations are not available. Consequently, often the dosing interval is too short to maintain adequate levels of loop diuretics in the tubular lumen. Note that *torsemide* has a longer $t_{1/2}$ than other agents available in the U.S. As the concentration of loop diuretic in the tubular lumen declines, nephrons begin to avidly reabsorb Na^+, which often nullifies the overall effect of the loop diuretic on total-body Na^+. This phenomenon of "postdiuretic Na^+ retention" can be overcome by restricting dietary Na^+ intake or by more frequent administration of the loop diuretic.

Therapeutic Uses

A major use of loop diuretics is in the treatment of acute pulmonary edema. A rapid increase in venous capacitance in conjunction with brisk natriuresis reduces left ventricular filling pressures and thereby rapidly relieves pulmonary edema. Loop diuretics also are used widely for treatment of chronic congestive heart failure (CHF) when diminution of extracellular fluid volume is desirable to minimize venous and pulmonary congestion (see Chapter 33). Diuretics cause a significant reduction in mortality and the risk of worsening heart failure, as well as an improvement in exercise capacity. Although *furosemide* is the most commonly used loop diuretic for the treatment of heart failure, patients with heart failure have fewer hospitalizations and better quality of life with *torsemide* than with *furosemide*, perhaps because of its more reliable absorption and due to other ancillary pharmacological effects (Buggey et al., 2015).

Although diuretics are used widely for treatment of hypertension (see Chapter 32), in patients with normal renal function, Na^+-K^+-$2Cl^-$ symport inhibitors are not considered first-line diuretics for the treatment of hypertension. This is due to the lower antihypertensive efficacy of loop diuretics in such patients and the lack of data demonstrating a reduction in cardiovascular events. However, in patients with a low GFR (<30 mL/min) or with resistant hypertension, loop diuretics are the diuretics of choice.

The edema of nephrotic syndrome often is refractory to less-potent diuretics, and loop diuretics often are the only drugs capable of reducing the massive edema associated with this renal disease. Loop diuretics also are employed in the treatment of edema and ascites of liver cirrhosis; however, care must be taken not to induce volume contraction. In patients with a drug overdose, loop diuretics can be used to induce forced diuresis to facilitate more rapid renal elimination of the offending drug. Loop diuretics, combined with isotonic saline administration to prevent volume depletion, are used to treat hypercalcemia. Loop diuretics interfere with the kidney's capacity to produce concentrated urine. Consequently, loop diuretics combined with hypertonic saline are useful for the treatment of life-threatening hyponatremia. Loop diuretics also are used to treat edema associated with chronic kidney disease (CKD), in which the dose-response curve may be right shifted, requiring higher doses of the loop diuretic.

Toxicity, Adverse Effects, Contraindications, Drug Interactions

Most adverse effects are due to abnormalities of fluid and electrolyte balance. Overzealous use of loop diuretics can cause serious depletion of total-body Na^+. This may manifest as hyponatremia or extracellular fluid volume depletion associated with hypotension, reduced GFR, circulatory collapse, thromboembolic episodes, and, in patients with liver disease, hepatic encephalopathy. Increased Na^+ delivery to the distal tubule, particularly when combined with activation of the RAS, leads to increased urinary K^+ and H^+ excretion, causing a hypochloremic alkalosis. If dietary K^+ intake is not sufficient, hypokalemia may develop, and this may induce cardiac arrhythmias, particularly in patients taking cardiac glycosides. Increased Mg^{2+} and Ca^{2+} excretion may result in hypomagnesemia (a risk factor for cardiac arrhythmias) and hypocalcemia (rarely leading to tetany). Loop diuretics should be avoided in postmenopausal osteopenic women, in whom increased Ca^{2+} excretion may have deleterious effects on bone metabolism.

Loop diuretics can cause ototoxicity that manifests as tinnitus, hearing impairment, deafness, vertigo, and a sense of fullness in the ears. Hearing impairment and deafness are usually, but not always, reversible. Ototoxicity occurs most frequently with rapid intravenous administration and least frequently with oral administration. To avoid ototoxicity, the rate of *furosemide* infusions should not exceed 4 mg/min. *Ethacrynic acid* appears to induce ototoxicity more often than do other loop diuretics and should be reserved for use only in patients who cannot tolerate other loop diuretics (e.g., due to sulfa allergies). Loop diuretics also can cause hyperuricemia (occasionally leading to gout) and hyperglycemia (infrequently precipitating diabetes mellitus) and can increase plasma levels of low-density lipoprotein (LDL) cholesterol and triglycerides while decreasing plasma levels of high-density lipoprotein (HDL) cholesterol. Other adverse effects include skin rashes, photosensitivity, paresthesias, bone marrow depression, and GI disturbances. Contraindications to the use of loop diuretics include severe Na^+ and volume depletion, hypersensitivity to sulfonamides (for sulfonamide-based loop diuretics), and anuria unresponsive to a trial dose of loop diuretic.

Drug interactions may occur when loop diuretics are administered with the following:

- Aminoglycosides, *carboplatin*, *paclitaxel*, and others (synergism of ototoxicity)
- Digitalis glycosides (increased *digitalis*-induced arrhythmias)
- *Lithium* (increased plasma levels of Li^+)
- *Propranolol* (increased plasma levels of *propranolol*)
- Sulfonylureas (hyperglycemia)
- *Cisplatin* (increased risk of diuretic-induced ototoxicity)
- NSAIDs (blunted diuretic response and salicylate toxicity with high doses of salicylates)
- *Probenecid* (blunted diuretic response)
- Thiazide diuretics (synergism of diuretic activity of both drugs, leading to profound diuresis)
- *Amphotericin B* (increased potential for nephrotoxicity and intensification of electrolyte imbalance)

Inhibitors of Na^+-Cl^- Symport: Thiazide-Type and Thiazide-Like Diuretics

The term *thiazide diuretics* generally refers to all inhibitors of Na^+-Cl^- symport, so named because the original inhibitors of Na^+-Cl^- symport were benzothiadiazine derivatives. The class now includes diuretics that are benzothiadiazine derivatives (*thiazide* or *thiazide-type diuretics*) and drugs that are pharmacologically similar to thiazide diuretics but differ structurally (*thiazide-like diuretics*). Table 29–5 lists diuretics in this drug class that are currently available in the U.S.

Mechanism and Site of Action

Thiazide diuretics inhibit NaCl transport in the DCT; the proximal tubule may represent a secondary site of action. Figure 29–5 illustrates the current model of electrolyte transport in the DCT. Transport is powered by a Na^+ pump in the basolateral membrane. Free energy in the electrochemical gradient for Na^+ is harnessed by a Na^+-Cl^- symporter in the luminal membrane that moves Cl^- into the epithelial cell against its electrochemical gradient. Cl^- then exits the basolateral membrane passively by a Cl^- channel. Thiazide diuretics inhibit the Na^+-Cl^- symporter (called *ENCC1* or *TSC*) that is expressed predominantly in kidney and localized to the apical membrane of DCT epithelial cells. Expression of the symporter is regulated by aldosterone. Mutations in the Na^+-Cl^- symporter cause a form of inherited hypokalemic alkalosis called Gitelman syndrome.

Effects on Urinary Excretion

Inhibitors of Na^+-Cl^- symport increase Na^+ and Cl^- excretion. However, thiazides are only moderately efficacious (i.e., maximum excretion of filtered Na^+ load is only 5%) because about 90% of the filtered Na^+ load is reabsorbed before reaching the DCT. Some thiazide diuretics also are weak inhibitors of carbonic anhydrase, an effect that increases HCO_3^- and phosphate excretion and probably accounts for their weak proximal tubular effects. Inhibitors of Na^+-Cl^- symport increase K^+ and titratable acid excretion by the same mechanisms discussed for loop diuresis. Acute thiazide administration increases uric acid excretion. However, uric acid excretion is reduced following chronic administration by similar mechanisms discussed for loop diuretics. Acute effects of inhibitors of Na^+-Cl^- symport on Ca^{2+} excretion are variable; when administered chronically, thiazide diuretics decrease Ca^{2+} excretion. The mechanism involves increased proximal reabsorption owing to volume depletion,

TABLE 29–5 ■ INHIBITORS OF Na^+-Cl^- SYMPORT (THIAZIDE DIURETICS)

DRUG	RELATIVE POTENCY	ORAL AVAILABILITY	$t_{1/2}$ (hours)	ROUTE OF ELIMINATION
Thiazide diuretics				
Bendroflumethiazide	10	~100%	3–3.9	~30% R, ~70% M
Chlorothiazide	0.1	9%–56% (dose-dependent)	~1.5	R
Hydrochlorothiazide	1	~70%	~2.5	R
Methyclothiazide	10	ID	ID	M
Thiazide-like diuretics				
Chlorthalidone	1	~65%	~47	~65% R, ~10% B, ~25% U
Indapamide	20	~93%	~14	M
Metolazone	10	~65%	8–14	~80% R, ~10% B, ~10% M

B, excretion of intact drug into bile; ID, insufficient data; M, metabolism; R, renal excretion of intact drug; U, unknown pathway of elimination.

as well as direct effects of thiazides to increase Ca^{2+} reabsorption in the DCT. Thiazide diuretics may cause mild magnesuria; long-term use of thiazide diuretics may cause magnesium deficiency, particularly in the elderly. Because inhibitors of Na^+-Cl^- symport inhibit transport in the cortical diluting segment, thiazide diuretics attenuate the kidney's ability to excrete dilute urine during water diuresis. However, because the DCT is not involved in the mechanism that generates a hypertonic medullary interstitium, thiazide diuretics do not alter the kidney's ability to concentrate urine during hydropenia. In general, inhibitors of Na^+-Cl^- symport do not affect RBF and only variably reduce GFR owing to increases in intratubular pressure. Thiazides have little or no influence on TGF.

ADME

Table 29–5 lists pharmacokinetic parameters of Na^+-Cl^- symport inhibitors. Note the wide range of half-lives for these drugs. As with loop diuretics, thiazides are secreted into the proximal tubule by the organic acid secretory pathway, which involves OAT1 and OAT3 on the basolateral membrane and multidrug resistance-associated protein 4 (MRP-4) on the luminal membrane (Ellison, 2019). Because thiazides must gain access to the tubular lumen to inhibit the Na^+-Cl^- symporter, drugs such as *probenecid* can attenuate the diuretic response to thiazides by competing for transport into the proximal tubule. However, plasma protein binding varies considerably among thiazide diuretics, and this parameter determines the contribution that filtration makes to tubular delivery of a specific thiazide.

Therapeutic Uses

Thiazide diuretics are used for the treatment of edema associated with diseases of the heart (CHF), liver (hepatic cirrhosis), and kidney (nephrotic syndrome, chronic renal failure, and acute glomerulonephritis). With the possible exceptions of *metolazone* and *indapamide*, most thiazide diuretics are ineffective when the GFR is less than 30 to 40 mL/min. Thiazide diuretics decrease blood pressure in hypertensive patients and are used widely for the treatment of hypertension in combination with other antihypertensive drugs (Tamargo et al., 2014) (see Chapter 32). Thiazide diuretics are inexpensive, as efficacious as other classes of antihypertensive agents, and well tolerated. Thiazides can be administered once daily, do not require dose titration, and have few contraindications. Moreover, thiazides have additive or synergistic effects when combined with other classes of antihypertensive agents. Although *hydrochlorothiazide* is the 10th most prescribed drug in the U.S. and is prescribed 20 times more often than *chlorthalidone* (Roush et al., 2015), there is strong evidence that *chlorthalidone* and other thiazide-like diuretics, such as *indapamide*, reduce blood pressure and cardiovascular events in hypertensive patients more so than does *hydrochlorothiazide* (Liang et al., 2017; Olde Engberink et al., 2015; Roush et al., 2015). This is likely due to the longer half-life of *thiazide-like* diuretics compared to *hydrochlorothiazide*, resulting in better 24-h control of arterial blood pressure by *chlorthalidone*.

Thiazide diuretics, which reduce urinary Ca^{2+} excretion, sometimes are employed to treat Ca^{2+} nephrolithiasis and may be useful for treatment of osteoporosis (see Chapter 52). Thiazide diuretics also are the mainstay for treatment of nephrogenic diabetes insipidus (DI), reducing urine volume by up to 50%. Although it may seem counterintuitive to treat a disorder of increased urine volume with a diuretic, thiazides reduce the kidney's ability to excrete free water: They increase proximal tubular water reabsorption (secondary to volume contraction) and block the ability of the DCT to form dilute urine. This last effect results in an increase in urine osmolality. Because other halides are excreted by renal processes similar to those for Cl^-, thiazide diuretics may be useful for the management of Br^- intoxication.

Toxicity, Adverse Effects, Contraindications, Drug Interactions

Thiazide diuretics rarely cause CNS (e.g., vertigo, headache), GI, hematological, and dermatological (e.g., photosensitivity, skin rashes, systemic lupus erythematosus) disorders. Thiazides are sulfonamides and can trigger sulfa allergic reactions. They can cause idiosyncratic acute transient myopia and acute angle-closure glaucoma (risk factors may include a history of sulfonamide allergy). The incidence of erectile dysfunction is greater with Na^+-Cl^- symport inhibitors than with several other antihypertensive agents (Grimm et al., 1997), but usually is tolerable. As with loop diuretics, most serious adverse effects of thiazides are related to abnormalities of fluid and electrolyte balance. These adverse effects include extracellular volume depletion, hypotension, hypokalemia, hyponatremia, hypochloremia, metabolic alkalosis, hypomagnesemia, hypercalcemia, and hyperuricemia. Thiazide diuretics have caused fatal or near-fatal hyponatremia (Rodenburg et al., 2013), and some patients are at recurrent risk of hyponatremia when rechallenged with thiazides.

Thiazide diuretics also decrease glucose tolerance and unmask latent diabetes mellitus (Scheen, 2018). The mechanism of impaired glucose tolerance appears to involve reduced insulin secretion and alterations in glucose metabolism. Hyperglycemia is reduced when K^+ or a K^+-sparing diuretic, such as *amiloride*, is given along with the thiazide diuretic (Brown et al., 2016). Importantly, thiazide-induced diabetes mellitus is not associated with the same cardiovascular disease risk as incident diabetes, and the benefits of thiazide diuretics outweigh the risks of worsening glucose control in both type 2 diabetics and those with new-onset diabetes (Scheen, 2018). Thiazide-induced hypokalemia also impairs the antihypertensive effect and cardiovascular protection afforded by thiazides in patients with hypertension. Thiazide diuretics also may increase plasma levels of low-density lipoprotein cholesterol, total cholesterol, and total triglycerides. Thiazide diuretics are contraindicated in individuals who are hypersensitive to sulfonamides. Thiazide diuretics may diminish the effects of uricosuric agents used to treat gout, sulfonylureas, and insulin and may increase the effects of anesthetics, *diazoxide*, digitalis glycosides, *lithium*, loop diuretics, and vitamin D. The effectiveness of thiazide diuretics may be reduced by NSAIDs, nonselective or selective COX-2 inhibitors, and bile acid sequestrants (reduced absorption of thiazides). *Amphotericin B* and corticosteroids increase the risk of hypokalemia induced by thiazide diuretics.

In a potentially lethal interaction, thiazide diuretic–induced K^+ depletion may contribute to fatal ventricular arrhythmias associated with drugs that prolong the QT interval (i.e., *quinidine*, *dofetilide*, *arsenic trioxide*; see also Chapter 34).

Inhibitors of Renal Epithelial Na^+ Channels: K^+-Sparing Diuretics

Triamterene and *amiloride* are the only two drugs of this class in clinical use. Both drugs cause small increases in NaCl excretion and usually are employed for their antikaliuretic actions to offset the effects of other diuretics that increase K^+ excretion. Consequently, *triamterene* and *amiloride*, along with *spironolactone* and *eplerenone* (described in the next section), often are classified as *potassium* (K^+)*–sparing diuretics*.

Mechanism and Site of Action

Both *triamterene* and *amiloride* are organic bases, are transported by the organic base secretory mechanism in the proximal tubule and have similar mechanisms of action (Figure 29–5). Principal cells in the late distal tubules and collecting ducts (particularly cortical collecting tubules) have, in their luminal membranes, ENaCs that provide a conductive pathway for Na^+ entry into the cell down the electrochemical gradient created by the basolateral Na^+ pump. The higher permeability of the luminal membrane for Na^+ depolarizes the luminal membrane but not the basolateral membrane, creating a lumen-negative transepithelial potential difference. This transepithelial voltage provides an important driving force for the secretion of K^+ into the lumen via K^+ channels (ROMK [Kir1.1] and BK channels) (Garcia and Kaczorowski, 2014) in the luminal membrane; however, the overall regulation of K^+ secretion in the late distal tubule and collecting duct involves multiple signaling mechanisms (Welling, 2013). Carbonic anhydrase inhibitors, loop diuretics, and thiazide diuretics increase Na^+ delivery to the late distal tubule and collecting duct, a situation that often is associated with increased K^+ and H^+ excretion.

Amiloride and *triamterene* block ENaCs in the luminal membrane of principal cells in late distal tubules and collecting ducts by binding to a site in the channel pore. ENaCs consist of three subunits (α, β, and γ)

TABLE 29–6 ■ INHIBITORS OF RENAL EPITHELIAL Na^+ CHANNELS (K^+-SPARING DIURETICS)

DRUG	RELATIVE POTENCY	ORAL BIOAVAILABILITY	$t_{1/2}$ (hours)	ROUTE OF ELIMINATION
Amiloride	1	15%–25%	~21	R
Triamterene	0.1	~50%	~4	M

M, metabolism; R, renal excretion of intact drug; however, triamterene is transformed into an active metabolite that is excreted in the urine.

(Kellenberger and Schild, 2015). Although the α subunit is sufficient for channel activity, maximal Na^+ permeability is induced when all three subunits are coexpressed in the same cell, probably forming a tetrameric structure consisting of two α subunits, one β subunit, and one γ subunit. Incompletely understood, complex mechanisms, including proteolytic cleavage, regulate epithelial Na^+ channel (ENaC) activation (Kellenberger and Schild, 2015).

Effects on Urinary Excretion

The late distal tubule and collecting duct have a limited capacity to reabsorb solutes; thus, Na^+ channel blockade in this part of the nephron increases Na^+ and Cl^- excretion rates only mildly (~2% of filtered load). Blockade of Na^+ channels hyperpolarizes the luminal membrane, reducing the lumen-negative transepithelial voltage. Because the lumen-negative potential difference normally opposes cation reabsorption and facilitates cation secretion, attenuation of the lumen-negative voltage decreases K^+, H^+, Ca^{2+}, and Mg^{2+} excretion rates. Volume contraction may increase reabsorption of uric acid in the proximal tubule; hence, chronic administration of *amiloride* and *triamterene* may decrease uric acid excretion. *Amiloride* and *triamterene* have little or no effect on renal hemodynamics and do not alter TGF.

ADME

Table 29–6 lists pharmacokinetic data for *amiloride* and *triamterene*. *Amiloride* is eliminated predominantly by urinary excretion of intact drug. *Triamterene* is metabolized in the liver to an active metabolite, 4-hydroxytriamterene sulfate, and this metabolite is excreted in urine. Therefore, *triamterene* toxicity may be enhanced in both hepatic disease and renal failure.

Therapeutic Uses

Because of the mild natriuresis induced by Na^+ channel inhibitors, these drugs seldom are used as sole agents in treatment of edema or hypertension; their major utility is in combination with other diuretics. Coadministration of a Na^+ channel inhibitor augments the diuretic and antihypertensive response to thiazide and loop diuretics. More important, the ability of Na^+ channel inhibitors to reduce K^+ excretion tends to offset the kaliuretic effects of thiazide and loop diuretics and results in normal plasma K^+ values.

Liddle syndrome (described later in this chapter) can be treated effectively with Na^+ channel inhibitors. Aerosolized *amiloride* has been shown to improve mucociliary clearance in patients with cystic fibrosis. By inhibiting Na^+ absorption from the surfaces of airway epithelial cells, *amiloride* augments hydration of respiratory secretions and thereby improves mucociliary clearance. *Amiloride* also is useful for *lithium*-induced nephrogenic DI because it blocks Li^+ transport into collecting tubule cells (Kortenoeven et al., 2009).

Toxicity, Adverse Effects, Contraindications, Drug Interactions

The most dangerous adverse effect of renal Na^+ channel inhibitors is hyperkalemia, which can be life threatening. Consequently, *amiloride* and *triamterene* are contraindicated in patients with hyperkalemia, as well as in patients at increased risk of developing hyperkalemia (e.g., patients with renal failure, patients receiving other K^+-sparing diuretics, patients taking angiotensin-converting enzyme inhibitors, or patients taking K^+ supplements). Even NSAIDs can increase the likelihood of hyperkalemia in patients receiving Na^+ channel inhibitors. Routine monitoring of the serum K^+ level is essential in patients receiving K^+-sparing diuretics. Cirrhotic patients are prone to megaloblastosis because of folic acid deficiency, and *triamterene*, a weak folic acid antagonist, may increase the likelihood of this adverse event. *Triamterene* also can reduce glucose tolerance and induce photosensitization and has been associated with interstitial nephritis and renal stones. Both drugs can cause CNS, GI, musculoskeletal, dermatological, and hematological adverse effects. The most common adverse effects of *amiloride* are nausea, vomiting, diarrhea, and headache; those of *triamterene* are nausea, vomiting, leg cramps, and dizziness.

Antagonists of Mineralocorticoid Receptors: Aldosterone Antagonists, K^+-Sparing Diuretics

Mineralocorticoids cause salt and water retention and increase K^+ and H^+ excretion by binding to specific mineralocorticoid receptors (MRs). Three MR antagonists are available in the U.S. (Table 29–7). *Spironolactone* and *eplerenone* have a steroidal structure, and *finerenone*, which was FDA approved in 2021, is a nonsteroidal MR antagonist. *Finerenone* and other nonsteroidal MR antagonists under development are sometimes referred to as "MR blockers" to differentiate them from *spironolactone* and *eplerenone*, which are widely called MR antagonists.

Mechanism and Site of Action

Epithelial cells in late distal tubule and collecting duct (particularly cortical collecting tubule) contain cytosolic MRs with high aldosterone affinity. When aldosterone binds to MRs, the MR-aldosterone complex translocates to the nucleus, where it regulates the expression of multiple gene products called aldosterone-induced proteins (AIPs) (Figure 29–6). AIPs affect the production, destruction, localization, and activation of multiple components of the system that mediates Na^+ reabsorption in late distal tubules and collecting ducts (Figure 29–6A). Consequently, transepithelial NaCl transport is enhanced, and the lumen-negative transepithelial voltage is increased. The latter effect increases the driving force for K^+ and H^+ secretion into the tubular lumen.

Drugs such as *spironolactone*, *eplerenone*, and *finerenone* competitively inhibit the binding of aldosterone to the MR. Unlike the MR-aldosterone complex, the MR-MRA antagonist complex is not able to induce the synthesis of AIPs. Because *spironolactone*, *eplerenone*, and *finerenone* block the biological effects of aldosterone, these agents also are referred to as *aldosterone antagonists*. MR antagonists are the only diuretics that do not require access to the tubular lumen to induce diuresis.

TABLE 29–7 ■ MINERALOCORTICOID RECEPTOR ANTAGONISTS (ALDOSTERONE ANTAGONISTS, K^+-SPARING DIURETICS)

DRUG	ORAL AVAILABILITY	$t_{1/2}$ (hours)	ROUTE OF ELIMINATION
Spironolactone	~65%	~1.6	M
Canrenone[a]	80%	3.7–22	M
Potassium canrenoate[a]	100%	3.7–22	M
Eplerenone	69%	~5	M
Finerenone	44%	~2	M

M, metabolism.

[a] Not available in the U.S.

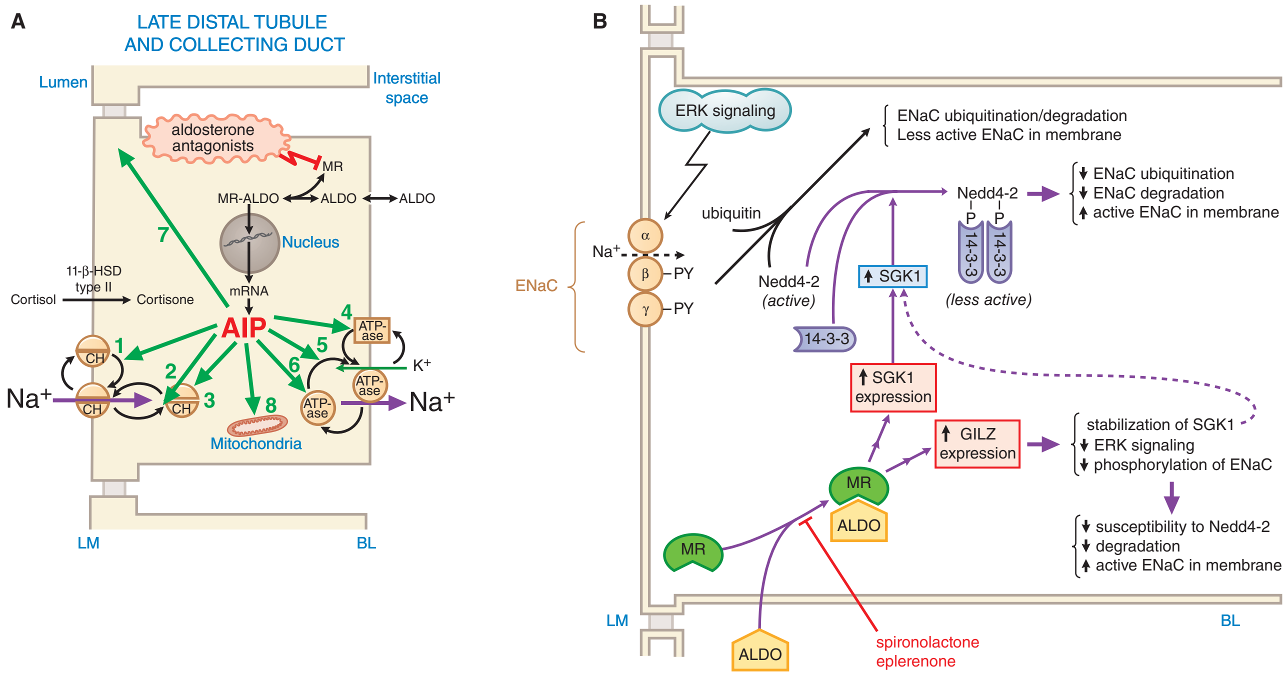

A
LATE DISTAL TUBULE AND COLLECTING DUCT
Lumen
Interstitial space
aldosterone antagonists
MR
MR-ALDO
ALDO
ALDO
Nucleus
7
11-β-HSD type II
Cortisol
Cortisone
mRNA
AIP
4
ATP-ase
1
CH
5
K+
2
6
ATP-ase
Na+
CH
CH
3
8
ATP-ase
Na+
Mitochondria
LM
BL
B
ERK signaling
ENaC ubiquitination/degradation
Less active ENaC in membrane
ubiquitin
Nedd4-2
P P
14-3-3
14-3-3
↓ ENaC ubiquitination
↓ ENaC degradation
↑ active ENaC in membrane
ENaC
Na+
α
β
γ
PY
PY
Nedd4-2 (active)
↑ SGK1
(less active)
14-3-3
↑ SGK1 expression
↑ GILZ expression
stabilization of SGK1
↓ ERK signaling
↓ phosphorylation of ENaC
↓ susceptibility to Nedd4-2
↓ degradation
↑ active ENaC in membrane
MR
ALDO
MR
LM
BL
ALDO
spironolactone
eplerenone

Figure 29–6 *Effects of aldosterone on late distal tubule and collecting duct and diuretic mechanism of aldosterone antagonists.*

A. *Overview of aldosterone's influences on Na$^+$ retention.* Via interaction with the mineralocorticoid receptor (MR), aldosterone affects several renal pathways that handle Na$^+$. Key to numbered items influenced by aldosterone (ALDO):

1. Activation of membrane-bound Na$^+$ channels
2. Na$^+$ channel (ENaC) removal from the membrane inhibited
3. *De novo* synthesis of Na$^+$ channels
4. Activation of membrane-bound Na$^+$,K$^+$-ATPase
5. Redistribution of Na$^+$,K$^+$-ATPase from cytosol to membrane
6. *De novo* synthesis of Na$^+$,K$^+$-ATPase
7. Changes in permeability of tight junctions
8. Increased mitochondrial production of ATP

Cortisol also has affinity for the mineralocorticoid receptor but is inactivated in the cell by 11-β-hydroxysteroid dehydrogenase (HSD) type II.

B. *Details of aldosterone's influences on membrane ENaC.* ERK signaling phosphorylates components of ENaC, making them susceptible to interaction with Nedd4-2, a ubiquitin-protein ligase that ubiquitinates ENaC, leading to its degradation. The Nedd4-2 interaction with ENaC occurs via several proline-tyrosine-proline (PY) motifs of ENaC. ALDO enhances expression of the serum and glucocorticoid-regulated kinase-1 (SGK1) and the glucocorticoid-induced leucine zipper protein (GILZ; TSC22D3). SGK1 phosphorylates and inactivates Nedd4-2; 14-3-3 dimers bind to the phosphorylated sites in Nedd4-2 and stabilize them. Phosphorylated Nedd4-2 no longer interacts well with the PY motifs of ENaC. As a result, ENaC is not ubiquitinated and remains in the membrane, leading to increased Na$^+$ entry into the cell. GILZ stabilizes SGK1, enhancing its effects, and decreases ERK signaling and ENaC phosphorylation, events that prime ENaC for degradation; these effects all lead to less ubiquitination and more active ENaC in the cell membranes of the distal tubule and collecting duct. For details see Ronzaud and Staub (2014) and Ronchetti et al. (2015). AIP, aldosterone-induced proteins; ALDO, aldosterone; CH, ion channel; BL, basolateral membrane; LM, luminal membrane; MR, mineralocorticoid receptor.

Both the β and γ subunits of ENaC have a specific region in their C terminus called the PY motif. The PY motif interacts with the ubiquitin ligase Nedd4-2 (Figure 29–6B), a protein that ubiquitinates ENaC and targets it for destruction by the proteasome. Aldosterone increases the expression of serum and glucocorticoid-stimulated kinase 1 (SKG-1); SGK-1 phosphorylates and inactivates Nedd4-2. Thus, aldosterone results in attenuated internalization and proteasome-mediated degradation of ENaC, leading to increased expression of ENaC in the luminal membrane. Liddle syndrome, an autosomal-dominant, monogenic disease characterized by sodium retention and severe hypertension, is caused by mutations in the PY motif of either the β or γ subunit of ENaC, leading to MR-independent overexpression of ENaC. Thus, Liddle syndrome is responsive to ENaC inhibitors but not to MR antagonists.

Effects on Urinary Excretion

The effects of MR antagonists on urinary excretion are similar to those induced by renal ENaC inhibitors. However, unlike Na$^+$ channel inhibitors, the clinical efficacy of MR antagonists is a function of endogenous aldosterone levels. The higher the endogenous aldosterone level, the greater the effects of MR antagonists on urinary excretion. MR antagonists have little or no effect on renal hemodynamics and do not alter TGF.

Other Actions

Spironolactone has some affinity toward progesterone and androgen receptors and thereby induces side effects such as gynecomastia, impotence, and menstrual irregularities. Owing to its 9,11-epoxide group, *eplerenone* has very low affinity for progesterone and androgen receptors (<1% and <0.1%, respectively) compared with *spironolactone*. Nonsteroidal MR blockers (e.g., *finerenone*) also have very low affinity for progesterone and androgen receptors. High *spironolactone* concentrations can interfere with steroid biosynthesis by inhibiting steroid hydroxylases, but these effects have limited clinical relevance.

ADME

Spironolactone is absorbed partially (~65%), is metabolized extensively (even during its first passage through the liver), undergoes enterohepatic recirculation, and is highly protein bound. Although *spironolactone* per se has a short $t_{1/2}$ (~1.6 h), it is metabolized to a number of active compounds (including canrenone; see discussion that follows) that have long half-lives. The $t_{1/2}$ of *spironolactone* is prolonged to 9 h in patients with cirrhosis. *Eplerenone* and *finerenone* have good oral availability and are eliminated primarily by metabolism by CYP3A4 to inactive metabolites, with a $t_{1/2}$ of about 5 h for *eplerenone* and 2 h for *finerenone*. Canrenone and K^+-canrenoate also are in clinical use (not available in the U.S.). Canrenoate is not active but is *converted* to canrenone. Due to first-pass metabolism in the gut wall and liver, *finerenone* has an oral availability of only 44%.

Therapeutic Uses

The MR antagonists often are coadministered with thiazide or loop diuretics in the treatment of edema and hypertension. Such combinations result in increased mobilization of edema fluid while causing lesser perturbations of K^+ homeostasis. MR antagonists are particularly useful in the treatment of resistant hypertension due to primary hyperaldosteronism (adrenal adenomas or bilateral adrenal hyperplasia) and of refractory edema associated with secondary aldosteronism (cardiac failure, hepatic cirrhosis, nephrotic syndrome, and severe ascites). MR antagonists are considered diuretics of choice in patients with hepatic cirrhosis. MR antagonists, added to standard therapy, substantially reduce morbidity and mortality in patients with heart failure with reduced ejection fraction (see Chapter 33) (D'Elia and Krum, 2014).

In patients with heart failure with reduced ejection fraction, MR antagonists reduce overall and cardiac mortality, sudden cardiac death, cardiac hospitalizations, and progression of heart failure and are recommended for patients with mild to marked heart failure severity (Flatt et al., 2016). In patients with heart failure with preserved ejection fraction, the use of MR antagonists remains controversial. In such patients, MR antagonists improve left ventricular diastolic function but do not improve exercise capacity, mortality, or hospitalization for heart failure (Edelmann et al., 2013; Li et al., 2018; Pitt et al., 2014). MR antagonists also may reduce ventricular arrhythmias and sudden cardiac death.

MR antagonists reduce proteinuria in patients with CKD, and the use of these drugs in kidney diseases is under intense investigation (Bauersachs et al., 2015). The nonsteroidal MR blocker *finerenone* improves a composite of renal outcomes and cardiovascular outcomes in patients with CKD and type 2 diabetes (Bakris et al., 2020). The improvement in cardiovascular outcomes with *finerenone* in type 2 diabetics with CKD is independent of preexisting atherosclerotic cardiovascular disease (Filippatos et al., 2020). *Finerenone* is FDA approved to decrease the risk of renal function decline, nonfatal myocardial infarction, hospitalization for heart failure, and death due to cardiovascular disease in adult patients with CKD associated with type 2 diabetes. In 2021, *finerenone* was FDA approved to reduce the risk of end-stage kidney disease, cardiovascular death, and heart failure in diabetic patients with CKD.

Spironolactone, but not *eplerenone*, is widely considered to be an antiandrogenic compound and has been used to treat hirsutism and acne; however, evidence for efficacy is weak (Brown et al., 2009), and these uses are not FDA approved. Biochemical studies suggested that *spironolactone* is a partial agonist of androgen receptors (Nirdé et al., 2001) and can exert antiandrogenic or androgenic effects depending on context (e.g., the prevailing levels of endogenous androgenic steroids). Indeed, a case report describes *spironolactone*-induced worsening of prostate cancer attributed to androgen receptor stimulation (Sundar and Dickinson, 2012).

Toxicity, Adverse Effects, Contraindications, Drug Interactions

Hyperkalemia is the principal risk of MR antagonists. Therefore, these drugs are contraindicated in patients with hyperkalemia and in those at increased risk of developing hyperkalemia. *Eplerenone* and *finerenone* are contraindicated in patients with creatinine clearance ≤30 mL/min and ≤25 mL/min, respectively. The starting dose of *finerenone* should be decreased from 20 mg to 10 mg in patients with creatinine clearance ≤60 mL/min. MR antagonists also can induce metabolic acidosis in cirrhotic patients. Salicylates may reduce the tubular secretion of canrenone and decrease diuretic efficacy of *spironolactone*. *Spironolactone* may alter the clearance of cardiac glycosides. Owing to its affinity for other steroid receptors, *spironolactone* may cause gynecomastia, impotence, decreased libido, and menstrual irregularities. *Spironolactone* also may induce diarrhea, gastritis, gastric bleeding, and peptic ulcers (the drug is contraindicated in patients with peptic ulcers). CNS adverse effects include drowsiness, lethargy, ataxia, confusion, and headache. *Spironolactone* may cause skin rashes and, rarely, Stevens-Johnson syndrome, toxic epidermal necrolysis, drug rash with eosinophilia and systemic symptoms, and blood dyscrasias. Strong inhibitors of CYP3A4 (e.g., *ketoconazole*, *itraconazole*) may increase plasma levels of *eplerenone* and *finerenone*, and such drugs should not be administered to patients taking *eplerenone* or *finerenone* and vice versa. Other than hyperkalemia and GI disorders, the rate of adverse events for *eplerenone* is similar to that of placebo.

Inhibitors of Sodium-Glucose Symport: SGLT2 Inhibitors, Gliflozins

Inhibitors of sodium-glucose cotransporter type 2 (SGLT2), also referred to as gliflozins, were developed for management of diabetes mellitus and are described in detail in Chapter 51. SGLT2 inhibitors are also efficacious diuretics. *Canagliflozin*, *dapagliflozin*, *empagliflozin,* and *ertugliflozin* are approved for treatment of diabetes in the U.S. *Ipragliflozin*, *luseogliflozin*, *remogliflozin*, and *tofogliflozin* are SGLT2 inhibitors used outside the U.S. Chemically, gliflozins consist of a glucose sugar (unmodified or modified) with a substituted diarylmethylene group attached to the β-position of the anomeric carbon.

Mechanism and Site of Action

SGLT2 is a low-affinity, yet high-capacity, sodium-glucose symporter (cotransporter) that is expressed in the apical membrane of the proximal tubule and is responsible for the reabsorption of approximately 90% of filtered glucose across the apical membrane (Ansary et al., 2019). SGLT1 is a high-affinity, but low-capacity, sodium-glucose cotransporter that is also expressed in the apical membrane of the proximal tubule but only reabsorbs approximately 10% of filtered glucose. Both SGLT2 and SGLT1 exploit the electrochemical gradient of Na^+ across the apical membrane to provide the free energy required to reabsorb the large quantities of glucose—the glomerular filtration barrier does not retain glucose—that are delivered to the proximal tubule via the ultrafiltrate. The transport of intracellular glucose across the renal epithelial basolateral membrane is mediated by the facilitative glucose transporter GLUT2. At prescribed doses, gliflozins selectively block SGLT2 and thereby reduce the reabsorption of filtered glucose by up to 50%. Because little glucose is reabsorbed by the more distal segments of the nephron, glucose that exits the proximal tubule functions as an osmotic diuretic, similar to *mannitol.* Because SGLT2 normally accounts for approximately 5% of total renal Na^+ reabsorption, inhibition of SGLT2 also decreases the reabsorption of Na^+ by the proximal tubule and thus delivers additional Na^+ to downstream nephron segments. Thus, the gliflozins, by inhibiting SGLT2, engage a dual or "hybrid" mechanism of diuretic action that combines an osmotic diuresis, due to increased glucose excretion, with attenuation of Na^+ reabsorption in the proximal tubule (Sarzani et al., 2020).

Effects on Urinary Excretion

In rats, acute administration of *luseogliflozin* increases glucose excretion by 450-fold, urine flow by 5-fold, and sodium excretion by 7-fold, but does not affect K^+ excretion (Ansary et al., 2017). Numerous preclinical and clinical studies confirm the diuretic and natriuretic effects of gliflozins (Ansary et al., 2019).

Effects on Renal Hemodynamics

In patients with diabetes mellitus, often there exists a state of glomerular hyperfiltration due to decreased vascular resistance of afferent arterioles and increased vascular resistance of efferent arterioles resulting in increased glomerular hydrostatic pressure (Cherney et al., 2014; Škrtić et al., 2014). Theoretically, by increasing NaCl delivery to the TAL, gliflozins should trigger TGF and thereby increase preglomerular vascular resistance and decrease glomerular hypertension. Indeed, *empagliflozin* decreases RBF and hyperfiltration in type 1 diabetics (Cherney et al., 2014), a response that is accompanied by an increase in the calculated preglomerular vascular resistance and a decrease in the estimated glomerular hydrostatic pressure (Škrtić et al., 2014).

Other Actions

Due to their diuretic and natriuretic effects, chronic treatment with gliflozins reduces plasma volume by approximately 7% and decreases systolic and diastolic blood pressures by approximately 2 to 4 and 1 to 2 mm Hg, respectively (Sarzani et al., 2020). The antihypertensive effects of gliflozins are mostly secondary to their diuretic activity.

ADME

Canagliflozin, *dapagliflozin*, *empagliflozin*, and *ertugliflozin* are readily absorbed (oral availabilities >65%), are highly protein bound (>85%), and have long $t_{1/2}$s (ranging from 11 to 17 h). The major route of elimination is via hepatic metabolism followed by excretion of metabolites in the urine and feces.

Therapeutic Uses

The role of gliflozins in the treatment of diabetes mellitus is described in detail in Chapter 51. Gliflozins combined with *metformin* are recommended as the preferred treatment regimen for type 2 diabetes. In type 2 diabetics, gliflozins reduce cardiovascular morality, nonfatal stroke, nonfatal myocardial infarction, hospitalization for heart failure, and total mortality (Neal et al., 2017; Wiviott et al., 2019; Zinman et al., 2015). In type 2 diabetes, gliflozins also protect against declining renal function, end-stage kidney disease, death due to kidney disease, and acute kidney injury (Neuen et al., 2019). The antihypertensive actions of gliflozins likely account for some of the beneficial effects of gliflozins to reduce cardiovascular disease and CKD. Relief of glomerular hypertension may also contribute to the effectiveness of gliflozins to prevent diabetic kidney disease. Because AngII preferentially constricts the postglomerular microcirculation, RAS blockers reduce postglomerular vascular resistance. The combination of a gliflozin and a RAS blocker may synergize to reduce glomerular hypertension and protect against diabetic nephropathy (Sarzani et al., 2020). The protective actions of gliflozins on the heart and kidneys may also be due in part to reduced hyperglycemia, inflammation, oxidative stress, and sympathetic nervous system activity (Ni et al., 2020; Sarzani et al., 2020).

Diuretics that act in the more distal segments of the nephron often upregulate transport systems in the proximal tubule, a phenomenon that contributes to diuretic resistance. This provides a rationale for combining gliflozins with loop or thiazide diuretics to provide for sequential nephron blockade to overcome diuretic resistance (Wilcox et al., 2020). Indeed, clinical studies demonstrate that gliflozins augment natriuresis induced by loop diuretics (Griffin et al., 2020; Wilcox et al., 2020). In patients with heart failure with reduced ejection fraction, a disease in which most patients are treated with loop diuretics, gliflozins reduce cardiovascular death and hospitalization regardless of presence or absence of diabetes (McMurray et al., 2019; Petrie et al., 2020).

Toxicity, Adverse Effects, Contraindications, Drug Interactions

Reproductive and urinary tract infections, although mild to moderate in severity, are not uncommon in patients treated with gliflozins. This may be due to elevated glucose concentrations in the urine, which provide a favorable environment for bacterial and fungal infections. Although rare, gliflozins can induce ketoacidosis, and serum ketone body levels and pH should be monitored. Gliflozins can reduce bone density and increase risk of bone fractures. Accordingly, gliflozins should be used with caution in patients at elevated risk of bone fractures. In some clinical trials, gliflozins appeared to increase the risk of lower limb amputation. However, a meta-analysis of randomized trials failed to reproduce this finding. Whether gliflozins as a class increase risk of amputation remains unclear. There is some evidence of gliflozin-induced tumorgenicity in animals and patients; however, at present, this remains controversial. Other adverse effects of gliflozins are related to their main pharmacological effects and include hypoglycemia, polyuria, dehydration, hyperphosphatemia, hypermagnesemia, and hyperkalemia, hypovolemia, and hypotension. Gliflozins are contraindicated in patients with severe renal impairment and end-stage renal disease. Regarding drug interactions, β-adrenoceptor blockers may mask symptoms of hypoglycemia, and fluoroquinolones, angiotensin-converting enzyme inhibitors, and other antidiabetic agents can increase the risk of hypoglycemia in patients taking gliflozins.

Inhibitors of the Nonspecific Cation Channel: Natriuretic Peptides

Four natriuretic peptides (NPs) are relevant with respect to human physiology: ANP, brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and urodilatin. The IMCD is a major site of action of NPs.

Three NPs—ANP, BNP, and CNP—share a common homologous 17-member amino acid ring formed by a disulfide bridge between cysteine residues, although they are products of different genes. Urodilatin, also structurally similar, arises from altered processing of the same precursor molecule as ANP and has four additional amino acids at the N terminus. ANP and BNP are produced by the heart in response to wall stretch; CNP is of endothelial and renal cell origin; urodilatin is found in the kidney and urine. Natriuretic peptide receptors (NPRs), classified as types A, B, and C, are membrane monospans. NPRA (binds ANP and BNP) and NPRB (binds CNP) have intracellular domains with guanylate cyclase activity and a protein kinase element. NPRC (binds all NPs) has a truncated intracellular domain and may help with NP clearance. The various NPs have somewhat overlapping effects, causing natriuresis, inhibition of production of renin and aldosterone, and vasodilation (the result of cGMP elevation in vascular smooth muscle). A human recombinant BNP, *nesiritide*, with the same 32–amino acid structure as the endogenous peptide produced by the ventricular myocardium, is available for clinical use in some countries but was discontinued in the U.S.

Mechanism and Site of Action

The IMCD is the final site along the nephron where Na^+ is reabsorbed. Up to 5% of the filtered Na^+ load can be reabsorbed here. The effects of nesiritide and other NPs are mediated via effects of cGMP on Na^+ transporters (Figure 29–7). Two types of Na^+ channels are expressed in IMCD.

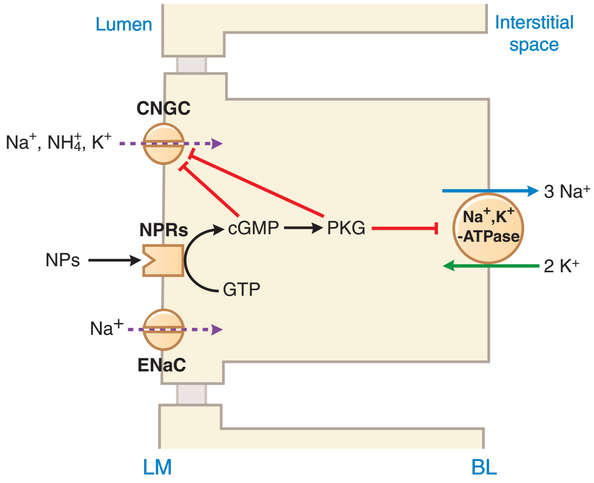


Figure 29–7 *The inner medullary collecting duct Na^+ transport and its regulation.* Na^+ enters the IMCD cell in one of two ways: via ENaC and through a CNGC that transports Na^+, K^+, and NH_4^+ and is gated by cGMP. Na^+ then exits the cell via the Na^+,K^+-ATPase. The CNGC is the primary pathway for Na^+ entry and is inhibited by NPs. NPs bind to cell surface NPRs A, B, and C. The A and B receptors are isoforms of particulate guanylyl cyclase that synthesize cGMP. The CNGC is inhibited by cGMP directly and indirectly through PKG. PKG activation also inhibits Na^+ exit via the Na^+,K^+-ATPase. ENaC is a low-conductance (4-pS) highly selective Na^+ channel that plays a minor role in IMCD Na^+ transport (see Figure 29–6). BL, basolateral membrane; LM, luminal membrane.

The *first* is a high-conductance, 28-pS, nonselective cyclic nucleotide-gated cation channel (CNGC). This channel is inhibited by intracellular cGMP and by NPs via their capacity to stimulate membrane-bound guanylyl cyclase activity and elevate cellular cGMP. The *second* type of Na^+ channel expressed in the IMCD is the low-conductance, 4-pS, highly selective Na^+ channel ENaC. The majority of Na^+ reabsorption in the IMCD is mediated via CNGC.

Effects on Urinary Excretion and Renal Hemodynamics

Nesiritide inhibits Na^+ transport in both the proximal and distal nephron, but its primary effect is in the IMCD. Urinary Na^+ excretion increases with *nesiritide*, but the effect may be attenuated by upregulation of Na^+ reabsorption in upstream segments of the nephron. GFR increases in response to *nesiritide* in normal subjects, but in treated patients with CHF, GFR may increase, decrease, or remain unchanged.

Other Actions

Administration of *nesiritide* decreases systemic and pulmonary resistances and left ventricular filling pressure and induces a secondary increase in cardiac output.

ADME

Natriuretic peptides are administered intravenously. *Nesiritide* has a distribution $t_{1/2}$ of 2 min and a mean terminal $t_{1/2}$ of 18 min. Clearance occurs via at least two mechanisms: internalization and subsequent degradation mediated by NPCR, and metabolism by extracellular proteases (Potter, 2011). There is no need to adjust the dose for renal insufficiency.

Therapeutic Uses

Human recombinant ANP (*carperitide*, available only in Japan) and BNP (*nesiritide*, no longer available in the U.S.) are the therapeutic agents of this class. In patients with acute decompensated CHF who have dyspnea with minimal activity or at rest, *nesiritide* reduces pulmonary capillary wedge pressure and improves short-term symptoms of dyspnea. However, the ASCEND-HF trial found that *nesiritide* does not change mortality and rehospitalization and has only a small effect on dyspnea (O'Connor et al., 2011). Thus, *nesiritide* is not recommended for routine use in the broad population of patients with acute heart failure (O'Connor et al., 2011).

Toxicity, Adverse Effects, Contraindications, Drug Interactions

Nesiritide can cause hypotension, and there are concerns about adverse renal effects. However, the ASCEND-HF trial did not demonstrate worsening of renal function in *nesiritide*-treated patients with heart failure (O'Connor et al., 2011).

Adenosine Receptor Antagonists

There are four adenosine receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3). A_1, A_{2A}, and A_{2B} receptors regulate aspects of renal physiology. The A_1 receptor is expressed in the proximal tubule and stimulates reabsorption of Na^+. Consequently, antagonists of A_1 receptors cause diuresis/natriuresis, yet are K^+ sparing. Several naturally occurring methylxanthines (e.g., caffeine, theophylline, and theobromine) are A_1 receptor antagonists (albeit nonselective) and consequently cause diuresis. *Pamabrom* is a mild diuretic consisting of a one-to-one mixture of 8-bromotheophylline and 2-amino-2-methyl-1-propanol; 8-bromotheophylline, a methylxanthine, is the active component of *pamabrom*. *Pamabrom* is the diuretic ingredient in several over-the-counter products marketed for relief of premenstrual syndrome. Little is known regarding the pharmacology, diuretic mechanism of action, and efficacy of *pamabrom*. However, because 8-bromotheophylline is a methylxanthine, it is possible that the mild diuresis induced by *pamabrom* is related to blockade of renal A_1 receptors.

Emerging Diuretics

Discoveries over the past few decades have revealed many of the intricate molecular mechanisms that regulate reabsorption and secretion by renal epithelial cells. Several components of these regulatory mechanisms are being targeted to create novel diuretics that may overcome diuretic resistance and improve patient outcomes (Cheng et al., 2017). Signal transduction kinases such as with-no-lysine kinases (WNKs), Ste20-related proline/alanine-rich kinase (SPAK), and oxidative stress-responsive 1 (OSR1) regulate the activity or expression of the Na^+-K^+-$2Cl^-$ symporter, the Na^+-Cl^- symporter, or ENaC, and inhibitors of these proteins may become useful diuretics. Transport by some renal epithelial cells requires the activity of basolateral Cl^- channels (ClC-Kb), apical inwardly rectifying K^+ channels (Kir1.1, aka ROMK), basolateral inwardly rectifying K^+ channels (Kir4.1), or basolateral Cl^--HCO_3^- antiporters (pendrin). Blockers of these channels may also become useful diuretics with unique properties. 8-Aminoguanosine and 8-aminoguanine increase sodium and glucose excretion while markedly reducing K^+ excretion, a unique diuretic profile that is likely due in part to inhibition of purine nucleoside phosphorylase (Jackson et al., 2016, 2018).

Clinical Use of Diuretics

Site and Mechanism of Diuretic Action

An understanding of the sites and mechanisms of action of diuretics enhances comprehension of the clinical aspects of diuretic pharmacology. Figure 29–5 provides a summary view of the sites and mechanisms of actions of diuretics.

The Role of Diuretics in Clinical Medicine

Figure 29–8 illustrates interrelationships among renal function, Na^+ intake, water homeostasis, distribution of extracellular fluid volume, and mean arterial blood pressure and suggests three fundamental strategies for mobilizing edema fluid:

- Correction of the underlying disease
- Restriction of Na^+ intake
- Administration of diuretics

Figure 29–9 presents a useful synthesis, Brater's algorithm, a logically compelling algorithm for diuretic therapy (specific recommendations for drug, dose, route, and drug combinations) in patients with edema caused by renal, hepatic, or cardiac disorders (Brater, 1998).

The clinical situation dictates whether a patient should receive diuretics and what therapeutic regimen should be used (type of diuretic, dose, route of administration, and speed of mobilization of edema fluid). Massive pulmonary edema in patients with acute left-sided heart failure is a medical emergency requiring rapid, aggressive therapy, including intravenous administration of a loop diuretic. In this setting, use of oral diuretics is inappropriate. Conversely, mild pulmonary and venous congestion associated with chronic heart failure is best treated with an oral loop or thiazide diuretic, the dosage of which should be titrated carefully to maximize the benefit-to-risk ratio. Loop and thiazide diuretics decrease morbidity and mortality in patients with heart failure (Faris et al., 2002). MR antagonists also demonstrate reduced morbidity and mortality in patients with heart failure receiving optimal therapy with other drugs.

Periodic administration of diuretics to cirrhotic patients with ascites may eliminate the necessity for or reduce the interval between paracenteses, adding to patient comfort and sparing protein reserves that are lost during paracenteses. Although diuretics can reduce edema associated with chronic renal failure, increased doses of more powerful loop diuretics usually are required. In nephrotic syndrome, diuretic response often is disappointing. In chronic renal failure and cirrhosis, edema will not pose an immediate health risk but can greatly reduce quality of life. In such cases, only partial removal of edema fluid should be attempted, and fluid should be mobilized slowly using a diuretic regimen that accomplishes the task with minimal perturbation of normal physiology.

Diuretic resistance refers to the condition in which the rate of diuresis/natriuresis is inadequate despite administration of what should be an adequate diuretic regimen (Cox and Testani, 2020). Diuretic resistance remains a major clinical challenge. Considerable attention has been directed toward the mechanisms of, and approaches to overcoming, diuretic resistance (Cox and Testani, 2020; Mullens et al., 2019; Wilcox et al., 2020). Pre-nephron (e.g., reduced RBF, hypoalbuminemia, excessive sodium intake, NSAIDs) or intra-nephron factors may contribute to diuretic resistance. Intra-nephron mechanisms are classified as pre–loop

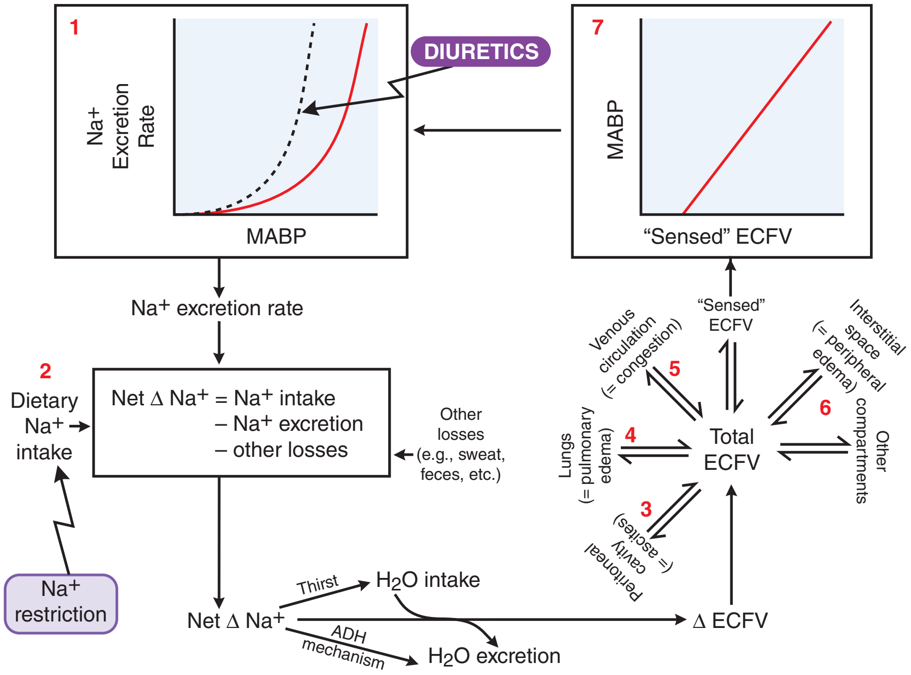


Figure 29–8 *Interrelationships among renal function, Na+ intake, water homeostasis, distribution of extracellular fluid volume, and mean arterial blood pressure.* Starting at upper left panel (#1), read this figure counterclockwise. Complex interrelationships exist among the cardiovascular system, kidneys, CNS, and capillary beds such that perturbations at one of these sites can affect all other sites. A primary law of the kidney is that Na^+ excretion is a steep function of mean arterial blood pressure (MABP) such that small increases in MABP cause marked increases in Na^+ excretion; this is known as the "pressure-natriuresis" relationship (upper left). Over any given time interval, the net change in total-body Na^+ is the dietary Na^+ intake minus the urinary excretion rate and other losses (lower left). If the pressure-natriuresis curve is right-shifted, a net positive Na^+ balance occurs, and the extracellular Na^+ concentration increases, thus stimulating water intake (thirst) and reducing urinary water output (via ADH release). These changes expand the extracellular fluid volume (ECFV), and the enlarged ECFV is distributed among many body compartments (lower right). ECFV on the arterial side of the circulation pressurizes the arterial tree ("sensed" ECFV) and increases MABP (upper right), thus increasing Na^+ excretion (and completing the loop). This loop cycles until net Na^+ accumulation is zero; i.e., in the long run, Na^+ intake must equal Na^+ loss.

These considerations explain the fundamental mechanisms of edema formation:

1. Rightward shift of renal pressure natriuresis curve.
2. Excessive dietary Na^+ intake.
3. Increased distribution of ECFV to peritoneal cavity (e.g., liver cirrhosis with increased hepatic sinusoidal hydrostatic pressure) leading to ascites formation.
4. Increased distribution of ECFV to lungs (e.g., left-sided heart failure with increased pulmonary capillary hydrostatic pressure) leading to pulmonary edema.
5. Increased distribution of ECFV to venous circulation (e.g., right-sided heart failure) leading to venous congestion.
6. Peripheral edema caused by altered Starling forces causing increased distribution of ECFV to interstitial space (e.g., diminished plasma proteins in nephrotic syndrome, severe burns, and liver disease).
7. Increased MABP resulting from "sensed" ECFV on the arterial side of the heart.

These perturbations leading to edema can be addressed by: **A.** Correcting the underlying disease; **B.** Administering diuretics to left-shift the renal pressure-natriuresis relationship; **C.** Restricting dietary Na^+ intake.

of Henle (e.g., increased sodium reabsorption by the proximal tubule, reduced GFR, albuminuria, competition for diuretic transport into the proximal tubule), loop of Henle (e.g., decreased response of the TAL to diuretics), or post–loop of Henle (e.g., hypertrophy and hyperfunction of the distal tubule) (Cox and Testani, 2020). NSAID coadministration is a common preventable cause of diuretic resistance. PG production, especially PGE_2, is an important counterregulatory mechanism in states of reduced renal perfusion (e.g., volume contraction, CHF, cirrhosis), characterized by activation of the renin-angiotensin-aldosterone system and sympathetic nervous system. NSAID administration can block PG-mediated effects that counterbalance the renin-angiotensin-aldosterone system and sympathetic nervous system, resulting in salt and water retention. Diuretic resistance also occurs with COX-2–selective inhibitors.

In chronic renal failure, a reduction in RBF decreases delivery of diuretics to the kidney, and accumulation of endogenous organic acids competes with loop diuretics for transport at the proximal tubule. Consequently, diuretic concentration at the active site in the tubular lumen is diminished. In nephrotic syndrome, binding of diuretics to luminal albumin was thought to limit response; however, the validity of this concept has been challenged. In hepatic cirrhosis, nephrotic syndrome, and heart failure, nephrons may have diminished diuretic responsiveness because of increased proximal tubular Na^+ reabsorption, leading to diminished Na^+ delivery to distal nephrons.

Faced with resistance to loop diuretics, the clinician has several options:

- Bed rest may restore drug responsiveness by improving the renal circulation.
- An increase in dose of loop diuretic may restore responsiveness; however, nothing is gained by increasing the dose above that which causes a near-maximal effect (the ceiling dose) of the diuretic.
- Administration of smaller doses more frequently or a continuous intravenous infusion of a loop diuretic will increase the length of time that an effective diuretic concentration is at the active site.
- Use of combination therapy to sequentially block more than one site in the nephron may result in a synergistic interaction between two diuretics. For example, a combination of a loop diuretic with a K^+-sparing or a thiazide diuretic may improve therapeutic response;

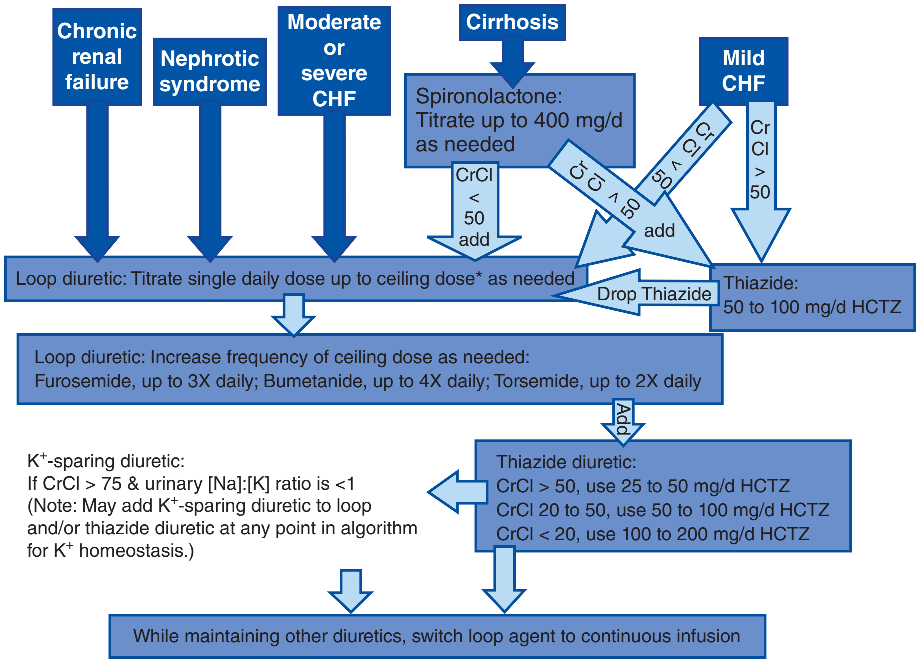

Chronic renal failure
Nephrotic syndrome
Moderate or severe CHF
Cirrhosis
Mild CHF
Spironolactone: Titrate up to 400 mg/d as needed
CrCl < 50 add
CrCl < 50
CrCl > 50 add
CrCl > 50
Loop diuretic: Titrate single daily dose up to ceiling dose* as needed
Drop Thiazide
Thiazide: 50 to 100 mg/d HCTZ
Loop diuretic: Increase frequency of ceiling dose as needed:
Furosemide, up to 3X daily; Bumetanide, up to 4X daily; Torsemide, up to 2X daily
Add
Thiazide diuretic:
CrCl > 50, use 25 to 50 mg/d HCTZ
CrCl 20 to 50, use 50 to 100 mg/d HCTZ
CrCl < 20, use 100 to 200 mg/d HCTZ
K+-sparing diuretic:
If CrCl > 75 & urinary [Na]:[K] ratio is <1
(Note: May add K+-sparing diuretic to loop and/or thiazide diuretic at any point in algorithm for K+ homeostasis.)
While maintaining other diuretics, switch loop agent to continuous infusion

Figure 29–9 *"Brater's algorithm" for diuretic therapy of chronic renal failure, nephrotic syndrome, CHF, and cirrhosis.* Follow algorithm until adequate response is achieved. If adequate response is not obtained, advance to the next step. For illustrative purposes, the thiazide diuretic used is hydrochlorothiazide (HCTZ). An alternative thiazide-type diuretic may be substituted with dosage adjusted to be pharmacologically equivalent to the recommended dose of HCTZ. *Do not combine two K^+-sparing diuretics due to the risk of hyperkalemia.* CrCl indicates creatinine clearance in milliliters per minute, and ceiling dose refers to the smallest dose of diuretic that produces a near-maximal effect. *Ceiling doses of loop diuretics and dosing regimens for continuous intravenous infusions of loop diuretics are disease-state specific. Doses are for adults only.

however, nothing is gained by the administration of two drugs of the same type. Thiazide diuretics with significant proximal tubular effects (e.g., metolazone) are particularly well suited for sequential blockade when coadministered with a loop diuretic.

- Reducing salt intake will diminish postdiuretic Na^+ retention, which can nullify previous increases in Na^+ excretion.
- Scheduling of diuretic administration shortly before food intake will provide effective diuretic concentration in the tubular lumen when salt load is highest.

Part II: Water Homeostasis and the Vasopressin System

Vasopressin Physiology

Arginine vasopressin (ADH in humans) is the main hormone that regulates body fluid osmolality. The hormone is released by the posterior pituitary whenever water deprivation causes an increased plasma osmolality or whenever the cardiovascular system is challenged by hypovolemia or hypotension. Vasopressin acts primarily in the renal collecting duct to increase the permeability of the cell membrane to water, thus permitting water to move passively down an osmotic gradient across the collecting duct into the extracellular compartment.

Vasopressin is a potent vasopressor/vasoconstrictor. It is also a neurotransmitter; among its actions in the CNS are apparent roles in the secretion of corticotropin (ACTH) and in regulation of the cardiovascular system, temperature, and other visceral functions. Vasopressin also promotes release of coagulation factors by vascular endothelium and increases platelet aggregability.

Anatomy of the Vasopressin System

The antidiuretic mechanism in mammals involves two anatomical components: a CNS component for synthesis, transport, storage, and release of vasopressin and a renal collecting duct system composed of epithelial cells that respond to vasopressin by increasing their water permeability. The CNS component of the antidiuretic mechanism is called the *hypothalamoneurohypophyseal system* and consists of neurosecretory neurons with perikarya located predominantly in two specific hypothalamic nuclei, the supraoptic nucleus (SON) and paraventricular nucleus (PVN). Long axons of magnocellular neurons in SON and PVN terminate in the neural lobe of the posterior pituitary (neurohypophysis), where they release vasopressin and oxytocin (see Figure 46–1).

Synthesis of Vasopressin

Vasopressin and oxytocin are synthesized mainly in the perikarya of magnocellular neurons in the SON and PVN. However, parvicellular neurons in the PVN also synthesize vasopressin, as do some non-CNS cells (see discussion that follows). Vasopressin synthesis appears to be regulated solely at the transcriptional level. In humans, a 168–amino acid preprohormone (Figure 29–10) is synthesized and then packaged into membrane-associated granules. The prohormone contains three domains: vasopressin (residues 1–9), vasopressin-neurophysin (residues 13–105), and vasopressin-glycopeptide (residues 107–145). The vasopressin domain is linked to the vasopressin-neurophysin domain through a GLY-LYS-ARG-processing signal, and the vasopressin-neurophysin is linked to the vasopressin-glycopeptide domain by an ARG-processing signal. In secretory granules, an endopeptidase, exopeptidase, monooxygenase, and lyase act sequentially on the prohormone to produce vasopressin, vasopressin-neurophysin (sometimes referred to as neurophysin II), and vasopressin-glycopeptide. The synthesis and transport of vasopressin depend on the preprohormone conformation. In particular, vasopressin-neurophysin binds vasopressin and is critical for correct processing, transport, and storage of vasopressin. Genetic mutations in either the signal peptide or vasopressin-neurophysin give rise to central DI.

Vasopressin also is synthesized by the heart and adrenal gland. In the heart, elevated wall stress increases vasopressin synthesis several-fold and may contribute to impaired ventricular relaxation and coronary vasoconstriction. Vasopressin synthesis in the adrenal medulla stimulates catecholamine secretion from chromaffin cells and may promote adrenal cortical growth and stimulate aldosterone synthesis.

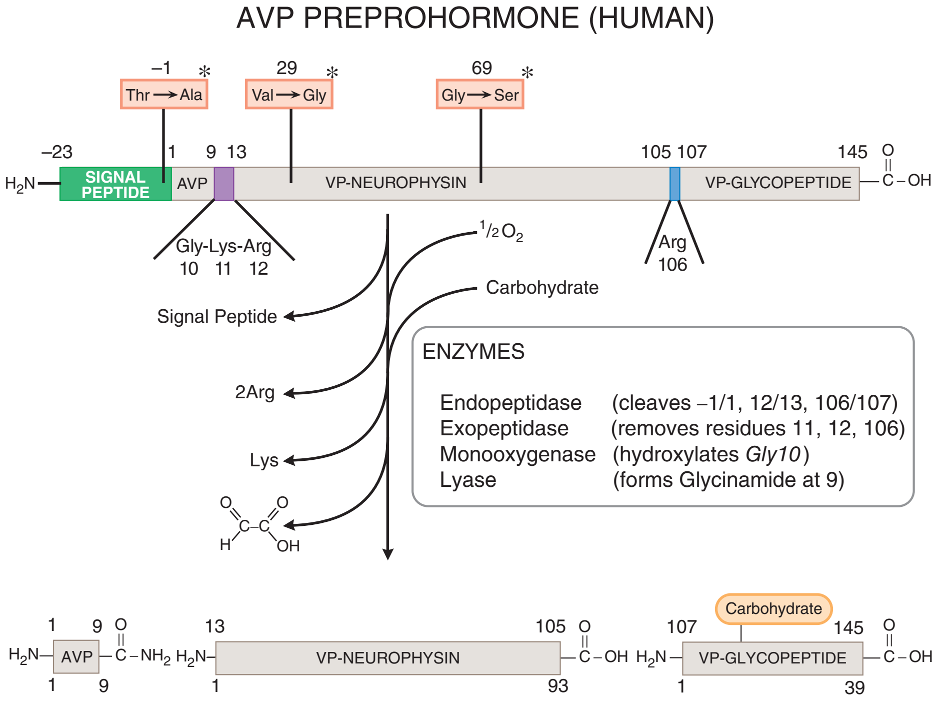


Figure 29–10 *Processing of human arginine vasopressin (AVP) preprohormone.* More than 40 mutations in the single gene on chromosome 20 that encodes AVP preprohormone give rise to central DI. *Boxes indicate mutations leading to central DI. DI, diabetes insipidus; VP, vasopressin.

Regulation of Vasopressin Secretion

Hyperosmolality. An increase in plasma osmolality is the principal physiological stimulus for vasopressin secretion by the posterior pituitary. The osmolality threshold for secretion is about 280 mOsm/kg. Below the threshold, vasopressin is barely detectable in plasma, and above the threshold, vasopressin levels are a steep and relatively linear function of plasma osmolality. Indeed, a 2% elevation in plasma osmolality causes a 2- to 3-fold increase in plasma vasopressin levels, which in turn causes increased solute-free water reabsorption, with an increase in urine osmolality. Increases in plasma osmolality above 290 mOsm/kg lead to an intense desire for water (thirst). Thus, the vasopressin system affords the organism longer thirst-free periods and, in the event that water is unavailable, allows the organism to survive longer periods of water deprivation. Above a plasma osmolality of approximately 290 mOsm/kg, plasma vasopressin levels exceed 5 pM. Since urinary concentration is maximal (~1200 mOsm/kg) when vasopressin levels exceed 5 pM, further defense against hypertonicity depends mainly on water intake rather than on decreases in urinary water loss.

Hepatic Portal Osmoreceptors. An oral salt load activates hepatic portal osmoreceptors, leading to increased vasopressin release. This mechanism augments plasma vasopressin levels even before the oral salt load increases plasma osmolality.

Hypovolemia and Hypotension. Vasopressin secretion is regulated hemodynamically by changes in effective blood volume or arterial blood pressure. Regardless of the cause (e.g., hemorrhage, Na^+ depletion, diuretics, heart failure, hepatic cirrhosis with ascites, adrenal insufficiency, or hypotensive drugs), reductions in effective blood volume or arterial blood pressure are associated with high circulating vasopressin concentrations. However, unlike osmoregulation, hemodynamic regulation of vasopressin secretion is exponential; that is, small decreases (5%) in blood volume or pressure have little effect on vasopressin secretion, whereas larger decreases (20%–30%) can increase vasopressin levels to 20 to 30 times normal (exceeding the vasopressin concentration required to induce maximal antidiuresis). Vasopressin is one of the most potent vasoconstrictors known, and the vasopressin response to hypovolemia or hypotension serves as a mechanism to stave off cardiovascular collapse during periods of severe blood loss or hypotension. Hemodynamic regulation of vasopressin secretion does not disrupt osmotic regulation; rather, hypovolemia/hypotension alters the set point and slope of the plasma osmolality–plasma vasopressin relationship.

Neuronal pathways that mediate hemodynamic regulation of vasopressin release are different from those involved in osmoregulation. Baroreceptors in the left atrium, left ventricle, and pulmonary veins sense blood volume (filling pressures), and baroreceptors in the carotid sinus and aorta monitor arterial blood pressure. Nerve impulses reach brainstem nuclei predominantly through the vagal trunk and glossopharyngeal nerve; these signals are ultimately relayed to the SON and PVN.

Hormones and Neurotransmitters. Vasopressin-synthesizing magnocellular neurons have a large array of receptors on both perikarya and nerve terminals; therefore, vasopressin release can be accentuated or attenuated by chemical agents acting at both ends of the magnocellular neuron (Iovino et al., 2014). Also, hormones and neurotransmitters can modulate vasopressin secretion by stimulating or inhibiting neurons in nuclei that project, either directly or indirectly, to the SON and PVN (Iovino et al., 2014). Because of these complexities, modulation of vasopressin secretion by most hormones or neurotransmitters is unclear. Several agents stimulate vasopressin secretion, including acetylcholine (by nicotinic receptors), histamine (by H_1 receptors), dopamine (by both D_1 and D_2 receptors), glutamine, aspartate, cholecystokinin, neuropeptide Y, substance P, vasoactive intestinal polypeptide, PGs, and AngII. Inhibitors of vasopressin secretion include ANP, γ-aminobutyric acid, and opioids (particularly dynorphin via κ receptors). The effects of AngII have received the most attention. AngII synthesized in the brain and circulating AngII may stimulate vasopressin release. Inhibition of the conversion of AngII to AngIII blocks AngII-induced vasopressin release, suggesting that AngIII is the main effector peptide of the brain RAS controlling vasopressin release.

Pharmacological Agents. Several drugs alter urine osmolality by stimulating or inhibiting vasopressin secretion. In most cases, the mechanism is not known. Stimulators of vasopressin secretion include *vincristine*, *cyclophosphamide*, tricyclic antidepressants, *nicotine*, *epinephrine*, and high doses of *morphine*. *Lithium*, which inhibits the renal effects of vasopressin, also enhances vasopressin secretion. Inhibitors of vasopressin secretion include *ethanol* (see also Chapter 27), *phenytoin*, low doses of *morphine*, glucocorticoids, *fluphenazine*, *haloperidol*, *promethazine*, *oxilorphan*, and *butorphanol*. *Carbamazepine* has a renal action to produce antidiuresis in patients with central DI but inhibits vasopressin secretion by a central action.

Vasopressin Receptors

Cellular vasopressin effects are mediated mainly by interactions of the hormone with the three types of receptors: V_{1a}, V_{1b}, and V_2. All are GPCRs. The V_{1a} receptor is the most widespread subtype of vasopressin receptor; it is found in vascular smooth muscle, adrenal gland, myometrium, bladder, adipocytes, hepatocytes, platelets, renal medullary interstitial cells, vasa recta in the renal microcirculation, epithelial cells in the renal cortical collecting duct, spleen, testis, and many CNS structures. V_{1b} receptors have a more limited distribution and are found in the anterior pituitary, several brain regions, pancreas, and adrenal medulla. V_2 receptors are located predominantly in principal cells of the renal collecting duct system but also are present on epithelial cells in TAL and on vascular endothelial cells.

Figure 29–11 summarizes the current model of V_1 receptor-effector coupling. Vasopressin binding to V_1 receptors activates the G_q-PLC-IP_3 pathway, thereby mobilizing intracellular Ca^{2+} and activating PKC, ultimately causing biological effects that include immediate responses (e.g., vasoconstriction, glycogenolysis, platelet aggregation, and ACTH release) and growth responses in smooth muscle cells.

Principal cells in renal collecting duct have V_2 receptors on their basolateral membranes that couple to G_s to stimulate adenylyl cyclase activity (Figure 29–12). The resulting activation of the cyclic AMP/PKA pathway triggers an increased rate of insertion of water channel–containing vesicles (WCVs) into the apical membrane and a decreased rate of endocytosis of WCVs from the apical membrane. Because WCVs contain preformed functional water channels (aquaporin 2), their net shift into apical membranes in response to V_2 receptor stimulation greatly increases water permeability of the apical membrane (Nejsum, 2005) (Figures 29–12 and 29–13).

V_2 receptor activation also increases urea permeability by 400% in the terminal portions of the IMCD. V_2 receptors increase urea permeability by activating a vasopressin-regulated urea transporter (termed *VRUT*, *UT1*, or *UTA1*), most likely by PKA-induced phosphorylation. Kinetics of vasopressin-induced water and urea permeability differ, and vasopressin-induced regulation of VRUT does not entail vesicular trafficking to the plasma membrane.

V_2 receptor activation also increases Na^+ transport in TAL and collecting duct. Increased Na^+ transport in TAL is mediated by three mechanisms that affect the Na^+-K^+-$2C1^-$ symporter: rapid phosphorylation of the symporter, translocation of the symporter into the luminal membrane, and increased expression of symporter protein. The multiple mechanisms by which vasopressin increases water reabsorption are summarized in Figure 29–14.

Renal Actions of Vasopressin

Several sites of vasopressin action in kidney involve both V_1 and V_2 receptors. V_1 receptors mediate contraction of mesangial cells in the glomerulus and contraction of vascular smooth muscle cells in vasa recta and efferent arterioles. V_1 receptor–mediated reduction of inner medullary blood flow contributes to the maximum concentrating capacity of the kidney. V_1 receptors also stimulate PG synthesis by medullary interstitial cells. Because PGE_2 inhibits adenylyl cyclase in collecting ducts, stimulation of PG synthesis by V_1 receptors may counterbalance V_2 receptor–mediated antidiuresis. V_1 receptors on principal cells in cortical collecting ducts may inhibit V_2 receptor–mediated water flux by activation of PKC. V_2 receptors mediate the most prominent response to vasopressin, which is increased water permeability of the collecting duct at concentrations as low as 50 fM. Thus, V_2 receptor–mediated effects of vasopressin occur

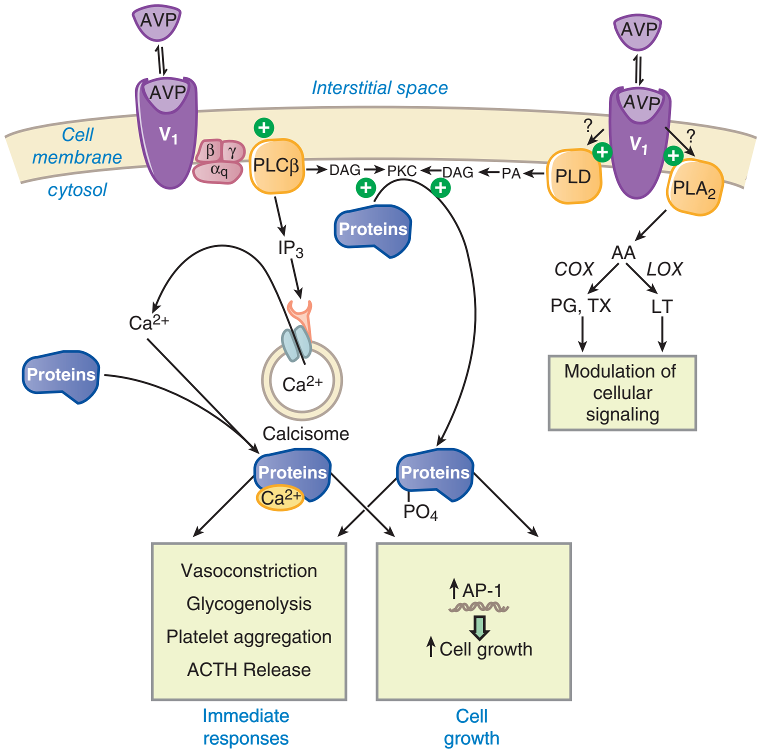


Figure 29–11 *Mechanism of V_1 receptor-effector coupling.* Binding of AVP to V_1 vasopressin receptors (V_1) stimulates membrane-bound phospholipases. Stimulation of G_q activates the PLCβ-IP_3/DAG-Ca^{2+}-PKC pathway. Activation of V_1 receptors also causes influx of extracellular Ca^{2+} by an unknown mechanism. PKC and Ca^{2+}/calmodulin-activated protein kinases phosphorylate cell-type–specific proteins, leading to cellular responses. A further component of the AVP response derives from the production of eicosanoids secondary to the activation of PLA_2; the resulting mobilization of arachidonic acid (AA) provides substrate for eicosanoid synthesis by the cyclooxygenase (COX) and lipoxygenase (LOX) pathways, leading to local production of prostaglandin (PG), thromboxane (TX) and leukotriene (LT), which may activate many signaling pathways, including those linked to G_s and G_q. AVP, arginine vasopressin; DAG, diacylglycerol; PA, phosphatidic acid; PKC, protein kinase C; PLA, phospholipase A; PLC, phospholipase C; PLD, phospholipase D.

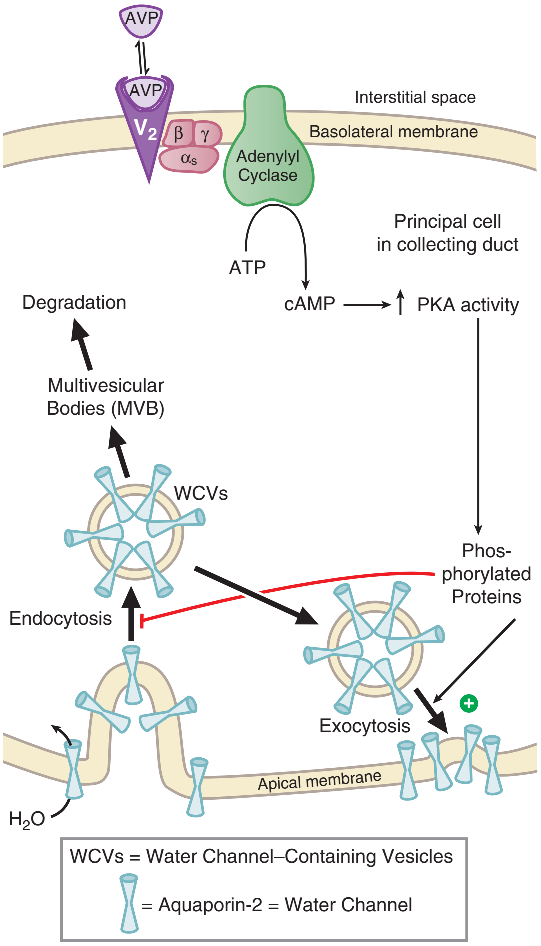


Figure 29–12 *Mechanism of V_2 receptor-effector coupling.* Binding of AVP to the V_2 receptor activates the G_s-adenylyl cyclase-cAMP-PKA pathway and shifts the balance of aquaporin 2 trafficking toward the apical membrane of the principal cell of the collecting duct, thus enhancing water permeability. Although phosphorylation of *Ser256* of aquaporin 2 is involved in V_2 receptor signaling, other proteins located in both the water channel–containing vesicles and the apical membrane of the cytoplasm also may be involved.

at concentrations far lower than are required to engage V_1 receptor–mediated actions. Other renal actions mediated by V_2 receptors include increased urea transport in the IMCD and increased Na^+ transport in the TAL; both effects contribute to the urine-concentrating ability of the kidney. V_2 receptors also increase Na^+ transport in cortical collecting ducts, and this may synergize with aldosterone to enhance Na^+ reabsorption during hypovolemia.

Pharmacological Modification of the Antidiuretic Response to Vasopressin

The NSAIDs, particularly *indomethacin*, enhance the antidiuretic response to vasopressin. Because PGs attenuate antidiuretic responses to vasopressin and NSAIDs inhibit PG synthesis, reduced PG production probably accounts for potentiation of vasopressin's antidiuretic response. *Carbamazepine* and *chlorpropamide* (not available in the U.S.) also enhance antidiuretic effects of vasopressin by unknown mechanisms. In rare instances, *chlorpropamide* can induce water intoxication. Several drugs inhibit the antidiuretic actions of vasopressin. *Lithium* is of particular importance because of its use in the treatment of manic-depressive disorders (Kishore and Ecelbarger, 2013). Acutely, Li^+ appears to reduce V_2 receptor–mediated stimulation of adenylyl cyclase. Also, Li^+ increases plasma levels of PTH, a partial antagonist to vasopressin. In most patients, the antibiotic *demeclocycline* attenuates the antidiuretic effects of vasopressin, probably owing to decreased accumulation and action of cyclic AMP (Kortenoeven et al., 2013).

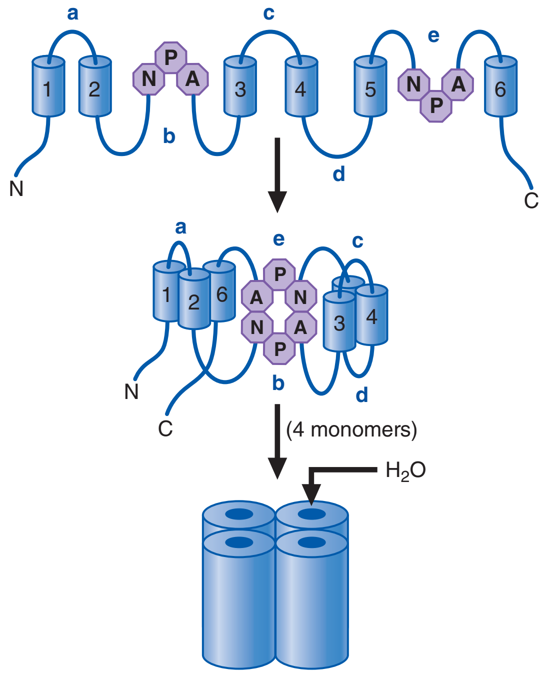


Figure 29–13 *Structure of aquaporins.* Aquaporins have six transmembrane domains, and the NH_2 and COOH termini are intracellular. Loops b and e each contain an asparagine-proline-alanine (NPA) sequence. Aquaporins fold with transmembrane domains 1, 2, and 6 in close proximity and transmembrane domains 3, 4, and 5 in juxtaposition. The long b and e loops dip into the membrane, and the NPA sequences align to create a pore through which water can diffuse. Most likely, aquaporins form a tetrameric oligomer. At least seven aquaporins are expressed at distinct sites in the kidney. Aquaporin 1, abundant in the proximal tubule and DTL, is essential for concentration of urine. Aquaporin 2, exclusively expressed in the principal cells of the connecting tubule and collecting duct, is the major vasopressin-regulated water channel. Aquaporin 3 and aquaporin 4 are expressed in the basolateral membranes of collecting duct principal cells and provide exit pathways for water reabsorbed apically by aquaporin 2. Aquaporin 7 is in the apical brush border of the straight proximal tubule. Aquaporins 6 to 8 are also expressed in kidney; their functions remain to be clarified. Vasopressin regulates water permeability of the collecting duct by influencing the trafficking of aquaporin 2 from intracellular vesicles to the apical plasma membrane (Figure 29–12). AVP-induced activation of the cAMP-PKA pathway also enhances expression of aquaporin 2 mRNA and protein; chronic dehydration thus causes upregulation of aquaporin 2 and water transport in the collecting duct.

Nonrenal Actions of Vasopressin

Cardiovascular System. The cardiovascular effects of vasopressin are complex. Vasopressin is a potent vasoconstrictor (V_1 receptor mediated), and resistance vessels throughout the circulation may be affected. Vascular smooth muscle in the skin, skeletal muscle, fat, pancreas, and thyroid gland appears most sensitive, with significant vasoconstriction also occurring in the GI tract, coronary vessels, and brain. Despite the potency of vasopressin as a direct vasoconstrictor, vasopressin-induced pressor responses *in vivo* are minimal and occur only with vasopressin concentrations significantly higher than those required for maximal antidiuresis. To a large extent, this is due to circulating vasopressin actions on V_1 receptors to inhibit sympathetic efferents and potentiate baroreflexes. In addition, V_2 receptors cause vasodilation in some blood vessels.

Vasopressin helps to maintain arterial blood pressure during episodes of severe hypovolemia/hypotension. The effects of vasopressin on the heart (reduced cardiac output and heart rate) are largely indirect and result from coronary vasoconstriction, decreased coronary blood flow, and alterations in vagal and sympathetic tone. Some patients with coronary insufficiency experience angina even in response to the relatively small amounts of vasopressin required to control DI, and vasopressin-

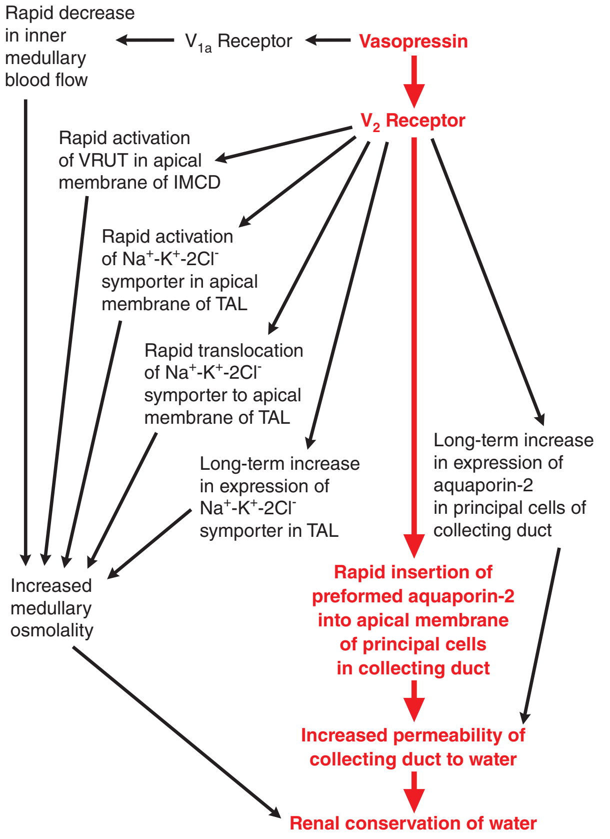

Rapid decrease in inner medullary blood flow
V_{1a} Receptor
Vasopressin
V_2 Receptor
Rapid activation of VRUT in apical membrane of IMCD
Rapid activation of Na^+-K^+-$2Cl^-$ symporter in apical membrane of TAL
Rapid translocation of Na^+-K^+-$2Cl^-$ symporter to apical membrane of TAL
Long-term increase in expression of Na^+-K^+-$2Cl^-$ symporter in TAL
Long-term increase in expression of aquaporin-2 in principal cells of collecting duct
Increased medullary osmolality
Rapid insertion of preformed aquaporin-2 into apical membrane of principal cells in collecting duct
Increased permeability of collecting duct to water
Renal conservation of water

Figure 29–14 *Mechanisms by which vasopressin increases the renal conservation of water.* Red and black arrows denote major and minor pathways, respectively.

induced myocardial ischemia has led to severe reactions and even death.

CNS. Vasopressin likely plays a role as a neurotransmitter or neuromodulator. Although vasopressin can modulate CNS autonomic systems controlling heart rate, arterial blood pressure, respiration rate, and sleep patterns, the physiological significance of these actions is unclear. While vasopressin is not the principal corticotropin-releasing factor, vasopressin may provide for sustained activation of the hypothalamic-pituitary-adrenal axis during chronic stress. Studies in both laboratory animals and humans indicated that vasopressin and oxytocin are key regulators of social and emotional behaviors (Benarroch, 2013). CNS effects of vasopressin appear to be mediated predominantly by V_1 receptors.

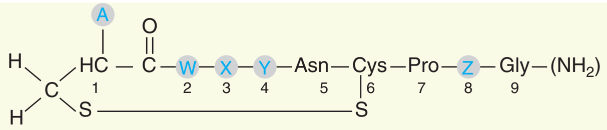
Blood Coagulation. Activation of V_2 receptors by *desmopressin* or *vasopressin* increases circulating levels of procoagulant factor VIII and of von Willebrand factor. These effects are mediated by extrarenal V_2 receptors. Presumably, vasopressin stimulates the secretion of von Willebrand factor and factor VIII from storage sites in vascular endothelium. However, because release of von Willebrand factor does not occur when *desmopressin* is applied directly to cultured endothelial cells or to isolated blood vessels, intermediate factors are likely to be involved.

Other Nonrenal Effects. At high concentrations, vasopressin stimulates smooth muscle contraction in the uterus (by oxytocin receptors) and GI tract (by V_1 receptors). Vasopressin is stored in platelets, and activation of V_1 receptors stimulates platelet aggregation. Also, activation of V_1 receptors on hepatocytes stimulates glycogenolysis.

Vasopressin Receptor Agonists

Several vasopressin-like peptides occur naturally across the animal kingdom (Table 29–8); all are nonapeptides. In all mammals except swine, the neurohypophyseal peptide is 8-arginine vasopressin, and the terms vasopressin, AVP, and ADH are used interchangeably. There are also a

TABLE 29–8 ■ VASOPRESSIN RECEPTOR AGONISTS


A
O
H
HC
C
W
X
Y
Asn
Cys
Pro
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(NH_2)
H
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S
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	A	W	X	Y	Z
Naturally occurring vasopressin-like peptides					
A. *Vertebrates*					
1. Mammals					
AVP[a] (humans and other mammals)	NH_2	Tyr	Phe	Gln	Arg
Lypressin[a] (pigs, marsupials)	NH_2	Tyr	Phe	Gln	Lys
Phenypressin (macropodids)	NH_2	Phe	Phe	Gln	Arg
2. Nonmammalian vertebrates					
Vasotocin	NH_2	Tyr	Ile	Gln	Arg
B. *Invertebrates*					
1. Arginine conopressin (*Conus striatus*)	NH_2	Ile	Ile	Arg	Arg
2. Lysine conopressin (*Conus geographicus*)	NH_2	Phe	Ile	Arg	Lys
3. Locust subesophageal ganglia peptide	NH_2	Leu	Ile	Thr	Arg
Synthetic vasopressin peptides					
A. *V_1-selective agonists*					
1. V_{1a}-selective: [Phe^2, Ile^3, Orn^8]AVP	NH_2	Phe	Ile	Gln	Orn
2. V_{1b}-selective: Deamino [D-3-(3′-pyridyl)-Ala^2]AVP	H	D-3-(3′-pyridyl)-Ala^2	Phe	Gln	Arg
B. *V_2-selective agonists*					
1. Desmopressin[a] (DDAVP)	H	Tyr	Phe	Gln	D-Arg
2. Deamino [Val^4, D-Arg^8]AVP	H	Tyr	Phe	Val	D-Arg
Nonpeptide agonist					
A. *OPC-51803*					

[a] Available for clinical use.

number of synthetic peptides with receptor-subtype specificity and one nonpeptide agonist.

Many vasopressin analogues were synthesized with the goal of increasing duration of action and selectivity for vasopressin receptor subtypes (V_1 vs. V_2 receptors, which mediate pressor responses and antidiuretic responses, respectively). Thus, the antidiuretic-to-vasopressor ratio for the V_2-selective agonist, DDAVP, also called *desmopressin*, is about 3000 times greater than that for vasopressin; thus, desmopressin is the preferred drug for the treatment of central DI. Substitution of valine for glutamine in position 4 further increases the antidiuretic selectivity, and the antidiuretic-to-vasopressor ratio for deamino [Val^4, D-Arg^8]AVP is about 11,000 times greater than that for vasopressin.

Increasing V_1 selectivity has proved more difficult than increasing V_2 selectivity. Vasopressin receptors in the adenohypophysis that mediate vasopressin-induced ACTH release are neither classical V_1 nor V_2 receptors. Because vasopressin receptors in the adenohypophysis appear to share a common signal-transduction mechanism with classical V_1 receptors and because many vasopressin analogues with vasoconstrictor activity release ACTH, V_1 receptors have been subclassified into V_{1a} (vascular/hepatic) and V_{1b} (pituitary) receptors (also called V_3 receptors). There are selective agonists for V_{1a} and V_{1b} receptors.

The chemical structure of oxytocin is closely related to that of vasopressin: Oxytocin is [Ile^3, Leu^8]AVP. With such structural similarities, it is not surprising that vasopressin and oxytocin agonists and antagonists can bind to each other's receptors. Therefore, most of the available peptide vasopressin agonists and antagonists have some affinity for oxytocin receptors; at high doses, they may block or mimic the effects of oxytocin.

Diseases Affecting the Vasopressin System

Diabetes Insipidus

Diabetes insipidus is a disease of impaired renal water conservation owing either to inadequate vasopressin secretion from the neurohypophysis (central DI) or to insufficient renal vasopressin response (nephrogenic DI). Very rarely, DI can be caused by an abnormally high degradation rate of vasopressin by circulating vasopressinases. Pregnancy may accentuate or reveal central or nephrogenic DI by increasing plasma levels of vasopressinase and by reducing renal sensitivity to vasopressin. Patients with DI excrete large volumes (>30 mL/kg per day) of dilute (<200 mOsm/kg) urine and, if their thirst mechanism is functioning normally, are polydipsic. Central DI can be distinguished from nephrogenic DI by administration of *desmopressin*, which will increase urine osmolality in patients with central DI but have little or no effect in patients with nephrogenic DI. DI can be differentiated from primary polydipsia by measuring plasma osmolality, which will be low to low-normal in patients with primary polydipsia and high to high-normal in patients with DI.

Central DI. Head injury, either surgical or traumatic, in the region of the pituitary or hypothalamus may cause central DI. Postoperative central DI may be transient, permanent, or triphasic (recovery followed by permanent relapse). Other causes include hypothalamic or pituitary tumors, cerebral aneurysms, CNS ischemia, and brain infiltrations and infections. Central DI may also be idiopathic or familial. Familial central DI usually is autosomal dominant (chromosome 20), and vasopressin deficiency occurs several months or years after birth and worsens gradually. Autosomal dominant central DI is linked to mutations in the vasopressin preprohormone gene that cause the prohormone to misfold and oligomerize improperly. Accumulation of mutant vasopressin precursor causes neuronal death, hence the dominant mode of inheritance. Rarely, familial central DI is autosomal recessive owing to a mutation in the vasopressin peptide itself that gives rise to an inactive vasopressin mutant.

Antidiuretic peptides are the primary treatment of central DI, with *desmopressin* the peptide of choice. For patients with central DI who cannot tolerate antidiuretic peptides because of side effects or allergic reactions, other treatment options are available. *Chlorpropamide*, an oral sulfonylurea, potentiates the action of small or residual amounts of circulating vasopressin and will reduce urine volume in more than half of all patients with central DI. Doses of 125 to 500 mg daily appear effective in patients with partial central DI. If polyuria is not controlled satisfactorily with *chlorpropamide* alone, addition of a thiazide diuretic to the regimen usually results in an adequate reduction in urine volume. *Carbamazepine* (800–1000 mg daily in divided doses) also reduces urine volume in patients with central DI. Long-term use may induce serious adverse effects; therefore, *carbamazepine* is used rarely to treat central DI. These agents are not effective in nephrogenic DI, which indicates that functional V_2 receptors are required for the antidiuretic effect. Because *carbamazepine* inhibits and *chlorpropamide* has little effect on vasopressin secretion, it is likely that *carbamazepine* and *chlorpropamide* act directly on the kidney to enhance V_2 receptor–mediated antidiuresis.

Nephrogenic DI. Nephrogenic DI may be congenital or acquired. Hypercalcemia, hypokalemia, postobstructive renal failure, Li^+, *foscarnet*, *clozapine*, *demeclocycline*, and other drugs can induce nephrogenic DI. As many as one in three patients treated with Li^+ may develop nephrogenic DI. X-linked nephrogenic DI is caused by mutations in the gene encoding the V_2 receptor, which maps to Xq28. Mutations in the V_2 receptor gene may cause impaired routing of the V_2 receptor to the cell surface, defective coupling of the receptor to G proteins, or decreased receptor affinity for vasopressin. Autosomal recessive and dominant nephrogenic DI result from inactivating mutations in aquaporin 2. These findings indicate that aquaporin 2 is essential for the antidiuretic effect of vasopressin in humans.

Although the mainstay of treatment of nephrogenic DI is assurance of an adequate water intake, drugs also can be used to reduce polyuria. *Amiloride* blocks Li^+ uptake by the Na^+ channel in the collecting duct system and may be effective in patients with mild-to-moderate concentrating defects. Thiazide *diuretics* reduce the polyuria of patients with DI and often are used to treat nephrogenic DI. In infants with nephrogenic DI, use of thiazides may be crucial because uncontrolled polyuria may exceed the child's capacity to imbibe and absorb fluids. It is possible that the natriuretic action of thiazides and resulting extracellular fluid volume depletion play an important role in thiazide-induced antidiuresis. The antidiuretic effects appear to parallel the thiazide's ability to cause natriuresis, and the drugs are given in doses similar to those used to mobilize edema fluid. In patients with DI, a 50% reduction of urine volume is a good response to thiazides. Moderate restriction of Na^+ intake can enhance the antidiuretic effectiveness of thiazides.

Several case reports have described the effectiveness of *indomethacin* in the treatment of nephrogenic DI; however, other PG synthase inhibitors (e.g., *ibuprofen*) appear to be less effective. The mechanism of the effect may involve a decrease in GFR, an increase in medullary solute concentration, or enhanced proximal fluid reabsorption. Also, because PGs attenuate vasopressin-induced antidiuresis in patients with at least a partially intact V_2 receptor system, some of the antidiuretic response to indomethacin may be due to diminution of the PG effect and enhancement of vasopressin effects on the principal cells of the collecting duct.

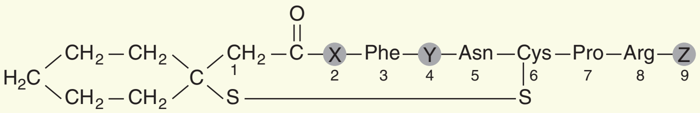
Syndrome of Inappropriate Secretion of Antidiuretic Hormone

Syndrome of inappropriate secretion of ADH (SIADH) is a disease of impaired water excretion with accompanying hyponatremia and hypoosmolality, caused by the *inappropriate* secretion of vasopressin. Clinical manifestations of plasma hypotonicity resulting from SIADH may include lethargy, anorexia, nausea and vomiting, muscle cramps, coma, convulsions, and death. A multitude of disorders can induce SIADH, including malignancies, pulmonary diseases, CNS injuries/diseases (e.g., head trauma, infections, and tumors), and general surgery.

Three drug classes are commonly implicated in drug-induced SIADH: psychotropic medications (e.g., selective serotonin reuptake inhibitors, *haloperidol*, and tricyclic antidepressants), sulfonylureas (e.g., *chlorpropamide*), and vinca alkaloids (e.g., *vincristine* and *vinblastine*). Other drugs strongly associated with SIADH include *clonidine*, *cyclophosphamide*, *enalapril*, *felbamate*, *ifosfamide*, *methyldopa*, *pentamidine*, and *vinorelbine*. Many other drugs have been implicated. In a normal individual, an elevation in plasma vasopressin per se does not induce plasma hypotonicity because the person simply stops drinking owing to an osmotically

TABLE 29–9 ■ VASOPRESSIN RECEPTOR ANTAGONISTS

Peptide antagonists



	X	Y	Z
A. *V_1-selective antagonists*			
V_{1a}-selective antagonist $d(CH_2)_5[Tyr(Me)^2]AVP$	Tyr—OMe	Gln	Gly (NH_2)
V_{1b}-selective antagonist dP $[Tyr(Me)^2]AVP^{a,b,c}$	Tyr—OMe	Gln	Gly (NH_2)
B. *V_2-selective antagonists*[a]			
1. des Gly-NH_2^9-$d(CH_2)_5$[D-Ile^2, Ile^4]AVP	D-Ile	Ile	—
2. $d(CH_2)_5$[D-Ile^2, Ile^4, Ala-NH_2^9]AVP	D-Ile	Ile	Ala(NH_2)

Nonpeptide antagonists

A. *V_{1a}-selective antagonists*
OPC-21268
SR 49059 (relcovaptan)

B. *V_{1b}-selective antagonists*
SSR 149415 (nelivaptan)

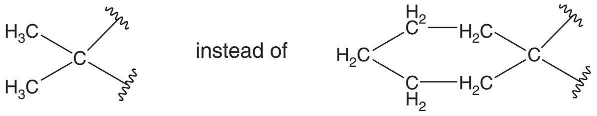
C. *V_2-selective antagonists*
SR 121463 (satavaptan)
VPA-985 (lixivaptan)
OPC-31260 (mozavaptan)
OPC-41061 (tolvaptan)[d]

D. *V_{1a}-/V_2-selective antagonists*
YM-471
YM 087 (conivaptan)[d]
JTV-605
CL-385004

[a] Also blocks V_{1a} receptor.

[b] V_2 antagonistic activity in rats; however, antagonistic activity may be less or nonexistent in other species. Also, with prolonged infusion may exhibit significant agonist activity.

[c]



[d] Available for clinical use in the U.S.

induced aversion to fluids. Therefore, plasma hypotonicity only occurs when excessive fluid intake (oral or intravenous) accompanies inappropriate secretion of vasopressin.

Treatment of hypotonicity in the setting of SIADH includes water restriction, intravenous administration of hypertonic saline, loop diuretics (which interfere with kidney's concentrating ability), and drugs that inhibit the effect of vasopressin to increase water permeability in collecting ducts. To inhibit vasopressin's action in collecting ducts, *demeclocycline*, a tetracycline, has been the preferred drug, but *tolvaptan* and *conivaptan*, V_2 receptor antagonists, are now available (see next section and Table 29–9).

Although Li^+ can inhibit the renal actions of vasopressin, it is effective in only a minority of patients, may induce irreversible renal damage when used chronically, and has a low therapeutic index. Therefore, Li^+ should be considered for use only in patients with symptomatic SIADH who cannot be controlled by other means or in whom tetracyclines are contraindicated (e.g., patients with liver disease). It is important to stress that the majority of patients with SIADH do not require therapy because plasma Na^+ stabilizes in the range of 125 to 132 mM; such patients usually are asymptomatic. Only when symptomatic hypotonicity ensues, generally when plasma Na^+ levels drop below 120 mM, should therapy with demeclocycline be initiated. Due to symptoms resulting from hypotonicity, which causes an influx of water into cells with resulting cerebral swelling, the goal of therapy is simply to increase plasma osmolality toward normal.

Other Water-Retaining States

In patients with CHF, cirrhosis, or nephrotic syndrome, *effective* blood volume often is reduced, and hypovolemia frequently is exacerbated by the liberal use of diuretics. Because hypovolemia stimulates vasopressin release, patients may become hyponatremic owing to vasopressin-mediated retention of water. The development of potent orally active V_2 receptor antagonists and specific inhibitors of water channels in the collecting duct has provided a new therapeutic strategy not only in patients with SIADH but also in the more common setting of hyponatremia in patients with heart failure, liver cirrhosis, and nephrotic syndrome.

Clinical Use of Vasopressin Agonists

Two antidiuretic peptides are available for clinical use in the U.S.:

- *Vasopressin* (synthetic 8-L-arginine vasopressin) is available as a sterile aqueous solution; it may be administered intravenously, subcutaneously, intramuscularly, intranasally, intraosseously (off label), intra-arterially, or endotracheally (off label; unreliable).
- *Desmopressin acetate* (synthetic DDAVP) is available as a sterile aqueous solution packaged for intravenous or subcutaneous injection, in a solution for intranasal administration with either a nasal spray pump or rhinal tube delivery system, and in tablets for oral administration.

Therapeutic Uses

The therapeutic uses of *vasopressin* and its congeners can be divided into two main categories according to the vasopressin receptor involved: V_1 receptor mediated and V_2 receptor mediated.

V_1 receptor–mediated therapeutic applications are based on the rationale that V_1 receptors cause GI and vascular smooth muscle contraction. *Vasopressin is the main agent used.* Vasodilatory shock is the only FDA-approved indication for *vasopressin*. *Vasopressin* levels in patients with vasodilatory shock are inappropriately low, and such patients are highly sensitive to the pressor actions of V_1 receptor agonists. Current recommendations for treatment of septic shock are to employ fluids and *norepinephrine* as first-line treatments, with the addition of *vasopressin* when necessary to achieve target blood pressure or to avoid excessive doses of *norepinephrine* (Demiselle et al., 2020; Shi et al., 2020). The use of *vasopressin* in other shock states, for example hemorrhagic (Gupta et al., 2017) and cardiogenic (Karami et al., 2020) shock, remains controversial. *Vasopressin* combined with *epinephrine* and steroids showed improved outcomes after in-hospital cardiac arrest (Layek et al., 2014). However, due to lack of external validation, current guidelines do not recommend this combination for treatment of cardiac arrest (Vallentin et al., 2020).

V_1 receptor–mediated GI smooth muscle contraction has been used to treat postoperative ileus and abdominal distension and to dispel intestinal gas before abdominal roentgenography to avoid interfering gas shadows. V_1 receptor–mediated vasoconstriction of the splanchnic arterial vessels reduces blood flow to the portal system and thereby attenuates pressure and bleeding in esophageal varices. Although endoscopic variceal banding ligation is the treatment of choice for bleeding esophageal varices, V_1 receptor agonists have been used in an emergency setting until endoscopy can be performed. Simultaneous administration of *nitroglycerin* with V_1 receptor agonists may attenuate the cardiotoxic effects of V_1 agonists while enhancing their beneficial splanchnic effects. Also, V_1 receptor agonists have been used during abdominal surgery in patients with portal hypertension to diminish the risk of hemorrhage during the procedure. V_1 receptor–mediated vasoconstriction has been used to reduce bleeding during acute hemorrhagic gastritis, burn wound excision, cyclophosphamide-induced hemorrhagic cystitis, liver transplant, cesarean section, and uterine myoma resection.

V_2 receptor–mediated therapeutic applications are based on the rationale that V_2 receptors cause water conservation and stimulate release of blood coagulation factors. *Desmopressin is the standard drug of choice.* Central, but not nephrogenic, DI can be treated with V_2 receptor agonists, and polyuria and polydipsia usually are well controlled by these agents. Some patients experience transient DI (e.g., in head injury or surgery in the area of the pituitary); however, therapy for most patients with DI is lifelong. *Desmopressin* is the drug of choice for the vast majority of patients. The duration of effect from a single intranasal dose is from 6 to 20 h; twice-daily administration is effective in most patients. The usual intranasal dosage in adults is 10 to 40 μg daily either as a single dose or divided into two or three doses. In view of the high cost of the drug and the importance of avoiding water intoxication, the schedule of administration should be adjusted to the minimal amount required. In some patients, chronic allergic rhinitis or other nasal pathology may preclude reliable peptide absorption following nasal administration. Oral administration of *desmopressin* in doses of 0.1 to 1.2 mg/d provides adequate *desmopressin* blood levels to control polyuria. Subcutaneous or intravenous administration of 2 to 4 μg daily of *desmopressin* also is effective in central DI.

Vasopressin has little, if any, place in the long-term therapy of DI because of its short duration of action and V_1 receptor–mediated side effects. *Vasopressin* can be used as an alternative to *desmopressin* in the initial diagnostic evaluation of patients with suspected DI and to control polyuria in patients with DI who recently have undergone surgery or experienced head trauma. Under these circumstances, polyuria may be transient, and long-acting agents may produce water intoxication.

Desmopressin is used in bleeding disorders. In most patients with type I von Willebrand disease (vWD) and in some with type IIn vWD, *desmopressin* will elevate von Willebrand factor and shorten bleeding time. However, *desmopressin* generally is ineffective in patients with types IIa, IIb, and III vWD. *Desmopressin* may cause a marked transient thrombocytopenia in individuals with type IIb vWD and is contraindicated in such patients. *Desmopressin* also increases factor VIII levels in patients with mild-to-moderate hemophilia A. *Desmopressin* is not indicated in patients with severe hemophilia A, those with hemophilia B, or those with factor VIII antibodies. In patients with renal insufficiency, *desmopressin* shortens bleeding time and increases circulating levels of factor VIII coagulant activity, factor VIII–related antigen, and ristocetin cofactor. It also induces the appearance of larger von Willebrand factor multimers. *Desmopressin* is effective in some patients with liver cirrhosis- or drug-induced (e.g., *heparin*, *hirudin*, and antiplatelet agents) bleeding disorders. *Desmopressin*, given intravenously at a dose of 0.3 μg/kg, increases factor VIII and von Willebrand factor for more than 6 h. *Desmopressin* can be given at intervals of 12 to 24 h depending on the clinical response and severity of bleeding. Tachyphylaxis to *desmopressin* usually occurs after several days (owing to depletion of factor VIII and von Willebrand factor storage sites) and limits its usefulness to preoperative preparation, postoperative bleeding, excessive menstrual bleeding, and emergency situations.

Another V_2 receptor–mediated therapeutic application is the use of *desmopressin* for primary nocturnal enuresis. Bedtime administration of *desmopressin* tablets provides a high response rate that is sustained with long-term use and that accelerates the cure rate. Intranasal *desmopressin* is no longer recommended for the treatment of primary nocturnal enuresis because of increased risk of hyponatremia. *Desmopressin* also relieves post–lumbar puncture headache, probably by causing water retention and thereby facilitating rapid fluid equilibration in the CNS.

ADME

When *vasopressin* and *desmopressin* are given orally, they are inactivated quickly by trypsin. Inactivation by peptidases in various tissues (particularly liver and kidney) results in a plasma $t_{1/2}$ of *vasopressin* of 17 to 35 min. Following intramuscular or subcutaneous injection, antidiuretic effects of *vasopressin* last 2 to 8 h. The $t_{1/2}$ of *desmopressin* is 75 min to 3.5 h.

Toxicity, Adverse Effects, Contraindications, Drug Interactions

Most adverse effects are mediated through V_1 receptor activation on vascular and GI smooth muscle; such adverse effects are much less common and less severe with *desmopressin* than with *vasopressin*. After injection of large doses of *vasopressin*, marked facial pallor due to cutaneous vasoconstriction is observed commonly. Increased intestinal activity is likely to cause nausea, belching, cramps, and an urge to defecate. *Vasopressin* should be administered with extreme caution in individuals suffering from vascular disease, especially coronary artery disease. Other cardiac complications include arrhythmia and decreased cardiac output. Peripheral vasoconstriction and gangrene were encountered in patients receiving large doses of *vasopressin*.

The major V_2 receptor–mediated adverse effect is water intoxication. Many drugs, including *carbamazepine*, *chlorpropamide*, *morphine*, tricyclic antidepressants, and NSAIDs, can potentiate the antidiuretic effects of these peptides. Several drugs, such as Li^+, *demeclocycline*, and *ethanol*, can attenuate the antidiuretic response to *desmopressin*. *Desmopressin* and *vasopressin* should be used cautiously in disease states in which a rapid increase in extracellular water may impose risks (e.g., in angina, hypertension, and heart failure) and should not be used in patients with acute renal failure. Patients receiving *desmopressin* to maintain hemostasis should be advised to reduce fluid intake. Also, it is imperative that these peptides not be administered to patients with primary or psychogenic polydipsia because severe hypotonic hyponatremia will ensue. Mild facial flushing and headache are the most common adverse effects. Allergic reactions ranging from urticaria to anaphylaxis may occur with *desmopressin* or *vasopressin*. Intranasal administration may cause local adverse effects in the nasal passages, such as edema, rhinorrhea, congestion, irritation, pruritus, and ulceration.

Clinical Use of Vasopressin Antagonists

Table 29–9 summarizes the selectivity of vasopressin receptor antagonists. Only *tolvaptan* and *conivaptan* are currently available in the U.S.

Therapeutic Uses

When the kidney perceives the arterial blood volume to be low (as in the disease states of CHF, cirrhosis, and nephrosis), AVP perpetuates a state of total-body salt and water excess. V_2 receptor antagonists or "aquaretics" may have a therapeutic role in these conditions, especially in patients with concomitant hyponatremia. They are also effective in hyponatremia associated with SIADH. Aquaretics increase renal free water excretion with little or no change in electrolyte excretion. Because they do not affect Na^+ reabsorption, they do not stimulate the TGF mechanism with its associated consequence of reducing GFR.

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Drug Facts for Your Personal Formulary: *Diuretics and Agents Regulating Renal Excretion*

Drug	Major Therapeutic Uses	Clinical Pharmacology and Tips
Carbonic Anhydrase Inhibitors		
Acetazolamide Dichlorphenamide	• Glaucoma • Epilepsy • Altitude sickness • Diuretic resistance • Metabolic alkalosis • Familial periodic paralysis	• Ineffective as diuretic monotherapy because effects on renal excretion are self-limiting • Dichlorphenamide drug of choice for familial periodic paralysis
Osmotic Diuretics		
Mannitol	• Elevated intraocular pressure • Elevated intracranial pressure • Dialysis disequilibrium syndrome • Diagnosis of bronchial hyperreactivity • Urologic irrigation • Management of some overdoses • Cystic fibrosis in adults	• Frequently used to treat or prevent acute kidney injuries, efficacy unclear • Expansion of extracellular fluid volume may cause pulmonary edema
Inhibitors of Na^+-K^+-$2Cl^-$ Symport (Loop Diuretics; High-Ceiling Diuretics)		
Bumetanide Ethacrynic acid Furosemide Torsemide	• Acute pulmonary edema • Edema associated with congestive heart failure, liver cirrhosis, chronic kidney disease, and nephrotic syndrome • Hyponatremia • Hypercalcemia • Hypertension	• Higher doses needed with impaired renal function • Torsemide superior to furosemide in heart failure • Increased risk of ototoxicity with ethacrynic acid compared to other loop diuretics • Risk for hypokalemia and arrhythmia when combined with QT-prolonging drugs • Ethacrynic acid first choice in sulfa allergy
Inhibitors of Na^+-Cl^- Symport (Thiazide Diuretics)		
Thiazide type Chlorothiazide Hydrochlorothiazide Methyclothiazide (not available in the U.S.) ***Thiazide-like*** Chlorthalidone Indapamide Metolazone	• Hypertension • Edema associated with congestive heart failure, liver cirrhosis, chronic kidney disease, and nephrotic syndrome • Nephrogenic diabetes insipidus	• Among first choice for treating hypertension • Thiazide-like have longer half-lives than thiazide-type and thus may be superior for hypertension • Higher doses needed for treating edema in patients with impaired renal function • Frequently combined with a loop diuretic to effect "sequential blockade" of tubular transport • Risk for hypokalemia and arrhythmia when combined with QT-prolonging drugs • Cause metabolic disturbances (e.g., elevate plasma glucose and cholesterol) • May cause severe hyponatremia in some patients
Inhibitors of Renal Epithelial Na^+ Channels (K^+-Sparing Diuretics)		
Amiloride Triamterene	• Hypertension • Edema associated with congestive heart failure, liver cirrhosis, and chronic kidney disease • Liddle syndrome • Lithium-induced nephrogenic diabetes insipidus	• Low efficacy as monotherapy for edema • Frequently combined with loop or thiazide diuretics to prevent hypokalemia and increase diuresis • Risk of hyperkalemia in renal insufficiency or when combined with angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists
Mineralocorticoid Receptor Antagonists (Aldosterone Antagonists; K^+-Sparing Diuretics)		
Eplerenone Spironolactone Finerenone (nonsteroidal MR antagonist)	• Hypertension • Edema associated with congestive heart failure, liver cirrhosis, chronic kidney disease • Primary hyperaldosteronism • Acute myocardial infarction (eplerenone) • Heart failure (in combination with standard therapy) • Polycystic ovary disease • Chronic kidney disease in diabetic patients (finerenone)	• Endogenous aldosterone levels determine efficacy • Can be combined with loop or thiazide diuretics to prevent hypokalemia and increase diuresis • Diuretics of choice for hypertension due to primary hyperaldosteronism and for edema due to secondary aldosteronism (e.g., heart failure, hepatic cirrhosis) • Finerenone reduces cardiovascular mortality and declining renal function in diabetic patients • High risk for hyperkalemia in chronic renal failure • Eplerenone and finerenone contraindicated with potent inhibitors of CYP3A4 (e.g., ketoconazole, itraconazole) • Spironolactone active metabolite has long half-life requiring slow dose adjustments (over days)

Drug Facts for Your Personal Formulary: *Diuretics and Agents Regulating Renal Excretion* (*continued*)

Drug	Major Therapeutic Uses	Clinical Pharmacology and Tips
Sodium-Glucose Symport Antagonists (SGLT2 Inhibitors; Gliflozins)		
Canagliflozin Dapagliflozin Empagliflozin, others	• Diabetes mellitus type 2 • Heart failure with reduced ejection fraction	• Reduction of cardiovascular mortality and declining renal function in diabetic patients • Augment natriuresis induced by loop diuretics • Rare necrotizing fasciitis of perineum • Hepatic metabolism, long half-life
Vasopressin Receptor Agonist		
V_1 receptor agonist Vasopressin	• Postoperative abdominal distention • Abdominal roentgenography • Bleeding • Cardiac arrest • Hypovolemic shock	• Contraindicated in nephrogenic diabetes insipidus • Not for long-term therapy of central diabetes insipidus • Use with extreme caution in patients with vascular disease
V_2 receptor agonist Desmopressin (DDAVP)	• Central diabetes insipidus • Primary nocturnal enuresis • Prevention of blood loss in patients with specific bleeding disorders	• Contraindicated in nephrogenic diabetes insipidus • Drug of choice for central diabetes insipidus • Can be administered orally at high doses • Major adverse effect is water intoxication
Vasopressin Receptor Antagonists		
Conivaptan Tolvaptan	• Treatment of hypervolemic and euvolemic hyponatremia	• Risk of too rapid correction with serious consequences (osmotic demyelination syndrome) • Close monitoring of serum Na^+ required

Tolvaptan is a selective oral V_2 receptor antagonist FDA approved for clinically significant hypervolemic and euvolemic hyponatremia. *Tolvaptan* is also approved to slow kidney function decline in adults at risk of rapidly progressing autosomal dominant polycystic kidney disease. *Conivaptan* is a nonselective V_{1a} receptor/V_2 receptor antagonist that is FDA approved for the treatment of hospitalized patients with euvolemic and hypervolemic hyponatremia. *Conivaptan* is available only for intravenous infusion. In heart failure patients, V_2 receptor antagonists improve decongestion and serum sodium and in the short term provide symptomatic improvement (Vinod et al., 2017); however, this does not translate into short-term or long-term improvements in important clinical outcomes such as mortality or hospitalization (Gunderson et al., 2019). The interaction of *tolvaptan* with loop diuretics is currently being studied in several clinical trials (Gunderson et al., 2019).

ADME

Tolvaptan has a $t_{1/2}$ of 2.8 to 12 h, and less than 1% is excreted in the urine. *Tolvaptan* is a substrate and inhibitor of P-glycoprotein and is eliminated entirely by CYP3A4 metabolism. *Conivaptan* is highly protein bound, has a terminal elimination $t_{1/2}$ of 5 to 12 h, is metabolized via CYP3A, and is partially excreted by the kidney.

Toxicity, Adverse Effects, Contraindications, Drug Interactions

The most dangerous adverse effect of V_2 receptor antagonists is due to their pharmacological action to increase free water excretion. This may correct hyponatremia too rapidly, resulting in serious and even fatal consequences (osmotic demyelination syndrome). Indeed, *tolvaptan* is labeled with a black-box warning against too rapid correction of hyponatremia and with the recommendation to initiate therapy in a hospital setting capable of close monitoring of serum Na^+. V_2 receptor antagonists should not be used with hypertonic saline. Antagonism of V_2 receptors can also cause polyuria, which likely explains the increased incidence of dehydration, hypotension, dizziness, pyrexia, increased thirst, and xerostomia with this class of drugs. Both *tolvaptan* and *conivaptan* can cause GI adverse effects. *Tolvaptan* can cause liver damage; therefore, administration of *tolvaptan* generally should be limited to 30 days, and *tolvaptan* should not be used in patients with liver disease. Both *tolvaptan* and *conivaptan* can induce headaches, hypokalemia, and hyperglycemia, and both are contraindicated in anuria (no benefit) and in patients receiving drugs that inhibit CYP3A4 (e.g., *clarithromycin*, *ketoconazole*). *Tolvaptan* and *conivaptan* are contraindicated in hypovolemic hyponatremia. *Tolvaptan* may increase the rate of hyperkalemia when it is given with angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and potassium-sparing diuretics.

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Chapter 30

Renin and Angiotensin

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THE RENIN-ANGIOTENSIN SYSTEM

- Classical RAS
- Newer Paradigms in the RAS

COMPONENTS OF THE RENIN-ANGIOTENSIN SYSTEM

- Renin
- Control of Renin Secretion
- Angiotensinogen
- Angiotensin-Converting Enzyme
- Angiotensin-Converting Enzyme 2
- Alternative Pathways for Angiotensin II Biosynthesis
- Angiotensin Peptides and Their Receptors
- Local (Tissue) Renin-Angiotensin System
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FUNCTIONS AND EFFECTS OF ANGIOTENSIN II

- Mechanisms by Which Angiotensin II Increases Peripheral Resistance
- Mechanisms by Which Angiotensin II Regulates Renal Function
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- Role of the RAS in Long-Term Maintenance of Arterial Blood Pressure Despite Extremes in Dietary Na^+ Intake
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INHIBITORS OF THE RENIN-ANGIOTENSIN SYSTEM

- Novel Agents for Targeting the RAS Pathway
- Angiotensin-Converting Enzyme Inhibitors
- Angiotensin II Receptor Blockers
- Direct Renin Inhibitors (DRIs)

THE RENIN-ANGIOTENSIN SYSTEM

The renin-angiotensin system (RAS) participates in the pathophysiology of numerous clinical disorders, including diabetic nephropathy, hypertension, congestive heart failure, and myocardial infarction (MI). This role has led to extensive study of the RAS and development of multiple ways to inhibit its actions. This chapter discusses the physiology, biochemistry, and cellular and molecular biology of classical and novel RAS components and pathways. The chapter also discusses the pharmacology and clinical utility of drugs that inhibit the RAS. Therapeutic applications of drugs covered in this chapter are also discussed in Chapters 31 to 33. The RAS is sometimes identified as the renin-angiotensin-aldosterone system. *Aldosterone*, a mineralocorticoid, is discussed in Chapter 50.

HISTORY

In 1898, Tiegerstedt and Bergman found that saline extracts of the kidney (ren-) contained a pressor substance that they named *renin*. In 1934, Goldblatt and colleagues found that renal artery constriction produced persistent hypertension in dogs. In 1940, Braun-Menéndez and colleagues in Argentina and Page and Helmer in the U.S. reported that renin was an enzyme that acted on a plasma protein substrate to catalyze the formation of a pressor peptide, which the Argentinian investigators named *hypertensin* and Page and Helman named *angiotonin*. This peptide was ultimately renamed *angiotensin,* and its plasma substrate was called *angiotensinogen*.

In the 1950s, two forms of angiotensin (Ang) were identified: a decapeptide (AngI) and an octapeptide (AngII) formed by the cleavage of AngI by angiotensin-converting enzyme (ACE), a zinc metalloproteinase discovered by Skeggs in 1956. AngII was the more active form; its synthesis by Schwyzer and Bumpus in 1957 made it available for study. Later research showed that the kidneys are an important site of aldosterone action; angiotensin stimulates its production in humans, and renin secretion increases with depletion of Na^+. Thus, the RAS became recognized as a mechanism that stimulates aldosterone synthesis and secretion and an important homeostatic mechanism in the regulation of blood pressure (BP) and electrolyte composition.

In the early 1970s, polypeptides were discovered that either inhibited the formation of AngII or blocked AngII receptors. These inhibitors revealed important physiological and pathophysiological roles for the RAS and inspired the development of an efficacious class of antihypertensive drugs: orally active ACE inhibitors (ACEIs). Studies with ACEIs uncovered roles for the RAS in the pathophysiology of hypertension, heart failure, vascular disease, and renal failure. Selective and competitive antagonists of AngII receptors were subsequently developed that yielded *losartan*, the first orally active, highly selective, and potent nonpeptide AngII receptor antagonist (Dell'Italia, 2011). Many other AngII receptor antagonists have been developed, followed by *aliskiren*, a direct renin inhibitor.

Classical RAS

Through the actions of AngII, the RAS participates in BP regulation, aldosterone release, Na^+-reabsorption from renal tubules, electrolyte and fluid homeostasis, and cardiovascular remodeling. AngII is derived from angiotensinogen in two proteolytic steps. First, the enzyme renin, released into the circulation from the juxtaglomerular (JG) cells, specialized smooth muscle cells in glomerular arterioles in the kidneys, cleaves the decapeptide AngI from the amino terminus of angiotensinogen (renin substrate). Then, ACE (also referred to as ACE1 for clarity), located on the endothelial cell lining of the vasculature (and in other cell types throughout the body), removes the carboxy-terminal dipeptide of AngI to produce the octapeptide AngII. Other enzymes, in particular chymase (expressed in mast cells and cardiac myocytes, among others), also participate in conversion of AngI to AngII. Figure 30–1 summarizes these enzymatic steps. AngII acts by binding to two distinct seven-membrane–spanning G protein-coupled receptors (GPCRs), AT_1 (also known as AGTR1) and AT_2 (also known as AGTR2). AGTR1, the more widely expressed and studied of the AngII GPCRs, is thought to mediate the majority of RAS-mediated physiological and pathophysiological effects.

Abbreviations

ACE: angiotensin-converting enzyme
ACEI: angiotensin-converting enzyme inhibitor
Ac-SDKP: *N*-acetyl-seryl-aspartyl-lysyl-proline
ACTH: corticotropin (formerly adrenocorticotropic hormone)
Ang: angiotensin
ARDS: acute respiratory distress syndrome
ARNi: angiotensin receptor–neprilysin inhibitors
ARB: angiotensin receptor blocker
BP: blood pressure
cAMP: cyclic AMP
CoV: coronavirus
COVID-19: coronavirus disease 2019
COX: cyclooxygenase
DRI: direct renin inhibitor
ECM: extracellular matrix
GFR: glomerular filtration rate
GI: gastrointestinal
GPCR: G protein-coupled receptor
GRK: G protein-coupled receptor kinases
HFrEF: heart failure with reduced ejection fraction
IRAP: insulin-regulated aminopeptidase
JG: juxtaglomerular
LDL: low-density lipoprotein
MD: macula densa
MI: myocardial infarction
MRGPRD: Mas-related G protein–coupled receptor D
NE: norepinephrine
NO: nitric oxide
NOS: nitric oxide synthase
NSAID: nonsteroidal anti-inflammatory drug
PAI-1: plasminogen activator inhibitor type 1
PG: prostaglandin
PI_3K: phosphoinositide 3-kinase
PL: phospholipase
PRA: plasma renin activity
PRC: plasma renin concentration
(pro)renin: renin and prorenin
PRR: (pro)renin receptor
RAS: renin-angiotensin system
RBF: renal blood flow
ROS: reactive O_2 species
SARS: severe acute respiratory syndrome
TGF: transforming growth factor
TPR: total peripheral resistance

Hence, the renin-ACE-AGTR1 axis represents an important opportunity for pharmacological intervention. The effects of ACE-AngII-AGTR1 signaling are counteracted by actions of ACE2, which converts AngI and AngII to other angiotensin peptides, whose actions generally oppose those of AngII. AGTR2 is widely expressed in many cell/tissue types throughout the body, especially in the vasculature, the heart, adipose tissue, and various endocrine tissues.

Newer Paradigms in the RAS

Discoveries regarding the RAS indicated that it was not only an endocrine system but also a paracrine, autocrine/intracrine hormonal system with several new components and pathways. The RAS includes local (tissue) RAS; alternative pathways for AngII synthesis (*ACE independent* and *renin independent*); a second ACE, ACE2, which converts AngI to peptides that include Ang(1–7), which acts primarily via the MAS1 receptor (a GPCR), whose activation opposes the vasoconstrictor effects of the ACE-AngII-AT_1 receptor axis; multiple biologically active angiotensin peptides such as Ang(1–9), AngIII, Ang(3–7), angiotensin A, and alamandine; multiple receptors for angiotensin (AT_1, AT_2, AT_4; MAS1; and MRGPRD [Mas-related G protein–coupled receptor D]); and the (pro)renin receptor (PRR). Differential activation of these multiple arms of the RAS may underlie pathophysiological outcomes in cardiovascular and renal disease (Campbell, 2014; Santos, 2014) and have been implicated in the pathobiology of coronavirus disease 2019 (COVID-19) (Sriram and Insel, 2020a).

Components of the Renin-Angiotensin System

Renin

Renin is the major determinant of the rate of AngII production; its secretion is regulated by several mechanisms (Figures 30–2 and 30–3). Renin is synthesized, stored, and secreted by exocytosis into the renal arterial circulation by the JG cells located in the walls of the afferent arterioles that enter the glomeruli. Renin, an aspartyl protease, cleaves the bond between residues 10 and 11 at the amino terminus of angiotensinogen to generate AngI. The active form of renin is a glycoprotein that contains 340 amino acids. The precursor gene for renin (*REN*) is also expressed in certain types of reproductive tissue, smooth muscle, numerous epithelial cell types (including alveolar epithelial cells, pancreatic ductal cells), and endothelial cells, indicating the possibility of localized RAS activation as many of these tissues/cells also express other RAS components.

Prorenin can be activated in two ways: *proteolytically,* by proconvertase 1 or cathepsin B enzymes that remove 43 amino acids (propeptide) from its amino terminus to uncover the active site of renin, and *nonproteolytically,* when prorenin binds to the PRR, resulting in conformational changes that unfold the propeptide and expose the active catalytic site of the enzyme (Nguyen and Danser, 2008). Both renin and prorenin are stored in the JG cells and, when released, circulate in the blood. The concentration of prorenin in the circulation is about 10-fold greater than that of the active enzyme. The $t_{1/2}$ of circulating renin is about 15 min.

Control of Renin Secretion

Renin is secreted by the granular cells within the JG apparatus and is regulated by the following pathways (Figure 30–2):

1. The macula densa (MD)
2. Intrarenal baroreceptors
3. β_1 Adrenergic receptors

The Macula Densa

The MD, which is adjacent to the JG cells, is important for salt and water regulation by the RAS. The MD is composed of specialized columnar epithelial cells in the wall of the cortical thick ascending limb that passes between the afferent and efferent arterioles of the glomerulus (see Chapter 29). The MD cells sense changes in the fluid composition in the tubule and send chemical (paracrine) signals to the JG cells that regulate renin release. Increases or decreases in NaCl flux across the MD inhibit or stimulate renin release, respectively.

Adenosine, ATP, and prostaglandins (PGs) modulate renin release (Figure 30–3). ATP and adenosine inhibit renin release when NaCl transport increases. ATP acts via P2Y2 receptors to enhance Ca^{2+} release, and adenosine acts via the A_1 adenosine receptor to inhibit adenylyl cyclase activity and cyclic AMP (cAMP) production. PGE_2 and PGI_2 stimulate renin release when NaCl transport decreases by enhancing cAMP formation. PG production is increased by inducible cyclooxygenase 2 (COX-2) and nNOS. Chronic dietary Na^+ restriction increases COX-2 and nNOS expression in MD cells; inhibition of either COX-2 or nNOS inhibits renin release (Peti-Peterdi and Harris, 2010).

Regulation by the MD is more dependent on the luminal concentration of Cl^- than Na^+. NaCl transport into the MD is mediated by the Na^+-K^+-$2Cl^-$ symporter (Figure 30–3). The half-maximal concentrations of Na^+

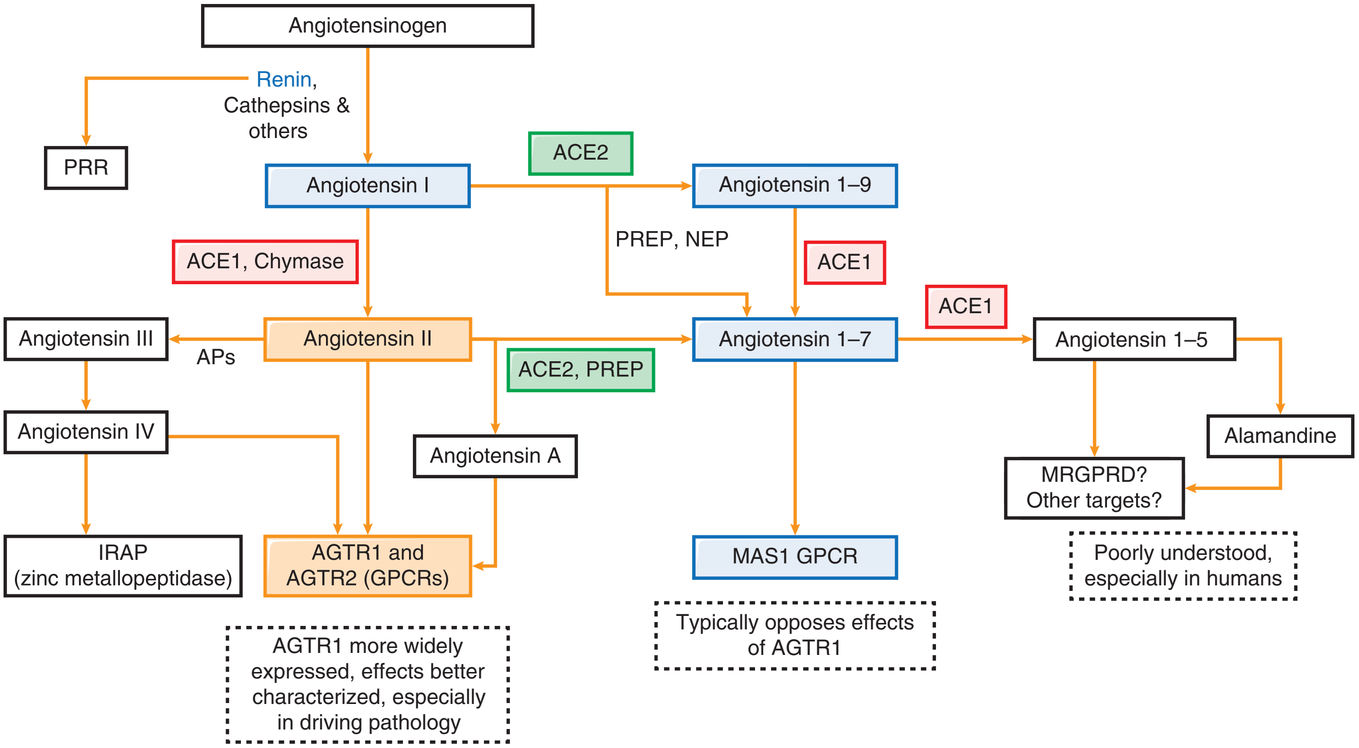
Angiotensinogen
Renin, Cathepsins & others
PRR
Angiotensin I
ACE2
Angiotensin 1–9
PREP, NEP
ACE1
ACE1, Chymase
ACE1
Angiotensin III
APs
Angiotensin II
Angiotensin 1–7
Angiotensin 1–5
ACE2, PREP
Angiotensin IV
Angiotensin A
Alamandine
MRGPRD? Other targets?
IRAP (zinc metallopeptidase)
AGTR1 and AGTR2 (GPCRs)
MAS1 GPCR
Poorly understood, especially in humans
Typically opposes effects of AGTR1
AGTR1 more widely expressed, effects better characterized, especially in driving pathology

Figure 30–1 *The RAS components and signaling pathway.* AGTR1 and 2, angiotensin II receptors 1 and 2; AP, aminopeptidase; PREP, prolyl endopeptidase; NEP, neprilysin, which also contributes to Ang(1–9) and AngII degradation (not shown), to a lesser degree than ACE1 or ACE2.

and Cl^- required for transport via this symporter are 2 to 3 and 40 mEq/L, respectively. Because the luminal concentration of Na^+ at the MD usually is much greater than the level required for half-maximal transport, physiological variations in luminal Na^+ concentrations at the MD have little effect on renin release (i.e., the symporter remains saturated with respect to Na^+). Conversely, physiological changes in Cl^- concentrations (20–60 mEq/L) profoundly affect MD-mediated renin release.

Intrarenal Baroreceptors

Increases and decreases in BP or renal perfusion pressure in the preglomerular vessels inhibit and stimulate renin release, respectively. The immediate stimulus is believed to be reduced tension within the afferent arteriole wall. Release of renal PGs and biomechanical coupling via stretch-activated ion channels may also contribute to renin release mediated by intrarenal baroreceptors.

β_1 Adrenergic Receptors

β_1 Adrenergic receptors are primarily regulated by the release of norepinephrine (NE) from postganglionic sympathetic nerves; activation of β_1 receptors on JG cells increases cAMP and enhances renin secretion. Treatment with β blockers reduces renin secretion and plasma renin activity (PRA).

Feedback Regulation

Renin release is subject to feedback regulation (Figures 30–2 and 30–3). Increased renin secretion enhances the formation of AngII, which stimulates AT_1 receptors on JG cells to inhibit renin release, an effect termed *short-loop negative feedback*. Inhibition of renin release by AngII-dependent increase in BP is termed *long-loop negative feedback*. AngII increases arterial BP via AT_1 receptors, a response that inhibits renin release by:

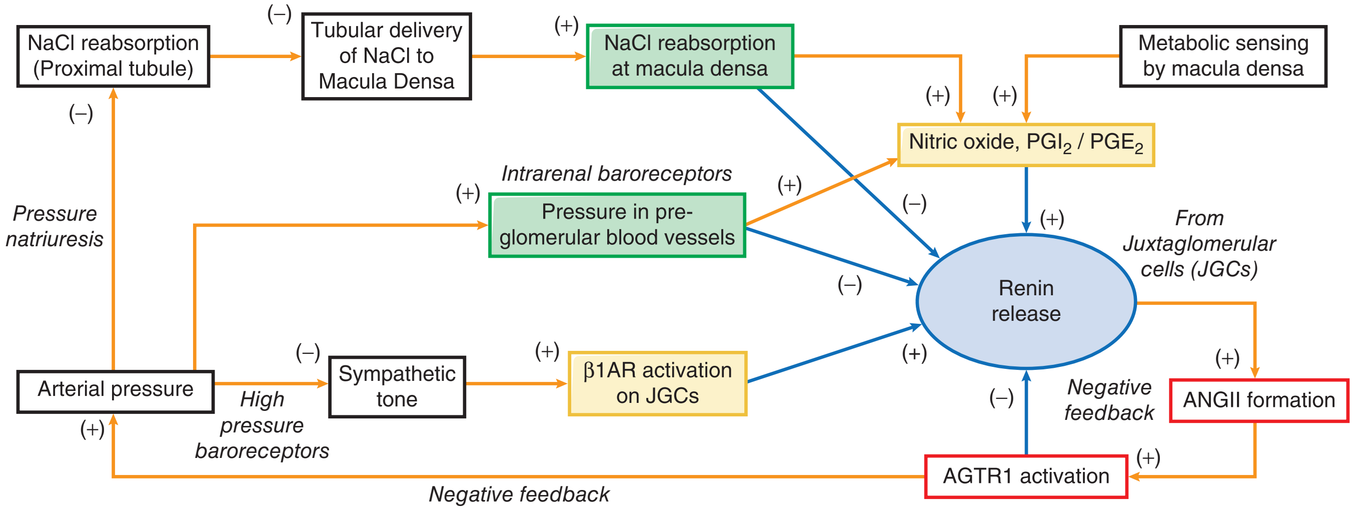
NaCl reabsorption (Proximal tubule)
(–)
Tubular delivery of NaCl to Macula Densa
(+)
NaCl reabsorption at macula densa
Metabolic sensing by macula densa
(–)
(+)
(+)
Nitric oxide, PGI2 / PGE2
Pressure natriuresis
Intrarenal baroreceptors
(+)
(+)
Pressure in pre-glomerular blood vessels
(–)
(+)
From Juxtaglomerular cells (JGCs)
(–)
Renin release
(–)
(+)
(+)
Arterial pressure
Sympathetic tone
β1AR activation on JGCs
(+)
(+)
High pressure baroreceptors
(–)
Negative feedback
ANGII formation
(+)
AGTR1 activation
Negative feedback

Figure 30–2 *Physiological pathways, feedback loops, and pharmacological regulation of the RAS.* Schematic portrayal of the three major physiological pathways regulating renin release. β1AR, β_1 adrenergic receptor. See text for details.

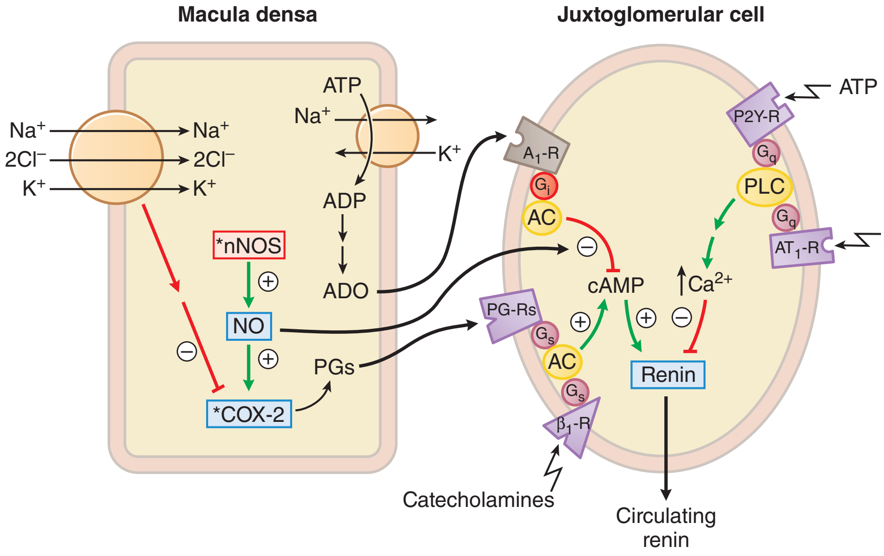

Figure 30–3 *Regulation of JG cell renin release by the macula densa (MD).* Mechanisms by which the MD regulates renin release. Changes in tubular delivery of NaCl to the MD cause signals to be conveyed to the JG cells (JGCs). Sodium depletion increases nNOS and COX-2 in the MD to enhance production of PGs. PGs and catecholamines stimulate adenylyl cyclase activity and cAMP production and hence renin release from the JGCs. Increased NaCl transport depletes ATP and increases adenosine (ADO) levels. Adenosine diffuses to the JGCs and inhibits cAMP production and renin release via G_i-coupled A_1 receptors. Increased NaCl transport in the MD augments the efflux of ATP, which may inhibit renin release by binding to P2Y receptors and activating the $G_{q/11}$-PLC-IP_3-Ca^{2+} pathway in JGCs. Circulating AngII also inhibits renin release on JGCs via G_{q11}-coupled AT_1 receptors. *Expression upregulated by chronic Na^+ depletion.

1. Activating high-pressure baroreceptors, thereby reducing renal sympathetic tone
2. Increasing pressure in the preglomerular vessels
3. Reducing NaCl reabsorption in the proximal tubule (pressure natriuresis), which increases tubular delivery of NaCl to the MD

Drugs That Affect Renin Secretion

Renin release is influenced by arterial BP, dietary salt intake, and pharmacological agents. Loop diuretics stimulate renin release and increase plasma renin concentration (PRC) by decreasing arterial BP and by blocking the reabsorption of NaCl at the MD. Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit PG synthesis and thereby decrease renin release. ACEIs, angiotensin receptor blockers (ARBs), and renin inhibitors interrupt both the short- and long-loop negative-feedback mechanisms and thereby increase renin release and PRC. Centrally acting sympatholytic drugs, as well antagonists of β_1 adrenergic receptors, decrease renin secretion by reducing β_1 adrenergic receptor activation of JG cells.

Angiotensinogen

Angiotensinogen, the substrate for renin, is a globular glycoprotein. AngI is cleaved by renin from the amino terminus of angiotensinogen. Human angiotensinogen contains 452 amino acids and is synthesized as preangiotensinogen, which has a 24– or 33–amino acid signal peptide. *AGT*, the precursor gene for angiotensinogen, is expressed in the liver and at substantial levels in the heart, brain, and smooth muscle cells, among others. Angiotensinogen is synthesized and secreted primarily by the liver, although angiotensinogen transcripts occur in many tissues, including the heart, kidneys, pancreas, lungs, adipocytes, and certain regions of the CNS. Synthesis of angiotensinogen is stimulated by inflammation, insulin, estrogens, glucocorticoids, thyroid hormone, and AngII. Pregnancy-related increases in estrogen increase plasma angiotensinogen levels several-fold.

Circulating levels of angiotensinogen are approximately equal to the K_m of renin for its substrate (~1 μM). Consequently, the rate of AngII synthesis, and therefore BP, can be influenced by changes in angiotensinogen levels. Oral contraceptives that contain estrogen increase circulating levels of angiotensinogen and can induce hypertension. Urinary angiotensinogen levels, considered an index for local intrarenal RAS activation, are elevated in patients with hypertension and progressive renal disease (Nishiyama and Kobori, 2018).

A missense mutation (M235T) in the angiotensinogen gene increases angiotensinogen plasma levels and has been implicated as a possible risk factor in several conditions (e.g., hypertension, preeclampsia, hypertrophic cardiomyopathy, and chronic kidney disease).

Angiotensin-Converting Enzyme

Angiotensin-converting enzyme (ACE, ACE1, kininase II, dipeptidyl carboxypeptidase, CD143, and several others) is a glycoprotein ectoenzyme with an apparent molecular weight of 170 kDa. Human ACE contains 1277 amino acid residues and has two homologous domains, each with a catalytic site and a Zn^{2+}-binding region. ACE has a large amino-terminal extracellular domain, a short carboxyl-terminal intracellular domain, and a 22–amino acid transmembrane hydrophobic region. ACE cleaves dipeptide units from substrates with diverse amino acid sequences. Preferred substrates have one free carboxyl group in the carboxyl-terminal amino acid, and proline must not be the penultimate amino acid; thus, the enzyme does not degrade AngII. ACE inactivates bradykinin and other potent vasodilator peptides. Slow conversion of AngI to AngII occurs in plasma, but rapid metabolism occurs *in vivo*, largely from the activity of membrane-bound ACE on the luminal surface of vascular endothelial cells (Guang et al., 2012).

An insertion/deletion polymorphism in intron 16 of the *ACE* gene produces large variation in serum ACE levels. The deletion allele, associated with higher levels of serum ACE and increased metabolism of bradykinin, may confer an increased risk of several disorders, including hypertension (and associated chronic kidney disease), and complications of pregnancy.

Angiotensin-Converting Enzyme 2

ACE2, a carboxypeptidase, cleaves one amino acid from the carboxyl terminal to convert AngII to Ang(1–7) and can convert AngI to Ang(1–9), which is then converted to Ang(1–7) by ACE, neprilysin, and endopeptidases (Santos, 2014). ACE2 contains a single catalytic domain that is

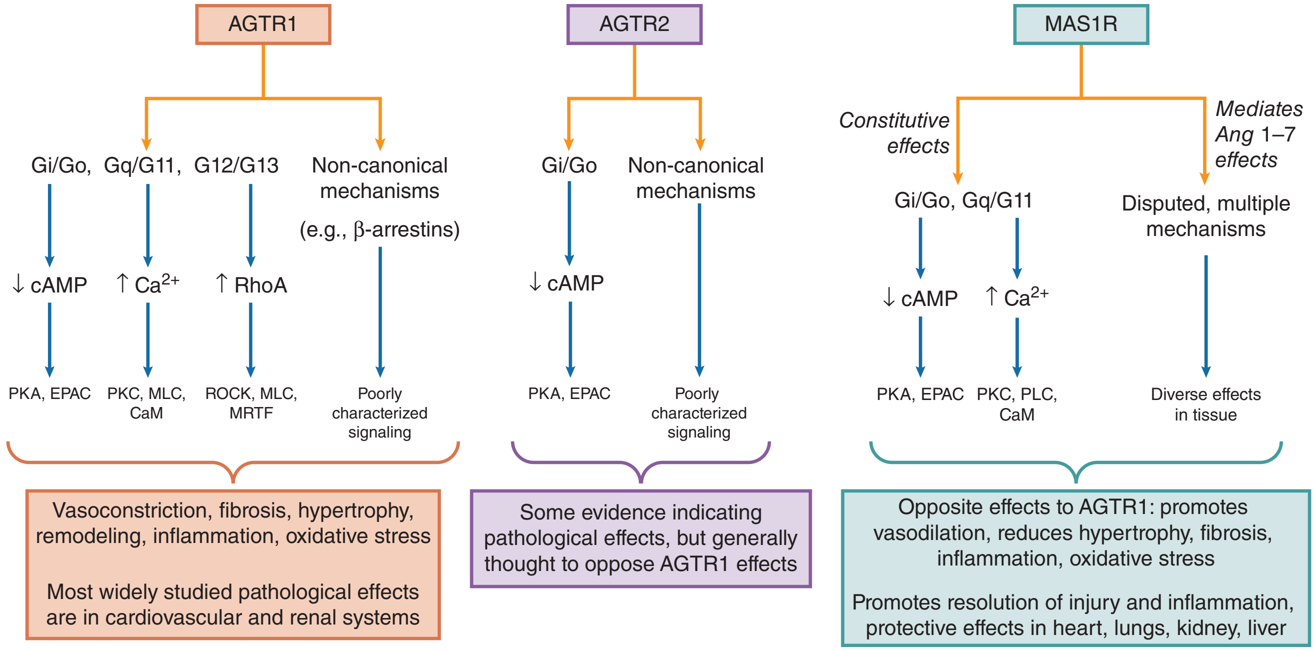
AGTR1
Gi/Go, Gq/G11, G12/G13
Non-canonical mechanisms
(e.g., β-arrestins)
↓ cAMP
↑ Ca2+
↑ RhoA
PKA, EPAC
PKC, MLC, CaM
ROCK, MLC, MRTF
Poorly characterized signaling
Vasoconstriction, fibrosis, hypertrophy, remodeling, inflammation, oxidative stress
Most widely studied pathological effects are in cardiovascular and renal systems
AGTR2
Gi/Go
Non-canonical mechanisms
↓ cAMP
PKA, EPAC
Poorly characterized signaling
Some evidence indicating pathological effects, but generally thought to oppose AGTR1 effects
MAS1R
Constitutive effects
Mediates Ang 1–7 effects
Gi/Go, Gq/G11
Disputed, multiple mechanisms
↓ cAMP
↑ Ca2+
PKA, EPAC
PKC, PLC, CaM
Diverse effects in tissue
Opposite effects to AGTR1: promotes vasodilation, reduces hypertrophy, fibrosis, inflammation, oxidative stress
Promotes resolution of injury and inflammation, protective effects in heart, lungs, kidney, liver

Figure 30–4 *Schematic diagram of opposing arms in the RAS.* Therapeutic interventions aim to inhibit the ACE-AngII-AT$_1$ receptor axis (orange) and enhance the ACE2-Ang(1–7)-Mas receptor axis (green). CaM, calmodulin; MLC, myosin light chain; MRTF, myocardin-related transcription factor A; PKA/PKC, protein kinase A/C; PLC, phospholipase C; ROCK, Rho kinase.

42% identical to the two catalytic domains of ACE. AngII is the preferred substrate for ACE2, with 400-fold higher affinity than AngI (Guang et al., 2012).

ACE2 opposes the actions of AngII by at least two mechanisms:

1. Decrease in AngII levels by metabolizing it to Ang(1–7), thus limiting effects of AngII
2. Increases in Ang(1–7), which via MAS1, opposes AngII actions (Figures 30–1 and 30–4)

ACE2 is not inhibited by standard ACEIs and has no effect on bradykinin. Reduced expression or deletion of ACE2 is associated with hypertension, defects in cardiac contractility, and elevated levels of AngII. Overexpression of the ACE2 gene decreases BP and prevents AngII-induced cardiac hypertrophy in hypertensive rats. ACE2 is protective against diabetic nephropathy through the Ang(1–7)/Mas receptor pathway (Varagic et al., 2014). Ang(1–9), which is generated from AngI by ACE2, may have vasodilating and protective effects by activating AT$_2$ receptors (Etelvino et al., 2014).

ACE2 metabolizes numerous peptides (including apelin, neurotensin, and ghrelin) and is a receptor for severe acute respiratory syndrome (SARS) coronaviruses (CoVs), including SARS-CoV-1 and SARS-CoV-2. The interaction with coronavirus spike proteins has led to efforts to use soluble ACE2 as a therapy to block entry and mitigate morbidity from coronavirus infections (Monteil et al., 2020).

Alternative Pathways for Angiotensin II Biosynthesis

AngII can be generated by ACE-independent pathways. Angiotensinogen is converted to AngI, or directly to AngII by cathepsin G and tonin. Enzymes that convert AngI to AngII include cathepsin G, chymostatin-sensitive AngII-generating enzyme, and chymase. Chymase contributes to tissue conversion of AngI and Ang(1–12) to AngII, particularly in the heart and kidneys. Mast cells are the major source of chymase (Ferrario et al., 2014).

Angiotensinases

Angiotensinases include aminopeptidases, endopeptidases, carboxypeptidases, and other peptidases that metabolize angiotensin peptides; none s specific.

Angiotensin Peptides and Their Receptors

Table 30–1 shows the sequence of the RAS peptides, and Table 30–2 lists the peptides, their receptors, and overall effects of the receptor-peptide interactions.

AngII-AT$_1$ Receptor Axis

Angiotensin II binds to the GPCRs AT$_1$ and AT$_2$. The hypertensive, renal, and hypertrophic effects of AngII are mediated by AT$_1$ receptors. Numerous polymorphisms have been identified in the AT$_1$ receptor gene, one of which (A1166C) has been associated with hypertension, hypertrophic cardiomyopathy, coronary artery vasoconstriction, and susceptibility to diabetic nephropathy (Zhuang et al., 2018). Preeclampsia may be associated with AT$_1$ polymorphisms or AT$_1$ agonistic autoantibodies, but this is a controversial area (Gathiram and Moodley, 2020).

AngII-AT$_1$ Receptor-Effector Coupling. AT$_1$ receptors link to numerous signal transduction systems to produce effects that vary with cell type and are a combination of primary and secondary responses. AT$_1$ receptors couple to several heterotrimeric G proteins, (e.g., G$_{q/11}$, G$_{12/13}$, and G$_i$) and are substrates for phosphorylation and desensitization by G

TABLE 30–1 ■ ANGIOTENSIN PEPTIDES AND THEIR SEQUENCES

PEPTIDE/PROTEIN	SEQUENCE
Angiotensinogen	**Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu- | - (~450 amino acids)**
Angiotensin I	**Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu**
Angiotensin 1–9	**Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His**
Angiotensin 1–7	**Asp-Arg-Val-Tyr-Ile-His-Pro**
Angiotensin 1–5	**Asp-Arg-Val-Tyr-Ile**
Angiotensin II	**Asp-Arg-Val-Tyr-Ile-His-Pro-Phe**
Angiotensin A	**Ala-Arg-Val-Tyr-Ile-His-Pro-Phe**
Angiotensin III	**Arg-Val-Tyr-Ile-His-Pro-Phe**
Angiotensin IV	**Val-Tyr-Ile-His-Pro-Phe**

protein-coupled receptor kinases (GRKs), interacting with β-arrestin, prior to being internalized.

In most cell types, AT_1 receptors couple to $G_{q/11}$ to activate the phospholipase (PL) Cβ-IP_3-Ca^{2+} pathway. Secondary responses to $G_{q/11}$ activation include activation of PKC, PLA_2, and PLD, eicosanoid production, Ca^{2+}-dependent and MAP kinases, and Ca^{2+}-calmodulin–dependent activation of nitric oxide synthase (NOS). Activation of G_i may occur and will reduce the activity of adenylyl cyclase, lowering cellular cAMP content. However, there also is evidence for $G_{q/11} \rightarrow G_s$ cross talk such that activation of the AT_1-$G_{q/11}$-PLC pathway can enhance cAMP production. The βγ subunits of G_i and activation of $G_{12/13}$ lead to activation of tyrosine kinases and low-molecular-weight ("small") G proteins such as Rho. Ultimately, the Jak/STAT pathway may be activated and transcriptional regulatory factors induced. By these mechanisms, AngII influences gene expression, including of genes that regulate cell growth and production of extracellular matrix (ECM) components. AngII/AT_1 also stimulate the activity of a membrane-bound NADH/NADPH oxidase that generates reactive oxygen species (ROS). ROS may contribute to certain biochemical effects (e.g., activation of MAP kinase, tyrosine kinase, and phosphatases; inactivation of nitric oxide [NO]) and physiological effects (acute effects on renal function, chronic effects on BP, vascular hypertrophy and inflammation) of AngII (Garrido and Griendling, 2009). The role of these many signaling pathways in mediating biological responses to AngII varies in different tissues and cell types. The AT_1 receptor is structurally flexible and may be activated by mechanical stress independent of AngII binding (Kim et al., 2012). Function of the AT_1 receptor may be modified by homodimerization or heterodimerization with GPCRs that include AT_2, bradykinin B_2, $β_2$ adrenergic, and apelin receptors (Takezako et al., 2017).

AngII-AT_2 Receptor Axis

Activation of the AT_2 receptors counteracts many of the effects of the AT_1 receptors by having antiproliferative, anti-inflammatory, vasodilatory, natriuretic, and antihypertensive effects (Figure 30–4). AT_2 receptors are more widely distributed in fetal tissues than in adults. AT_2 receptor expression is increased and AT_2 receptors have been implicated in cardiovascular and renal disorders (Jones et al., 2008; Kaschina et al., 2017; Ocaranza et al., 2020; Santos et al., 2019). AT_2 receptor signaling is mediated by G protein-dependent ($G_{iα2}$ and $G_{iα3}$) and G protein-independent pathways. Effects of AT_2 receptor activation include activation of phosphotyrosine phosphatases that inhibit MAP kinases and ERK1/2; inhibition of Ca^{2+} channel functions; and increases in NO, cyclic GMP, and bradykinin production. AT_2 receptors can bind AT_1 receptors to antagonize and reduce their expression. AT_2 receptors can also form heterodimers with bradykinin B_2 receptors, an interaction that can enhance NO production (Jones et al., 2008; Padia and Carey, 2013) and may dimerize with MAS receptors (Patel and Hussain, 2018).

Angiotensin (1–7)/Mas Receptor Axis

The ACE2/Ang(1–7)/Mas receptor axis is a negative regulator of the pressor, profibrotic, and anti-natriuretic effects of the ACE-AngII-AT_1 receptor axis of the RAS (Figure 30–4). Ang(1–7) is generated in several ways (Figure 30–1), including from:

- AngII by ACE2;
- AngII by carboxypeptidases;
- AngI by endopeptidases; and
- AngI in two steps: by ACE2 to Ang(1–9) and then to Ang(1–7) by ACE or neprilysin.

The antihypertensive effects of Ang(1–7) occur via binding to Mas receptors, but Ang(1–7) can also bind and activate AT_2 receptors (Gironacci et al., 2014). Activation of Mas receptors by Ang(1–7) induces vasodilation, stimulates a phosphoinositide 3-kinase (PI_3K)/Akt pathway that promotes NO production, potentiates the vasodilatory effects of bradykinin, and inhibits AngII-induced activation of ERK1/2 and NFκB. Ang(1–7) has antiangiogenic, antiproliferative, and antithrombotic effects and is renoprotective and cardioprotective in cardiac ischemia and heart failure (Fraga-Silva et al., 2013; Santos et al., 2019). Activation of Mas receptors in the brain is associated with improved memory and cognition (Gironacci et al., 2014). Mice with knockout of the *Mas* gene have increased vascular resistance and cardiac dysfunction (Santos et al., 2019).

TABLE 30–2 ■ RAS PEPTIDES AND THEIR RECEPTORS

RECEPTOR	RAS PEPTIDE	EFFECT
AT_1	AngII, AngIII, AngA, Ang(1–12)	Vasoconstriction, hypertrophy, proliferation, angiogenesis, fibrosis, inflammation, nephropathy
AT_2	AngII, AngIII, Ang(1–7), Ang(1–9), AngA	Vasodilation, anti-hypertrophy, reduction of fibrosis, natriuresis; some contradictory pathological effects
MAS1	Ang(1–7)	Vasodilation, anti-hypertrophy, reduction of fibrosis, resolution of inflammation and injury, natriuresis
MRGPRD	Alamandine	Less well understood; believed to have similar effects to MAS1: vasodilation, anti-hypertrophy, reduction of fibrosis
IRAP	AngIV, Ang(3–7)	Neuroprotection, cognition, renal vasodilation, natriuresis
PRR	Prorenin, renin	Hypertrophy, fibrosis, apoptosis

The capacity of Ang(1–7) to counterbalance actions of AngII may depend on the relative activities of ACE-AngII-AT_1 receptors and ACE2-Ang(1–7)-Mas receptors (Figure 30–4 and Table 30–2; Forrester et al., 2018; Ocaranza et al., 2020; Santos et al., 2019). Enhancing the ACE2-Ang(1–7)-Mas receptor pathway using ACE2 activators or Mas receptor agonists could provide new ways to modulate the RAS in cardiovascular and renal disease and other settings.

Angiotensin III

Angiotensin III, also called Ang(2–8), can be formed by the action of aminopeptidase A on AngII or by the action of ACE on Ang(2–10). AngIII binds to both AT_1 and AT_2 receptors, causing effects qualitatively like those of AngII. AngII and AngIII stimulate aldosterone secretion with equal potency; however, AngIII is less efficacious in elevating BP (25%) and stimulating the adrenal medulla (10%). Data from model systems imply that AngIII and the shorter angiotensin-derived peptides have significant activity, especially at the AT_2 receptor, and that there may be instances where AngIII is the active endogenous ligand (Bosnyak et al., 2011).

Angiotensin IV/AT_4 Receptor Axis

Angiotensin IV [Ang(3–8)] is formed from AngIII through the catalytic action of aminopeptidase N. Central and peripheral actions of AngIV are mediated through a specific AT_4 receptor that was identified as IRAP (insulin-regulated aminopeptidase) (Figure 30–1 and Table 30–2). This single transmembrane protein receptor colocalizes with the glucose transporter type 4. AT_4 receptors are detectable in numerous tissues, e.g., heart, vasculature, adrenal cortex, and brain regions, that process sensory and motor functions. AngIV-dependent AT_4 receptor activation regulates cerebral blood flow, is neuroprotective, and facilitates long-term potentiation, memory consolidation, and cognition (Wright et al., 2013). AngIV binding to the AT_4 receptor inhibits the catalytic activity of IRAP and enables accumulation of neuropeptides linked to memory potentiation. Other actions include renal vasodilation, natriuresis, neuronal differentiation, inflammation, and ECM remodeling. AngIV analogues have been developed as potential therapeutics for Alzheimer's disease and head injury (Wright and Harding, 2019).

Other Physiologically Active Angiotensin Peptides

Other biologically active angiotensin peptides and their receptors have been identified (Tables 30–1 and 30–2). These peptides include

Ang (1-9), alamandine, AngA, Ang(3-7), and proangiotensin/Ang(1–12) (Ferrario et al., 2014; Forrester et al., 2018; Ocaranza et al., 2020; Santos et al., 2019). Ang(1–9) is generated from AngI by ACE2, carboxypeptidase A, and cathepsin. Ang(1–9) has cardioprotective and antipressor effects reportedly mediated through binding to AT_2 receptors and the release of NO (Etelvino et al., 2014) and may have a role in heart failure (Basu et al., 2017). Alamandine is produced from Ang(1–7) by the decarboxylation of the N-terminal Asp_1 residue into Ala_1. Alamandine acts through MrgD, a GPCR, to mediate vasodilatory and antifibrotic effects similar to Ang(1–7). Alamandine is an ACE substrate and may be an ACEI. Alamandine is elevated in patients with chronic renal disease (Etelvino et al., 2014).

Angiotensin A is an octapeptide produced by decarboxylation of the Asp_1 residue of AngII into Ala_1. AngA binds to both AT_1 and AT_2 receptors and has effects similar to, but less potent than, AngII. AngA is reported to be elevated in patients with end-stage renal disease (Ferrario et al., 2014). Ang(3–7) is generated from AngIV and binds AT_4 receptors (Wright et al., 2013). Proangiotensin or Ang(1–12) is generated from angiotensinogen through a non-renin pathway and can be converted to AngII by chymase. Ang(1–12) can bind to the AT_1 receptors and may be a precursor for autocrine/intracrine production of AngII (Ferrario et al., 2014).

Local (Tissue) Renin-Angiotensin System

Local RAS is a tissue-based AngII-producing system that plays a role in hypertrophy, inflammation, remodeling, and apoptosis. ACE is present on the luminal face of vascular endothelial cells throughout the circulation; circulating (pro)renin can bind the PRR in the arterial wall in tissues to locally generate AngII (Campbell, 2014; Paul et al., 2006). Tissue RAS is also an autocrine and intracrine mechanism that can generate AngII and other angiotensin peptides independently of the renal/hepatic-based system (Nehme et al., 2019). Many tissues—including the brain, pituitary, blood vessels, heart, lung, kidney, and adrenal gland—express renin, angiotensinogen, ACE and ACE2, chymase, PRR, angiotensin I, II, III, IV, Ang(1–7), and AT_1, AT_2, and Mas receptors (Campbell, 2014; Ferrario et al., 2014). Activation of the local RAS components is tissue specific and may affect pathophysiology. However, the existence and role of tissue RAS in the brain remain controversial (Saravi et al., 2021). The potential role of local RAS and impact of RAS inhibition in solid tumors is an emerging area of interest (Almutlaq et al., 2021).

RAS components, in particular AngII, can be upregulated during inflammation and tissue injury. This underscores the role of AngII in pathobiology, with inflammation/injury driving RAS activity, which further promotes inflammation in a positive feedback loop (Sriram and Insel, 2020a). Evidence for local RAS activity is most notable in the heart, vasculature, and lungs (Campbell, 2014; Forrester et al., 2018).

The (Pro)Renin Receptor

(Pro)renin/PRR binding enhances tissue-RAS activity and exerts organ-specific localized actions that can be independent of AngII production (Ichihara and Yatabe, 2019; Ramkumar and Kohan, 2019). PRR is widely expressed in tissues throughout the body. The PRR gene is on the X chromosome and named *ATP6ap2* (ATPase-6 accessory protein 2). Knockout of the PRR gene is lethal, indicating its important role in development. Human mutations in the PRR gene are associated with intellectual disability and epilepsy (Nguyen and Danser, 2008). Human PRR, an approximately 37 kDa (350–amino acid) transmembrane protein, possesses an N-terminal extracellular domain that binds (pro)renin, a transmembrane domain, and a cytosolic domain associated with vacuolar-H^+-ATPase (V-ATPase) activity. Nephron-specific deletion of PRR in mice does not affect the RAS but produces renal concentration defects and distal renal tubular acidosis because of decreased V-ATPase activity (Trepiccione et al., 2016). Moreover, mice that overexpress human PRR in neurons have an ERK and NOX-4–dependent, angiotensin-independent increase in BP in response to intracerebroventricular infusion of (pro)renin (Peng et al., 2018). Together, such findings support the idea that (pro)renin and PRR can produce effects independent of the RAS.

The PRR binds (pro)renin with nanomolar affinity and high specificity, increases catalytic activity of renin, and induces activation of (pro)renin by unfolding its pro-segment, exposing the enzymatic cleft. Bound, activated (pro)renin catalyzes the conversion of angiotensinogen to AngI and, in turn, AngII formation. PRR binding of (pro)renin also induces AngII-independent signaling events, including activation of ERK1/2, p38, tyrosine kinases, COX-2, transforming growth factor (TGF)-β gene expression, and plasminogen activator inhibitor type 1 (PAI-1) (Ichihara and Yatabe, 2019; Nguyen and Danser, 2008; Peters, 2017). Such effects are not blocked by ACEIs or AT_1 receptor antagonists and may contribute to fibrosis, nephrosis, and end-organ damage (Kaneshiro et al., 2007).

Circulating plasma concentrations of (pro)renin are elevated 100-fold in diabetic patients and associated with increased risk of nephropathy, renal fibrosis, and retinopathy (Nguyen and Danser, 2008). Blockade of PRR by a peptide antagonist, known as "handle region peptide," reportedly protected animals from diabetic nephropathy and retinopathy, but the efficacy and specificity of this peptide have not been confirmed by others (Danser, 2015).

As noted above, in addition to its RAS-mediated actions, the PRR participates in RAS-independent functions, including as an accessory protein essential for V-ATPase activity, which is required for intracellular acidity, receptor-mediated endocytosis, and activation of lysosomal and autophagosomal enzymes. Cardiomyocyte-specific and podocyte-specific PRR knockout mice develop lethal organ-specific failure due to loss of V-ATPase and dysregulation of intracellular acidification and autophagy (Binger and Muller, 2013). PRR also participates in the activation of Wnt/β-catenin and Wnt/planar cell polarity signaling pathways and antagonizes ELABELA/apelin actions (Chen and Xu, 2020). Recent work has implicated PRR as a novel biomarker and potential therapeutic target for a variety of cancers (Wang J et al., 2020). PRR reportedly can regulate low-density lipoprotein (LDL) uptake and metabolism and may have a role in disorders of glucose and lipid metabolism (Ramkumar and Kohan, 2019). Silencing PRR expression in hepatocytes decreased cellular LDL uptake by decreasing expression of LDL receptor and sortelin 1 protein, a regulator of LDL uptake and metabolism and a PRR-interacting protein (Lu et al., 2016).

Functions and Effects of Angiotensin II

Angiotensin II increases total peripheral resistance (TPR) and alters renal function and cardiovascular structure (Figure 30–5). Modest increases in plasma AngII concentration acutely raise BP; on a molar basis, AngII is about 40 times more potent than NE: the EC_{50} of AngII for acutely raising arterial BP is approximately 0.3 nM. Intravenous injection of a moderate dose of AngII raises systemic BP within seconds, but it peaks rapidly and returns to normal within minutes (Figure 30–6). This rapid pressor response to AngII is due to an increase in TPR—a response that helps maintain arterial BP in the face of acute hypotensive challenges (e.g., blood loss or vasodilation). Although AngII increases cardiac contractility directly (by opening voltage-gated Ca^{2+} channels in cardiac myocytes) and increases heart rate indirectly (by facilitating sympathetic tone, enhancing adrenergic neurotransmission, and provoking adrenal catecholamine release), the rapid increase in arterial BP activates a baroreceptor reflex that decreases sympathetic tone and increases vagal tone. Thus, depending on the physiological state, AngII may increase, decrease, or not change cardiac contractility, heart rate, and cardiac output. Changes in cardiac output therefore contribute little, if at all, to the rapid pressor response induced by AngII.

AngII also causes a slow pressor response that helps to stabilize arterial BP over the long term. A continuous infusion of initially subpressor doses of AngII gradually increases arterial BP; the maximum effect requires days to achieve. This slow pressor response probably is mediated by a decrease in renal excretory function that shifts the renal pressure–natriuresis curve to the right (see the next section). AngII stimulates the synthesis of endothelin 1 and superoxide anion, which may contribute to the slow pressor response.

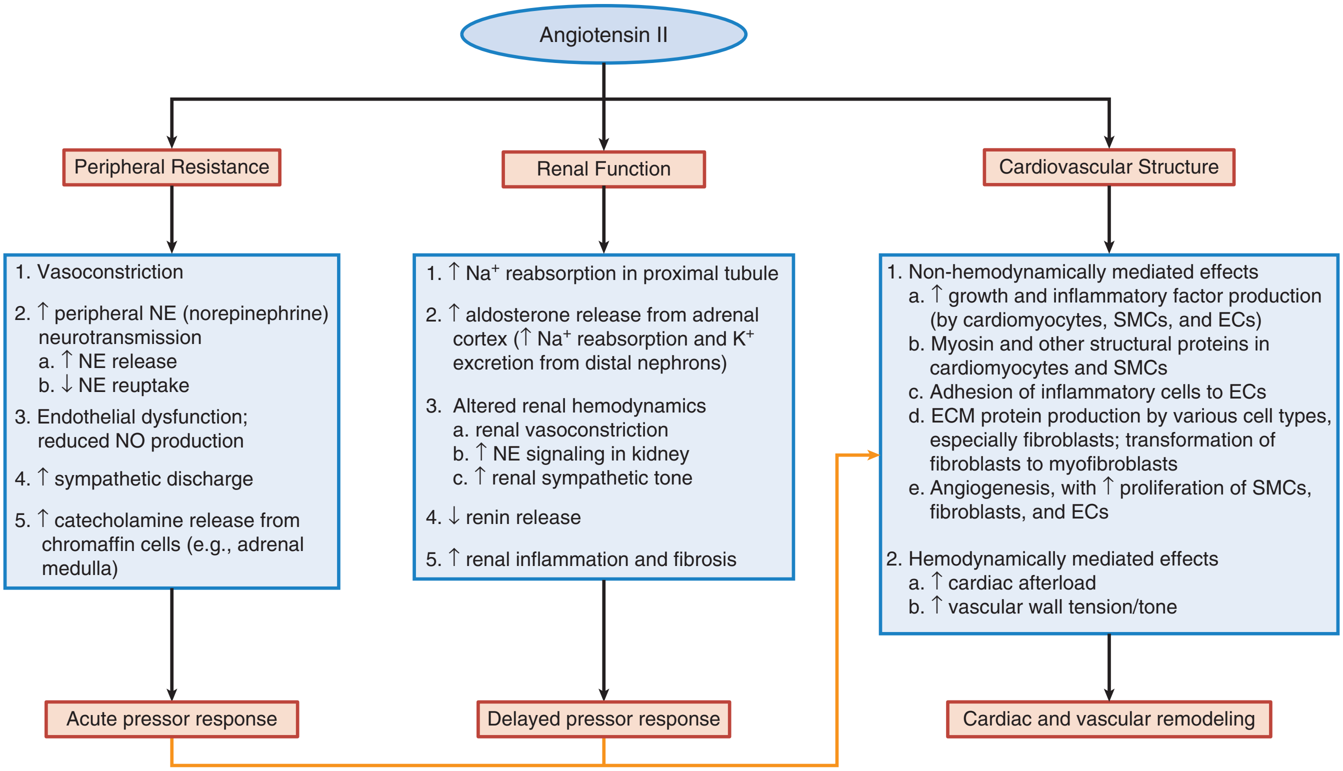
Angiotensin II
Peripheral Resistance
Renal Function
Cardiovascular Structure
1. Vasoconstriction
2. ↑ peripheral NE (norepinephrine) neurotransmission
a. ↑ NE release
b. ↓ NE reuptake
3. Endothelial dysfunction; reduced NO production
4. ↑ sympathetic discharge
5. ↑ catecholamine release from chromaffin cells (e.g., adrenal medulla)
1. ↑ Na$^+$ reabsorption in proximal tubule
2. ↑ aldosterone release from adrenal cortex (↑ Na$^+$ reabsorption and K$^+$ excretion from distal nephrons)
3. Altered renal hemodynamics
a. renal vasoconstriction
b. ↑ NE signaling in kidney
c. ↑ renal sympathetic tone
4. ↓ renin release
5. ↑ renal inflammation and fibrosis
1. Non-hemodynamically mediated effects
a. ↑ growth and inflammatory factor production (by cardiomyocytes, SMCs, and ECs)
b. Myosin and other structural proteins in cardiomyocytes and SMCs
c. Adhesion of inflammatory cells to ECs
d. ECM protein production by various cell types, especially fibroblasts; transformation of fibroblasts to myofibroblasts
e. Angiogenesis, with ↑ proliferation of SMCs, fibroblasts, and ECs
2. Hemodynamically mediated effects
a. ↑ cardiac afterload
b. ↑ vascular wall tension/tone
Acute pressor response
Delayed pressor response
Cardiac and vascular remodeling

Figure 30–5 *Major physiological effects of AngII.* AngII exerts physiological and pathological effects on both the renal and cardiovascular systems, providing a rationale for the therapeutic benefits of RAS inhibition. EC, endothelial cell; SMC, smooth muscle cell.

In addition to its effects on arterial BP, AngII stimulates remodeling of the cardiovascular system, causing hypertrophy of vascular and cardiac cells and increased synthesis and deposition of collagen by cardiac fibroblasts, as discussed in subsequent sections of this chapter.

Mechanisms by Which Angiotensin II Increases Total Peripheral Resistance

AngII increases peripheral resistance by direct and indirect effects on blood vessels.

Direct Vasoconstriction

AngII constricts precapillary arterioles and, to a lesser extent, postcapillary venules by activating AT$_1$ receptors on vascular smooth muscle cells and stimulating the $G_{q/11}$-PLC-IP$_3$-Ca^{2+} pathway. AngII also exerts vasoconstrictive effects due to signaling in endothelial cells, likely due to the presence of AGTR1 receptors in caveolae. AGTR1 activation is associated with an increase in ROS production and a decrease in endothelial NOS activity, contributing to endothelial dysfunction (Eguchi et al., 2018; Forrester et al., 2018; Gomolak and Didion, 2014). Direct vasoconstriction is strongest in the kidneys, somewhat less in the splanchnic bed, much less in vessels of the brain, and even weaker in pulmonary and skeletal muscle vessels. Nevertheless, high circulating concentrations of AngII may decrease cerebral and coronary blood flow. The effects of AngII on vasoconstriction may also be mediated by AGTR2 receptors, though this is controversial (Forrester et al., 2018).

Enhancement of Peripheral Noradrenergic Neurotransmission

Angiotensin II, binding to AT$_1$ receptors, augments NE release from sympathetic nerve terminals by inhibiting the reuptake of NE into nerve terminals and by enhancing the vascular response to NE (see Chapter 14). High concentrations of the peptide directly stimulate ganglion cells.

Effects on the CNS

Angiotensin II increases sympathetic tone. Small amounts of AngII infused into the vertebral arteries increase arterial BP via effects of AngII on circumventricular nuclei that are not protected by the blood-brain barrier (e.g., area postrema, subfornical organ, organum vasculosum of the lamina terminalis). Circulating AngII also attenuates baroreceptor-mediated reductions in sympathetic discharge, thereby increasing arterial BP. The CNS is affected by bloodborne AngII and AngII formed within the brain, which contains all components of the RAS. AngII also has a centrally mediated dipsogenic (thirst) effect and enhances vasopressin release from the neurohypophysis.

Release of Catecholamines from the Adrenal Medulla

AngII stimulates the release of catecholamines from the adrenal medulla by promoting Ca^{2+} entry secondary to depolarization of chromaffin cells.

Mechanisms by Which Angiotensin II Regulates Renal Function

Angiotensin II has pronounced effects on renal function, reducing the urinary excretion of Na$^+$ and water while increasing the excretion of K$^+$. The overall effect of AngII on the kidneys is to shift the renal pressure–natriuresis curve to the right (Figure 30–7), as discussed subsequently.

Direct Effects of AngII on Na$^+$ Reabsorption in the Renal Tubules

Very low concentrations of AngII stimulate Na$^+$/H$^+$ exchange in the proximal tubule—an effect that increases Na$^+$, Cl$^-$, and bicarbonate reabsorption (see also Chapter 29). Approximately 20% to 30% of the bicarbonate handled by the nephron may be affected by this mechanism. AngII also increases the expression of the Na$^+$-glucose symporter in the proximal tubule. Paradoxically, high concentrations of AngII may inhibit proximal tubule Na$^+$ transport. AngII also directly stimulates the Na$^+$-K$^+$-2Cl$^-$ symporter in the thick ascending limb. The proximal tubule secretes angiotensinogen, and the connecting tubule releases renin, so a paracrine tubular RAS may contribute to Na$^+$ reabsorption.

Release of Aldosterone from the Adrenal Cortex

Angiotensin II stimulates the zona glomerulosa of the adrenal cortex to increase the synthesis and secretion of aldosterone and augments

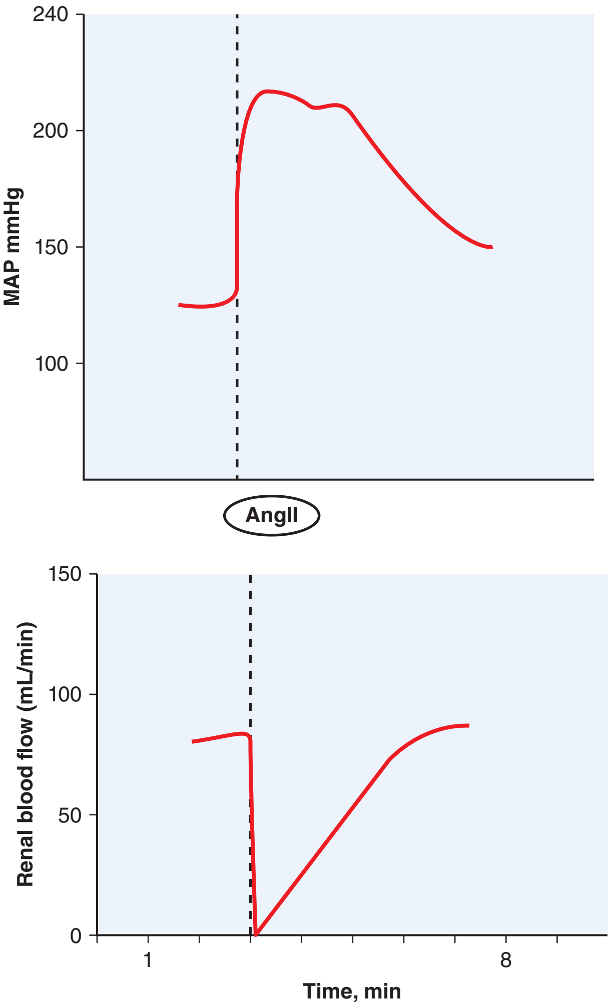

Figure 30–6 *Effect of an intravenous bolus of AngII on arterial BP and RBF.* Angiotensin was added at the time indicated by the dashed vertical line.

responses to other stimuli (e.g., corticotropin [ACTH], K^+). Increased output of aldosterone is elicited by AngII concentrations that have little or no acute effect on BP. Aldosterone acts on the distal and collecting tubules to cause retention of Na^+ and excretion of K^+ and H^+. The stimulant effect of AngII on aldosterone synthesis and release is enhanced under conditions of hyponatremia or hyperkalemia and is reduced when concentrations of Na^+ and K^+ in the plasma are altered in the opposite directions.

Altered Renal Hemodynamics

Angiotensin II reduces renal blood flow (RBF) and renal excretory function by directly constricting the renal vascular smooth muscle, by enhancing renal sympathetic tone (a CNS effect), and by facilitating renal adrenergic transmission (an intrarenal effect). AngII-induced vasoconstriction of preglomerular microvessels is enhanced by endogenous adenosine via signal transduction systems activated by AT_1 and the adenosine A_1 receptor, respectively.

Angiotensin II influences the glomerular filtration rate (GFR) by several mechanisms:

- Constriction of the afferent arterioles, which reduces intraglomerular pressure and reduces GFR
- Contraction of mesangial cells, which decreases the capillary surface area within the glomerulus available for filtration and thereby decreases GFR
- Constriction of efferent arterioles, which increases intraglomerular pressure and tends to increase GFR

Normally, AngII slightly reduces GFR; however, with renal artery stenosis, the effects of AngII on the efferent arteriole predominate so that AngII increases GFR. Thus, blockade of the RAS may cause acute renal failure in patients with bilateral renal artery stenosis or in patients with unilateral stenosis who have only a single kidney.

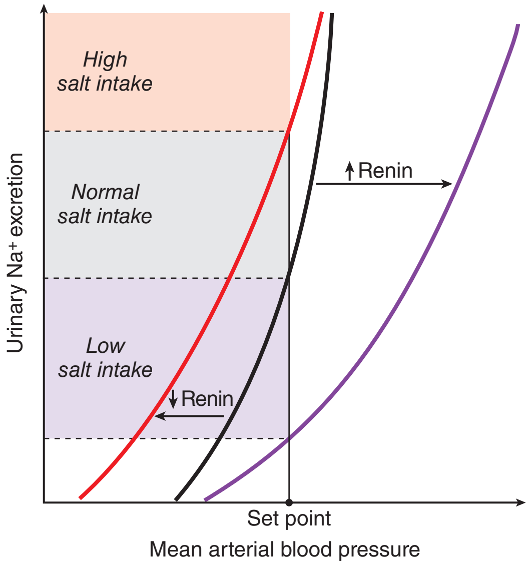

Figure 30–7 *Pressure-natriuresis curve: effects of Na^+ intake on renin release (AngII formation) and arterial BP.* Inhibition of the RAS will cause a large drop in blood pressure in Na^+-depleted individuals. (Modified with permission from Jackson EK, Branch RA, Margolius HS, Oates JA. Physiological functions of the renal prostaglandin, renin, and kallikrein systems. In: Seldin DW, Giebisch GH, eds. *The Kidney: Physiology and Pathophysiology*. Vol 1. Lippincott Williams & Wilkins, Philadelphia, **1985**, 624.)

Mechanisms by Which Angiotensin II Alters Cardiovascular Structure

Pathological alterations involving cardiovascular hypertrophy and remodeling increase morbidity and mortality. The cells involved include vascular smooth muscle cells, cardiac myocytes, endothelial cells, immune cells, and fibroblasts. AngII induces hypertrophy of cardiac myocytes; stimulates the migration, proliferation, and hypertrophy of vascular smooth muscle and endothelial cells; increases ECM production by vascular smooth muscle cells; and increases ECM production by cardiac fibroblasts. Moreover, AngII promotes the release of proinflammatory factors (e.g., cytokines and chemokines) that induce paracrine effects on neighboring cells (e.g., TGF-β produced by cardiac myocytes stimulates transformation of fibroblasts into myofibroblasts). The recruitment of immune cells contributes further to this pro-remodeling, inflammatory, and hypertrophic phenotype. Secreted factors from immune cells contribute to cardiac myocyte hypertrophy along with pathological effects in other cell types. AngII signaling also enhances coagulopathy through interaction with the coagulation cascade and increases immune cell adhesion to endothelial cells, thus further enhancing vascular pathology. Hence, cardiac remodeling by AngII results from a complex interplay of multiple cell types (Figure 30–8) (Forrester et al., 2018; Sriram and Insel, 2020a). In addition, AngII alters ECM formation and degradation indirectly by increasing aldosterone production and mineralocorticoid receptor activation. The adverse cardiovascular remodeling induced by AngII can be reduced but not entirely eliminated by mineralocorticoid receptor antagonists.

Hemodynamically Mediated Effects of Angiotensin II on Cardiovascular Structure

In addition to the direct effects of AngII on cardiovascular structure, changes in cardiac preload (volume expansion owing to Na^+ retention) and afterload (increased arterial BP) probably contribute to cardiac hypertrophy and remodeling. Arterial hypertension also contributes to hypertrophy and remodeling of blood vessels.

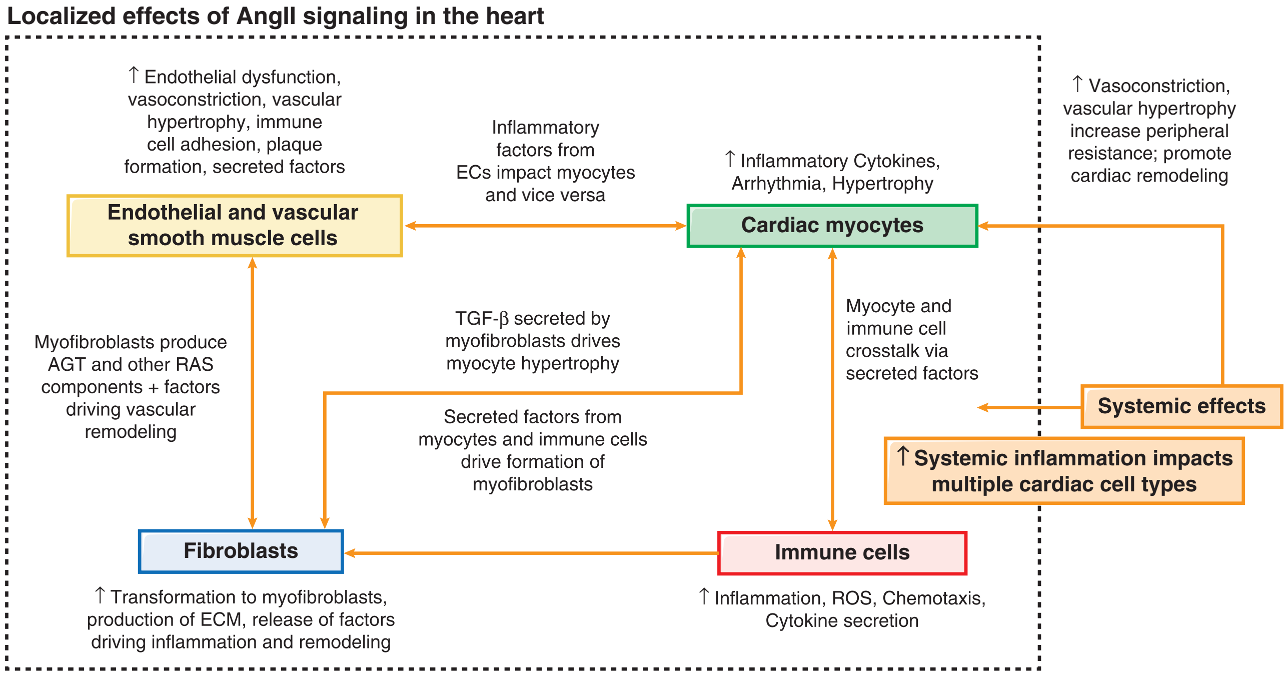
Localized effects of AngII signaling in the heart
↑ Endothelial dysfunction, vasoconstriction, vascular hypertrophy, immune cell adhesion, plaque formation, secreted factors
Endothelial and vascular smooth muscle cells
Inflammatory factors from ECs impact myocytes and vice versa
↑ Inflammatory Cytokines, Arrhythmia, Hypertrophy
Cardiac myocytes
↑ Vasoconstriction, vascular hypertrophy increase peripheral resistance; promote cardiac remodeling
Myofibroblasts produce AGT and other RAS components + factors driving vascular remodeling
TGF-β secreted by myofibroblasts drives myocyte hypertrophy
Myocyte and immune cell crosstalk via secreted factors
Systemic effects
Secreted factors from myocytes and immune cells drive formation of myofibroblasts
↑ Systemic inflammation impacts multiple cardiac cell types
Fibroblasts
Immune cells
↑ Transformation to myofibroblasts, production of ECM, release of factors driving inflammation and remodeling
↑ Inflammation, ROS, Chemotaxis, Cytokine secretion

Figure 30–8 *Impact of AngII signaling on cell types that contribute to cardiac hypertrophy.* The effects of AngII involve the interaction of multiple cell types and processes that include inflammation, remodeling, and hypertrophy, with cardiac myocytes interacting with other cells via secreted factors. Besides these localized effects are systemic effects of AngII—increased peripheral resistance and systemic inflammation—that contribute to hypertrophy. AGT, angiotensinogen; ECs, endothelial cells; ECM, extracellular matrix; ROS, reactive oxygen species.

Role of the RAS in Long-Term Maintenance of Arterial Blood Pressure Despite Extremes in Dietary Na^+ Intake

Arterial BP is a major determinant of Na^+ excretion. This is illustrated graphically by plotting urinary Na^+ excretion versus mean arterial BP (Figure 30–7), a plot known as the *renal pressure–natriuresis curve*. Over the long term, Na^+ excretion must equal Na^+ intake; therefore, the set point for long-term levels of arterial BP can be obtained as the intersection of a horizontal line representing Na^+ intake with the renal pressure–natriuresis curve. The RAS plays a major role in maintaining a constant set point for long-term levels of arterial BP despite extreme changes in dietary Na^+ intake. When dietary Na^+ intake is low, renin release is stimulated, and AngII acts on the kidneys to shift the renal pressure–natriuresis curve to the right. Conversely, when dietary Na^+ is high, renin release is inhibited, and the withdrawal of AngII shifts the renal pressure–natriuresis curve to the left. When modulation of the RAS is blocked by drugs, changes in salt intake markedly affect long-term levels of arterial BP.

Other Effects of the RAS

Expression of the RAS is required for the development of normal kidney morphology, particularly the maturational growth of the renal papilla. AngII causes a marked anorexigenic effect and weight loss; high circulating levels of AngII may contribute to the anorexia, wasting, and cachexia of heart failure (Yoshida et al., 2013).

The RAS in SARS Infections and Pulmonary Pathobiology

The COVID-19 pandemic has brought attention to the biology of coronaviruses. Understanding how coronaviruses infect humans can provide insights into therapeutic approaches focused on mitigating host pathobiology. ACE2 is the viral receptor for SARS-CoV-1 (which causes SARS) and SAR-CoV-2 (which causes COVID-19). The viral spike proteins bind to ACE2, followed by viral endocytosis and subsequent infection (Sriram and Insel, 2020a). Given the high expression of ACE2 in respiratory epithelia, particularly in alveolar pneumocytes (especially type II pneumocytes), both SARS viruses can cause severe alveolar injury, leading to acute respiratory distress syndrome (ARDS) and systemic organ failure in the most serious cases.

Interaction of the SARS-CoV spike proteins with ACE2 is thought to be a critical component of SARS-CoV pathobiology. This interaction leads to ACE2 internalization and decrease in ACE2 activity (along with a decrease in ACE2 gene expression). The functional activities of ACE2 in the RAS pathway are thus blunted (Figures 30–1 and 30–4) (reviewed in Sriram and Insel, 2020a). The result is an imbalance in the actions of ACE1 versus ACE2 with respect to the peptides they generate and receptors that are activated: less ACE2 relative to ACE1 implies greater abundance of AngII (with less degradation of AngII by ACE2) and, hence, increased activation of AngII receptors (in particular AGTR1, which is highly expressed in many pulmonary cell types) and less activation of MAS1. This imbalance leads to a bias toward the injury- and inflammation-promoting effects of AngII, including apoptosis in the alveolar epithelium, fibrosis by fibroblasts, and inflammatory and remodeling effects in endothelial cells that mediate alveolar-capillary communication. A positive feedback loop of inflammation and injury occurs, which can produce severe pathology when coupled with a rapid immune/inflammatory response (Figure 30–9; Sriram and Insel, 2020a, 2021). Such inflammatory signaling can couple with coagulation signaling, leading to “thromboinflammation,” a characteristic of SARS infections and other forms of ARDS (Sriram and Insel, 2021). Studies with SARS-infected ACE2-knockout mice (Kuba et al., 2005) and with nonreplicating viral particles expressing ACE2-binding spike proteins (Lei et al., 2021) are examples of evidence supporting this mechanism.

Concerns emerged in the early stages of the COVID-19 pandemic that RAS inhibitors may be harmful by enhancing ACE2 expression, thereby promoting viral entry of SARS-CoV-2. However, subsequent analysis of data for ACE2 expression in animals and humans treated with RAS inhibitors questioned the validity of this idea (reviewed in Sriram and Insel, 2020b). Subsequent epidemiological studies in COVID-19 patients have indicated either no effect or a beneficial effect of using RAS inhibitors (e.g., Lopes et al., 2021; Zhang et al., 2020). Thus, patients prescribed

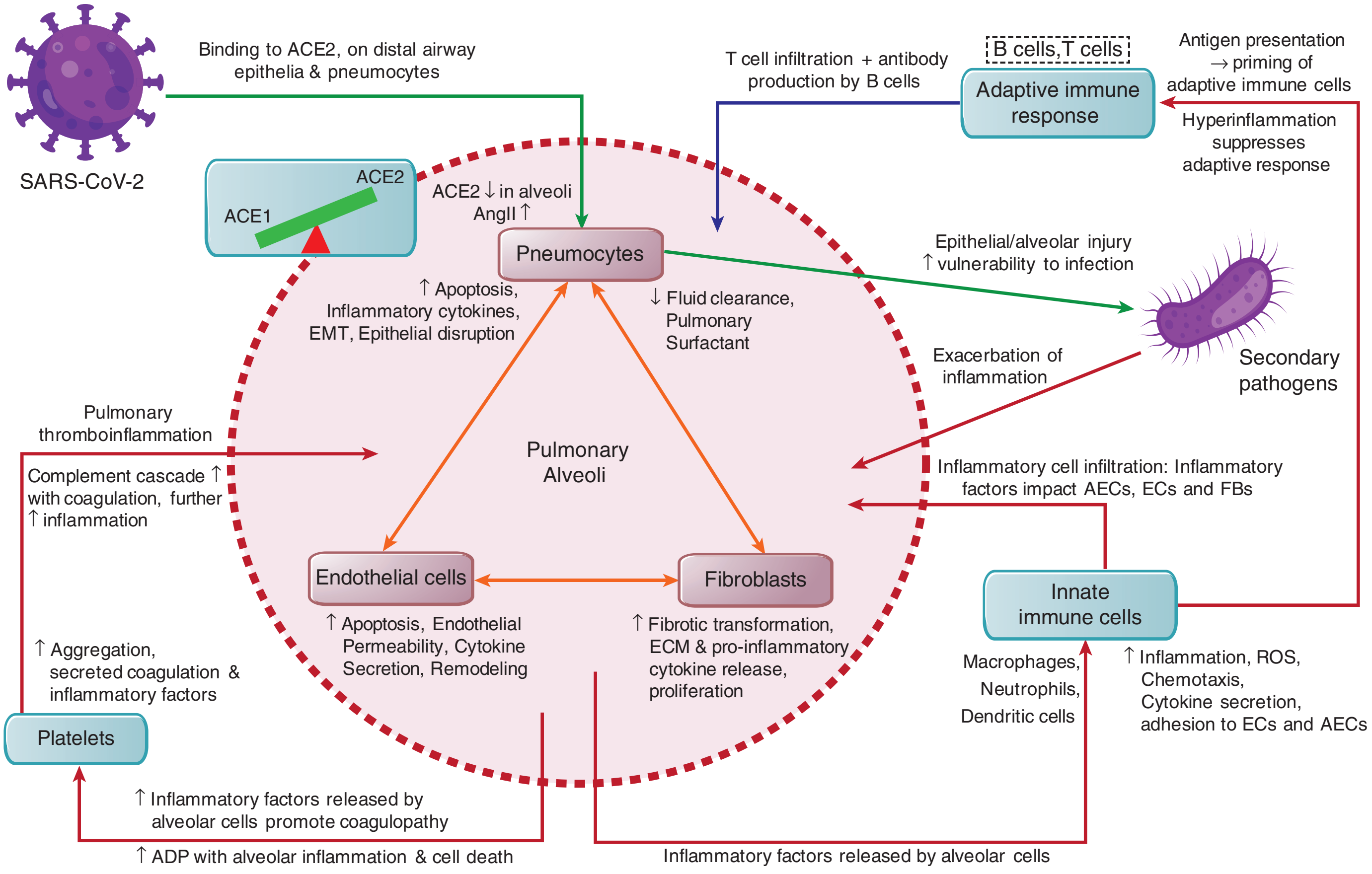
SARS-CoV-2
Binding to ACE2, on distal airway epithelia & pneumocytes
T cell infiltration + antibody production by B cells
B cells,T cells
Adaptive immune response
Antigen presentation → priming of adaptive immune cells
Hyperinflammation suppresses adaptive response
ACE2
ACE1
ACE2 ↓ in alveoli
AngII ↑
Pneumocytes
Epithelial/alveolar injury
↑ vulnerability to infection
↑ Apoptosis, Inflammatory cytokines, EMT, Epithelial disruption
↓ Fluid clearance, Pulmonary Surfactant
Exacerbation of inflammation
Secondary pathogens
Pulmonary thromboinflammation
Pulmonary Alveoli
Complement cascade ↑ with coagulation, further ↑ inflammation
Inflammatory cell infiltration: Inflammatory factors impact AECs, ECs and FBs
Endothelial cells
Fibroblasts
Innate immune cells
↑ Aggregation, secreted coagulation & inflammatory factors
↑ Apoptosis, Endothelial Permeability, Cytokine Secretion, Remodeling
↑ Fibrotic transformation, ECM & pro-inflammatory cytokine release, proliferation
Macrophages, Neutrophils, Dendritic cells
↑ Inflammation, ROS, Chemotaxis, Cytokine secretion, adhesion to ECs and AECs
Platelets
↑ Inflammatory factors released by alveolar cells promote coagulopathy
↑ ADP with alveolar inflammation & cell death
Inflammatory factors released by alveolar cells

Figure 30–9 *A model for how COVID-19 pulmonary pathobiology is mediated by dysregulation of RAS signaling.* SARS-CoV-2 binds to ACE2, with resulting functional inhibition of ACE2 resulting in an imbalance of ACE1- versus ACE2-mediated effects, with proinflammatory effects of the ACE1-AngII-AGTR1 axis promoting pathobiology. ADP, adenosine diphosphate; AECs alveolar epithelial cells; ECM, extracellular matrix; EMT, epithelial mesenchymal transition; FBs, fibroblasts; ROS, reactive oxygen species.

RAS inhibitors should remain on these drugs, irrespective of COVID-19. In addition, RAS inhibitors are being explored as therapeutic agents for COVID-19 (e.g., Sriram et al., 2020; Zhang et al., 2020).

Angiotensin II has also been implicated as a contributor to other lung diseases, such as pulmonary fibrosis, pulmonary hypertension, and ARDS, due to the range of pathological effects on pulmonary cell types shown in Figure 30–9 (Sriram and Insel, 2020a; Wang et al., 2019). Accordingly, inhibitors of RAS signaling, including ACEIs, ARBs, and novel molecules such as soluble ACE2 (discussed subsequently), are under investigation for use in such conditions.

Effects of RAS on Immune Cells

Angiotensin II has effects on immune cells, primarily via AGTR1 receptors that promote inflammation, enhancing tissue injury, remodeling, and fibrosis, especially in the heart, vascular smooth muscle, and kidneys (Crowley and Rudemiller, 2017; Forrester et al., 2018). Most notable is the activation of macrophages (promoting macrophage infiltration, ROS production, and proinflammatory cytokine release). Similar effects occur in T cells, in particular, CD4$^+$ cells; AngII also enhances T-cell proliferation. Proinflammatory effects of the RAS have been noted in the CNS, likely via AGTR1 activation of macrophages (Hammer et al., 2017). AGTR1 activation may induce differentiation of hematopoietic stem cells and monocytes, promoting a migratory, proinflammatory, and adhesive phenotype. These effects are in addition to the ability of activated immune cells to stimulate expression of angiotensinogen (particularly in the liver and kidney), further enhancing AngII production and establishing a pathological feedback loop (Satou et al., 2018). Despite the evidence for AngII as a promoter of a proinflammatory, pathological phenotype, certain paradoxical effects have been noted. These include the ability of AGTR1 activation on immune cells to suppress inflammation (Crowley and Rudemiller, 2017), suggesting a possible biphasic response, dependent on the physiological context. The use of RAS inhibitors for treating inflammatory conditions, such as arthritis, atherosclerosis, and pancreatitis, is an active area of research (Ranjbar et al., 2019).

Besides actions of AGTR1, ACE may have effects on immune cell function independent of AngII signaling, including by cleaving certain peptide antigens presented to CD8$^+$ T cells, leading to modulation of immune surveillance—a phenomenon that is poorly understood and merits further study (Crowley and Rudemiller, 2017). Limited evidence indicates a potential anti-inflammatory role for AGTR2 and MAS1 receptors on immune cells that may counteract the effects of AGTR1 (Forrester et al., 2018; Santos et al., 2019).

Inhibitors of the Renin-Angiotensin System

Drugs that interfere with the RAS play a prominent role in the treatment of cardiovascular disease. Besides β_1 blockers that inhibit renin release, the following three classes of inhibitors of the RAS are utilized therapeutically (Figure 30–10):

1. ACE inhibitors
2. Angiotensin II receptor blokcers
3. Direct renin inhibitors

All these drug classes reduce the actions of AngII and lower BP, but each class has different effects on components of the RAS (Table 30–3). Angiotensin II receptor blockers can be combined with a neprilysin inhibitor, as shown in Figure 30–10, and discussed in subsequent sections. Representative structures of agents that inhibit the RAS and reduce effects of AngII are shown in Figure 30–12, near the end of this chapter.

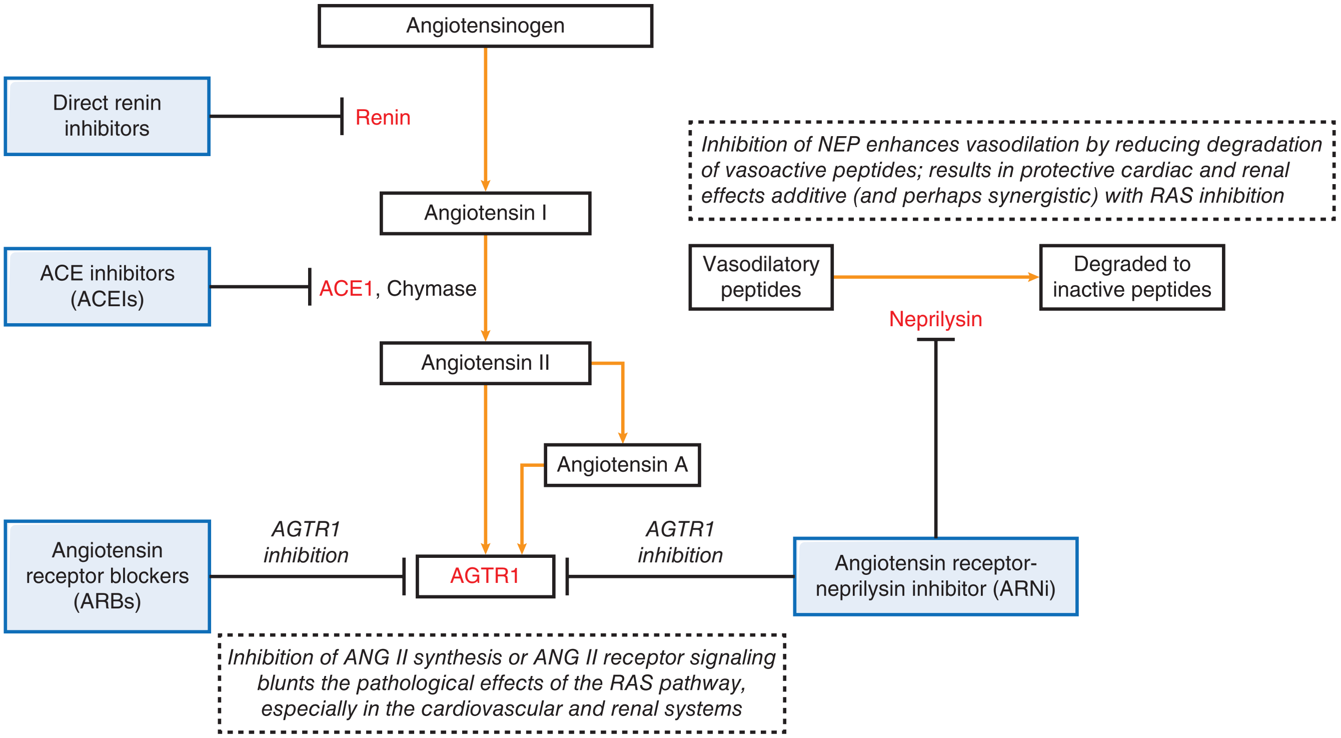
Angiotensinogen
Direct renin inhibitors
Renin
Inhibition of NEP enhances vasodilation by reducing degradation of vasoactive peptides; results in protective cardiac and renal effects additive (and perhaps synergistic) with RAS inhibition
Angiotensin I
ACE inhibitors (ACEIs)
ACE1, Chymase
Vasodilatory peptides
Degraded to inactive peptides
Neprilysin
Angiotensin II
Angiotensin A
Angiotensin receptor blockers (ARBs)
AGTR1 inhibition
AGTR1
AGTR1 inhibition
Angiotensin receptor-neprilysin inhibitor (ARNi)
Inhibition of ANG II synthesis or ANG II receptor signaling blunts the pathological effects of the RAS pathway, especially in the cardiovascular and renal systems

Figure 30–10 *Classes of RAS inhibitors with currently approved drugs.* NEP, neprilysin.

Novel Agents for Targeting the RAS Pathway

Besides approved inhibitors of the canonical RAS pathway, other novel agents have been identified as antagonists of the RAS, or that curb its pathological effects. As described earlier, MAS1 is considered the primary receptor by which Ang(1–7) exerts effects that counter those of AngII, providing a rationale for the therapeutic use of Ang(1–7) analogues and/or MAS1 agonists. Numerous preclinical studies have been conducted using Ang(1–7) (including a purified synthetic form, TXA127), along with early clinical trials in humans that indicate excellent drug tolerance (Sriram et al., 2020). AVE 0991, a small-molecule, nonpeptide MAS1 agonist has also been tested in preclinical animal studies, but not as yetin humans. Thus, clinical testing of MAS1 as a target is in its very early stages.

An alternative means of targeting the RAS pathway and the pathological effects of AngII is to promote ACE2 activity and in turn enhance the generation of peptides that signal via MAS1 and counteract actions of AngII. Soluble, recombinant human ACE2 (rhACE2) has been tested in preclinical studies, reducing inflammation and injury in various cell and tissue types, both *ex vivo* and *in vivo* (Sriram and Insel, 2020a; Sriram et al., 2020). A synthetic rhACE2, GSK2586881, has been tested in early-stage clinical trials for pulmonary hypertension (ClinicalTrials.gov identifier: NCT03177603) and acute lung injury (NCT01597635), with limited therapeutic benefit but excellent drug safety. rhACE2 is also being tested in COVID-19, as both a decoy for the virus and to directly ameliorate inflammation and injury, especially in the lung.

Biased agonists of AGTR1 may represent another novel means to mitigate pathological effects of AngII/AGTR1. TRV027 is an example of one such biased AGTR1 agonist that has been tested in preclinical models and patients. This compound and other AGTR1 biased agonists are discussed in greater detail below in the section on ARBs. Agonists for AGTR2 are also in development. Such agonists exerted anti-inflammatory and antifibrotic effects in preclinical studies and have been well tolerated in early-stage clinical trials (Colafella et al., 2019). Figure 30–11 summarizes novel compounds that target the RAS pathway.

TABLE 30–3 ■ EFFECTS OF ANTIHYPERTENSIVE AGENTS ON COMPONENTS OF THE RAS

	DRIs	ACEIs	ARBs	DIURETICS	β_1 BLOCKERS
PRC	↑	↑	↑	↑	↓
PRA	↓	↑	↑	↑	↓
AngI	↓	↑	↑	↑	↓
AngII	↓	↓	↑	↑	↓
ACE activity	↔	Inhibition	↔		
Aldosterone	↓	↓	↓	↑	↓/↔
Bradykinin	↔	↑	↔		
AT_1R	↔	↔	Inhibition		
AT_2R	↔	↔	Stimulation		

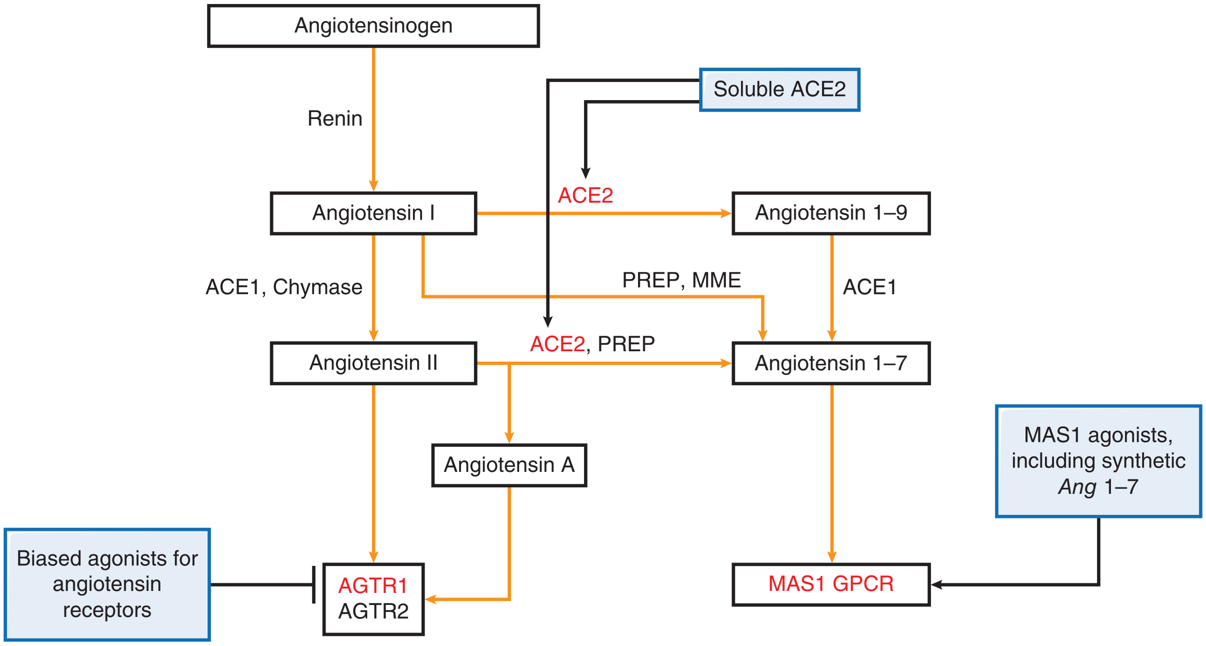

Figure 30–11 *Novel RAS-targeting agents.* MME, membrane metalloendopeptidase; PREP, prolyl endopeptidase.

Angiotensin-Converting Enzyme Inhibitors

HISTORY

In the 1960s, Ferreira and colleagues found that venom from the Brazilian pit viper (*Bothrops jararaca*) contains factors that intensify vasodilator responses to bradykinin. These bradykinin-potentiating factors are peptides that inhibit kininase II, an enzyme that inactivates bradykinin. Erdös and coworkers established that ACE and kininase II are the same enzyme, which catalyzes both the synthesis of AngII and the destruction of bradykinin. A nonapeptide, teprotide, the snake venom peptide that inhibits kininase II and ACE, was synthesized and tested in human subjects. It lowered BP in many patients with essential hypertension and exerted beneficial effects in patients with heart failure. Captopril, an orally effective ACE inhibitor, was developed by analysis of the inhibitory action of teprotide, inference about the action of ACE on its substrates, and analogy with carboxypeptidase A, which is inhibited by D-benzylsuccinic acid. Ondetti, Cushman, and colleagues argued that inhibition of ACE might be produced by succinyl amino acids corresponding to the dipeptide cleaved by ACE. This led to the synthesis of carboxy alkanoyl and mercapto alkanoyl derivatives that are potent competitive inhibitors of ACE.

Pharmacological Effects

The ACEIs block the conversion of AngI to AngII. Inhibition of AngII production lowers BP and enhances natriuresis. ACE has many substrates; thus, its inhibition has multiple consequences, including inhibition of bradykinin degradation, which has beneficial antihypertensive and protective effects. ACEIs increase the circulating levels of the stem cell regulator Ac-SDKP (*N*-acetyl-seryl-aspartyl-lysyl-proline), which may contribute to the cardioprotective effects of ACEIs. ACEIs increase renin release and the rate of formation of AngI by interfering with both short- and long-loop negative feedbacks on renin release (Figure 30–2). The AngI that accumulates is directed into alternative metabolic routes, resulting in increased production of vasodilator peptides [e.g., Ang(1–9) and Ang(1–7)] (Figure 30–1).

Clinical Pharmacology

The ACEIs can be classified into three broad groups based on chemical structure:

1. Sulfhydryl-containing ACEIs structurally related to *captopril*
2. Dicarboxyl-containing ACEIs structurally related to *enalapril* (e.g., *lisinopril*, *benazepril*, *quinapril*, *moexipril*, *ramipril*, *trandolapril*, *perindopril*; Figure 30–12)
3. Phosphorus-containing ACEIs structurally related to *fosinopril*

Many ACEIs are ester-containing prodrugs that are 100 to 1000 times less potent but have better oral bioavailability than the active molecules. Currently, 11 ACEIs are available for clinical use in the U.S. They differ with regards to potency, pharmacokinetics, and whether ACE inhibition is primarily an effect of the drug or an active metabolite.

All ACEIs block the conversion of AngI to AngII and have similar therapeutic indications, adverse effect profiles, and contraindications. Because hypertension usually requires long-term treatment, quality-of-life issues are an important consideration in comparing antihypertensive drugs. With the exception of *fosinopril*, *trandolapril*, and *quinapril* (which display balanced elimination by the liver and kidneys), ACEIs are predominantly cleared by the kidneys. Impaired renal function thus significantly diminishes the plasma clearance of most ACEIs, and dosages of these drugs should be reduced in patients with renal impairment. *Elevated PRA renders patients hyperresponsive to ACEI-induced hypotension. Thus, initial dosages of all ACEIs should be reduced in patients with high plasma levels of renin (e.g., patients with heart failure and during salt depletion, including diuretic use).* ACEIs differ markedly in tissue distribution; it is possible that this difference could be exploited to selectively inhibit certain local (tissue) RAS.

Captopril. *Captopril* is a potent ACE inhibitor (K_i = 1.7 nM). Orally administered *captopril* is absorbed rapidly and has a bioavailability of approximately 75% but is reduced by 25% to 30% with food. Peak concentrations in plasma occur within an hour, and the drug is cleared rapidly ($t_{1/2}$ of ~2 h). Most of the drug is eliminated in urine, 40% to 50% as *captopril*, and the rest as captopril disulfide dimers and captopril–cysteine disulfide. The oral dose of *captopril* ranges from 6.25 to 150 mg 2 to 3 times daily, with 6.25 mg thrice daily or 25 mg twice daily appropriate to initiate therapy for heart failure or hypertension, respectively.

Enalapril. *Enalapril maleate* is a prodrug that is hydrolyzed by esterases in the liver to produce enalaprilat, the active dicarboxylic acid. *Enalaprilat* is a potent inhibitor of ACE (K_i = 0.2 nM). *Enalapril* is absorbed rapidly when given orally; its oral bioavailability is approximately 60% and not reduced by food. Peak concentrations of *enalapril* in plasma occur within

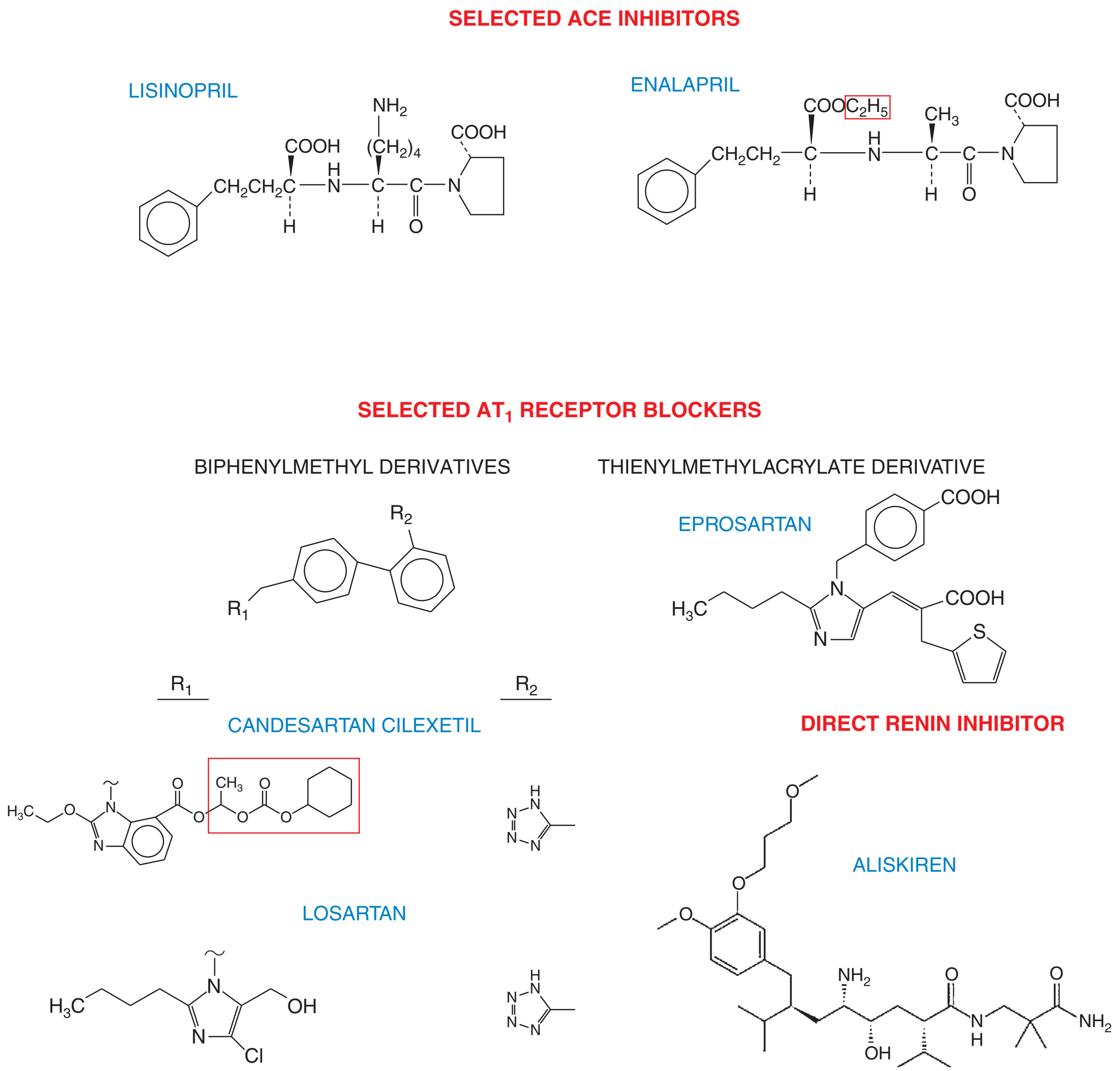
SELECTED ACE INHIBITORS
LISINOPRIL
NH2
COOH
(CH2)4
COOH
CH2CH2C
H
N
C
C
N
H
H
O
ENALAPRIL
COOC2H5
CH3
COOH
CH2CH2
C
H
N
C
C
N
H
H
O
SELECTED AT1 RECEPTOR BLOCKERS
BIPHENYLMETHYL DERIVATIVES
R2
R1
THIENYLMETHYLACRYLATE DERIVATIVE
EPROSARTAN
COOH
H3C
N
N
COOH
S
R1
R2
CANDESARTAN CILEXETIL
H3C
O
N
N
O
O
CH3
O
O
O
H
N
N
N
N
DIRECT RENIN INHIBITOR
O
O
ALISKIREN
O
NH2
OH
O
N
H
O
NH2
LOSARTAN
H3C
N
N
OH
Cl
H
N
N
N
N

Figure 30–12 *Structures of representative RAS inhibitors. Enalapril* and *candesartan cilexetil* are prodrugs that are relatively inactive until *in vivo* esterases remove the region within the red box, replacing it with a hydrogen atom to form the active drug.

an hour; *enalaprilat* concentrations peak after 3 to 4 h. *Enalapril* has a $t_{1/2}$ of approximately 1.3 h, but *enalaprilat*, because of tight binding to ACE, has a plasma $t_{1/2}$ of approximately 11 h. Intact *enalapril* and *enalaprilat* are excreted by the kidneys. The oral dosage of *enalapril* ranges from 2.5 to 40 mg daily, with 2.5 and 5 mg daily appropriate to initiate therapy for heart failure and hypertension, respectively.

Enalaprilat. *Enalaprilat* is not absorbed orally but is available for intravenous administration when oral therapy is not appropriate. For hypertensive patients, the dosage is 0.625 to 1.25 mg given intravenously over 5 min. This dosage may be repeated every 6 h.

Lisinopril. *Lisinopril* is the lysine analogue of *enalaprilat*; unlike *enalapril*, *lisinopril* itself is active. *In vitro*, *lisinopril* is a slightly more potent ACE inhibitor than is *enalaprilat*. *Lisinopril* is absorbed slowly, variably, and incompletely (~30%) after oral administration (and not reduced by food); peak concentrations in plasma are achieved in about 7 h. It is excreted unchanged by the kidney with a plasma $t_{1/2}$ of about 12 h. The oral dosage of *lisinopril* ranges from 5 to 40 mg daily (single or divided dose), with 5 and 10 mg daily appropriate for the initiation of therapy for heart failure and hypertension, respectively. A daily dose of 2.5 mg with close medical supervision is recommended for patients with heart failure who are hyponatremic or have renal impairment.

Benazepril. Cleavage of the ester moiety by hepatic esterases transforms *benazepril*, a prodrug, into *benazeprilat*. *Benazepril* is absorbed rapidly but incompletely (37%) after oral administration (only slightly reduced by food). *Benazepril* is nearly completely metabolized to *benazeprilat* and to the glucuronide conjugates of *benazepril* and *benazeprilat*, which are excreted into the urine and bile; peak concentrations of *benazepril* and *benazeprilat* in plasma are achieved in 0.5 to 1 and 1 to 2 h, respectively. *Benazeprilat* has an effective plasma $t_{1/2}$ of 10 to 11 h. Except for the lungs, *benazeprilat* does not accumulate in tissues. The oral dosage of *benazepril* ranges from 5 to 80 mg daily (single or divided dose).

Fosinopril. Cleavage of the ester moiety by hepatic esterases transforms *fosinopril* into *fosinoprilat*. *Fosinopril* is absorbed slowly and incompletely (36%) after oral administration (the rate but not extent is reduced by food). *Fosinopril* is largely metabolized to *fosinoprilat* (75%) and to its glucuronide conjugate, which are excreted in both the urine and the bile. Peak concentrations of *fosinoprilat* in plasma occur in about 3 h. *Fosinoprilat* has an effective plasma $t_{1/2}$ of approximately 11.5 h, which is not significantly altered by renal impairment. The oral dosage of *fosinopril* ranges from 10 to 80 mg daily (single or divided dose). The initial dose is reduced to 5 mg daily in patients with Na^+ or water depletion or renal failure.

Trandolapril. An oral dose of *trandolapril* is absorbed without reduction by food and produces plasma levels of *trandolapril* (10% bioavailability) and *trandolaprilat* (70% bioavailability). *Trandolaprilat* is approximately 8 times more potent than *trandolapril* as an ACEI. Glucuronides of *trandolapril* and de-esterification products are recovered in the urine (33%, mostly *trandolaprilat*) and feces (66%). Peak concentrations of *trandolaprilat* in plasma are achieved in 4 to 10 h.

Trandolaprilat displays biphasic elimination kinetics, with an initial $t_{1/2}$ of about 10 h (the major component of elimination), followed by a more prolonged $t_{1/2}$ (owing to slow dissociation of *trandolaprilat* from tissue ACE). Plasma clearance of *trandolaprilat* is reduced by renal and hepatic insufficiency. The oral dosage ranges from 1 to 8 mg daily (single or divided dose). The initial dose is 0.5 mg in patients who are taking a diuretic or who have renal impairment.

Quinapril. Cleavage of the ester moiety by hepatic esterases transforms *quinapril*, a prodrug, into *quinaprilat*, a conversion that is reduced in patients with diminished liver function. *Quinapril* is absorbed rapidly (with peak concentrations in 1 h); the rate, but not extent, of oral absorption (60%) may be reduced by food. *Quinaprilat* and other metabolites of *quinapril* are excreted in the urine (61%) and feces (37%). Peak concentrations of *quinaprilat* in plasma are achieved in about 2 h. The initial $t_{1/2}$ of *quinaprilat* is about 2 h; a prolonged terminal $t_{1/2}$ (~25 h) may be due to its high-affinity binding to tissue ACE. The oral dosage of *quinapril* ranges from 5 to 80 mg daily.

Ramipril. Orally administered *ramipril* is absorbed rapidly (peak concentrations in 1 h). The rate but not extent of its oral absorption (50%–60%) is reduced by food. *Ramipril* is metabolized to *ramiprilat* by hepatic esterases and to inactive metabolites that are excreted predominantly by the kidneys. Peak concentrations of *ramiprilat* in plasma are achieved in approximately 3 h. *Ramiprilat* displays triphasic elimination kinetics ($t_{1/2}$ values: 2–4, 9–18, and >50 h). This triphasic elimination is due to extensive distribution in tissues (initial $t_{1/2}$), clearance of free *ramiprilat* from plasma (intermediate $t_{1/2}$), and dissociation of *ramiprilat* from tissue ACE (long terminal $t_{1/2}$). The oral dosage of *ramipril* ranges from 1.25 to 20 mg daily (single or divided dose).

Moexipril. The antihypertensive activity of *moexipril* derives from its de-esterified metabolite, *moexiprilat*. *Moexipril* is absorbed incompletely; bioavailability as *moexiprilat* is about 13% and markedly decreased by food. The time to peak plasma concentration of *moexiprilat* is approximately 1.5 h; the elimination $t_{1/2}$ varies between 2 and 12 h. The recommended dosage range is 7.5 to 30 mg daily (single or divided doses) and is halved in patients taking diuretics or who have renal impairment.

Perindopril. *Perindopril erbumine* is a prodrug that is transformed to *perindoprilat* by hepatic esterases. The oral bioavailability of *perindopril* (75%) is not affected by food, but bioavailability of *perindoprilat* is reduced by about 35%. *Perindopril* is also converted to inactive metabolites that are excreted predominantly by the kidneys. Peak concentrations of *perindoprilat* in plasma are achieved in 3 to 7 h. *Perindoprilat* displays biphasic elimination kinetics with half-lives of 3 to 10 h (the major component of elimination) and 30 to 120 h (owing to slow dissociation of *perindoprilat* from tissue ACE). The oral dosage ranges from 2 to 16 mg daily (single or divided dose).

Therapeutic Uses of ACEIs

ACEIs are effective in the treatment of hypertension, cardiovascular disease, heart failure, and diabetic nephropathy.

ACEIs in Hypertension. Inhibition of ACE lowers systemic vascular resistance and mean, diastolic, and systolic BPs in hypertensive states except in primary aldosteronism (see Chapters 32 and 50) (Table 30–3). The initial change in BP tends to be positively correlated with PRA and AngII plasma levels prior to treatment. However, some patients may show a sizable reduction in BP that correlates poorly with pretreatment values of PRA. Perhaps increased local (tissue) production of AngII or increased responsiveness of tissues to normal levels of AngII makes some hypertensive patients sensitive to ACEIs despite normal PRA.

The long-term fall in systemic BP in hypertensive individuals treated with ACEIs is accompanied by a leftward shift in the renal pressure–natriuresis curve (Figure 30–7) and a reduction in TPR (from variable participation of different vascular beds). The kidney is a notable exception: Because renal vessels are extremely sensitive to vasoconstriction by AngII, ACEIs increase RBF via vasodilation of the afferent and efferent arterioles. Increased RBF occurs without increased GFR; thus, the filtration fraction is reduced.

The ACEIs produce systemic arteriolar dilation and increased compliance of large arteries, which contributes to a reduction of systolic pressure. Cardiac function in patients with uncomplicated hypertension generally is little changed, although stroke volume and cardiac output may increase slightly with sustained treatment. Baroreceptor function and cardiovascular reflexes are not compromised; responses to postural changes and exercise are little impaired. Even with substantial lowering of BP, heart rate and plasma catecholamine concentrations generally only increase slightly (if at all), perhaps reflecting an alteration of baroreceptor function with increased arterial compliance and decreased tonic influence of AngII on the sympathetic nervous system.

Aldosterone secretion is reduced, but not seriously impaired, by ACEIs. Aldosterone secretion is maintained at adequate levels by other steroidogenic stimuli, such as ACTH and K^+. Their activity on the zona glomerulosa of the adrenal cortex requires very small trophic or permissive amounts of AngII, which remain present because ACE inhibition is never complete. Excessive retention of K^+ and hyperkalemia can occur in patients taking supplemental K^+, in patients with renal impairment, or in patients taking other medications that reduce K^+ excretion.

ACEIs can normalize BP in approximately 50% of patients with mild-to-moderate hypertension. Ninety percent of such patients will be controlled by the combination of an ACEI and a Ca^{2+} channel blocker, a β_1 adrenergic receptor blocker, or a diuretic (see Chapter 32). Diuretics augment the antihypertensive response to ACEIs by rendering the patient's BP renin-dependent. Several ACEIs are marketed in fixed-dose combinations with a thiazide diuretic or Ca^{2+} channel blocker for the management of hypertension.

ACEIs in Left Ventricular Systolic Dysfunction. *Unless contraindicated, ACEIs should be given to all patients with impaired left ventricular systolic function whether or not they have symptoms of overt heart failure* (see Chapter 33). Several large clinical studies have demonstrated that inhibition of ACE in patients with systolic dysfunction prevents or delays the progression of heart failure, decreases the incidence of sudden death and MI, decreases hospitalization, and improves quality of life. Inhibition of ACE generally reduces afterload and systolic wall stress and increases cardiac output, cardiac index, and stroke volume. In systolic dysfunction, AngII decreases arterial compliance; this is reversed by ACE inhibition. Heart rate generally is reduced. Systemic BP falls, sometimes steeply at the outset, but tends to return toward initial levels. Renovascular resistance falls sharply and RBF increases. Natriuresis occurs because of improved renal hemodynamics, reduced stimulus to the secretion of aldosterone by AngII, and diminished direct renal effects of AngII. The decrease in excess volume of body fluids, venodilation, and increased capacity of the venous bed reduce venous return to the heart.

The response to ACEIs also involves reductions of pulmonary arterial pressure, pulmonary capillary wedge pressure, and left atrial and left ventricular filling volumes and pressures. Consequently, preload and diastolic wall stress are diminished. The improved hemodynamic performance results in increased exercise tolerance and suppression of the sympathetic nervous system. Cerebral and coronary blood flows usually are well maintained, even when systemic BP is reduced. In heart failure, ACEIs reduce ventricular dilation and tend to restore the heart to its normal elliptical shape. ACEIs may reverse ventricular remodeling via changes in preload/afterload by preventing the growth effects of AngII on myocytes and by attenuating cardiac fibrosis induced by AngII and aldosterone.

ACEIs in Acute MI. Beneficial effects of ACEIs in acute MI are observed especially in hypertensive and diabetic patients. Unless contraindicated (e.g., cardiogenic shock or severe hypotension), ACEIs should be started during the acute phase of MI and can be administered along with thrombolytics, aspirin, and β adrenergic receptor antagonists (ACE Inhibitor Myocardial Infarction Collaborative Group, 1998). In high-risk patients (e.g., large infarct, systolic ventricular dysfunction), ACE inhibition should be continued long term (see Chapters 31 and 33).

ACEIs in Patients Who Are at High Risk of Cardiovascular Events. ACEIs benefit patients at high risk of cardiovascular events (Heart Outcomes Prevention Study Investigators, 2000). ACEIs decrease the rate of MI, stroke, and death in patients who do not have left ventricular dysfunction but have evidence of vascular disease or diabetes and one other risk factor for cardiovascular disease. In patients with coronary artery disease but without heart failure, ACE inhibition reduces cardiovascular disease death and MI (European Trial, 2003).

ACEIs in Diabetes Mellitus and Renal Failure. Diabetes mellitus is the leading cause of chronic renal disease. In patients with diabetic nephropathy, ACEIs prevent or delay disease progression. The renoprotective effects of ACEIs are in part independent of BP reduction. ACEIs can also decrease progression of retinopathy in type 1 diabetics and attenuate the progression of renal insufficiency in patients with nondiabetic nephropathies (Ruggenenti et al., 2010).

Several mechanisms participate in the renal protective effects of ACEIs. By decreasing arterial BP and dilating renal efferent arterioles, ACEIs reduce the increased glomerular capillary pressure that induces glomerular injury. ACEIs also increase the permeability selectivity of the filtering membrane, thereby diminishing exposure of the mesangium to proteinaceous factors that may stimulate mesangial cell proliferation and matrix production, which contribute to expansion of the mesangium in diabetic nephropathy. Because AngII is a growth factor, reduced intrarenal levels of AngII may attenuate mesangial cell growth and matrix production. ACEIs increase Ang(1–7) levels by preventing its metabolism by ACE; Ang(1–7), via Mas receptors, has protective and antifibrotic effects (Santos, 2014; Santos et al., 2019). In diabetes, AngII, via AT_1 receptors in renal podocytes, leads to activation of protein kinase signaling cascades, cytoskeletal rearrangements, retraction of podocyte processes, and reduction in proteins of the slit diaphragm, all resulting in increased permeability of the renal epithelium to proteins (proteinuria). ACEIs reduce these effects of AngII (Márquez et al., 2015).

Adverse Effects of ACEIs

The ACEIs are generally well tolerated. The drugs do not alter plasma concentrations of uric acid or Ca^{2+}, may improve insulin sensitivity and glucose tolerance in patients with insulin resistance, and decrease cholesterol and lipoprotein (a) levels in proteinuric renal disease.

Hypotension. A steep fall in BP may occur following the first dose of an ACEI in patients with elevated PRA. One should exercise care in patients who are salt depleted, on multiple antihypertensive drugs, or have congestive heart failure.

Cough. In 5% to 20% of patients, ACEIs induce a bothersome, dry cough mediated by the accumulation in the lungs of bradykinin, substance P, or PGs. Thromboxane antagonism, *aspirin*, and iron supplementation reduce cough induced by ACEIs. Dose reduction or switching to an ARB is sometimes effective. The cough disappears, usually within 4 days, if ACEIs are stopped.

Hyperkalemia. Significant K^+ retention is rarely encountered in patients with normal renal function. However, ACEIs may cause hyperkalemia in patients with renal insufficiency or diabetes or if used with K^+-sparing diuretics, K^+ supplements, β receptor blockers, or NSAIDs.

Acute Renal Failure. Inhibition of ACE can induce acute renal insufficiency in patients with bilateral renal artery stenosis, stenosis of the artery to a single remaining kidney, heart failure, or volume depletion owing to diarrhea or diuretics.

Angioedema. In 0.1% to 0.5% of patients, ACEIs induce rapid swelling in the nose, throat, mouth, glottis, larynx, lips, and/or tongue. Once ACEIs are stopped, angioedema disappears within hours; the patient's airway should be protected, and if necessary, *epinephrine*, an antihistamine, or a glucocorticoid should be administered. African Americans have a 4.5 times greater risk of ACEI-induced angioedema than Caucasians. Although rare, angioedema of the intestine (visceral angioedema) characterized by emesis, watery diarrhea, and abdominal pain has also been reported. ACEI-associated angioedema is a class effect: Patients who develop this adverse event should not be prescribed other ACEIs.

Fetopathic Potential. *If a pregnancy is diagnosed, it is imperative that ACEIs be discontinued.* ACEIs and ARBs have been associated with renal developmental defects when administered in the third trimester of pregnancy, and potentially earlier. The fetopathic effects may be due in part to fetal hypotension. This possible adverse effect should be discussed with women of childbearing potential, as should the necessity of birth control measures.

Skin Rash. The ACEIs occasionally cause a maculopapular rash that may itch but that may resolve spontaneously or with antihistamines.

Other Side Effects. Extremely rare but reversible side effects include dysgeusia (an alteration in or loss of taste), neutropenia (symptoms include sore throat and fever), glycosuria (spillage of glucose into the urine in the absence of hyperglycemia), anemia, and hepatotoxicity.

Drug Interactions. Antacids and other drugs (e.g., lanthanum, a phosphate binder) may reduce the bioavailability of ACEIs; capsaicin may worsen ACEI-induced cough; NSAIDs, including aspirin, may reduce the antihypertensive response to ACEIs; and K^+-sparing diuretics and K^+ supplements may exacerbate ACEI-induced hyperkalemia. ACEIs may increase plasma levels of digoxin and lithium and hypersensitivity reactions to allopurinol and other drugs. Coadministration of dipeptidyl peptidase-IV inhibitors (e.g., sitagliptin), alteplase, everolimus, and pregabalin may increase the risk of developing angioedema.

Angiotensin II Receptor Blockers

HISTORY

Attempts to develop therapeutically useful AngII receptor antagonists began in the 1970s. Initial efforts concentrated on angiotensin peptide analogues. Saralasin, 1-sarcosine, 8-isoleucine AngII, and other 8-substituted angiotensins are potent AngII receptor antagonists but are not of clinical value because they lack oral bioavailability and have (unacceptable) partial agonist activity. A breakthrough came in the 1980s with the synthesis of a series of imidazole-5–acetic acid derivatives that attenuated pressor responses to AngII in rats. Two compounds, S-8307 and S-8308, proved to be highly specific, albeit very weak, nonpeptide AngII receptor antagonists that were devoid of partial agonist activity (Dell'Italia, 2011). Through a series of stepwise modifications, *losartan*, an orally active, potent, and selective nonpeptide AT_1 receptor antagonist, was developed and approved for clinical use in the U.S. in 1995. Since then, seven additional AT_1 receptor antagonists (see Drug Facts for Your Personal Formulary table) have been approved. These AT_1 receptor antagonists are devoid of partial agonist activity, but structural modifications as minor as a methyl group can transform a potent antagonist into an agonist (Perlman et al., 1997).

Pharmacological Effects

AngII receptor blockers (ARBs) bind to AT_1 receptors with high affinity and are greater than 10,000-fold more selective for the AT_1 than the AT_2 receptor. Although binding of ARBs to AT_1 receptors is competitive, the inhibition by ARBs of biological responses to AngII often is functionally insurmountable. Insurmountable antagonism has the theoretical advantage of sustained receptor blockade even with increased levels of endogenous ligand and missed doses of drug. ARBs inhibit most biological effects of AngII, including: (1) vascular smooth muscle contraction; (2) rapid pressor responses; (3) slow pressor responses; (4) thirst;

(5) vasopressin release; (6) aldosterone secretion; (7) adrenal catecholamine release; (8) enhancement of noradrenergic neurotransmission; (9) increases in sympathetic tone; (10) changes in renal function; and (11) cellular hypertrophy and hyperplasia. ARBs reduce arterial BP in animals with renovascular and genetic hypertension, as well as in transgenic animals that overexpress the renin gene. ARBs, however, have little effect on arterial BP in animals with low-renin hypertension (e.g., rats with NaCl/deoxycorticosterone-induced hypertension) (Csajka et al., 1997).

Do ARBs Have Therapeutic Efficacy Equivalent to That of ACEIs?

Although ARBs and ACEIs both block the RAS, they differ in important aspects:

- *ARBs reduce AT_1 receptor activation more effectively than do ACEIs.* ACEIs reduce ACE-mediated biosynthesis of AngII but do not inhibit AngII generation via chymase and other ACE-independent AngII-producing pathways. ARBs block the actions of AngII via AT_1 receptors irrespective of the biochemical pathway leading to AngII formation.
- *Unlike ACEIs, ARBs permit activation of AT_2 receptors.* ACEIs increase renin release but block the conversion of AngI to AngII. ARBs also stimulate renin release; however, with ARBs, this translates into a several-fold increase in circulating levels of AngII. Because ARBs block AT_1 receptors, this increased level of AngII can activate AT_2 receptors.
- *ACEIs increase Ang(1–7) levels.* ACE is involved in the clearance of Ang(1–7), so inhibition of ACE increases Ang(1–7) levels. With ARBs, it is unclear if there is a clinically meaningful impact on Ang(1-7) levels.
- *ACEIs can increase the levels of ACE substrates, including bradykinin and Ac-SDKP.*

Whether the pharmacological differences between ARBs and ACEIs result in significant differences in therapeutic outcomes is an open question. Meta-analyses indicate some differences; thus, further investigation is needed. Such analyses indicate similar efficacy for treating primary hypertension, with an advantage for ARBs with respect to frequency of adverse events (Li et al., 2014; Chen et al., 2021). Meta-analysis of patients with heart failure with reduced ejection fraction (HFrEF) indicated that both drug classes improve heart failure at high doses, but only ARBs reduce heart failure–related hospitalization (Turgeon et al., 2019). ARBs are preferable to ACEIs in reducing risk of renal failure in diabetic patients with albuminuria (Wang et al., 2018). Clinical trial evidence does not support combination therapy with ARBs and ACEIs for patients with hypertension, heart failure, or nephropathy (Makani et al., 2013; ONTARGET Investigators, 2008; Saglimbene et al., 2018).

Clinical Pharmacology

Oral bioavailability of ARBs generally is low (<50%) except for *azilsartan* (~60%) and *irbesartan* (~70%), and protein binding is high (>90%).

Candesartan Cilexetil. *Candesartan cilexetil*, an inactive ester prodrug, is hydrolyzed to the active form, *candesartan*, during gastrointestinal (GI) tract absorption (Figure 30–12). Peak plasma levels are obtained 3 to 4 h after oral administration; the plasma $t_{1/2}$ is about 9 h. Plasma clearance of *candesartan* is by renal elimination (33%) and biliary excretion (67%). The plasma clearance of *candesartan* is affected by renal insufficiency but not by mild-to-moderate hepatic insufficiency. *Candesartan cilexetil* should be administered orally once or twice daily for a total daily dose of 4 to 32 mg.

Eprosartan (no longer available in the U.S.). Peak plasma levels are obtained 1 to 2 h after oral administration; the plasma $t_{1/2}$ is 5 to 9 h. *Eprosartan* is metabolized in part to a glucuronide conjugate. Clearance is by renal elimination and biliary excretion and is affected by both renal and hepatic insufficiency. The recommended dosage of *eprosartan* is 400 to 800 mg/d in one or two doses.

Irbesartan. Peak plasma levels are obtained about 1.5 to 2 h after oral administration; the plasma $t_{1/2}$ is 11 to 15 h. *Irbesartan* is metabolized in part to a glucuronide conjugate. The parent compound and its glucuronide conjugate are cleared by renal elimination (20%) and biliary excretion (80%). The plasma clearance of *irbesartan* is unaffected by either renal or mild-to-moderate hepatic insufficiency. The oral dosage of *irbesartan* is 150 to 300 mg once daily.

Losartan. Approximately 14% of an oral dose of *losartan* is converted by CYP2C9 and CYP3A4 to the 5-carboxylic acid metabolite, EXP 3174, which is more potent than *losartan* as an AT_1 receptor antagonist. Peak plasma levels of *losartan* and EXP 3174 occur approximately 1 to 3 h after oral administration; the plasma half-lives are 2.5 and 6 to 9 h, respectively. The plasma clearances of *losartan* and EXP 3174 are via the kidney and liver (metabolism and biliary excretion) and are affected by hepatic but not renal insufficiency. *Losartan* should be administered orally once or twice daily for a total daily dose of 25 to 100 mg. *Losartan* is a competitive antagonist of the thromboxane A_2 receptor and attenuates platelet aggregation. EXP 3179, another metabolite of *losartan* without angiotensin receptor effects, has multiple potential antioxidant and antifibrotic actions that may be mediated by inhibition of protein kinase C (Wenzel et al., 2009).

Olmesartan Medoxomil. *Olmesartan medoxomil*, an inactive ester prodrug, is hydrolyzed to the active form, *olmesartan*, during GI tract absorption. Peak plasma levels are obtained 1.4 to 2.8 h after oral administration; the plasma $t_{1/2}$ is 10 to 15 h. Plasma clearance of *olmesartan* is via renal elimination and biliary excretion. Although renal impairment and hepatic disease decrease the plasma clearance of *olmesartan*, no dose adjustment is required in patients with mild-to-moderate renal or hepatic impairment. The oral dosage of *olmesartan medoxomil* is 20 to 40 mg once daily.

Telmisartan. Peak plasma levels are obtained 0.5 to 1 h after oral administration; the plasma $t_{1/2}$ is about 24 h. *Telmisartan* is cleared from the circulation mainly by biliary secretion of intact drug. The plasma clearance of *telmisartan* is affected by hepatic but not renal insufficiency. The recommended oral dosage of *telmisartan* is 40 to 80 mg once daily.

Valsartan. Peak plasma levels occur 2 to 4 h after oral administration; food decreases absorption. The plasma $t_{1/2}$ is about 9 h. *Valsartan* is cleared from the circulation by the liver (~70% of total clearance); hepatic insufficiency reduces clearance. The oral dosage is 80 to 320 mg once daily.

Azilsartan Medoxomil. The prodrug is hydrolyzed in the GI tract into the active form, *azilsartan*. The drug is available in 40- and 80-mg once-daily doses. At the recommended dose of 80 mg once a day, *azilsartan medoxomil* is superior to *valsartan*, *olmesartan*, and *telmisartan* in lowering BP. Bioavailability of *azilsartan* is about 60% and is not affected by food. Peak plasma concentrations (C_{max}) are achieved within 1.5 to 3 h. The elimination $t_{1/2}$ is approximately 11 h. *Azilsartan* is metabolized mostly by CYP2C9 into inactive metabolites. Elimination of the drug is 55% in feces and 42% in urine. About 15% of the dose is eliminated unchanged in urine. Plasma clearance is not affected by renal or hepatic insufficiency.

Angiotensin Receptor–Neprilysin Inhibitors (ARNis). A combination of *sacubitril* and *valsartan*, marketed as Entresto (generic name: LCZ696), combines the AT_1 receptor antagonistic moiety of *valsartan* with the neprilysin inhibitor moiety of *sacubitril*. The complex (*sacubitril*, *valsartan*, Na^+, and water [1:1:3:2.5]) dissociates into *sacubitril* and *valsartan* after oral administration. *Sacubitril* is about 60% bioavailable and highly protein bound (94%–97%). *Sacubitril* is further metabolized by esterases into the active metabolite LBQ657, which has a $t_{1/2}$ of 11 h. The neprilysin inhibitor blocks the breakdown of natriuretic peptides (atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide), AngI and AngII, endothelin 1, adrenomedullin, and bradykinin. The drug combination lowers vascular resistance and increases blood flow. In a large randomized clinical trial, the drug combination was superior to *enalapril* and decreased the risk of deaths from cardiovascular causes and heart failure by 20% (McMurray et al., 2014).

Meta-analyses of clinical trials have further assessed the therapeutic benefit of ARNis, including in comparison with other RAS inhibitors. ARNis reduced hospitalization and death from heart failure compared to RAS inhibitors alone in patients with HFrEF (Solomon et al., 2016) and promoted reversal of cardiac remodeling (Wang et al., 2019). Benefits of ARNis compared to ACE inhibition alone have also been observed in patients with decompensated HFrEF (PIONEER-HF trial; Ambrosy et al., 2020) and HFrEF after MI, including reduced heart failure hospitalization and mortality from cardiovascular events, albeit with an increased incidence of hypotension (PARADISE-MI trial; Jering et al., 2021). ARNis also appear to have advantages compared to ARBs in treating hypertension (Zhao et al., 2017) and greater BP-lowering effects than an ACEI or ARB for patients with both heart failure and chronic kidney disease (Kang et al., 2020). ARNis may have beneficial effects on renal function compared to ACEIs/ARBs in heart failure patients (Chen et al., 2020; Kang et al., 2020). A recent meta-analysis of clinical trials that included patients with heart failure with preserved ejection fraction indicates a reduced rate of hospitalization with ARNi treatment, although no reduction in mortality, an observation potentially confounded by challenges with patient classification (Kuno et al., 2020).

Entresto is approved for treatment of HFrEF at a recommended dose of 100 to 400 mg daily, divided into two doses. Entresto is contraindicated in patients with a history of angioedema during ACEI or ARB use. The drug should not be used in conjunction with an ARB (since Entresto contains an ARB) or ACEI (as combined neprilysin inhibition plus ACE inhibition likely increases the risk of angioedema). It also should not be used in conjunction with *aliskiren* in diabetic patients.

A New Class of ARBs in Development. The *β-arrestin–biased AT$_1$ receptor blocker* TRV027 (and others) binds to the AT$_1$ receptor and blocks G protein-coupled signaling while engaging β-arrestin. β-Arrestin, an adaptor protein, participates in receptor desensitization and internalization. In animal models, the β-arrestin–biased AT$_1$ receptor ligand increased myocyte contractility and protected against apoptosis (Kim et al., 2012). In phase II clinical studies, TRV027 decreased mean arterial pressure and was well tolerated. However, treatment with TRV027 did not improve clinical status in patients with acute heart failure (Pang et al., 2017).

Therapeutic Uses of ARBs

Angiotensin receptor blockers are approved for the treatment of hypertension. ARBs are renoprotective in type 2 diabetes mellitus and may be drugs of choice for renoprotection in diabetic patients.

Irbesartan and *losartan* are approved for diabetic nephropathy, *losartan* is approved for stroke prophylaxis, and *valsartan* and *candesartan* are approved for heart failure and to reduce cardiovascular mortality in clinically stable patients with left ventricular failure or left ventricular dysfunction following MI. ARBs and ACEIs have comparable efficacy in lowering BP, and ARBs have a favorable adverse effect profile. ARBs also are available as fixed-dose combinations with *hydrochlorothiazide* or *amlodipine* (see also Chapters 29, 32, and 33).

The Losartan Intervention for Endpoint (LIFE) Reduction in Hypertension Study demonstrated the superiority of an ARB compared with a β$_1$ adrenergic receptor antagonist in reducing stroke in hypertensive patients with left ventricular hypertrophy (Dahlöf et al., 2002). The ELITE (Evaluation of Losartan in the Elderly) study and a follow-up study (ELITE II) concluded that in elderly patients with heart failure, *losartan* is as effective as *captopril* in improving symptoms (Pitt et al., 2000). The VALIANT (Valsartan in Acute Myocardial Infarction) trial demonstrated that *valsartan* was as effective in reducing all-cause mortality as *captopril* in patients with MI complicated by left ventricular systolic dysfunction (Pfeffer et al., 2003). Both *valsartan* and *candesartan* reduce mortality and morbidity in patients with heart failure (Makani et al., 2013). ACEIs are used as first-line agents for the treatment of HFrEF. ARBs are used to treat such patients who cannot tolerate or have unsatisfactory responses to ACEIs. However, the more recent positive results from trials testing an ARNi (*sacubitril-valsartan* [Entresto]) have suggested that ARNis may be a standard of care in this setting (Rossignol et al., 2019).

Adverse Effects

ARBs are generally well tolerated. The incidence of angioedema and cough with ARBs is less than with ACEIs. ARBs have teratogenic potential and should be discontinued in pregnancy. ARBs can cause hypotension, oliguria, progressive azotemia, or acute renal failure in patients whose arterial BP or renal function is highly dependent on the RAS (e.g., renal artery stenosis). ARBs may cause hyperkalemia in patients with renal disease or those taking K$^+$ supplements or K$^+$-sparing diuretics. ARBs enhance the BP-lowering effect of other antihypertensive drugs, a desirable effect but one that may necessitate dosage adjustment. There are rare postmarketing reports of anaphylaxis, abnormal hepatic function, hepatitis, neutropenia, leukopenia, agranulocytosis, pruritus, urticaria, hyponatremia, alopecia, and vasculitis.

Direct Renin Inhibitors (DRIs)

Angiotensinogen is the only specific substrate for renin. DRIs inhibit the cleavage of AngI from angiotensinogen by renin, the rate-limiting enzymatic reaction for the generation of AngII. *Aliskiren* is the only DRI approved for clinical use.

HISTORY

Earlier inhibitors of renin were orally inactive peptide analogues of the prorenin propeptide or analogues of the renin-substrate cleavage site. Orally active, first-generation renin inhibitors (*enalkiren*, *zankiren*, CGP38560A, and *remikiren*) were effective in reducing AngII levels, but none of them succeeded in clinical trials due to their low potency, poor bioavailability, and short $t_{1/2}$. Low-molecular-weight renin inhibitors were designed based on molecular modeling and crystallographic structural information of renin-substrate interaction (Wood et al., 2003). This led to the development of *aliskiren*, a renin inhibitor that is approved for the treatment of hypertension. *Aliskiren* has BP-lowering effects akin to those of ACEIs and ARBs.

Pharmacological Effects

Aliskiren, a low-molecular-weight nonpeptide, is a competitive inhibitor of renin. It binds the active site of renin to block conversion of angiotensinogen to AngI, thus reducing the production of AngII. Aliskiren has a 10,000-fold higher affinity for renin (IC$_{50}$ ~0.6 nM) than other aspartic peptidases. In healthy volunteers, *aliskiren* (40–640 mg/d) dose-dependently decreases BP, PRA, AngI, and AngII levels but increases PRC by 16- to 34-fold due to the loss of the short-loop negative feedback by AngII (Figure 30–2). *Aliskiren* also decreases plasma and urinary aldosterone levels and enhances natriuresis (Nussberger et al., 2002).

Clinical Pharmacology

Aliskiren is recommended as a single oral dose of 150 or 300 mg/d. Bioavailability of *aliskiren* is low (~2.5%), but its high affinity compensates for this low bioavailability. Peak plasma concentrations are reached within 3 to 6 h; the $t_{1/2}$ is 20 to 45 h. Steady state in plasma is achieved in 5 to 8 days. Plasma protein binding is 50% and is independent of concentration. *Aliskiren* is a substrate for P-glycoprotein, which contributes to low bioavailability. Fatty meals significantly decrease *aliskiren* absorption. Hepatic metabolism is minimal. Elimination is mostly as unchanged drug in feces; approximately 25% of the absorbed dose appears in the urine as the parent drug.

Therapeutic Uses of Aliskiren

Aliskiren is an efficacious antihypertensive agent (Musini et al., 2017; Wang GM et al., 2020; see Chapter 32), with similar efficacy as ACEIs and ARBs and similar rates of serious adverse events. Definitive evidence for its utility in blunting diabetic complications or in patients with heart failure has not been obtained (Zheng et al., 2017; Louvis and Coulson, 2018; Luo and Chen, 2019) and thus, *aliskiren* is not approved for these indications

Adverse Effects and Contraindications

Aliskiren is well tolerated. Adverse events are mild or comparable to placebo and include mild GI symptoms, such as diarrhea at high doses

Drug Facts for Your Personal Formulary: *Inhibitors of the RAS*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Angiotensin-Converting Enzyme Inhibitors • Inhibit the conversion of AngI to AngII		
Captopril Lisinopril Enalapril Benazepril Quinapril Ramipril Moexipril	• Hypertension • Acute myocardial infarction • Heart failure • Diabetic nephropathy	• Antihypertensive effects potentiated by inhibition of ACE-catalyzed degradation of bradykinin • Antihypertensive effects potentiated by increase in Ang(1–7) levels and activation of Ang(1–7)/Mas receptor pathway • Increase PRC and PRA • Adverse effects include cough in 5%–20% of patients, angioedema, hypotension, hyperkalemia, skin rash, neutropenia, anemia, fetopathic syndrome • Contraindicated in renal artery stenosis; use with caution in impaired renal function or hypovolemia • Contraindicated in pregnancy
Enalaprilat (IV)		• Intravenous administration
Fosinopril Trandolapril Perindopril		• Undergo both hepatic and renal elimination; use with caution in renal or hepatic impairment
Angiotensin Receptor Blockers • Block AT_1 receptors		
Losartan Valsartan Eprosartan Irbesartan Candesartan Olmesartan Telmisartan Azilsartan	• Hypertension • Heart failure • Diabetic nephropathy	• Increase PRC and PRA • Adverse effects include hyperkalemia and hypotension • Contraindicated in patients with renal insufficiency • Contraindicated in pregnancy
Angiotensin Receptor–Neprilysin Inhibitor Combination • Blocks AT_1 receptors and neprilysin		
Sacubitril-valsartan (Entresto)	• Heart failure	• Superior to ACEI in heart failure patients • Adverse effects and contraindications same as valsartan • Contraindicated in combination with other ARB or ACEI
Direct Renin Inhibitors • Inhibit renin and thus the conversion of angiotensinogen to AngI		
Aliskiren	• Hypertension	• Increases PRC but decreases PRA • Contraindicated in combination with ARB or ACEI • Contraindicated in diabetic nephropathy, pregnancy, or renal insufficiency

(600 mg daily), abdominal pain, dyspepsia, and gastroesophageal reflux; headache; dizziness; fatigue; upper respiratory tract infection; back pain; angioedema; and cough (much less common than with ACEIs). Other adverse effects include rash, hypotension, hyperkalemia in diabetics on combination therapy, elevated uric acid, renal stones, and gout. *Aliskiren* is contraindicated in pregnancy. Concomitant use with ACEIs and ARBs in patients with diabetes is contraindicated. *Aliskiren* should be avoided in patients with creatinine clearance less than 60 mL/min.

Drug Interactions. *Aliskiren* reduces absorption of *furosemide* by 50%. *Irbesartan* reduces the C_{max} of *aliskiren* by 50%. *Aliskiren* plasma levels are increased by drugs that inhibit P-glycoprotein, such as *ketoconazole*, *atorvastatin*, and *cyclosporine*. During *aliskiren* treatment, the PRA assay will be inhibited by persistence of *aliskiren* in this *ex vivo* reaction, whereas the renin concentration radioimmunoassay will not be inhibited.

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Chapter 31

Treatment of Ischemic Heart Disease

Thomas Eschenhagen

ISCHEMIC HEART DISEASE: A SHORT INTRODUCTION

PATHOPHYSIOLOGY OF MYOCARDIAL ISCHEMIA

PHARMACOTHERAPY OF ISCHEMIC HEART DISEASE

- Organic Nitrates
- Ca^{2+} Channel Blockers
- β Blockers
- Antiplatelet, Anti-integrin, and Antithrombotic Agents
- Other Antianginal Agents

THERAPEUTIC STRATEGIES

- Stable Coronary Artery Disease
- Acute Coronary Syndromes
- Claudication and Peripheral Vascular Disease

MECHANOPHARMACOLOGICAL THERAPY: DRUG-ELUTING ENDOVASCULAR STENTS

Ischemic Heart Disease: A Short Introduction

Ischemic heart disease comprises pathologies that lead to myocardial ischemia, a pathological reduction in blood supply and, by extension, oxygenation of myocardial tissue. The main symptom of myocardial ischemia is angina pectoris. This stage of cell injury is, in principle, reversible. Myocardial infarction (MI) refers to the complete stoppage of blood supply and oxygenation, resulting in the onset of irreversible cell injury, also called cell death or necrosis (Oakes, 2021).

The pathophysiological understanding of ischemic heart disease has seen major changes over the past two decades—from a concept of localized calcification causing progressive constrictions of coronary arteries, ischemia, and exercise-induced angina pectoris to a systemic inflammatory disease of the arteries, including the coronaries (thus the name coronary artery disease [CAD]). A key finding in this change of paradigm was that most infarct-causing occlusions occur at small-to-medium plaques ("active plaques") by thrombosis rather than at hemodynamically relevant stenoses by progressive narrowing. Thus, in addition to the mere size of an obstructing plaque, the inflammatory activity of the atherosclerotic process, the stability of the plaque, and platelet reactivity appear to determine the prognosis (concept of the "vulnerable plaque"; Libby et al., 2002).

Atherosclerosis encompasses increased lipid deposition in the subendothelial space (early plaque), endothelial dysfunction with decreased production of nitric oxide (NO), less vasodilation and increased risk of platelet adhesion, influx of lipid scavenger cells (mainly macrophages), necrosis, sterile inflammation, proliferation of smooth muscle cells, and calcification and narrowing of the blood vessel by increasing plaque formation. If the endothelium covering the plaque or the cell layer enclosing the necrotic core of the plaque disrupts, thrombogenic materials such as collagen are presented to the bloodstream, causing platelet adhesion, fibrin deposition, thrombus formation, and closure of the blood vessel. It is increasingly recognized that platelets not only play a (mechanical) role in thrombus formation but also are an integral part of the immune response by stimulating neutrophil function (e.g., the formation of neutrophil extracellular traps [NETs]; Döring et al., 2017).

Triggering factors can be not only acute inflammation (e.g., influenza) but also blood pressure peaks during physical exercise or emotional stress (e.g., demonstrated during a life-threatening emergency and in avid fans during football games). Importantly, the process is dynamic, and the net thrombus formation is the result of the balance between thrombosis and thrombolysis by the fibrinolytic system (plasminogen). The degree and duration of coronary obstruction and thereby of the ischemia of downstream myocardium (and its size) determine the degree of necrosis of muscle tissue, that is, infarct size.

Taken together, important factors that determine the progress of CAD are the concentration of lipids in the blood, endothelial function, blood pressure (as a mechanical factor predisposing to plaque rupture), the activity of the inflammatory system, and the reactivity of pro- and antithrombotic systems. Patients with CAD, broadly, are advised to follow two sets of guidelines: (1) lifestyle (control body weight and blood pressure by exercising regularly, eating healthy, and not smoking), and (2) medications (take *aspirin*, statins, β adrenergic receptor antagonists [β blockers], and annual vaccinations against influenza). The widespread implementation of this combination drug regimen and the considerably improved treatment of acute coronary syndromes (ACSs) likely account for the continuous reduction in MIs and age-corrected cardiovascular lethality in Western countries (−42% between 2000 and 2011; Mozaffarian et al., 2015).

On the electrocardiogram (ECG), MI can be accompanied by ST-segment elevation (STEMI, indicating large transmural cell death) or not (non-STEMI, indicating smaller, not transmural cell death). The incidence of the classical large STEMI has been declining over the years as that of smaller non-STEMI has increased. While part of the latter may be explained by changes in definition (from ECG to a mainly troponin-based definition of MI), the observation raises the hypothesis that the dominant pathogenesis of acute coronary thrombosis may have changed from the rupture of lipid-rich, inflammatory plaques (in the prestatin era) to the erosion of stable plaques (Libby and Pasterkamp, 2015). Coronary imaging studies in patients treated for an ACS have shown that, over an observation period of 3.4 years, half of clinical events were associated with the large culprit lesion and half with nonculprit lesions. Although, in the latter, a "thin-capped" morphology and small lumen (signs of a "vulnerable plaque") were predictive of an event, only 5% of them gave rise to a clinical event (Stone et al., 2011). This indicates that the plaque phenotype is dynamic over time and that mechanisms beyond plaque rupture must play an additional role. These considerations have not yet led to new approved treatments but are the basis for active drug development. Examples of anti-inflammatory interventions with beneficial effects on the progression of CAD are the interleukin-1β antibody *canakinumab* (Ridker et al., 2017) and low-dose *colchicine* (Nidorf et al., 2020).

Antiplatelet agents, fibrinolytic drugs, anticoagulants, and statins (HMG-CoA [3-hydroxy-3-methylglutaryl coenzyme A] reductase inhibitors) are systematically discussed in Chapters 36 and 37. This chapter concentrates on the pharmacotherapy for angina pectoris and myocardial ischemia.

Abbreviations

ACE: angiotensin-converting enzyme
ACEI: angiotensin-converting enzyme inhibitor
ACS: acute coronary syndrome
ALDH2: mitochondrial aldehyde dehydrogenase
ARB: angiotensin receptor blocker
AV: atrioventricular
CAD: coronary artery disease
COX-1: cyclooxygenase isoform 1
CYP: cytochrome P450
ECG: electrocardiogram
EMA: European Medicines Agency
eNOS: endothelial NOS
FFA: free fatty acid
GI: gastrointestinal
Gp: glycoprotein
GTN: glyceryl trinitrate (nitroglycerin)
HCM: hypertrophic cardiomyopathy
HCN: hyperpolarization-activated cyclic nucleotide–gated
HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A
iNOS: inducible NOS
IP_3: inositol 1,4,5-trisphosphate
ISDN: isosorbide dinitrate
ISMN: isosorbide-5-mononitrate
LDL: low-density lipoprotein
MI: myocardial infarction
NET: neutrophil extracellular trap
nNOS: neuronal NOS
NO: nitric oxide
NOS: nitric oxide synthase
PAD: peripheral arterial disease
PDE: cyclic nucleotide phosphodiesterase
Pgp: P-glycoprotein
PLC: phospholipase C
rTPA: recombinant tissue plasminogen activator
SA: sinoatrial
STEMI: ST-segment elevation myocardial infarction
TxA_2: thromboxane A_2

Pathophysiology of Myocardial Ischemia

Myocardial ischemia leads to angina pectoris—chest pain behind the sternum, which, in the sense of the term, means a "strangling feeling in the chest." Myocardial ischemia is due to an imbalance in the myocardial oxygen supply-demand relationship. This imbalance may be caused by an increase in myocardial oxygen demand (which is determined by heart rate, ventricular contractility, and ventricular wall tension) or by a decrease in myocardial oxygen supply (primarily determined by coronary blood flow, but occasionally modified by the oxygen-carrying capacity of the blood), or sometimes by both (Figure 31–1). Because blood flow is proportional to the fourth power of the artery's luminal radius, the progressive decrease in vessel radius that characterizes coronary atherosclerosis can impair coronary blood flow and lead to symptoms of angina when myocardial O_2 demand increases, as with exertion (the so-called typical and most prevalent form of angina pectoris). In some patients, anginal symptoms may occur without any increase in myocardial O_2 demand, but rather as a consequence of an abrupt reduction in blood flow, as might result from coronary thrombosis (unstable angina or ACS) or localized vasospasm (variant or Prinzmetal angina). Regardless of the precipitating factors, the sensation of angina is similar in most patients. Typical angina is experienced as a heavy, pressing substernal discomfort (rarely described as a "pain"), often radiating to the left shoulder, flexor aspect of the left arm, jaw, or epigastrium. However, a significant minority of patients note discomfort in a different location or of a different character. Women, the elderly, and diabetics are more likely to experience myocardial ischemia with atypical symptoms. In most patients with typical angina, whose symptoms are provoked by exertion, the symptoms are relieved by rest or by administration of sublingual *nitroglycerin*.

Angina pectoris is a common symptom, affecting 8 million Americans (Mozaffarian et al., 2015). Angina pectoris may occur in a stable pattern over many years or may become unstable, increasing in frequency or severity and even occurring at rest. In typical stable angina, the pathological substrate is usually fixed atherosclerotic narrowing of an epicardial coronary artery, on which exertion or emotional stress superimposes an increase in myocardial O_2 demand. In variant angina, focal or diffuse coronary vasospasm episodically reduces coronary flow. Patients also may display a mixed pattern of angina with the addition of altered vessel tone on a background of atherosclerotic narrowing. In most patients with unstable angina, a local platelet deposition at an atherosclerotic plaque decreases coronary blood flow. As outlined above, this can occur as a result of a ruptured ("vulnerable") plaque or plaque erosion. Superimposed thrombosis may lead to the complete abrogation of blood flow.

Myocardial ischemia also may be *silent*, with electrocardiographic, echocardiographic, or radionuclide evidence of ischemia appearing in the absence of symptoms. While some patients have only silent ischemia, most patients who have silent ischemia have symptomatic episodes as well. The precipitants of silent ischemia appear to be the same as those of symptomatic ischemia. The *ischemic burden* (i.e., the total time a patient is ischemic each day) is greater in many patients than was recognized previously. In most trials, the agents that are efficacious in typical angina also are efficacious in reducing silent ischemia. β Blockers appear to be more

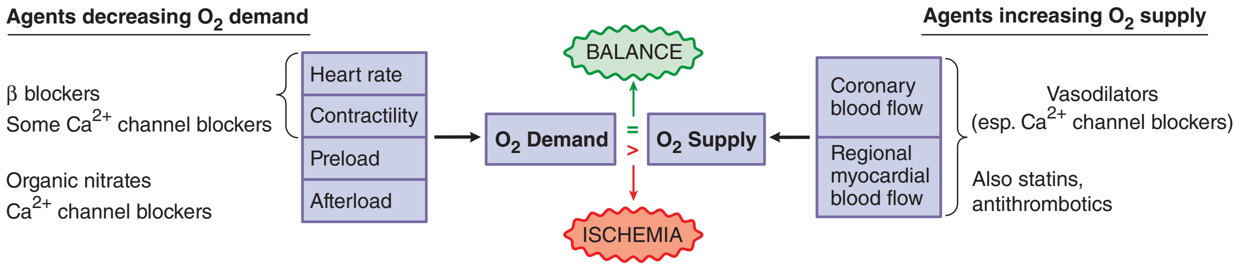

Agents decreasing O₂ demand
β blockers
Some Ca²⁺ channel blockers
Organic nitrates
Ca²⁺ channel blockers
Heart rate
Contractility
Preload
Afterload
O₂ Demand
BALANCE
=
>
ISCHEMIA
O₂ Supply
Coronary blood flow
Regional myocardial blood flow
Agents increasing O₂ supply
Vasodilators
(esp. Ca²⁺ channel blockers)
Also statins, antithrombotics

Figure 31–1 *Pharmacological modification of the major determinants of myocardial O_2 supply.* When myocardial O_2 requirements exceed O_2 supply, an ischemic episode results. This figure shows the primary hemodynamic sites of action of pharmacological agents that can reduce O_2 demand (left side) or enhance O_2 supply (right side). Some classes of agents have multiple effects. Stents, angioplasty, and coronary bypass surgery are mechanical interventions that increase O_2 supply. Both pharmacotherapy and mechanotherapy attempt to restore a dynamic balance between O_2 demand and O_2 supply.

effective than the Ca^{2+} channel blockers in the prevention of episodes. Therapy directed at abolishing all silent ischemia has not been shown to be of additional benefit over conventional therapy.

Pharmacotherapy of Ischemic Heart Disease

The principal pharmacological agents used in the treatment of angina are nitrovasodilators, β blockers (see Chapter 14), and Ca^{2+} channel blockers. In patients with typical exercise-induced angina due to CAD, these antianginal agents improve the balance of myocardial O_2 supply and O_2 demand principally by reducing myocardial O_2 demand: by decreasing heart rate, myocardial contractility, or ventricular wall stress. Increased O_2 supply by dilating the coronary vasculature may play an additional role and is the major effect of nitrovasodilators and Ca^{2+} channel blockers in variant angina.

By contrast, the principal therapeutic goal in ACSs with unstable angina is to prevent or reduce thrombus formation and increase myocardial blood flow; strategies include the use of antiplatelet agents and *heparin*, often accompanied by efforts to restore flow by mechanical means, including percutaneous coronary interventions using coronary stents, or (less commonly) emergency coronary bypass surgery. The principal therapeutic aim in variant or Prinzmetal angina is to prevent coronary vasospasm.

Antianginal agents may provide prophylactic or symptomatic treatment, but β blockers also reduce mortality, apparently by decreasing the incidence of sudden cardiac death associated with myocardial ischemia and infarction. The chronic use of organic nitrate vasodilators, which are highly efficacious in treatment of angina, is not associated with improvements in cardiac mortality, and some investigators have suggested that chronic use of *nitroglycerin* may have adverse cardiovascular effects (Parker, 2004).

Besides symptomatic relief from angina pain conferred by antianginal drugs, patients with CAD should be treated with drugs that can reduce the progression of atherosclerosis and reduce the risk of coronary thrombosis and MI. *Aspirin* is used routinely in patients with myocardial ischemia, and daily *aspirin* at low doses reduces the incidence of clinical events (Fihn et al., 2012). The optimal recommended dose is 75 to 100 mg/d (Collet et al., 2020). Oral ADP receptor antagonists such as *clopidogrel* are recommended as an alternative in patients with *aspirin* intolerance but should not routinely be used in addition to *aspirin* in patients with stable disease because of an unfavorable risk-benefit ratio (Fihn et al., 2012). However, recent guidelines recommend dual platelet inhibition in patients with high ischemic risk and without high bleeding risk (Collet et al., 2020). Dual platelet inhibition is routinely given in patients who underwent coronary artery stenting. The recommended time (generally 12 months) varies depending on the intervention (e.g., bare metal vs. drug-eluting stent) and the risk profile of patients. The newer ADP receptor antagonists *prasugrel* and *ticagrelor* have a more useful pharmacokinetic profile and seem to have a better benefit/risk ratio than clopidogrel in the postintervention treatment phase (Cannon et al., 2010; Wiviott et al., 2007) but are not generally recommended as alternatives to *clopidogrel* in patients with stable CAD. Concomitant treatment with a proton pump inhibitor is recommended in patients receiving single or dual antiplatelet therapy with a high risk of gastrointestinal bleeding (e.g., age >75; Knuuti et al., 2020; Li et al., 2017). Statins reduce mortality in patients with CAD. Although high-risk patients (including those with high plasma low-density lipoprotein (LDL) cholesterol levels) have the greatest absolute benefit, the relative risk reduction of approximately 25% appears largely independent of baseline cholesterol blood levels. Statins should therefore be given to all patients with CAD. Combination with *ezetimibe* or a PCSK9 inhibitor (antibody) should be considered in patients who are not reaching their LDL cholesterol goal with statins or statins plus *ezetimibe*, respectively, and are at very high ischemic risk (Knuuti et al., 2020). It is unclear whether angiotensin-converting enzyme (ACE) inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) (see Chapter 30) reduce mortality or other end points in patients with CAD when given routinely in addition to *aspirin*, statins, and β blockers, but they are recommended for subgroups of patients with CAD with reduced left ventricular systolic function, hypertension, diabetes, or chronic kidney disease (Collet et al., 2020).

Coronary artery bypass surgery and percutaneous coronary interventions such as angioplasty and coronary artery stent deployment commonly complement pharmacological treatment. In some subsets of patients, percutaneous or surgical revascularization may have a survival advantage over medical treatment alone (Kappetein et al., 2011). However, a large, randomized trial in patients with stable CAD and signs of recurrent ischemia failed to provide evidence for superiority of an early invasive strategy (Maron et al., 2020). Intracoronary drug delivery using drug-eluting coronary stents represents an intersection of mechanical and pharmacological approaches in the treatment of CAD.

Organic Nitrates

The organic nitrate agents are prodrugs that are sources of NO. NO activates the soluble isoform of guanylyl cyclase, thereby increasing intracellular levels of cGMP. In turn, cGMP promotes the dephosphorylation of the myosin light chain and the reduction of cytosolic Ca^{2+} and leads to the relaxation of smooth muscle cells in a broad range of tissues (see Figures 3–13, 3–17, and 48–7). The NO-dependent relaxation of vascular smooth muscle leads to vasodilation; NO-mediated guanylyl cyclase activation also inhibits platelet aggregation and relaxes smooth muscle in the bronchi and gastrointestinal (GI) tract.

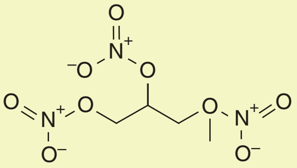
The broad biological response to nitrovasodilators reflects the existence of endogenous NO-modulated regulatory pathways. The endogenous synthesis of NO in humans is catalyzed by a family of nitric oxide synthases (NOSs) that oxidize the amino acid L-arginine to form NO, plus L-citrulline as a coproduct. There are three distinct mammalian NOS isoforms: *neuronal NOS (nNOS), endothelial NOS (eNOS),* and *inducible NOS (iNOS)* (see Chapter 3), and they are involved in processes as diverse as neurotransmission, vasomotion, and immunomodulation. In several vascular disease states, pathways of endogenous NO-dependent regulation appear to be deranged (Dudzinski et al., 2006).

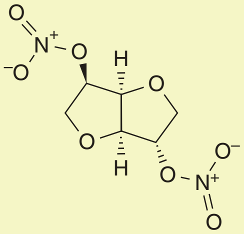
HISTORICAL PERSPECTIVE

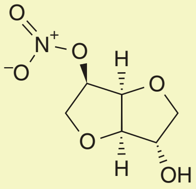
Nitroglycerin was first synthesized in 1846 by Sobrero, who observed that a small quantity placed on the tongue elicited a severe headache. The explosive properties of *nitroglycerin* also were soon noted, and control of this unstable compound for military and industrial use was not realized until Alfred Nobel devised a process to stabilize the *nitroglycerin* and patented a specialized detonator in 1863. The vast fortune that Nobel accrued from the *nitroglycerin* detonator patent provided the funds later used to establish the Nobel prizes. In 1857, T. Lauder Brunton of Edinburgh (no relation to the editor of this volume) administered *amyl nitrite*, a known vasodepressor, by inhalation and noted that anginal pain was relieved within 30 to 60 sec. The action of *amyl nitrite* was transitory, however, and the dosage was difficult to adjust. Subsequently, William Murrell surmised that the action of *nitroglycerin* mimicked that of *amyl nitrite* and established the use of sublingual *nitroglycerin* for relief of the acute anginal attack and as a prophylactic agent to be taken prior to exertion. The empirical observation that organic nitrates could dramatically and safely alleviate the symptoms of angina pectoris led to their widespread acceptance by the medical profession. Indeed, Alfred Nobel himself was prescribed *nitroglycerin* by his physicians when he developed angina in 1890. Basic investigations defined the role of NO in both the vasodilation produced by nitrates and endogenous vasodilation. The importance of NO as a signaling molecule in the cardiovascular system and elsewhere was recognized by the awarding of the 1998 Nobel Prize in Medicine/Physiology to the pharmacologists Robert Furchgott, Louis Ignarro, and Ferid Murad.

TABLE 31–1 ■ ORGANIC NITRATES AVAILABLE FOR CLINICAL USE

AGENT	PREPARATIONS, DOSES, ADMINISTRATION[a]	
Nitroglycerin (glyceryl trinitrate)	T: 0.3–0.6 mg as needed	O: 2.5–5 cm, topically every 4–8 h
	S: 0.4 mg per spray as needed	D: 1 disk (2.5–15 mg) for 12–16 h/d
	C: 2.5–9 mg 2–4 times daily	IV: 10–20 μg/min; ↑ 10 μg/min to max of 400 μg/min
	B: 1 mg every 3–5 h	
Isosorbide dinitrate	T: 2.5–10 mg every 2–3 h	T(O): 5–40 mg every 8 h
	T(C): 5–10 mg every 2–3 h	C: 40–80 mg every 12 h
Isosorbide-5-mononitrate	T: 10–40 mg twice daily	C: 60–120 mg daily


Nitroglycerin (glyceryl trinitrate, GTN)


Isosorbide dinitrate (ISDN)


Isosorbide-5-mononitrate (ISMN)

[a] B, buccal (transmucosal) tablet; C, sustained-release capsule or tablet; D, transdermal disk or patch; IV, intravenous injection; O, ointment; S, lingual spray; T, tablet for sublingual use; T(C), chewable tablet; T(O), oral tablet or capsule.

Chemistry

Organic nitrates are polyol esters of nitric acid, whereas organic nitrites are esters of nitrous acid (Table 31–1). Nitrate esters (—C—O—NO_2) and nitrite esters (—C—O—NO) are characterized by a sequence of carbon-oxygen-nitrogen, whereas nitro compounds possess carbon-nitrogen bonds (C—NO_2). Thus, glyceryl trinitrate (GTN; *nitroglycerin*) is not a nitro compound, and it is erroneously called *nitroglycerin*; however, this nomenclature is both widespread and official. Amyl nitrite is a highly volatile liquid that must be administered by inhalation and is of limited therapeutic utility. Organic nitrates of low molecular mass (such as GTN) are moderately volatile, oily liquids, whereas the high-molecular-mass nitrate esters (e.g., erythrityl tetranitrate, isosorbide dinitrate (ISDN), and isosorbide mononitrate) are solids. In the pure form (without an inert carrier such as lactose), *nitroglycerin* is explosive. The organic nitrates and nitrites, collectively termed *nitrovasodilators*, must be metabolized (reduced) to produce gaseous NO, which appears to be the active principle of this class of compounds. NO gas also can be directly administered by inhalation.

Pharmacological Properties

Mechanism of Action. Nitrites, organic nitrates, nitroso compounds, and a variety of other nitrogen oxide–containing substances (including *nitroprusside*; see further in the chapter) lead to the formation of the reactive gaseous free radical NO and related NO-containing compounds. NO gas also may be administered by inhalation. Surprisingly, more than 140 years after its introduction in the therapy of angina pectoris, the mode of action of organic nitrates is still incompletely understood (Mayer and Beretta, 2008). Established mechanisms of GTN bioactivation and action include a nonenzymatic reaction with L-cysteine, formation of nitrite and NO by ALDH2 (mitochondrial aldehyde dehydrogenase) (Chen et al., 2002), activation of soluble guanylyl cyclase, and generation of cGMP. The bioactivation of other nitrovasodilators such as ISDN and isosorbide-5-mononitrate (ISMN) is ALDH2 independent, suggesting the involvement of other enzymes, such as cytochrome P450s (CYPs), xanthine oxidoreductase, and cytosolic ALDH isoforms (Munzel et al., 2014). The action of NO on soluble guanylyl cyclase seems to be elicited in substantial part by S-nitrosothiol. The different ALDH2 dependence of GTN and ISDN is clinically relevant because individuals of Asian origin carry an inactive ALDH2 variant and do not respond adequately to GTN but do respond to ISDN (Stamler, 2008).

The NO-stimulated elevation of cGMP activates protein kinase G and modulates the activities of cyclic nucleotide PDEs (PDEs 2, 3, and 5) in a variety of cell types. In smooth muscle, the net result is reduced phosphorylation of myosin light chain, reduced Ca^{2+} concentration in the cytosol, and relaxation (see Figure 48–7). Reduced phosphorylation of myosin light chain is the result of decreased myosin light-chain kinase activity and increased myosin light-chain phosphatase activity and promotes vasorelaxation and smooth muscle relaxation in many tissues. cGMP is a substrate for PDE5, whose inhibition by *sildenafil* and related compounds potentiates the action of nitrovasodilators (see *Toxicity and Untoward Responses*).

Hemodynamic Effects. The nitrovasodilators promote relaxation of vascular smooth muscle. For reasons not understood, GTN dilates large blood vessels (>200-μm diameter) more potently than small vessels, explaining why low doses of GTN preferentially dilate veins and conductance arteries and leave the tone of the small-to-medium arterioles (that regulate resistance) unaffected. This profile has important consequences for the antianginal efficacy of nitrovasodilators. At low-to-medium doses, preferential venodilation decreases venous return, leading to a fall in left and right ventricular chamber size and end-diastolic pressures, reduced wall stress, and thereby reduced cardiac O_2 demand (see discussion that follows). Systemic vascular resistance and arterial pressure are not or only mildly decreased, leaving coronary perfusion pressure unaffected. Heart rate remains unchanged or may increase slightly in response to a decrease in blood pressure. Pulmonary vascular resistance and cardiac output are slightly reduced. Doses of GTN that do not alter systemic arterial pressure may still produce arteriolar dilation in the face and neck, resulting in a facial flush, or dilation of meningeal arterial vessels, causing headache.

Higher doses of organic nitrates cause further venous pooling and may decrease arteriolar resistance as well, thereby decreasing systolic and diastolic blood pressure and causing pallor, weakness, dizziness, and activation of compensatory sympathetic reflexes. This can happen to such an extent that coronary flow is compromised, and the sympathetic increase in myocardial O_2 demand overrides the beneficial action of the nitrovasodilators, leading to ischemia. In addition, sublingual *nitroglycerin* administration may produce bradycardia and hypotension, probably owing to activation of the Bezold-Jarisch reflex.

In patients with autonomic dysfunction and an inability to increase sympathetic outflow (multiple-system atrophy and pure autonomic failure are the most common forms, much less commonly seen in the autonomic dysfunction associated with diabetes), the fall in blood pressure consequent to the venodilation produced by nitrates cannot be compensated. In these clinical contexts, nitrates may reduce arterial pressure and coronary perfusion pressure significantly, producing potentially life-threatening hypotension (nitrate syncope) and even aggravating angina.

ADME. As outlined previously, nitrovasodilators differ in their dependence on ALDH2 for bioactivation (note ALDH2 deficiency in many Asians). In addition, their pharmacokinetic profiles exhibit therapeutically relevant differences in sublingual resorption, onset of action, and half-life (Table 31–1).

Nitroglycerin. Peak concentrations of GTN are found in plasma within 4 min of sublingual administration; the drug has a $t_{1/2}$ of 1 to 3 min. The onset of action of GTN may be even more rapid if delivered as a sublingual spray rather than as a sublingual tablet. Glyceryl dinitrate metabolites, which have about one-tenth the vasodilator potency, appear to have half-lives of about 40 min.

Isosorbide Dinitrate. Sublingual administration of ISDN produces maximal plasma concentrations of the drug by 6 min, and the fall in concentration is rapid ($t_{1/2}$ of about 45 min). The primary initial metabolites, isosorbide-2-mononitrate and ISMN, have longer half-lives (3–6 h) and are presumed to contribute to the therapeutic efficacy of the drug. ISDN is therefore suitable both for standby and sustained therapy.

Isosorbide-5-Mononitrate. This agent is available in tablet form. ISMN does not undergo significant first-pass metabolism, so it has high bioavailability after oral administration, but its onset of action is too slow for acute treatment of angina.

Inhaled NO. Nitric oxide gas administered by inhalation appears to exert most of its therapeutic effects on the pulmonary vasculature because of the rapid inactivation of NO by hemoglobin in the blood. It is approved for the treatment of pulmonary hypertension in hypoxemic neonates, in whom it has reduced morbidity and mortality (Bloch et al., 2007).

Mechanisms of Antianginal Efficacy of Organic Nitrates

When GTN is injected directly into the coronary circulation of patients with CAD, anginal attacks (induced by electrical pacing) are not aborted even when coronary blood flow is increased. In contrast, sublingual administration of GTN does relieve anginal pain in the same patients, indicating that the major antianginal effect of nitrovasodilators is mediated by preload reduction rather than coronary artery dilation.

This interpretation is supported by studies in exercising patients showing that angina occurs at the same value of the *triple product* (Aortic pressure × Heart rate × Ejection time, which is roughly proportional to myocardial consumption of O_2) with or without *nitroglycerin*. Thus, the beneficial effect of *nitroglycerin* has to result from reduced cardiac O_2 demand rather than an increase in the delivery of O_2 to ischemic regions of myocardium. However, these results do not preclude the possibility that a favorable redistribution of blood flow to ischemic subendocardial myocardium may contribute to relief of pain in a typical anginal attack, and they do not preclude the possibility that direct coronary vasodilation may be the major effect of *nitroglycerin* in situations where vasospasm compromises myocardial blood flow.

Effects on Myocardial O_2 Requirements. The major determinants of myocardial O_2 consumption are left ventricular wall tension, heart rate, and myocardial contractility (Figure 31–1). Ventricular wall tension is affected by preload and afterload. *Preload* is determined by the diastolic pressure that distends the ventricle (ventricular end-diastolic pressure). Increasing end-diastolic volume augments the ventricular wall tension (by the law of Laplace, tension is proportional to pressure times radius). Increasing venous capacitance with nitrates decreases venous return to the heart, decreases ventricular end-diastolic volume, and thereby decreases O_2 consumption. An additional benefit of reducing preload is that it increases the pressure gradient for perfusion across the ventricular wall, which favors subendocardial perfusion. *Afterload* is the impedance against which the ventricle must eject. In the absence of aortic valvular disease, afterload is related to peripheral resistance. Decreasing peripheral arteriolar resistance reduces afterload and thus myocardial work and O_2 consumption. The nitrate-induced increase in distensibility of the large conductance arteries such as the aorta may play an additional role.

Nitrovasodilators preferentially decrease preload by dilating venous capacitance vessels. The decrease in afterload is generally small and mainly observed at higher doses. The effect on aortic stiffness appears complex (Soma et al., 2000). NO and nitrovasodilators can directly modulate the inotropic or chronotropic state of the heart via cGMP and its stimulatory effect on PDE2 (thereby reducing cAMP) or an inhibitory effect on the cAMP-specific PDE3 (thereby increasing cAMP). An inotropic response depends on the extent to which the cyclic nucleotide phosphodiesterase (PDE) isoforms are expressed in the appropriate cells and in the proper subcellular compartment (Steinberg and Brunton, 2001). Small NO concentrations favor a positive inotropic effect (Kojda et al., 1997); however, the effect size is small and its significance unclear. Because nitrates affect several of the primary determinants of myocardial O_2 demand, their net effect usually is to decrease myocardial O_2 consumption. In addition, an improvement in the lusitropic state of the heart may be seen with more rapid early diastolic filling. This may be secondary to the relief of ischemia rather than primary, or it may be due to a reflex increase in sympathetic activity. Nitrovasodilators also increase cGMP in platelets, with consequent inhibition of platelet function. While this may contribute to their antianginal efficacy, the effect appears to be modest. Earlier evidence for a negative interaction of nitrates with *heparin* were not reproduced in larger studies in patients (Koh et al., 1995).

Effects on Total and Regional Coronary Blood Flow. When considering the effect of vasodilators in the ischemic heart, it is important to realize that myocardial ischemia itself is a powerful stimulus to coronary vasodilation and part of an autoregulatory mechanism. In the presence of atherosclerotic coronary artery narrowing, ischemia distal to the lesion stimulates vasodilation of downstream resistance arterioles and thereby helps maintain adequate perfusion of the ischemic area under rest. If the stenosis is severe, much of the capacity to dilate is used to maintain resting blood flow. Further dilation may not be possible, neither under exercise nor with therapeutically applied vasodilators. In contrast, nonselective vasodilators such as adenosine or dipyridamole (which inhibits adenosine transmembrane transport and thereby increases extracellular concentrations) can worsen the perfusion of ischemic areas by dilating the relatively constricted arterioles of the healthy myocardium, leading to redistribution of blood flow away from the ischemic myocardium ("steal phenomenon"). Accordingly, dipyridamole is not used therapeutically but can be used as a stress test to provoke angina pectoris (Bodi et al., 2007). The same mechanism probably explains the potential of dihydropyridines with a fast onset of action such as nifedipine to provoke angina pectoris.

Nitrovasodilators, in contrast, do not have a major effect on the smaller resistance arteries (and therefore do not cause steal phenomena) but can dilate the large, epicardial sections of the coronary arteries upstream of a stenosis and also in a stenosis (concept of the "dynamic stenosis"; Brown et al., 1981) and thereby increase blood flow distal to the narrowing. Collateral flow to ischemic regions also is increased. As outlined previously, GTN also reduces wall stress that opposes blood flow to the subendocardium, which is particularly sensitive to ischemia.

In patients with angina owing to coronary artery spasm, the capacity of nitrovasodilators to dilate epicardial coronary arteries, particularly regions affected by spasm, is the primary mechanism of their beneficial effect.

Other Effects. The nitrovasodilators also relax smooth muscles of the bronchial tract, the gallbladder, biliary ducts, and sphincter of Oddi and the GI tract. Spontaneous motility is decreased by nitrates both *in vivo* and *in vitro*. The effect may be transient and incomplete *in vivo*, but abnormal "spasm" frequently is reduced. Indeed, many incidences of atypical chest pain and "angina" are due to biliary or esophageal spasm, and these also can be relieved by nitrates. Nitrates can also relax ureteral and uterine smooth muscle, but these responses are of uncertain clinical significance.

Tolerance

Frequently repeated or continuous exposure to high doses of nitrovasodilators leads to tolerance, that is, marked attenuation in the magnitude of most of their pharmacological effects. The magnitude of tolerance is a function of dosage and frequency of use. Tolerance may result from a reduced capacity of the vascular smooth muscle to convert *nitroglycerin* to NO, *true vascular tolerance*, or to the activation of mechanisms extraneous to the vessel wall, *pseudotolerance* (Munzel et al., 1995). Multiple mechanisms have been proposed to account for nitrate tolerance, including volume expansion, neurohumoral activation, cellular depletion of sulfhydryl groups, and the generation of free radicals (Parker and Parker, 1998). A reactive intermediate formed during the generation of NO from organic nitrates may itself damage and inactivate the enzymes of the activation pathway (Munzel et al., 1995; Parker, 2004). Inactivation of ALDH2 (Sydow et al., 2004) and S-nitrosylation of soluble guanylyl cyclase (Sayed et al., 2008) are seen in models of nitrate tolerance and could explain cross-tolerance to different (nitro)vasodilators. Other changes observed in the setting of *nitroglycerin* tolerance include an enhanced response to vasoconstrictors such as angiotensin II, serotonin, and phenylephrine. Prolonged administration of GTN is associated with plasma volume expansion, which may be reflected by a decrease in hematocrit. Unfortunately, attempts to prevent nitrate tolerance based on these mechanisms (e.g., antioxidants, coapplication of vasodilators or diuretics) failed in clinical trials.

A clinically important lesson of research on nitrate tolerance is that prolonged treatment with nitrates may not only induce a loss of response to nitrates, but also actually increase the risk of angina in the interval (Parker et al., 1995). A special form of GTN tolerance is observed in individuals exposed to GTN in the manufacture of explosives. If protection is inadequate, workers may experience severe headaches, dizziness, and postural weakness during the first several days of employment ("Monday disease"). Tolerance then develops and can lead to organic nitrate dependence. Workers without demonstrable organic vascular disease have been reported to have an increase in the incidence of ACSs during the 24- to 72-h periods away from the work environment. It seems prudent not to withdraw nitrates abruptly from a patient who has received such therapy chronically.

Therapy should be designed to prevent tolerance. High doses should be avoided and therapy interrupted for 8 to 12 h daily, which allows the return of efficacy. In patients with exertional angina, it is usually most convenient to omit dosing at night either by adjusting dosing intervals of oral or buccal preparations or by removing cutaneous GTN. Patients whose anginal pattern suggests its precipitation by increased left ventricular filling pressures (e.g., in association with orthopnea or paroxysmal nocturnal dyspnea) may benefit from continuing nitrates at night and omitting them during a quiet period of the day. Some patients develop an increased frequency of nocturnal angina when a nitrate-free interval is employed using GTN patches; such patients may require another class of antianginal agent during this period. Continuous intravenous administration of GTN regularly induces tolerance and should therefore be avoided. Tolerance also has been seen with ISMN and ISDN; an eccentric twice-daily dosing schedule appears to maintain efficacy (Parker and Parker, 1998). *Molsidomine*, a direct NO donor, is approved in many European countries and is claimed to induce less tolerance than the organic nitrates, but the supporting evidence is weak. A clinical study failed to demonstrate beneficial effects of *molsidomine* on endothelial dysfunction (Barbato et al., 2015).

Toxicity and Untoward Responses

Untoward responses to the therapeutic use of organic nitrates are almost all secondary to actions on the cardiovascular system. Headache is common and can be severe, usually decreasing over a few days if treatment is continued and often controlled by decreasing the dose. Transient episodes of dizziness, weakness, and other manifestations associated with postural hypotension may develop, particularly if the patient is standing immobile, and may progress occasionally to loss of consciousness, a reaction that appears to be accentuated by alcohol. It also may be seen with very low doses of nitrates in patients with autonomic dysfunction. Even in severe nitrate syncope, positioning and other measures that facilitate venous return are the only therapeutic measures required. All the organic nitrates occasionally can produce drug rash.

Interaction of Nitrates With PDE5 Inhibitors. Erectile dysfunction is a frequently encountered problem whose risk factors parallel those of CAD. Thus, many men desiring therapy for erectile dysfunction already may be receiving (or may require, especially if they increase physical activity) antianginal therapy. The combination of sildenafil and other PDE5 inhibitors with organic nitrate vasodilators can cause extreme hypotension.

Cells in the corpus cavernosum produce NO during sexual arousal in response to nonadrenergic, noncholinergic neurotransmission (Burnett et al., 1992). NO stimulates the formation of cGMP, which leads to relaxation of smooth muscle of penile arteries that fill the corpus cavernosum, leading to engorgement of the corpus cavernosum and erection. The accumulation of cGMP is enhanced by inhibition of the cGMP-specific PDE5 family. *Sildenafil* and congeners inhibit PDE5 and have been demonstrated to improve erectile function in patients with erectile dysfunction. Not surprisingly, PDE5 inhibitors have assumed the status of widely used recreational drugs. *Sildenafil* is also FDA and European Medicines Agency (EMA) approved in patients with pulmonary arterial hypertension in whom the drug decreased pulmonary vascular resistance and enhanced exercise capacity. PDE5 inhibitors also are being studied in patients with congestive heart failure, but a large trial in patients with preserved ejection fraction failed (Redfield et al., 2013; Chapter 33). *Tadalafil* and *vardenafil* share similar therapeutic efficacy and side effect profiles with *sildenafil*; *tadalafil* has a longer time to onset of action and a longer therapeutic $t_{1/2}$ than the other PDE5 inhibitors (see Table 49–2). *Sildenafil* has been the most thoroughly characterized of these compounds, but all three PDE5 inhibitors are contraindicated for patients taking organic nitrate vasodilators, and the PDE5 inhibitors should be used with caution in patients taking α or β blockers (see Chapter 14).

The side effects of *sildenafil* and other PDE5 inhibitors are largely predictable based on their effects on PDE5. Headache, flushing, and rhinitis may be observed, as well as dyspepsia owing to relaxation of the lower esophageal sphincter. *Sildenafil* and *vardenafil* also weakly inhibit PDE6, the enzyme involved in photoreceptor signal transduction (see Figure 74–9), and can produce visual disturbances, most notably changes in the perception of color hue or brightness. In addition to visual disturbances, sudden one-sided hearing loss has also been reported. *Tadalafil* inhibits PDE11, a widely distributed PDE isoform, but the clinical importance of this effect is not clear. The most important toxicity of all these PDE5 inhibitors is hemodynamic. When given alone to men with severe CAD, these drugs induce only a modest (<10%) decrease of blood pressure (Herrmann et al., 2000). However, PDE5 inhibitors and nitrates act synergistically to cause profound increases in cGMP and dramatic reductions in blood pressure (>25 mm Hg). *PDE5 inhibitors should therefore not be prescribed to patients receiving any form of nitrate* (Cheitlin et al., 1999); in prescribing nitrates, the physician should warn the patient that PDE5 inhibitors and nitrates must not be used concurrently and that no PDE5 inhibitor should be used in the 24 h prior to initiating nitrate therapy. A period of longer than 24 h may be needed following administration of a PDE5 inhibitor for safe use of nitrates, especially with *tadalafil*, due to its prolonged $t_{1/2}$. If patients develop significant hypotension following combined administration of *sildenafil* and a nitrate, fluids and α adrenergic receptor agonists may be used for support.

Sildenafil, *tadalafil*, and *vardenafil* are metabolized via CYP3A4, and their toxicity may be enhanced in patients who receive inhibitors of this enzyme, including macrolide and imidazole antibiotics, and antiretroviral agents (see individual chapters and Chapter 7). PDE5 inhibitors also may prolong cardiac repolarization by blocking the I_{Kr}. Although these interactions and effects are important clinically, the overall incidence and profile of adverse events observed with PDE5 inhibitors, when used without nitrates, are consistent with the expected background frequency of the same events in the treated population. In patients with CAD whose exercise capacity indicates that sexual activity is unlikely to precipitate angina and who are not currently taking nitrates, the use of PDE5 inhibitors can be considered.

Therapeutic Uses

Stable Angina Pectoris. Diseases that predispose to CAD and angina should be treated as part of a comprehensive therapeutic program with the primary goal being to prolong life. Conditions such as hypertension, anemia, thyrotoxicosis, obesity, heart failure, cardiac arrhythmias, and acute emotional stress can precipitate anginal symptoms in many patients. Patients should be counseled to stop smoking, lose weight, and maintain a low-fat, high-fiber diet; hypertension and hyperlipidemia should be corrected; and daily *aspirin* (or *clopidogrel* if *aspirin* is not tolerated) and statins (see Chapter 37) should be prescribed. Exposure to sympathomimetic agents (e.g., those in nasal decongestants and other sources) and serotonin receptor agonists used in the treatment of migraine (*sumatriptan* and similar) should be avoided. The use of drugs that modify the perception of pain is a poor approach to the treatment of angina because the underlying myocardial ischemia is not relieved.

Table 31–1 lists the preparations and dosages of the nitrites and organic nitrates. The rapidity of onset, the duration of action, and the likelihood of developing tolerance are related to the method of administration.

Short-Acting Nitrates for Standby Therapy. GTN is the most used drug for the rapid release of angina and can be applied as tablets, capsules, sublingual powder, spray, and aerosol. The onset of action is within 1 to 2 min (fastest with the spray), and the effects are undetectable by 1 h after administration. An initial dose of 0.3 mg GTN often relieves pain within 3 min. ISDN, but not ISMN, is an alternative to GTN. It has a slower onset of action (3–4 min) but a longer duration (>1 h). Anginal pain may be prevented when the drugs are used prophylactically immediately prior to exercise or stress. The smallest effective dose should be prescribed. Patients should be instructed to seek medical attention immediately if three tablets of GTN taken over a 15-min period do not relieve a sustained attack because this situation may be indicative of MI, unstable angina, or another cause of the pain.

Longer-Acting Nitrates for the Prophylaxis of Angina. Nitrates can also be used to provide prophylaxis against anginal episodes in patients who have more than occasional angina. However, such patients should be offered revascularizing therapy. Moreover, chronic treatment with nitrates is not associated with a prognostic benefit and may induce tolerance and endothelial dysfunction as discussed previously. Nitrates must therefore be considered a second choice compared to β blockers. Sustained-release oral preparations of ISDN, ISMN, and GTN are available. Sustained-release ISDN and ISMN are typically given in two doses administered 6 to 7 h apart, followed by a nitrate-free interval of at least 8 h.

Variant (Prinzmetal) Angina. The large coronary arteries normally contribute little to coronary resistance. However, in variant angina, coronary constriction results in reduced blood flow and ischemic pain. Multiple mechanisms have been proposed to initiate vasospasm, including endothelial cell injury. Whereas long-acting nitrates alone are occasionally efficacious in abolishing episodes of variant angina, additional therapy with Ca^{2+} channel blockers usually is required. Ca^{2+} channel blockers, but not nitrates, have been shown to influence mortality and the incidence of MI favorably in variant angina; they should generally be included in therapy.

Congestive Heart Failure. The utility of nitrovasodilators to relieve pulmonary congestion and to increase cardiac output in congestive heart failure is addressed in Chapter 33.

Unstable Angina Pectoris (Acute Coronary Syndromes [ACS], see discussion that follows). Resistance to nitrates classifies angina symptoms as "unstable" and is a characteristic feature of ACSs, typically caused by transient or permanent thrombotic occlusion of coronary vessels. Nitrates do not modify this process specifically and are second-line drugs.

Ca^{2+} Channel Blockers

Voltage-gated Ca^{2+} channels (L-type or slow channels) mediate the entry of extracellular Ca^{2+} into smooth muscle and cardiac myocytes and sinoatrial (SA) and atrioventricular (AV) nodal cells in response to electrical depolarization. In both smooth muscle and cardiac myocytes, Ca^{2+} is a trigger for contraction, albeit by different mechanisms. Ca^{2+} channel antagonists, also called *Ca^{2+} entry blockers* or *Ca^{2+} channel blockers*, inhibit Ca^{2+} influx. In vascular smooth muscle, this leads to relaxation, especially in arterial beds, in cardiac myocytes to negative inotropic effects. All Ca^{2+} channel blockers exert these two principal actions, but the ratio differs according to the class, as does the presence of chronotropic and dromotropic effects.

HISTORICAL PERSPECTIVE

The work in the 1960s of Fleckenstein and colleagues led to the concept that drugs can alter cardiac and smooth muscle contraction by blocking the entry of Ca^{2+} into myocytes (Fleckenstein et al., 1969). Godfraind and associates showed that the effect of the diphenylpiperazine analogues in preventing agonist-induced vascular smooth muscle contraction could be overcome by raising the concentration of Ca^{2+} in the extracellular medium (Godfraind et al., 1986). Hass and Hartfelder reported in 1962 that *verapamil*, a coronary vasodilator, possessed negative inotropic and chronotropic effects that were not seen with other vasodilatory agents, such as GTN. In 1967, Fleckenstein suggested that the negative inotropic effect resulted from inhibition of excitation-contraction coupling and that the mechanism involved reduced movement of Ca^{2+} into cardiac myocytes. *Verapamil* was the first clinically available Ca^{2+} channel blocker; it is a congener of *papaverine*. Many other Ca^{2+} entry blockers with a wide range of structures are now available.

Chemistry

The multiple Ca^{2+} channel blockers that are approved for clinical use have diverse chemical structures. Clinically used Ca^{2+} channel blockers include the phenylalkylamine *verapamil*, the benzothiazepine *diltiazem*, and numerous dihydropyridines, including *amlodipine*, *clevidipine*, *felodipine*, *isradipine*, *lercanidipine*, *nicardipine*, *nifedipine*, *nimodipine*, and *nisoldipine*. The structures and relative specificities of representative drugs are shown in Table 31–2. Although these drugs are commonly grouped together as "calcium channel blockers," there are fundamental differences among *verapamil*, *diltiazem*, and the dihydropyridines with respect to pharmacodynamics, drug interactions, and toxicities.

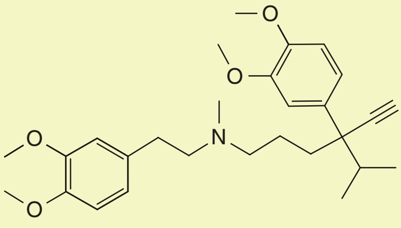
Mechanisms of Action

An increased concentration of cytosolic Ca^{2+} causes increased contraction in both cardiac and vascular smooth muscle cells. In cardiac myocytes, the entry of extracellular Ca^{2+} causes a larger Ca^{2+} release from intracellular stores (Ca^{2+}-induced Ca^{2+} release) and thereby initiates the contraction twitch. In smooth muscle cells, entry of Ca^{2+} plays a dominant role, but the release of Ca^{2+} from intracellular storage sites also contributes to contraction of vascular smooth muscle, particularly in some vascular beds. In contrast to cardiac muscle, smooth muscles typically contract tonically. Cytosolic Ca^{2+} concentrations can be increased by diverse contractile stimuli in vascular smooth muscle cells. Many hormones and autocoids increase Ca^{2+} influx through so-called receptor-operated channels, whereas increases in external concentrations of K^+ and depolarizing electrical stimuli increase Ca^{2+} influx through voltage-gated, or "potential operated," channels. The Ca^{2+} channel blockers produce their effects by binding to the α_1 subunit of the L-type voltage-gated Ca^{2+} channels and reducing Ca^{2+} flux through the channel. The vascular and cardiac effects of some of the Ca^{2+} channel blockers are summarized in the next section and in Table 31–2.

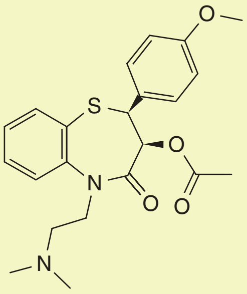
Voltage-gated channels contain domains of homologous sequence that are arranged in tandem within a single large subunit. In addition to the major channel-forming subunit (termed α_1), Ca^{2+} channels contain

TABLE 31–2 ■ COMPARATIVE CV EFFECTS OF Ca^{2+} CHANNEL BLOCKERS[a]

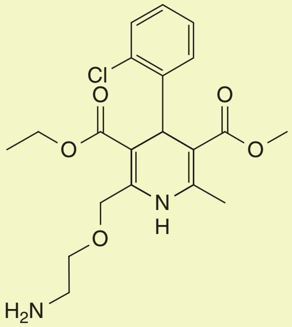
DRUG CLASS: EXAMPLE	VASODILATION	↓ CARDIAC CONTRACTILITY	↓ AUTOMATICITY (SA NODE)	↓ CONDUCTION (AV NODE)
Phenylalkylamine: Verapamil	4	4	5	5
Benzothiazepine: Diltiazem	3	2	5	4
Dihydropyridine[b]: Amlodipine	5	1	1	0



Verapamil



Diltiazem



Amlodipine

[a]Relative effects are ranked from *no effect* (0) to *prominent* (5).

[b]See text for individual characteristics of the numerous dihydropyridines.

several other associated subunits (termed α_2, β, γ, and δ) (Schwartz, 1992). Voltage-gated Ca^{2+} channels have been divided into at least three subtypes based on their conductances and sensitivities to voltage (Schwartz, 1992; Tsien et al., 1988). The channels best characterized to date are the L, N, and T subtypes. Only the L-type channel is sensitive to the dihydropyridine Ca^{2+} channel blockers. All approved Ca^{2+} channel blockers bind to the α_1 subunit of the L-type Ca^{2+} channel, which is the main pore-forming unit of the channel. This approximately 250,000-Da subunit is associated with a disulfide-linked $\alpha_2\delta$ subunit of about 140,000 Da and a smaller intracellular β subunit. The α_1 subunits share a common topology of four homologous domains, each of which is composed of six putative transmembrane segments (S1–S6). The α_2, δ, and β subunits modulate the α_1 subunit (see Figure 14–2). The phenylalkylamine Ca^{2+} channel blocker verapamil binds to transmembrane segment 6 of domain IV (IVS6), the benzothiazepine Ca^{2+} channel blocker diltiazem binds to the cytoplasmic bridge between domain III (IIIS) and domain IV (IVS), and the dihydropyridine Ca^{2+} channel blockers (*nifedipine* and several others) bind to transmembrane segments of both domains III and IV. These three separate receptor sites are linked allosterically.

Pharmacological Actions

Vascular Tissue. Depolarization of vascular smooth muscle cells depends primarily on the influx of Ca^{2+}. At least three distinct mechanisms may be responsible for contraction of vascular smooth muscle cells. First, voltage-gated Ca^{2+} channels open in response to depolarization of the membrane, and extracellular Ca^{2+} moves down its electrochemical gradient into the cell. After closure of Ca^{2+} channels, a finite period of time is required before the channels can open again in response to a stimulus. Second, agonist-induced contractions that occur without depolarization of the membrane result from stimulation of the G_q-phospholipase C (PLC)-IP_3 (inositol 1,4,5-trisphosphate) pathway, resulting in the release of intracellular Ca^{2+} from the sarcoplasmic reticulum (Chapter 3). Emptying of intracellular Ca^{2+} stores may trigger further influx of extracellular Ca^{2+} (store-operated Ca^{2+} entry), but its relevance in smooth muscle is unresolved. Third, receptor-operated Ca^{2+} channels allow the entry of extracellular Ca^{2+} in response to receptor occupancy. An increase in cytosolic Ca^{2+} results in enhanced binding of Ca^{2+} to calmodulin. The Ca^{2+}-calmodulin complex in turn activates myosin light-chain kinase, with resulting phosphorylation of the myosin light chain. Such phosphorylation promotes interaction between actin and myosin and leads to sustained contraction of smooth muscle. Ca^{2+} channel blockers inhibit the voltage-dependent Ca^{2+} channels in vascular smooth muscle and decrease Ca^{2+} entry. All Ca^{2+} channel antagonists relax arterial smooth muscle and thereby decrease arterial resistance, blood pressure, and cardiac afterload. Although experimentally large conductance veins of pig appear similarly or even more sensitive to Ca^{2+} channel blockers than arteries (Magnon et al., 1995), Ca^{2+} channel blockers do not affect cardiac preload significantly when given at normal doses in patients. This suggests that capacitance veins that determine venous return to the heart are resistant to the relaxing effect of Ca^{2+} channel antagonists.

Cardiac Cells. The mechanisms of excitation-contraction coupling in cardiac myocytes of the working myocardium differ from those in vascular smooth muscle in that increases in intracellular Ca^{2+} are fast and transient (Chapter 34). They are initiated by a fast and short (<5 msec) Na^+ influx through voltage-gated Na^+ channels that causes depolarization of the membrane and opening of L-type Ca^{2+} channels. Repolarizing K^+ currents terminate the cardiac action potential and Ca^{2+} influx. Within the cardiac myocyte, Ca^{2+} binds to troponin C, relieving the inhibitory effect of the troponin complex on the contractile apparatus and permitting productive interaction of actin and myosin, leading to contraction. By inhibiting Ca^{2+} influx, Ca^{2+} channel blockers reduce the peak size of the systolic Ca^{2+} transient and thereby produce a negative inotropic effect. Although this is true for all classes of Ca^{2+} channel blockers, the greater degree of peripheral vasodilation seen with the dihydropyridines is accompanied by a baroreceptor reflex–mediated increase in sympathetic tone sufficient to overcome the negative inotropic effect.

In the SA and AV nodes, depolarization largely depends on the movement of Ca^{2+} through the slow channel (and not on the opening of Na^+ channels as in working myocardium). The effect of a Ca^{2+} channel blocker on AV conduction and on the rate of the sinus node pacemaker depends on whether the agent delays the recovery of the slow channel (Schwartz, 1992). Although *nifedipine* reduces the slow inward current in a dose-dependent manner, it does not affect the rate of recovery of the slow Ca^{2+} channel. Although *nifedipine* has clear negative chronotropic effects in isolated preparations (at ~5-fold higher concentrations than needed for negative inotropy), at doses used clinically, *nifedipine* does not directly affect pacemaking or conduction through the AV node. Rather, it stimulates the heart indirectly by eliciting reflex sympathetic activation in response to a lowering of blood pressure (Figure 31–2).

In contrast, *verapamil* not only reduces the magnitude of the Ca^{2+} current through the slow channel but also decreases the rate of recovery of the channel. In addition, channel blockade caused by *verapamil* (and to a lesser

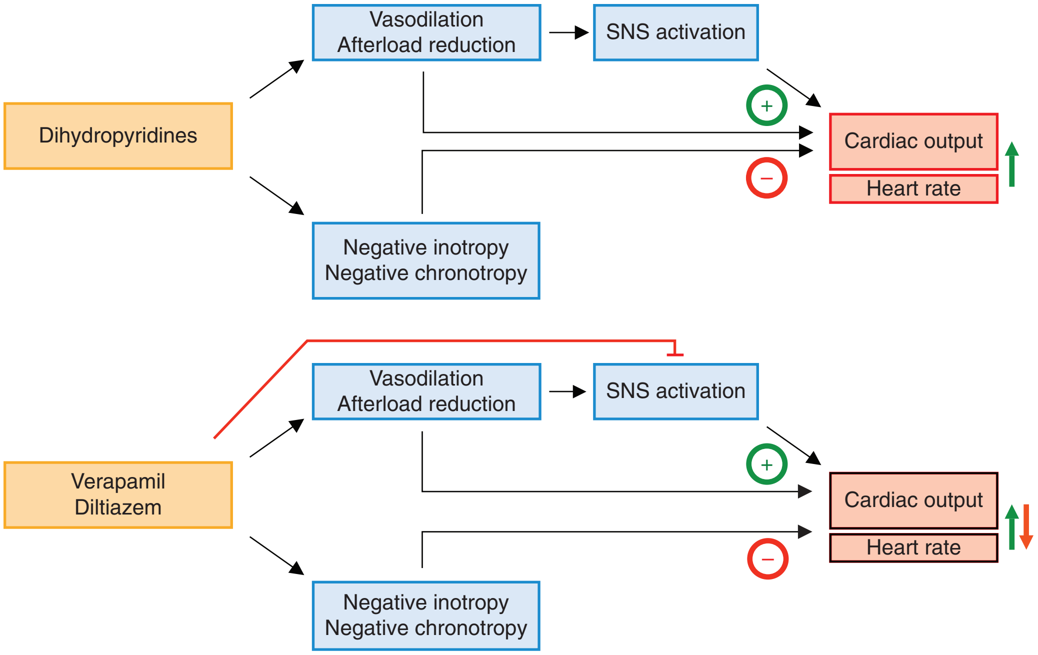


Figure 31–2 *Comparison of the integrated actions of Ca^{2+} channel blockers.* Due to different potencies and efficacies at various sites of action within the cardiovascular system, dihydropyridines produce integrated effects that are not identical to those of *verapamil* and *diltiazem*. *Verapamil* can have direct inhibitory effects on the sympathetic nervous system (SNS). The thickness of the arrow indicates the relative strength of the effect.

extent by *diltiazem*) is enhanced as the frequency of stimulation increases, a phenomenon known as *frequency dependence* or *use dependence*. *Verapamil* and *diltiazem* depress the rate of the sinus node pacemaker and slow AV conduction at clinically used doses; the latter effect is the basis for their use in the treatment of supraventricular tachyarrhythmias (see Chapter 34). *Verapamil* also inhibits fast Na^+ and repolarizing K^+ currents (I_{Kr}). The contribution of these actions to the clinical profile is unclear, but note that *verapamil*, despite the effect on I_{Kr}, has not been associated with torsades des pointes arrhythmias, as have other I_{Kr} blockers.

Integrated Cardiovascular Effects of Different Ca^{2+} Channel Antagonists. The hemodynamic profiles of the Ca^{2+} channel blockers approved for clinical use differ and depend mainly on the ratio of vasodilating and negative inotropic and chronotropic effects on the heart (Figure 31–2). The dihydropyridines dilate blood vessels at several-fold lower concentrations than those required for decreasing myocardial force; the ratio is close to one for *diltiazem* and *verapamil*. The published selectivity values differ widely, depending on the type of blood vessel and the mode of precontraction used for the comparison (Table 31–2 and Figure 31–3). The differences between the relatively vasoselective dihydropyridines and the essentially nonselective *diltiazem* and *verapamil* have important consequences because the decrease in arterial blood pressure elicits reflex sympathetic activation, resulting in the stimulation of heart rate, AV conduction velocity, and myocardial force, just the opposite of the direct effect of Ca^{2+} channel blockers. While direct and indirect effects normally balance each other in the case of *verapamil* and *diltiazem*, sympathetic stimulation often prevails in dihydropyridines, causing an increase in heart rate and contractility. Cardiac depressant effects of dihydropyridines may be unmasked, though, in the presence of β blockers and in patients with heart failure.

The dihydropyridines in clinical use—*amlodipine, clevidipine, felodipine, isradipine, lercanidipine, nicardipine, nifedipine, nimodipine*, and *nisoldipine*—share most pharmacodynamic properties. Differences regarding vascular selectivity or subvascular selectivity have been intensely studied in the past, but claims of large vasoselectivity factors were based on indirect comparisons (Godfraind et al., 1992). Overall, the clinical relevance of vasoselectivity ratios appears questionable; actual differences are probably not great (Figure 31–3). In any event, the drugs exert their antianginal effect mainly by peripheral arterial vasodilation and afterload reduction and not by coronary artery dilation (exception in variant angina).

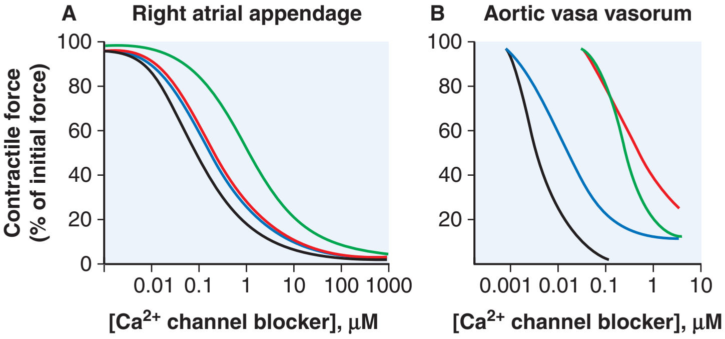


Figure 31–3 *Potency of Ca^{2+} channel blockers at different sites.* Effects were assessed on the contractile force of human right atrial appendages (**A**) and human arteries from aortic vasa vasorum precontracted with high-K^+ concentrations (**B**). *Felodipine* (black), *nifedipine* (blue), and *amlodipine* (green) are more potent on vascular muscle, inhibiting contraction of atrial muscle at concentrations about 10 times higher than those needed to reduce contraction in vascular tissue. *Verapamil* (red) inhibits atrial muscle force development at 20% of the concentration required to reduce contraction in vascular tissue. The vascular selectivity of the various Ca^{2+} channel blockers (EC_{50} [half-maximal effective concentration] on atrial appendage/EC_{50} on vasa vasorum) are as follows: felodipine, 12; nifedipine, [illegible]; amlodipine, 5; verapamil, 0.2. Figure is based on data of Angus et al., 2000.)

Verapamil, like the dihydropyridines, causes little effect on venous return and preload but has more direct negative inotropic and chronotropic effects than the dihydropyridines at doses that produce arteriolar dilation and afterload reduction (Figure 31–2). Thus, the consequences of a reflex increase in adrenergic tone are generally offset by the direct cardiodepressant effects of the drug. In patients without heart failure, oral administration of *verapamil* reduces peripheral vascular resistance and blood pressure with minimal changes in heart rate. Ventricular performance is not impaired and may improve, especially if ischemia limits its performance. In contrast, in patients with heart failure, intravenous *verapamil* can cause a marked decrease in contractility and left ventricular function. The antianginal effect of *verapamil*, like that of all Ca^{2+} channel blockers, is due primarily to a reduction in myocardial O_2 demand. The negative dromotropic effect has no relevance for the improvement of exercise but can cause second-degree AV block, particularly when given in combination with β blockers (contraindicated). *Diltiazem*'s effects lie in between those of dihydropyridines and *verapamil*.

The effects of Ca^{2+} channel blockers on diastolic ventricular relaxation (the lusitropic state of the ventricle) are complex. *Nifedipine*, *diltiazem*, and *verapamil* impaired parameters of ventricular relaxation in dogs, especially when given into the coronary arteries (Walsh and O'Rourke, 1985). However, reflex stimulation of sympathetic tone accelerates relaxation, which may outweigh a direct negative lusitropic effect. Likewise, a reduction in afterload will improve the lusitropic state. In addition, if ischemia is improved, the negative lusitropic effect will be reduced. The sum total of these effects in any given patient cannot be determined *a priori*.

ADME and Drug Interactions. Ca^{2+} channel blockers exhibit clinically relevant differences in pharmacokinetics (Figure 31–4). Immediate-release *nifedipine* is quickly absorbed after oral intake and produces only a briefly elevated blood level of the drug ($t_{1/2}$ ~1.8 h) that is associated with an abrupt decrease in blood pressure, reflex activation of the sympathetic nervous system, and tachycardia. This can cause a typical flush and can increase the risk of angina pectoris by abruptly decreasing coronary perfusion pressure concomitantly with tachycardia. Sustained-release preparations of *nifedipine* reduce fluctuations of plasma concentration. By contrast, *amlodipine* has slow absorption and a prolonged effect. With a plasma $t_{1/2}$ of 35 to 50 h, plasma levels and effect increase over 7 to 10 days of daily administration of a constant dose, resulting in a C_p with modest peaks and troughs. Such a profile allows the body to adapt and is associated with less reflex tachycardia. *Felodipine*, *nitrendipine*, *lercanidipine*, and *isradipine* have similar profiles for chronic treatment (Table 31–2). *Clevidipine* is available in the U.S. and Switzerland for intravenous administration and has a very rapid ($t_{1/2}$ ~2 min) onset and offset of action. It is metabolized by esterases in blood. It may be useful in controlling blood pressure in severe or perioperative hypertension as an alternative to GTN, *sodium nitroprusside*, or *nicardipine*.

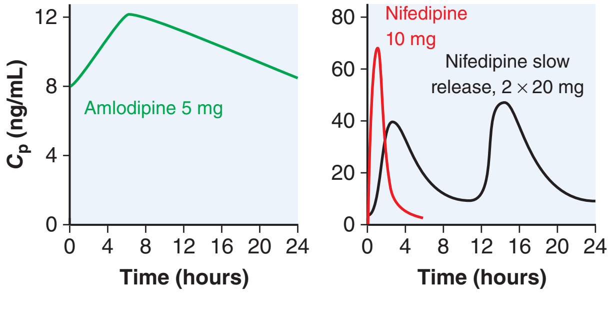


Figure 31–4 *Minimizing daily fluctuations in C_p values of Ca^{2+} channel blockers.* Graphs show plasma levels (C_p values) of *amlodipine* (left) and of *nifedipine* (right) in immediate-release (red) and slow-release (black) preparations; doses were administered at zero time. Plasma levels of *amlodipine* and *nifedipine* slow-release formulations were assessed after repeated application; thus, C_p values do not start at zero. Note the much smaller differences between trough and peak plasma concentrations in case of *amlodipine* compared to the rapid and brief pulse in plasma concentration of immediate-release *nifedipine* and the relatively large fluctuations even with the slow-release form of *nifedipine*. The plasma $t_{1/2}$ of *amlodipine* is about 39 h; that of *nifedipine* is about 1.8 h. A large fluctuation in C_p may result in adverse effects at the maximum and lack of efficacy at the minimum (see Figure 2–9A). (For original data, see Bainbridge et al., 1993; Debbas et al., 1986; and van Harten et al., 1987.)

The bioavailability of all Ca^{2+} channel blockers is reduced, in some cases markedly, by first-pass metabolism by CYP3A4 enzymes in the intestinal epithelium and the liver. This has two consequences:

- The bioavailability of these drugs may be increased by strong inhibitors of CYP3A4, such as macrolide and imidazole antibiotics, antiretroviral agents, and grapefruit juice (see Chapter 5). Bioavailability is reduced by inducers of CYP3A4, such as *rifampin*, *carbamazepine*, and hypericum (St. John's wort).
- Some Ca^{2+} channel blockers (particularly *verapamil*) are strong CYP3A4 inhibitors and cause clinically relevant drug interactions with other CYP3A4 substrates, such as *simvastatin* and *atorvastatin*.

Moreover, *verapamil* is a relatively efficient inhibitor of the intestinal and renal ABC transport protein P-glycoprotein (Pgp) (also called MDR1 and ABCB1; see Chapter 5) and can thereby increase plasma levels of *digoxin*, *cyclosporine*, and *loperamide* and other agents that are exported by Pgp. The same mechanism operates at the blood-brain barrier, leading to high exposure of the central nervous system (cave central actions of *loperamide*). This high potential of *verapamil* for drug-drug interactions is a clear disadvantage and one of the reasons for its declining use. In patients with hepatic cirrhosis, the bioavailabilities and half-lives of the Ca^{2+} channel blockers may be increased, and dosage should be decreased accordingly. The half-lives of these agents also may be longer in older patients.

Toxicity and Untoward Responses. The profile of adverse reactions to the Ca^{2+} channel blockers varies among the drugs in this class. Immediate-release capsules of *nifedipine* often cause headache, flushing, and dizziness and can actually worsen myocardial ischemia. Dizziness and flushing are much less of a problem with the sustained-release formulations and with the dihydropyridines having a long $t_{1/2}$ and providing more constant plasma drug concentrations. Peripheral edema may occur in some patients with Ca^{2+} channel blockers but is not the result of generalized fluid retention; rather, it most likely results from increased hydrostatic pressure in the lower extremities owing to precapillary dilation and reflex postcapillary constriction (Epstein and Roberts, 2009). *Lercanidipine* appears to cause less peripheral edema than other dihydropyridines (Makarounas-Kirchmann et al., 2009). Other adverse effects of these drugs are due to actions in nonvascular smooth muscle. For example, Ca^{2+} channel blockers can cause or aggravate gastroesophageal reflux. Constipation is a common side effect of *verapamil* but occurs less frequently with other Ca^{2+} channel blockers. Urinary retention is a rare adverse effect. Uncommon adverse effects include rash and elevations of liver enzymes.

Although bradycardia, transient asystole, and exacerbation of heart failure have been reported with *verapamil*, these responses usually have occurred after intravenous administration of *verapamil* in patients with disease of the SA node, with AV nodal conduction disturbances, or in the presence of β blockade. The use of *verapamil* with a β blocker is contraindicated because of the increased propensity for AV block or severe depression of ventricular function. Patients with ventricular dysfunction, SA or AV nodal conduction disturbances, and systolic blood pressures below 90 mm Hg should not be treated with *verapamil* or *diltiazem*, particularly intravenously. *Verapamil* may also exacerbate AV nodal conduction disturbances observed with *digoxin*, both for pharmacodynamic and pharmacokinetic reasons (Pgp inhibition; see previous discussion). When used with *quinidine*, *verapamil* may cause excessive hypotension, again due to pharmacodynamic and pharmacokinetic reasons (*quinidine* is a CYP3A4 substrate and Pgp inhibitor).

Therapeutic Uses

Variant Angina. Variant angina results from reduced blood flow (a consequence of transient localized vasoconstriction) rather than increased

O_2 demand. Drug-induced causes (e.g., cocaine, amphetamines, *sumatriptan*, and related antimigraine drugs) should be excluded. Ca^{2+} channel blockers are effective in about 90% of patients (Collet et al., 2020). These agents are considered first-line treatment and may be combined with nitrovasodilators (Amsterdam et al., 2014).

Exertional Angina. Ca^{2+} channel blockers also are effective in the treatment of exertional, or exercise-induced, angina. Numerous double-blind, placebo-controlled studies have shown that these drugs decrease the number of anginal attacks and attenuate exercise-induced ST-segment depression, but evidence for life-prolonging efficacy is lacking. They are therefore considered the drugs of choice if β blockers do not achieve sufficient symptomatic benefit or are not tolerated (Knuuti et al., 2020).

The Ca^{2+} channel blockers reduce the *double product*, Heart rate × Systolic blood pressure, an approximate index of myocardial O_2 demand. Because these agents reduce the level of the double product at a given external workload and because the value of the double product at peak exercise is not altered, the beneficial effect of Ca^{2+} channel blockers likely derives from a decrease in O_2 demand rather than an increase in coronary flow.

Concurrent therapy of a dihydropyridine with a β blocker has proven more effective than either agent given alone in exertional angina, presumably because the β blocker suppresses reflex tachycardia. This concurrent drug therapy is particularly attractive because the dihydropyridines do not delay AV conduction and will not enhance the negative dromotropic effects associated with β receptor blockade. In contrast, the concurrent administration of *verapamil* or *diltiazem* with a β blocker is contraindicated for the potential for AV block, severe bradycardia, and decreased left ventricular function.

Unstable Angina (Acute Coronary Syndrome). In the past, Ca^{2+} channel blockers were routinely administered in patients presenting with unstable angina and ACS without persistent ST elevation. Reports about trends for harm with immediate-release *nifedipine* or *nifedipine* infusion in the absence of β blockers have led to the recommendation not to use dihydropyridines without concurrent therapy with β blockers. *Verapamil* and *diltiazem* are recommended only for patients who continue to show signs of ischemia, do not tolerate β blockers, have no clinically significant left ventricular dysfunction, and show no signs of disturbed AV conduction (Amsterdam et al., 2014).

Other Uses. The use of *verapamil* and *diltiazem* (but not dihydropyridines) as antiarrhythmic agents in supraventricular tachyarrhythmias is discussed in Chapter 34; their use for the treatment of hypertension is discussed in Chapter 32. Ca^{2+} channel blockers are contraindicated in patients with heart failure with reduced ejection fraction, but *amlodipine* and *felodipine* did not worsen the prognosis and can therefore be administered if indicated for other reasons (Chapter 33). *Verapamil* improves left ventricular outflow obstruction and symptoms in patients with hypertrophic cardiomyopathy (HCM). *Diltiazem* has shown early promising results in a clinical study in asymptomatic HCM mutation carriers (Ho et al., 2015). *Verapamil* also has been used in the prophylaxis of migraine headaches but is considered a second-choice drug. *Nimodipine* has been approved for use in patients with neurological deficits secondary to cerebral vasospasm after the rupture of a congenital intracranial aneurysm (Hanggi et al., 2017). *Nifedipine*, *diltiazem*, *amlodipine*, and *felodipine* appear to provide symptomatic relief in Raynaud disease. The Ca^{2+} channel blockers cause relaxation of the myometrium *in vitro* and may be effective in reducing preterm uterine contractions in preterm labor (see Chapter 48). A recent study suggests that *verapamil* can delay the progression of early stages of type 1 diabetes by improving the survival of pancreatic beta cells (Ovalle et al., 2018).

β Blockers

β Blockers are the only drug class that is effective in reducing the severity and frequency of attacks of exertional angina and in improving survival in patients who have had an MI. They are therefore recommended as first-line treatment of patients with stable CAD (Knuuti et al., 2020) and unstable angina/ACS (Hamm et al., 2011). Recent meta-analyses raised doubts about the mortality-reducing effects of β blockers in the MI reperfusion era (Bangalore et al., 2014); however, some of the results, such as the slightly increased heart failure frequency in patients receiving β blockers, contradicted numerous well-controlled prospective trials (see Chapter 33) and raised doubts about the validity of the analysis. Thus, the issue has not been definitively resolved. β Blockers are not useful for vasospastic angina and, if used in isolation, may worsen that condition. β Blockers appear equally effective in the treatment of exertional angina (Fihn et al., 2012; Knuuti et al., 2020), but very short-acting agents or drug formulations giving rise to large fluctuations of plasma concentrations (e.g., unformulated *metoprolol*) should be avoided for treatment of chronic CAD.

The effectiveness of β blockers in the treatment of exertional angina is attributable primarily to a fall in myocardial O_2 consumption at rest and during exertion. The decrease in myocardial O_2 consumption is due to a negative chronotropic effect (particularly during exercise), a negative inotropic effect, and a reduction in arterial blood pressure (particularly systolic pressure) during exercise. A decrease in heart rate prolongs the time of myocardial perfusion during diastole. Moreover, there is evidence that β blockers can increase blood flow toward ischemic regions by increasing coronary collateral resistance and preventing blood from being shunted away from the ischemic myocardium during maximal coronary vasodilation (Billinger et al., 2001), a "reverse steal or Robin Hood phenomenon" (see previous discussion).

Not all actions of β blockers are beneficial in all patients. The decreases in heart rate and contractility cause increases in the systolic ejection period and left ventricular end-diastolic volume; these alterations tend to increase O_2 consumption. However, the net effect of β blockade usually is to decrease myocardial O_2 consumption, particularly during exercise. In patients with limited cardiac reserve who are critically dependent on adrenergic stimulation, β blockade can result in profound decreases in left ventricular function. Despite this, several β blockers demonstrably reduce mortality in patients with congestive heart failure, and β blockers have become standard therapy for many such patients (see Chapters 14 and 33).

Numerous β blockers are approved for clinical use. β Blockers commonly used for the treatment of angina are β_1-selective and without intrinsic sympathomimetic activity (e.g., *atenolol*, *bisoprolol*, or *metoprolol*, Table 31–3). Chapter 14 presents their pharmacology in detail.

Antiplatelet, Anti-integrin, and Antithrombotic Agents

Antiplatelet agents represent the cornerstone of therapy for ACS (Amsterdam et al., 2014; Collet et al., 2020) and are systematically discussed in Chapter 36. They interfere either with two signaling pathways (thromboxane A2 [TxA2] and ADP) that cooperatively promote platelet aggregation in an auto- and paracrine manner or with a major common pathway of platelet aggregation, the glycoprotein (Gp) IIb/IIIa fibrinogen receptor. *Aspirin* inhibits platelet aggregation by irreversibly inactivating the thromboxane-synthesizing cyclooxygenase isoform 1 (COX-1) in platelets, thereby reducing production of TxA_2. *Aspirin*, given at doses of 160 to 325 mg at the onset of treatment of ACS, improves survival (Yeghiazarians et al., 2000). The thienopyridines are ADP receptor ($P2Y_{12}$ receptor) antagonists that block the pro-aggregatory effect of ADP, which is stored in vesicles within platelets and released when platelets adhere to prothrombotic structures. The pro-aggregatory synergism of TxA_2 and ADP on platelet aggregation and thrombus formation accounts for the potentiating effect of adding a thienopyridine to *aspirin*.

The addition of the thienopyridine *clopidogrel* to *aspirin* therapy reduces mortality in patients with ACS. Newer thienopyridines (*prasugrel, ticagrelor, cangrelor*) with favorable pharmacokinetic properties have been approved for the treatment of ACS. All three appear superior to *clopidogrel* in treating patients with ACS; contributing factors likely include faster onset of action and less variable pharmacokinetics. *Ticagrelor* is a direct, reversible $P2Y_{12}$ receptor antagonist, whereas *clopidogrel* and *prasugrel* are both prodrugs. The hepatic activation of *prasugrel* is more stable and faster than that of *clopidogrel*. *Cangrelor* is the first $P2Y_{12}$ receptor antagonist for intravenous application, producing very rapid inhibition of platelet aggregation. Recent guidelines recommend *ticagrelor* and

TABLE 31–3 ■ β BLOCKERS COMMONLY USED IN ISCHEMIC HEART DISEASE

AGENT	β_1-SELECTIVE	VASODILATION	HALF-LIFE (h)	STANDARD DOSE (mg)[a]	DOSE ADAPTATION IN KIDNEY FAILURE	CYP DEPENDENCE
Atenolol	Yes	No	6–10	1 × 50–100	Yes	No
Bisoprolol	Yes	No	10–12	2.5–10	No	No
Carvedilol	No	Yes	6–10	2 × 12.5–50	No	CYP2D6
Metoprolol succinate[b]	Yes	No	>12[b]	1 × 47.5–190	No	CYP2D6

[a] Doses of β blockers need to be individually adapted according to the heart rate response, which can vary widely between individuals. Evidence for fixed target doses such as in the treatment of heart failure are lacking for patients with ischemic heart disease. In general and particularly in the elderly, treatment should be started with the lowest dose, which is to be increased in 2- to 4-weekly intervals until heart rate decreases and angina pectoris symptoms improve.

[b] Metoprolol itself has a half-life of 3–5 h and is therefore not sufficiently long acting for the desired 24-h efficacy. It is therefore recommended to use only slow-release preparations. The numbers presented here apply to metoprolol succinate in a slow-release formulation (zero order of kinetics). β Blockers should never be stopped acutely, but their dose should be slowly reduced to avoid excessive increases in heart rate, relapse of angina pectoris, and cardiac arrhythmia such as atrial fibrillation.

prasugrel as the primary choice in patients with ACS and *clopidogrel* as an alternative in patients who cannot receive the former or are on oral anticoagulation therapy (e.g., for stroke prevention in atrial fibrillation). The place of *cangrelor* is not yet fully defined (Collet et al., 2020). *Cangrelor* is approved as adjunct treatment for reducing the risk of periprocedural MI, repeat coronary revascularization, and stent thrombosis in patients who have not been treated with a $P2Y_{12}$ platelet inhibitor and are not being given a GpIIb/IIIa inhibitor.

The optimal timing of the initiation of dual platelet treatment is controversial and depends on the likely clinical course. If conservative treatment is likely and the patient is not at an increased risk of bleeding, *aspirin* and a parenteral anticoagulant (see discussion that follows) should be given as soon as possible, with the addition of a $P2Y_{12}$ receptor antagonist as soon as the diagnosis of non-STEMI has been made. Dual platelet inhibition for 1 year is currently recommended for all patients after non-STEMI or STEMI and revascularization, independently of the type of revascularization and type of stent used (Collet et al., 2020). Due to the irreversible (*aspirin, clopidogrel*, and *prasugrel*) or prolonged (*ticagrelor*) modes of action, the risk of bleeding remains increased for extended periods after withdrawal of these drugs. Nonemergency major noncardiac surgeries should therefore be postponed for 5 (*ticagrelor*, *clopidogrel*) or 7 days (*prasugrel*, *aspirin*) after intake of the last dose.

Anti-integrin agents directed against the platelet integrin GpIIb/IIIa (including *abciximab*, *tirofiban*, and *eptifibatide*) are highly effective by blocking the final effector pathway of platelet aggregation; however, these agents have a small therapeutic index and must be administered parenterally. Meta-analyses of studies in patients with ACS showed that the use of GpIIb/IIIa inhibitors in addition to *heparin* was associated with about a 10% reduction in mortality, but with an increase in bleeding. Because most of these trials were conducted before the widespread use of the newer and more effective thienopyridines *prasugrel* and *ticagrelor*, the current value of the GpIIb/IIIa antagonists is not clear. Guidelines recommend them in patients on *prasugrel* or *ticagrelor* only in bailout situations (Amsterdam et al., 2014; Collet et al., 2020).

Heparin, in its unfractionated form and as low-molecular-weight *heparin* (e.g., *enoxaparin, dalteparin*), also reduces symptoms and prevents infarction in unstable angina (Yeghiazarians et al., 2000) and is currently recommended as first choice (Collet et al., 2020). *Fondaparinux*, a heparinoid pentasaccharide, antithrombin III–dependent factor Xa inhibitor, is recommended only in cases of an expected delay in mechanical coronary interventions. Thrombin inhibitors, such as *hirudin* or *bivalirudin*, directly inhibit even clot-bound thrombin, are not affected by circulating inhibitors, and function independently of antithrombin III. *Bivalirudin* provides no benefit over *heparin* in ACS (Valgimigli et al., 2015). Thrombolytic agents such as recombinant tissue plasminogen activator (rTPA) (*alteplase*) are of no benefit in unstable angina. The new oral anticoagulants (factor IIa inhibitor *dabigatran* and factor Xa inhibitors *rivaroxaban*, *apixaban*, and *edoxaban*) are recommended in patients with ACS and concomitant atrial fibrillation in addition to the dual antiplatelet therapy ("triple therapy"), but higher bleeding rates need to be considered (Collet et al., 2020). The addition of very-low-dose *rivaroxaban* (2 × 2.5 mg) to *aspirin* has been successfully tested for long-term prevention in patients with stable CAD (Eikelboom et al., 2017). *Vorapaxar*, first in its class, inhibits the protease-activated receptor-1 (PAR-1), the primary receptor for thrombin, which is a potent activator of platelets. *Vorapaxar* was shown to significantly reduce thrombotic cardiovascular events in patients with a history of MI or with peripheral artery disease.

Other Antianginal Agents

Ranolazine

Ranolazine is FDA and EMA approved as a second-line agent for the treatment of chronic angina. The drug may be used with a variety of other agents, including β blockers, Ca^{2+} channel blockers, ACEIs, ARBs, and therapeutic agents for lowering lipids and reducing platelet aggregation.

Mechanism of Action. The mechanism of *ranolazine*'s therapeutic efficacy in angina is uncertain. Its anti-ischemic and antianginal effects occur independently of reductions in heart rate and arterial blood pressure or changes in coronary blood flow. *Ranolazine* inhibits several cardiac ion fluxes, including I_{Kr} and I_{Na}. Preferential inhibition of late I_{Na} may explain its cardiac effects (Hasenfuss and Maier, 2008). The late I_{Na} contributes to arrhythmias in patients with the rare long QT 3 syndrome (see Chapter 34) and is increased in heart failure and ischemia. Reduction of the late I_{Na} could explain in part the elevated cytosolic Na^+ concentrations in cardiac myocytes in these conditions, leading to higher diastolic Ca^{2+} concentrations, Ca^{2+} overload, arrhythmias, and problems with diastolic relaxation.

Other mechanisms of action have been proposed. *Ranolazine* reduces cardiac fatty acid oxidation and stimulates glucose metabolism without inhibiting carnitine palmityl transferase 1 (McCormack et al., 1998). However, the effect is small, occurs at *ranolazine* concentrations more than 5-fold higher than do therapeutic effects, and can be assessed in the absence of fatty acid oxidation (Belardinelli et al., 2006). *Ranolazine* has weak β receptor blocking activity (Letienne et al., 2001) that may contribute to its antianginal activity.

Ranolazine did not improve the prognosis of patients with incomplete revascularization after percutaneous coronary intervention (Weisz et al., 2016), nor did it affect symptoms or measures of left ventricular diastolic function or dimensions in patients with HCM (Olivotto et al., 2018). These data question the therapeutic value of the compound.

ADME and Adverse Effects. *Ranolazine*, supplied as extended-release tablets, is administered without regard to meals at 500 to 1000 mg twice daily; higher doses are poorly tolerated. The drug's oral bioavailability is about 75%; inhibitors of Pgp (e.g., *digoxin*, *cyclosporine*; see Chapter 5) can increase absorption of *ranolazine* and increase exposure to both *ranolazine* and the competing drug. *Ranolazine*'s terminal $t_{1/2}$ is about 7 h; with repeated dosing, a steady-state C_p is reached in 3 days. *Ranolazine* is metabolized mainly by CYP3A4 and to a lesser extent by CYP2D6; unchanged drug (5%) and metabolites are excreted in the urine.

Ranolazine should not be used together with strong CYP3A4 inhibitors (e.g., macrolide and imidazole antibiotics, HIV protease inhibitors), and doses need to be limited when moderate CYP3A4 inhibitors such as *verapamil, diltiazem*, and *erythromycin* are used in combination. Inducers of CYP3A4 (e.g., *rifampin, carbamazepine*, and hypericum) can decrease *ranolazine* plasma levels, requiring dose adjustment. *Ranolazine* can affect plasma levels of other CYP3A4 substrates, including doubling levels of *simvastatin* and its active metabolite and requiring dose adjustment; dose reduction may be needed for other CYP3A4 substrates (e.g., *lovastatin*), especially for those with a narrow therapeutic range (e.g., *cyclosporine*, *tacrolimus*, *sirolimus*). Coadministration of *ranolazine* may increase exposure to other substrates of CYP2D6, such as tricyclic antidepressant drugs and antipsychotic agents.

The most frequent adverse effects are dizziness, headache, nausea, and constipation. Some CNS effects (e.g., dizziness, blurry vision, and confusional state) are reminiscent of class I antiarrhythmics. QT prolongation must be considered, but no torsades des pointes arrhythmias or related events have been reported.

Ivabradine

Ivabradine is EMA approved for treating stable angina and heart failure in patients in whom β blockers are not tolerated or are insufficiently effective in reducing heart rate and is FDA approved only for the treatment of heart failure (Chapter 33). *Ivabradine* is a selective blocker of hyperpolarization-activated hyperpolarization-activated cyclic nucleotide–gated (HCN) ion channels involved in the generation of automaticity in the SA node. By reducing the pacemaker current I_f through HCN channels, the compound dose dependently reduces heart rate and, differently from β blockers, does not affect cardiac contractile force. The antianginal effect is explained solely by reduction of heart rate and thereby O_2 demand (Figure 31–1).

A typical, often transient, side effect are phosphenes, transient enhanced lightness in restricted areas of the visual field, that are explained by effects on retinal HCN channels (3%–5% of cases). In a recent study in patients with chronic angina and normal left ventricular function, the addition of *ivabradine* to β blockers did not confer benefit but was associated with a trend for more cardiovascular end points and an increase in symptomatic bradycardia, atrial fibrillation, and QT prolongation (Fox et al., 2014). The data raise doubts about the hypothesis that heart rate reduction per se is associated with better cardiovascular outcome and has led to restrictions on use of *ivabradine* (e.g., contraindication for concurrent therapy with *verapamil* or *diltiazem*).

Nicorandil

Nicorandil is a nitrate ester of nicotinamide developed as an antianginal agent and currently is approved in many Asian and European countries for the treatment of stable angina pectoris. *Nicorandil* is not available in the U.S.

Mechanism of Action and Pharmacological Effects. *Nicorandil* has nitrate-like (cGMP-dependent) properties and acts as an agonist at ATP-sensitive potassium (K_{ATP}) channels. Its vasodilating action is potentiated by PDE5 inhibitors and only partially blocked by inhibitors of K_{ATP} channels, such as *glibenclamide*, suggesting that both properties participate in *nicorandil*'s effect. *Nicorandil* dilates both arterial and venous vascular beds, leading to decreases in afterload and preload of the heart. In the absence of direct effects on contractile force of the ventricles, the decrease in afterload causes cardiac output to increase. The last effect is stronger than that seen after administration of nitrovasodilators and partially explained by (reflex) tachycardia. Thus, the hemodynamic profile of *nicorandil* lies in between that of nitrovasodilators and dihydropyridine Ca^{2+} channel blockers. Its antianginal effect is described to be stable, but early studies reported a clear decrease or loss of antianginal effect after 2 weeks of oral treatment (Meany et al., 1989; Rajaratnam et al., 1999).

Experimental and clinical studies indicated that *nicorandil* has cardioprotective effects (Matsubara et al., 2000), mimicking that of ischemic preconditioning, a phenomenon where short periods of ischemia preceding prolonged stopping of perfusion (as in MI) reduce myocardial injury. While the exact mechanisms are not fully understood, a central role of mitochondrial K_{ATP} channels is assumed (Ardehali and O'Rourke, 2005; Sato et al., 2000). Retrospective studies indicated a survival-prolonging effect of chronic treatment with *nicorandil* in patients with stable CAD, but sufficiently powered prospective studies are lacking.

ADME and Adverse Effects. *Nicorandil* is rapidly absorbed after sublingual or oral administration and has a short $t_{1/2}$ (1 h), which does not provide relevant trough levels at the usual regimen of twice-daily dosing at 20 mg/dose. Besides nitrate-like headache and hypotension (note contraindication of concurrent PDE5 inhibitors), *nicorandil* has been associated with the appearance of ulcerations. They were first described in 1997 (Boulinguez et al., 1997) as large, painful, buccal apthosis and seem to extend to a 40% to 60% increased risk of GI ulcerations and perforations (Lee et al., 2015).

Trimetazidine

Trimetazidine was developed as an antianginal agent. *Trimetazidine* is approved in several European countries but not in the U.S. Its effect is thought to be due to inhibition of long-chain 3-ketoacyl coenzyme A thiolase, the final enzyme in the free fatty acid (FFA) β-oxidation pathway. This leads to a partial shift from FFA to glucose oxidation in the heart, which provides less ATP but requires less O_2 and may therefore be beneficial in ischemia (Ussher et al., 2014). Numerous small studies provided evidence for the efficacy of the compound to reduce angina and increase exercise tolerance, particularly in patients with diabetes and heart failure (e.g., Tuunanen et al., 2008). However, a recent, adequately powered trial in patients after percutaneous coronary intervention showed no benefit of adding *trimetazidine* to standard care (Ferrari et al., 2020).

Trimetazidine can cause GI upset, nausea, and vomiting, and rarely, it has been associated with thrombocytopenia, agranulocytosis, and liver dysfunction. More important, *trimetazidine* may increase the risk of movement disorders such as Parkinson's disease, particularly in older patients with decreased kidney function. This serious effect has led to use restrictions by the EMA and the recommendation to use *trimetazidine* only as second-line treatment of stable angina in patients inadequately controlled by or intolerant to first-line antianginal therapies.

Therapeutic Strategies

Stable Coronary Artery Disease

Guidelines

Task forces from the American College of Cardiology and the American Heart Association (Fihn et al., 2012) and the European Society of Cardiology (Knuuti et al., 2020) have published guidelines that are useful in the selection of appropriate initial therapy for patients with chronic stable angina pectoris. All patients with CAD should receive at least one drug for angina relief in addition to fast- and short-acting nitrovasodilators (GTN, ISDN) and, for event prevention, *aspirin* and a statin. ACEIs should be considered in patients with CAD who have left ventricular dysfunction or diabetes (Table 31–4).

The evidence for clinically relevant differences between the three main classes of antianginal drugs is not compelling. A meta-analysis of publications that compared two or more antianginal therapies concluded that β blockers are associated with fewer episodes of angina per week and a lower rate of withdrawal due to adverse events than is *nifedipine*. However, differences did not extend to Ca^{2+} channel blockers other than *nifedipine*. Of note, no significant differences were observed in outcome between *long-acting* nitrates, Ca^{2+} channel blockers, and β blockers. Nevertheless, guidelines recommend that β blockers be considered first-line treatment of chronic angina relief; Ca^{2+} channel blockers with heart rate–lowering effects (*diltiazem, verapamil*) are alternatives. Dihydropyridines should be considered in patients who do not tolerate β blockers. In case of persistent angina, a combination of a dihydropyridine and a β blocker should be considered.

Second-Line Treatment

Longer-acting organic nitrates/nitrate formulation (e.g., cutaneous GTN) or *ranolazine* and, in non-U.S. countries, *ivabradine, trimetazidine*, and *nicorandil* may be considered as adjunct therapy in patients whose angina is not adequately controlled by first-line drugs. β Blockers can block the

TABLE 31-4 ■ MANAGEMENT OF PATIENTS WITH STABLE CORONARY ARTERY DISEASE

TREATMENT LEVEL	ANGINA RELIEF	EVENT PREVENTION
All patients	Short-acting nitrates as standby medication	Education: Lifestyle management, control of risk factors
First-line treatment	β Blockers or diltiazem/verapamil	Aspirin + statins; consider ACEIs or ARBs
	Long-acting dihydropyridine if heart rate is low or there are issues of intolerance/contraindications	
	β Blockers + dihydropyridines if angina persists	
	For vasospastic angina, consider dihydropyridines or long-acting nitrates; avoid β blockers	
Second-line treatment (first line in some cases, according to comorbidities and tolerance)	Add or switch to ivabradine, long-acting nitrates, nicorandil, ranolazine,[a] or trimetazidine[a]	Consider clopidogrel in cases of aspirin intolerance
Invasive therapy	Consider angiography and stenting or coronary artery bypass grafting	

[a]In patients with diabetes mellitus.

Source: Adapted from the European Society for Cardiology Guidelines; for details, see Knuuti et al., 2020.

baroreceptor-mediated reflex tachycardia and positive inotropic effects that may occur with nitrates, whereas nitrates, by increasing venous capacitance, can attenuate the increase in left ventricular end-diastolic volume associated with β blockade. Concurrent administration of nitrates also can alleviate the increase in coronary vascular resistance associated with blockade of β adrenergic receptors. *Ranolazine* and *trimetazidine* have a direct effect on the myocardium and likely act independently of hemodynamic effects. They can therefore be well combined with all other antianginal drugs where permitted. *Ivabradine* is a possible alternative to β blockers but is associated with toxicity when added to β blockers, *verapamil*, or *diltiazem* (Fox et al., 2014).

Ca^{2+} Channel Blockers and Nitrates. In severe exertional or vasospastic angina, the combination of a nitrate and a Ca^{2+} channel blocker may provide additional relief over that obtained with either type of agent alone. Because nitrates primarily reduce preload, whereas Ca^{2+} channel blockers reduce afterload, the net effect on reduction of O_2 demand should be additive; however, excessive vasodilation and hypotension can occur.

Acute Coronary Syndromes

The term *ACS* refers to chest pain with or without MI (i.e., myocardial necrosis). The latter diagnosis is essentially based on the presence or absence of increases in plasma levels of cardiac troponin (I or T). With tests becoming more and more sensitive, the number of MI diagnoses has increased, while that of unstable angina (i.e., chest pain without necrosis) has decreased. The term *unstable angina pectoris* is used for angina symptoms that present for the first time, change their usual pattern, occur at rest, or are resistant to nitrates.

Common to most clinical presentations of ACS is a disruption of a coronary plaque, leading to local platelet aggregation and thrombosis at the arterial wall, with subsequent partial or total occlusion of the vessel. Less commonly, vasospasm in minimally atherosclerotic coronary vessels may account for unstable angina. The pathophysiological principles that underlie therapy for exertional angina—which are directed at decreasing myocardial O_2 *demand*—have limited efficacy in the treatment of ACSs characterized by an insufficiency of myocardial O_2 (blood) *supply*. The most important interventions are as follows:

- Antiplatelet agents, including *aspirin* and thienopyridines (e.g., *clopidogrel, prasugrel,* or *ticagrelor*)
- Antithrombin agents such as *heparin* or *fondaparinux*
- Anti-integrin therapies that directly inhibit platelet aggregation mediated by glycoprotein GpIIb/IIIa
- Primary angioplasty with percutaneously deployed intracoronary stents or, if not possible for logistical reasons, fibrinolysis with rTPA (*alteplase*), *reteplase,* or *tenecteplase*.
- Coronary bypass surgery for selected patients

The β blockers reduce O_2 consumption and arrhythmias and have been associated with a moderate reduction in mortality in ACS but should be avoided in patients with compromised ventricular function or decreased blood pressure (Collet et al., 2020). Nitrates are useful in reducing vasospasm and in reducing myocardial O_2 consumption by decreasing ventricular wall stress. Intravenous administration of *nitroglycerin* allows high concentrations of the drug to be attained rapidly. Because *nitroglycerin* is degraded rapidly, the dose can be titrated quickly and safely using intravenous administration. If coronary vasospasm is present, intravenous *nitroglycerin* is likely to be effective, although the addition of a Ca^{2+} channel blocker may be required to achieve complete control in some patients. If a patient has consumed a PDE5 inhibitor within the preceding 24 h, there is a risk of profound hypotension, and nitrates should be withheld in favor of an alternate antianginal therapy.

While these principles apply to the entire group of patients with ACS, specific treatment algorithms and the value of different drug classes in ACS depend on the exact diagnosis and should be chosen according to recent guidelines (Collet et al., 2020).

STEMI is generally due to a complete obstruction of a large coronary artery. The mainstay in these patients is immediate reperfusion by primary angioplasty and stenting or, in the absence of invasive options, fibrinolytic therapy.

Non-STEMI can present with variable symptoms and electrocardiographic signs and is likely due to transient obstructions of larger coronary arteries or occlusion of small branches, leading to disseminated myocardial necrosis. Primary angioplasty is also indicated in these patients.

Unstable angina is differentiated from non-STEMI by the absence of increased plasma troponin concentrations. These patients have a better long-term prognosis and benefit less from early invasive procedures and intensified antiplatelet therapy. Mainstays are β blockers and nitrovasodilators (in the absence of contraindications such as hypotension). Short-acting Ca^{2+} channel blockers (e.g., *nifedipine*; see Figure 31–4) should normally be avoided in ACS because of a strong reflex activation of the sympathetic nervous system, but they are the first choice if vasospasm is the underlying cause.

Claudication and Peripheral Vascular Disease

Most patients with peripheral vascular disease also have CAD, and the therapeutic approaches for peripheral and coronary arterial diseases overlap (Gerhard-Herman et al., 2017). Mortality in patients with peripheral vascular disease is most commonly due to cardiovascular disease (Regensteiner and Hiatt, 2002), and treatment of CAD remains the central focus of therapy. Many patients with advanced peripheral arterial disease (PAD) are more limited by the consequences of peripheral ischemia than by myocardial ischemia. In the cerebral circulation, arterial disease may be manifest as stroke or transient ischemic attacks. The painful symptoms of PAD in the lower extremities (claudication) typically are provoked by exertion, with increases in skeletal muscle O_2 demand exceeding blood flow that is impaired by proximal stenoses. When flow to the extremities becomes critically limiting, peripheral ulcers and rest pain from tissue ischemia can become debilitating.

Most of the therapies shown to be efficacious for treatment of CAD also have a salutary effect on progression of PAD (Hirsch et al., 2006). Antiplatelet therapy with *aspirin* (75–325 mg) or *clopidogrel* (75 mg), statins, and antihypertensive treatment are recommended in all patients with PAD (Gerhard-Herman et al., 2017). Oral anticoagulation is ineffective and increases bleeding risks. Interestingly, neither intensive treatment of diabetes mellitus nor antihypertensive therapy appears to alter the progression of symptoms of claudication. Other risk factor and lifestyle modifications remain cornerstones of therapy for patients with claudication; physical exercise, rehabilitation, and smoking cessation (possibly supported by drug treatment with *varenicline* or *bupropion*) have proven efficacy.

Cilostazol is specifically used in the treatment of lower extremity claudication. *Cilostazol* is an inhibitor of PDE3 and promotes accumulation of intracellular cAMP in many cells, including blood platelets. *Cilostazol*-mediated increases in cAMP inhibit platelet aggregation and promote vasodilation. The drug is metabolized by CYP3A4 and has important drug interactions with other drugs metabolized via this pathway (see Chapter 7). *Cilostazol* has been mainly studied in Asian populations and seems to improve symptoms of claudication, but the effect on cardiovascular mortality remains unclear (Bedenis et al., 2014). As a PDE3 inhibitor, *cilostazol* is in the same drug class as *milrinone*, which had been used orally as an inotropic agent for patients with heart failure. *Milrinone* therapy was associated with an increase in sudden cardiac death, and the oral form of the drug was withdrawn from the market. Concerns about several other inhibitors of PDE3 (*inamrinone, flosequinan*) followed. *Cilostazol* therefore is labeled as being contraindicated in patients with heart failure, although it is not clear that *cilostazol* itself leads to increased mortality in such patients. *Cilostazol* has been reported to increase nonsustained ventricular tachycardia; headache is the most common side effect.

Pentoxifylline is a methylxanthine derivative that is called a *rheologic modifier* for its effects on increasing the deformability of red blood cells. However, the effects of *pentoxifylline* on lower extremity claudication appear to be modest and not sufficiently supported by prospective evidence (Salhiyyah et al., 2015). Its use is not recommended anymore, similar to chelation therapy or B-complex vitamins (Gerhard-Herman et al., 2017). In contrast, all patients with PAD should be vaccinated against influenza.

Mechanopharmacological Therapy: Drug-Eluting Endovascular Stents

Intracoronary stents can ameliorate angina and reduce adverse events in patients with ACSs. However, the long-term efficacy of intracoronary stents is limited by subacute luminal restenosis within the stent, which, in bare metal stents, occurs in 20% to 30% of patients during the first 6 to 9 months of follow-up (Collet et al., 2020). The pathways that lead to "in-stent restenosis" are complex, but smooth muscle proliferation within the lumen of the stented artery is a common pathological finding. Local antiproliferative therapies at the time of stenting have been explored over many years; several drug-eluting stents and, more recently, biodegradable stents have been introduced in the market. The drugs currently used in intravascular stents are *paclitaxel*, *sirolimus* (*rapamycin*), and the two *sirolimus* derivatives *everolimus* and *zatarolimus*. *Paclitaxel* is a tricyclic diterpene that inhibits cellular proliferation by binding to and stabilizing polymerized microtubules. *Sirolimus* is a hydrophobic macrolide that binds to the cytosolic immunophilin FKBP12; the FKBP12-*sirolimus*

Drug Facts for Your Personal Formulary: *Coronary Artery Disease*

Drug	Therapeutic Uses	Major Toxicity and Clinical Pearls
Organic Nitrates		
Glyceryl trinitrate (GTN, nitroglycerin) Isosorbide dinitrate (ISDN) Isosorbide mononitrate (ISMN)	• Angina (sublingual) • Acute pulmonary edema (IV) • Acute hypertension (IV)	• NO-mediated vasodilation of large (venous, arterial) > small (resistance) vessels → preferential preload reduction without steal effect • Short-acting formulations of GTN or ISDN are standby drugs for all patients with CAD • First choice for vasospastic angina, along with Ca^{2+} channel blockers • Second choice for the prevention of exertional angina (longer-acting formulations) • Adverse effects: headache, dizziness, postural hypotension, syncope • Tolerance after >16 h (leave nitrate-free interval of >8 h) • Do not use concurrently with PDE5 inhibitor
Molsidomine	• Angina	• Direct NO donor • Second choice for the prevention of angina • Adverse effects same as above • No documented advantage over GTN/ISDN/ISMN
Inhaled NO	• Pulmonary hypertension in neonates	• Relatively selective effect on pulmonary vascular bed
Ca^{2+} Channel Blockers		
Dihydropyridines Amlodipine Felodipine Lercanidipine Nifedipine Nitrendipine ***Others*** Diltiazem Verapamil	• Angina • Hypertension • Rate control in atrial fibrillation (verapamil, diltiazem)	• Preferential arterial vasodilation → afterload reduction • First choice for vasospastic angina (dihydropyridines) • Second choice for preventing exertional angina • Immediate-release nifedipine and short-acting dihydropyridines can cause tachycardia and hypotension and trigger angina • Diltiazem and verapamil can ↓ heart rate and AV conduction; should not be used with β blockers • CYP3A4-mediated drug interactions with verapamil and diltiazem • Other unwanted effects: peripheral edema (dihydropyridines, less with lercanidipine), obstipation (verapamil)

Drug Facts for Your Personal Formulary: *Coronary Artery Disease* (*continued*)

Drug	Therapeutic Uses	Major Toxicity and Clinical Pearls
β Blockers		
Atenolol Bisoprolol Carvedilol Metoprolol Nadolol Nebivolol Many others	• Angina • Heart failure • Hypertension • Widely used for other indications (prevention of arrhythmias, rate control in atrial fibrillation, migraine, etc.)	• First choice for prevention of exertional angina • Only antianginal drug class with proven prognostic benefits in CAD • Adverse effects: bradycardia, AV block, bronchospasm, peripheral vasoconstriction, worsening of acute heart failure, depression, worsening of psoriasis • Polymorphic CYP2D6 metabolism (metoprolol) • Additional vasodilation (carvedilol, nebivolol)
Ranolazine		
	• Angina	• Inhibits late Na^+ and other cardiac ion currents • Has weak β-blocking and metabolic effects • Second choice in the prevention of exertional angina • CYP3A4-dependent metabolism
Ivabradine		
	• Angina • Heart failure	• Selectively ↓ heart rate by inhibiting HCN currents in SA node • Second choice in the prevention of exertional angina; approved in patients not tolerating β blockers or having heart rate >75 under β blockers • Unwanted effects: bradycardia, QT prolongation, atrial fibrillation, phosphenes • Contraindication: combination with diltiazem or verapamil
Nicorandil		
	• Angina	• Dual nitrate-like and I_{KATP}-stimulatory action • Hemodynamic profile between nitrates and dihydropyridines; ↓ afterload more than nitrates • Second choice in the prevention of exertional angina • Adverse effects: hypotension, headache, buccal and GI ulcers • Do not combine with PDE5 inhibitor
Trimetazidine		
	• Angina	• Metabolic shift from fatty acid to glycolytic metabolism in the heart • Second choice in the prevention of exertional angina • May increase the incidence of Parkinson's disease
Antiplatelet, Anti-integrin, and Antithrombotic Drugs		
Aspirin $P2Y_{12}$ receptor antagonists (clopidogrel, prasugrel, ticagrelor cangrelor [IV])	• Prevention of thrombotic events (MI, stroke) • Acute coronary syndromes • Prevention of stent thrombosis	• ↓ Platelet aggregation by inhibiting COX-1–mediated TxA_2 production (aspirin) or ADP receptors ($P2Y_{12}$ receptor antagonists) • Oral use only: clopidogrel, prasugrel, ticagrelor • Irreversible action: aspirin, clopidogrel, prasugrel • Prodrugs: clopidogrel, prasugrel • Variable, CYP2C9-dependent metabolism (clopidogrel) • Withdraw 5–7 days before surgery • First choice in non-STEMI and STEMI • Dual platelet inhibition after stenting
Abciximab Eptifibatide Tirofiban	• Percutaneous coronary interventions	• Antibody (abciximab) or small molecule antagonists at platelet GpIIb/IIIa receptor • Parenteral use only • Highly efficient platelet inhibition • Therapeutic value in the era of highly effective dual platelet inhibition unclear
Heparin Low-molecular-weight heparins (e.g., enoxaparin, dalteparin)	• Acute coronary syndromes • Percutaneous coronary interventions	• Endogenous polysaccharide, inhibits thrombin (factor IIa) and factor Xa in an antithrombin III–dependent manner • Parenteral use only • Heparin: short $t_{1/2}$, complex pharmacokinetics, low bioavailability after subcutaneous injection • Low-molecular-weight heparin: longer half-life, renal excretion; accumulation in renal insufficiency • Heparin-induced thrombocytopenia
Fondaparinux	• Acute coronary syndromes • Percutaneous coronary interventions	• Synthetic pentasaccharide, antithrombin III–dependent, factor Xa inhibitor • Most favorable efficacy-safety ratio
Bivalirudin Lepirudin	• Percutaneous coronary interventions (bivalirudin) • Heparin-induced thrombocytopenia (HIT II) recombinant lepirudin	• Direct thrombin (factor IIa) inhibitors • Parenteral use only • Advantage of bivalirudin over heparin unclear

complex inhibits the protein kinase mTOR, the mammalian target of rapamycin (see Figure 39–2), thereby inhibiting cell cycle progression (see Figure 69–2). *Paclitaxel* and *sirolimus* differ markedly in their mechanisms of action but share common chemical properties as hydrophobic small molecules. Stent-induced damage to the vascular endothelial cell layer can lead to thrombosis. The inhibition of cellular proliferation by *paclitaxel* and *sirolimus* or derivatives affects vascular smooth muscle cell proliferation and thereby markedly reduces the rate of restenosis compared with bare metal stents. Drug-eluting stents are therefore recommended over bare metal stents independently of the type of intervention (Collet et al., 2020). Dual antiplatelet therapy (e.g., *aspirin* and *ticlopidine*) is recommended for 1 year after intracoronary stenting with drug-eluting stents, similar to bare metal stents. Evidence for the benefit of even longer periods is limited.

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Chapter 32

Treatment of Hypertension

Thomas Eschenhagen

EPIDEMIOLOGY AND TREATMENT ALGORITHMS
- Principles of Antihypertensive Therapy

INHIBITORS OF THE RENIN-ANGIOTENSIN SYSTEM
- Angiotensin-Converting Enzyme Inhibitors
- AT_1 Receptor Blockers
- Direct Renin Inhibitors

Ca^{2+} CHANNEL BLOCKERS

DIURETICS
- Benzothiadiazines and Related Compounds
- Other Diuretic Antihypertensive Agents
- K^+-Sparing Diuretics
- Diuretic-Associated Drug Interactions

SYMPATHOLYTIC AGENTS
- β Blockers
- α_1 Blockers
- Combined α_1 and β Blockers
- Centrally Acting Sympatholytic Drugs

VASODILATORS
- Hydralazine
- K_{ATP} Channel Openers: Minoxidil
- Sodium Nitroprusside
- Diazoxide

NONPHARMACOLOGICAL THERAPY OF HYPERTENSION

SELECTION OF ANTIHYPERTENSIVE DRUGS IN INDIVIDUAL PATIENTS

ACUTE ANTIHYPERTENSIVE TREATMENT

RESISTANT HYPERTENSION

Epidemiology and Treatment Algorithms

Hypertension is the most common cardiovascular disease. Elevated arterial pressure causes hypertrophy of the left ventricle and pathological changes in the vasculature. As a consequence, hypertension is the principal cause of stroke; a major risk factor for coronary artery disease (CAD) and its associated complications, myocardial infarction (MI) and sudden cardiac death; and a major contributor to heart failure, renal insufficiency, and dissecting aneurysm of the aorta. The prevalence of hypertension increases with age; for example, about 50% of people between the ages of 60 and 69 years old have hypertension, and the prevalence further increases beyond age 70. According to a survey in the U.S., 81.5% of those with hypertension are aware they have it, 74.9% are being treated, yet only 52.5% are considered controlled (Go et al., 2014). The success of hypertension treatment programs, such as one organized in a large integrated healthcare delivery system in the U.S. (Jaffe et al., 2013), shows that these figures can be substantially improved by electronic hypertension registries tracking hypertension control rates, regular feedback to providers, development and frequent updating of an evidence-based treatment guideline, promotion of single-pill combination therapies, and follow-up blood pressure checks. Between 2001 and 2009, this program increased the number of patients with a diagnosis of hypertension by 78%, as well as the proportion of subjects meeting target blood pressure goals from 44% to more than 84% (Jaffe et al., 2013).

The definition of hypertension and treatment goals have evolved over the years according to results of intervention studies. The SPRINT study in nondiabetics with increased cardiovascular risk was prematurely stopped because the group of patients treated with antihypertensives to a systolic blood pressure target of 120 mmHg experienced a 25% lower rate of cardiovascular end points and total mortality than the group targeted to 140 mmHg (SPRINT Research Group, 2015). The rate of adverse effects such as hypotension and worsening of renal function were higher in the intensified treatment group, but this did not translate to a signal for real harm. The consequences of these results on the recent American Heart Association (AHA)/American College of Cardiology (ACC) (Whelton et al., 2018) and European Society of Cardiology (ESC) guidelines (Williams et al., 2018) slightly differ (Bakris et al., 2019; Table 32–1). In the U.S., hypertension is now defined as a blood pressure of 130/80 mmHg or higher, and in Europe, it is defined as a blood pressure of 140/90 mmHg or higher. These criteria characterize a group of patients whose risk of hypertension-related cardiovascular disease is high enough to merit medical attention. The risk of both fatal and nonfatal cardiovascular disease in adults is lowest with systolic blood pressures of less than 120 mmHg and diastolic blood pressures less than 80 mmHg; these risks increase incrementally as systolic and diastolic blood pressures rise. Recognition of this continuously increasing risk prevents a simple definition of hypertension (Table 32–1). Although many early clinical trials classified the severity of hypertension by diastolic pressure, progressive elevations of systolic pressure are similarly predictive of adverse cardiovascular events; at every level of diastolic pressure, risks are greater with higher levels of systolic blood pressure. Indeed, in patients more than 50 years old, systolic blood pressures predict adverse outcomes better than diastolic pressures. Pulse pressure, defined as the difference between systolic and diastolic pressure, may add additional predictive value (Franklin et al., 2009; Pastor-Barriuso et al., 2003). This may be at least in part due to higher-than-normal pulse pressure indicating adverse remodeling of blood vessels, representing an accelerated decrease in blood vessel compliance ("stiffening") normally associated with aging and atherosclerosis. Isolated systolic hypertension (increased systolic and normal diastolic blood pressure) in younger people (particularly men) is strongly associated with smoking. In elderly people, it indicates stiffening of the large arteries.

High blood pressure in the presence of pathological changes in certain target organs heralds a worse prognosis than the same level of blood pressure in a patient lacking these findings. For instance, retinal

Abbreviations

ACC: American College of Cardiology
ACE: angiotensin-converting enzyme
AHA: American Heart Association
Aldo: aldosterone
AngII: angiotensin II
ARB: angiotensin receptor blocker
AT_1: type 1 receptor for angiotensin II
AV: atrioventricular
β blocker: β adrenergic receptor antagonist
BP: blood pressure
CAD: coronary artery disease
COX-2: cyclooxygenase 2
DOPA: 3,4-dihydroxyphenylalanine
ENaC: epithelial Na^+ channel
ESC: European Society of Cardiology
GI: gastrointestinal
HDL: high-density lipoprotein
LDL: low-density lipoprotein
MI: myocardial infarction
MRA: mineralocorticoid receptor antagonist
NE: norepinephrine
NO: nitric oxide
NSAID: nonsteroidal anti-inflammatory drug
RAS: renin-angiotensin system
SA: sinoatrial

hemorrhages, exudates, and papilledema in the eyes indicate a far worse short-term prognosis for a given level of blood pressure. Left ventricular hypertrophy defined by electrocardiogram, or more sensitively by echocardiography or cardiac magnetic resonance imaging, is associated with a substantially worse long-term outcome that includes a higher risk of sudden cardiac death. The risk of cardiovascular disease, disability, and death in hypertensive patients also is increased markedly by concomitant cigarette smoking, diabetes, or elevated low-density lipoprotein (LDL); the coexistence of hypertension with these risk factors increases cardiovascular morbidity and mortality to a degree that is compounded by each additional risk factor.

The purpose of treating hypertension is to decrease cardiovascular risk and to improve life expectancy. Effective pharmacological treatment of patients with hypertension decreases morbidity and mortality from cardiovascular disease, particularly the risk of stroke, heart failure, and CAD (Rosendorff et al., 2015). The reduction in risk of MI may be less significant.

Principles of Antihypertensive Therapy

Nonpharmacological therapy (lifestyle-related changes) is an important component of treatment of all patients with hypertension (Whelton et al., 2018; Williams et al., 2018). In some grade 1 hypertensives (Figure 32–1), blood pressure may be adequately controlled by a combination of weight loss (in overweight individuals), restricting sodium intake (to <5 g/d), increasing aerobic exercise (>30 min/d), moderating consumption of alcohol (ethanol/day ≤20–30 g in men [two drinks], ≤10–20 g in women [one drink]), smoking cessation, and increased consumption of fruits, vegetables, and low-fat dairy products.

The majority of patients require drug therapy for adequate blood pressure control (Figure 32–1). Current guidelines from cardiovascular societies differ slightly in their definitions and treatment targets, but the principles are the same (Bakris et al., 2019; Table 32–1). One of the important common recommendations is early use of single-pill combination therapy (Figure 32–2).

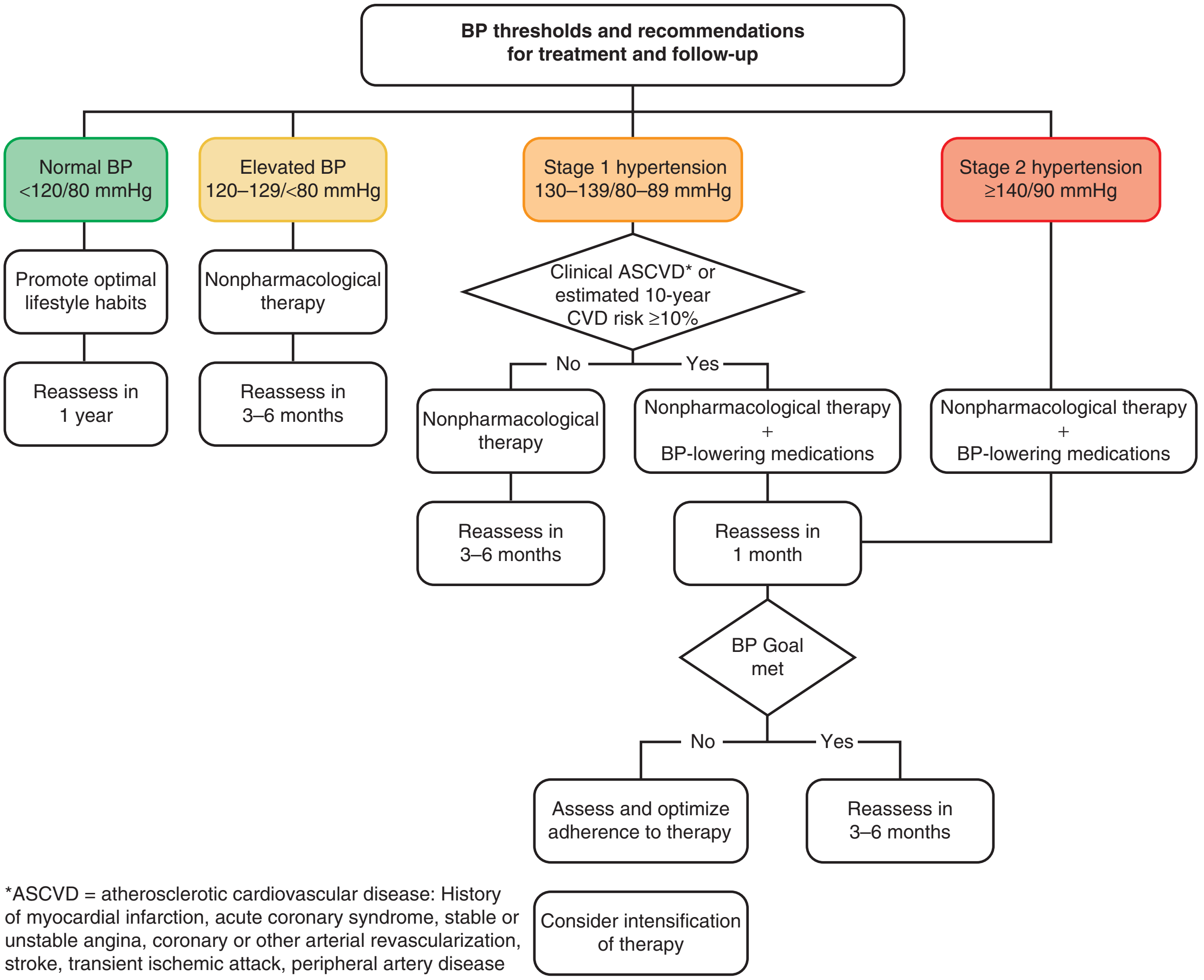
TABLE 32–1 ■ COMPARISON OF DEFINITIONS AND TREATMENT GOALS OF HYPERTENSION IN THE U.S. AND EUROPE

GUIDELINE DEFINITIONS OF HYPERTENSION	BLOOD PRESSURE (BP; mmHg)	
	U.S.	EUROPE
Office/clinic BP	≥130 systolic and/or ≥80 diastolic	≥140 systolic and/or ≥90 diastolic
Daytime mean	≥130 systolic and/or ≥80 diastolic	≥135 systolic and/or ≥85 diastolic
Nighttime mean	≥110 systolic and/or ≥65 diastolic	≥120 systolic and/or ≥70 diastolic
24-h mean	≥125 systolic and/or ≥75 diastolic	≥130 systolic and/or ≥80 diastolic
Home BP mean	≥130 systolic and/or ≥80 diastolic	≥135 systolic and/or ≥85 diastolic
BP targets for treatment	<130/80 mmHg	Systolic target <140 mmHg and close to 130 mmHg
Initial combination therapy	Initial single-pill combination in patients >20/10 mmHg above BP goal	Initial single-pill combination in patients ≥140/90 mmHg
BP requiring intervention	>130/80 mmHg	≥140/90 mmHg
Importance of home BP monitoring	• Take BP at home, twice in the morning and twice in the evening, in the week before clinic • Check BP machine annually	
Therapy	• Restrict β blockers to patients with comorbidities or other indications • Single-pill combinations as initial therapy	
Follow-up	• Detect poor adherence and focus on improvement • BP telemonitoring and digital health solutions recommended	

Source: Adapted with permission from Bakris G, Ali W, Parati G. ACC/AHA versus ESC/ESH on hypertension guidelines: JACC guideline comparison. *J Am Coll Cardiol*, 2019, 73:3018–3026. Copyright © 2019 by the American College of Cardiology Foundation. Published by Elsevier.

Arterial pressure is the product of cardiac output and peripheral vascular resistance (Figure 32–3). Drugs lower blood pressure by actions on peripheral resistance, cardiac output, or both. Drugs may decrease the cardiac output by inhibiting myocardial contractility or by decreasing ventricular filling pressure. Reduction in ventricular filling pressure may be achieved by actions on the venous tone or on blood volume via renal effects. Drugs can decrease peripheral resistance by acting on smooth muscle to cause relaxation of resistance vessels or by interfering with the activity of systems that produce constriction of resistance vessels (e.g., the sympathetic nervous system, the renin-angiotensin system [RAS]). Lowering of body sodium content (by diuretics or low-salt diet) can indirectly lower peripheral resistance, possibly by reducing the response to vasoconstrictors and/or by reducing T-cell–mediated inflammation (Titze, 2015). In patients with isolated systolic hypertension, complex hemodynamics in a rigid arterial system contribute to increased blood pressure; drug effects may be mediated not only by changes in peripheral resistance but also via effects on large artery stiffness.

Antihypertensive drugs can be classified according to their sites or mechanisms of action (Table 32–2, Figure 32–3). The hemodynamic consequences of long-term treatment with antihypertensive agents (Table 32–3) provide a rationale for potential complementary effects of


BP thresholds and recommendations for treatment and follow-up
Normal BP <120/80 mmHg
Elevated BP 120–129/<80 mmHg
Stage 1 hypertension 130–139/80–89 mmHg
Stage 2 hypertension ≥140/90 mmHg
Promote optimal lifestyle habits
Nonpharmacological therapy
Clinical ASCVD* or estimated 10-year CVD risk ≥10%
Reassess in 1 year
Reassess in 3–6 months
No
Yes
Nonpharmacological therapy
Nonpharmacological therapy + BP-lowering medications
Nonpharmacological therapy + BP-lowering medications
Reassess in 3–6 months
Reassess in 1 month
BP Goal met
No
Yes
Assess and optimize adherence to therapy
Reassess in 3–6 months
Consider intensification of therapy

*ASCVD = atherosclerotic cardiovascular disease: History of myocardial infarction, acute coronary syndrome, stable or unstable angina, coronary or other arterial revascularization, stroke, transient ischemic attack, peripheral artery disease

Figure 32–1 *Treatment algorithm for adults with hypertension.* Algorithm is based on recommendations of the American Heart Association and the American College of Cardiology (Whelton et al., 2018). *Patients with diabetes or chronic kidney disease are automatically put in the high-risk category. BP, blood pressure; CVD, cardiovascular disease.

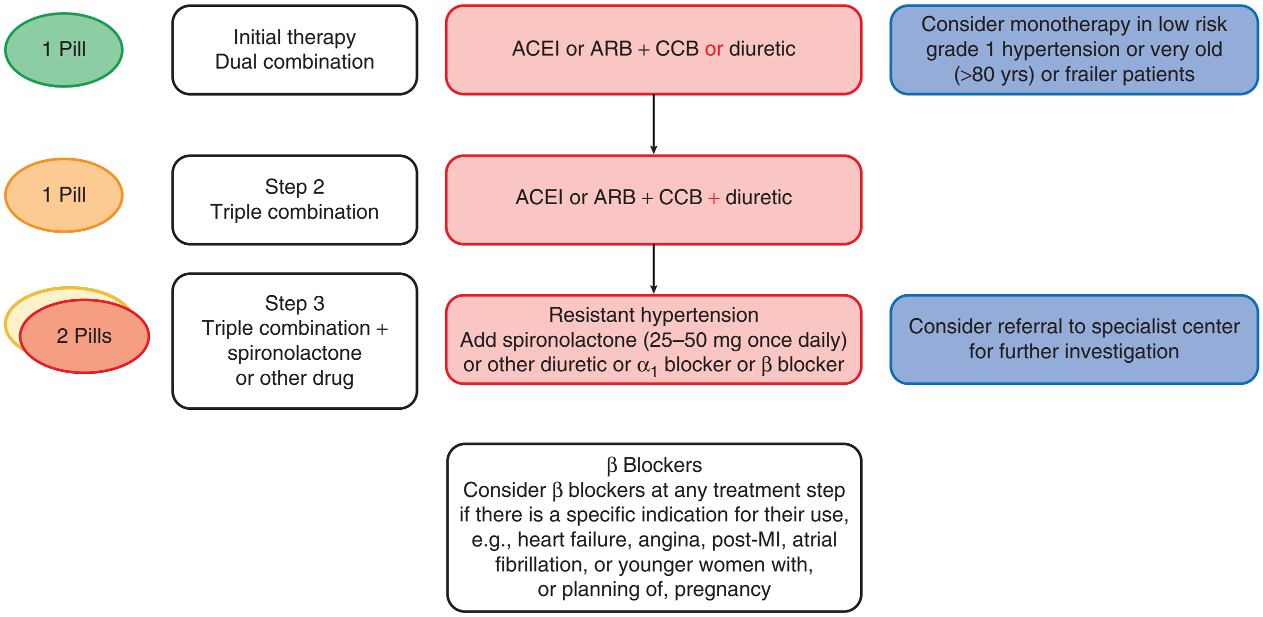

1 Pill
Initial therapy Dual combination
ACEI or ARB + CCB or diuretic
Consider monotherapy in low risk grade 1 hypertension or very old (>80 yrs) or frailer patients
1 Pill
Step 2 Triple combination
ACEI or ARB + CCB + diuretic
2 Pills
Step 3 Triple combination + spironolactone or other drug
Resistant hypertension Add spironolactone (25–50 mg once daily) or other diuretic or α1 blocker or β blocker
Consider referral to specialist center for further investigation
β Blockers
Consider β blockers at any treatment step if there is a specific indication for their use, e.g., heart failure, angina, post-MI, atrial fibrillation, or younger women with, or planning of, pregnancy

Figure 32–2 *Core drug treatment strategy for patients with uncomplicated hypertension.* The recommendation is based on the ESC guidelines for the treatment of hypertension (Williams et al., 2018). The algorithm is also appropriate for most patients with hypertension-mediated organ disease, cerebrovascular disease, diabetes, or peripheral artery disease. ACEI, ACE inhibitor; CCB, calcium channel blocker.

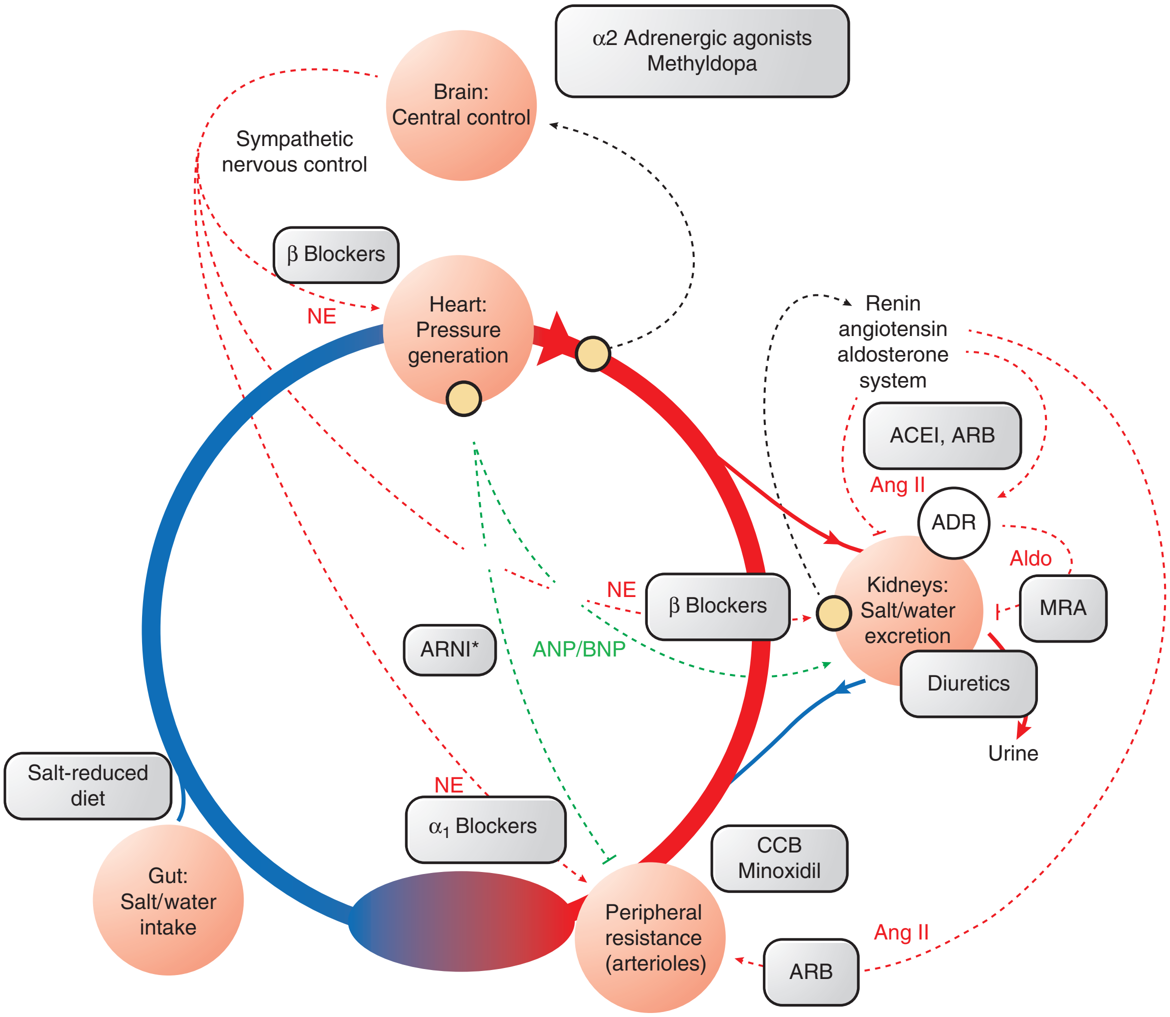


Figure 32–3 *Principles of blood pressure regulation and its modification by drugs.* Cardiac output and peripheral arteriolar resistance, the major determinants of arterial blood pressure, are regulated by myriad mechanisms, including the sympathetic nervous system (SNS) (main peripheral neurotransmitter NE), the balance between salt intake by the intestine (GI) and salt excretion by the kidneys, the renin-angiotensin-aldosterone system (RAAS) (main agonists AngII and aldosterone [Aldo]), and natriuretic peptides produced in the heart (atrial natriuretic peptide [ANP] and brain natriuretic peptide [BNP]). Sensors (yellow circles) provide afferent input on pressure in the heart and great vessels and on salt concentrations in the kidney. Note positive feedback between the SNS and RAAS via β_1-stimulated renin release and AngII-stimulated NE release (the latter not shown here for graphical reasons). Drug classes are indicated in grey boxes at their main site of action. Arrows indicate blood pressure-increasing (red) and -decreasing (green) effects. Neprilysin inhibitors (*sacubitril* in combination with *valsartan*, angiotensin receptor–neprilysin inhibitor [ARNI]) reduce blood pressure and are approved for heart failure (Chapter 33) but not for the treatment of hypertension. ACEI, ACE inhibitor; ADR, adrenal gland.*ARNI are not approved for the treatment of hypertension. CCB, calcium channel blocker.

concurrent therapy with two or more drugs. Concurrent use of drugs from different classes is a strategy for achieving effective control of blood pressure while minimizing dose-related adverse effects.

It generally is not possible to predict the responses of individuals with hypertension to any specific drug. For example, for some antihypertensive drugs, about two-thirds of patients will have a meaningful clinical response, whereas about one-third of patients will not respond to the same drug. Racial origin and age may have modest influence on the likelihood of a favorable response to a particular class of drugs. Polymorphisms in genes involved in the metabolism of antihypertensive drugs have been identified in the CYPs (phase I metabolism) and in phase II metabolism, such as catechol-*O*-methyltransferase (see Chapters 5 and 7). While these polymorphisms can change the pharmacokinetics of specific drugs quite markedly (e.g., five times higher plasma concentrations of metoprolol in CYP2D6 poor metabolizers), differences in efficacy are smaller (Rau et al., 2009) and of unknown clinical relevance. Polymorphisms that influence pharmacodynamic responses to antihypertensive drugs, including angiotensin-converting enzyme (ACE) inhibitors and diuretics, have also been identified, but evidence for clinically meaningful differences in drug response is sparse. Genome-wide scanning has identified several genetic variants associated with hypertension, but the effect sizes are much smaller than that of clinically established risk factors such as overweight.

While it is firmly established that the primary goal in the treatment of people with hypertension is the sustained reduction in mean blood pressure *per se* (Law et al., 2009), differences between antihypertensive drug classes may be relevant. This applies to unwanted effects and compliance, salutary effects on specific diseases, and most importantly, their efficacy to improve cardiovascular outcome. Current guidelines therefore differentiate among first-line antihypertensives (ACE inhibitors, angiotensin receptor blockers [ARBs], calcium channel blockers, and diuretics) that can be used in monotherapy or in different combinations, second-line drugs (*spironolactone*, β blockers [β adrenergic receptor antagonist], α_1 blockers; Figure 32–2), and antihypertensives for special indications (e.g., pregnancy, terminal kidney failure).

TABLE 32–2 ■ CLASSES OF ANTIHYPERTENSIVE DRUGS

Renin-angiotensin antagonists (Chapter 30)

- *Angiotensin-converting enzyme inhibitors*: benazepril, captopril,[a] enalapril, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, trandolapril
- *AngII receptor blockers*: candesartan, eprosartan, irbesartan, losartan, olmesartan, telmisartan, valsartan, azilsartan
- *Direct renin inhibitor*: aliskiren

Ca^{2+} channel blockers (Chapter 31): amlodipine, levamlodipine, clevidipine,[b] diltiazem, felodipine, isradipine, lercanidipine, nicardipine, nifedipine,[c] nisoldipine, verapamil

Diuretics (Chapter 29)

- *Thiazides and related agents*: chlorothiazide, chlorthalidone, hydrochlorothiazide, indapamide
- *Loop diuretics*: bumetanide, furosemide, torsemide
- *K^+-sparing diuretics*: amiloride, triamterene, MRA spironolactone

Sympatholytic drugs (Chapters 13 and 14)

- *Ganglionic blocking agents*: trimethaphan, mecamylamine
- *β Blockers*: atenolol, bisoprolol, esmolol,[b] nadolol, nebivolol, propranolol, acebutolol, betaxolol, metoprolol,[d] timolol
- *α Blockers*: prazosin, terazosin, doxazosin, phentolamine, metyrosine,[e] phenoxybenzamine[f]
- *Mixed α/β blockers*: labetalol, carvedilol
- *Centrally acting sympatholytic agents*: clonidine, guanfacine, methyldopa, moxonidine, reserpine

Vasodilators (Chapters 31 and 32)

- *Arterial*: diazoxide, fenoldopam, hydralazine, minoxidil
- *Arterial and venous*: nitroprusside

[a] Short duration of action makes captopril not a preferred choice for chronic treatment.
[b] Clevidipine and esmolol have an ultra-short half-life and are only approved for acute treatment.
[c] Only extended-release nifedipine is approved for hypertension.
[d] Only extended-release metoprolol (preferably as succinate salt with zero order of kinetics) should be used for the treatment of chronic hypertension.
[e] Metyrosine is a tyrosine hydroxylase inhibitor only indicated for pheochromocytoma, not for essential hypertension.
[f] Phenoxybenzamine is an irreversible inhibitor of α adrenergic receptors and is only approved in the treatment of pheochromocytoma.

Inhibitors of the Renin-Angiotensin System

Angiotensin II is an important regulator of cardiovascular function (see Chapter 30). The capacity to reduce the effects of angiotensin II (AngII) with pharmacological agents has been an important advance in the treatment of hypertension and its sequelae. Chapter 30 presents the basic physiology of the RAS and the pharmacology of inhibitors of the RAS. Table 30–2 summarizes the effects of a variety of antihypertensive agents on components of the RAS and warrants careful study. Drugs of this class are currently the most frequently prescribed antihypertensives worldwide.

Angiotensin-Converting Enzyme Inhibitors

The ability to reduce levels of AngII with orally effective ACE inhibitors represents an important advance in the treatment of hypertension. *Captopril* was the first such agent to be developed for the treatment of hypertension. Since then, *enalapril*, *lisinopril*, *quinapril*, *ramipril*, *benazepril*, *moexipril*, *fosinopril*, *trandolapril*, and *perindopril* have become available. These drugs are useful for the treatment of hypertension because of their efficacy and a favorable adverse effect profile that enhances patient adherence. Chapter 30 presents the pharmacology of ACE inhibitors in detail.

The ACE inhibitors appear to confer a special advantage in the treatment of patients with diabetes, slowing the development and progression of diabetic glomerulopathy. They also are effective in slowing the progression of other forms of chronic renal disease, such as glomerulosclerosis, which coexists with hypertension in many patients. An ACE inhibitor is the preferred initial agent in these patients. Patients with hypertension and ischemic heart disease are also candidates for treatment with ACE inhibitors. Administration of ACE inhibitors in the immediate post-MI period has been shown to improve ventricular function and reduce morbidity and mortality, particularly in patients with reduced left ventricular function (see Chapter 31).

The endocrine consequences of inhibiting the biosynthesis of AngII are of importance in several facets of hypertension treatment. Because ACE inhibitors blunt the rise in aldosterone concentrations in response to Na^+ loss, the normal role of aldosterone to oppose diuretic-induced natriuresis is diminished. Consequently, ACE inhibitors tend to enhance the efficacy of diuretic drugs. This means that even very small doses of diuretics may substantially improve the antihypertensive efficacy of ACE inhibitors; conversely, the use of high doses of diuretics together with ACE inhibitors may lead to excessive reduction in blood pressure and to Na^+ loss in some patients.

TABLE 32–3 ■ HEMODYNAMIC EFFECTS OF LONG-TERM ADMINISTRATION OF ANTIHYPERTENSIVE AGENTS

	HEART RATE	CARDIAC OUTPUT	TOTAL PERIPHERAL RESISTANCE	PLASMA VOLUME	PLASMA RENIN ACTIVITY
ACE inhibitors	↔	↔	↓	↔	↑
AT_1 receptor blockers	↔	↔	↓	↔	↑
Renin inhibitor	↔	↔	↓	↔	↓ (but renin ↑)
Ca^{2+} channel blockers	↓ or ↑	↓ or ↑	↓	–↑	–↑
Diuretics	↔	↔	↓	–↓	↑
Sympatholytic agents					
Centrally acting	– ↓	–↓	↓	– ↑	–↓
$α_1$ Blockers	–↑	–↑	↓	–↑	↔
β Blockers					
No ISA	↓	↓	–↓	–↑	↓
ISA[a]	↓↑	↔	↓	–↑	–↓
Arteriolar vasodilators	↑	↑	↓	↑	↑

↑, increased; ↓, decreased; –↑, increased or no change; –↓, decreased or no change; ↔, unchanged; ISA, intrinsic sympathomimetic activity.
[a] During rest, ISA may increase resting heart rate; during exercise, β adrenergic antagonism predominates, attenuating heart rate acceleration by NE. The profile has long-term adverse effects on the cardiovascular system, making these β blockers with ISA obsolete.

Attenuation of aldosterone production by ACE inhibitors also influences K^+ homeostasis; there is a small and clinically unimportant rise in serum K^+ when these agents are used alone in patients with normal renal function. However, substantial retention of K^+ can occur in some patients with renal insufficiency. Furthermore, the potential for developing hyperkalemia should be considered when ACE inhibitors are used with other drugs that can cause K^+ retention, including the K^+-sparing diuretics (*amiloride*, *triamterene*, and the MRAs *spironolactone* and *eplerenone*), nonsteroidal anti-inflammatory drugs (NSAIDs), K^+ supplements, and β blockers. Some patients with diabetic nephropathy may be at greater risk of hyperkalemia.

There are several cautions in the use of ACE inhibitors in patients with hypertension. Cough is a common (~5%) adverse effect and a reason to switch to ARBs. Angioedema of the head/neck and intestines is a rare but serious and potentially fatal adverse effect of the ACE inhibitors. Patients starting treatment with these drugs should be explicitly warned to discontinue their use with the advent of any signs of angioedema. Due to the risk of severe fetal adverse effects, ACE inhibitors are contraindicated during pregnancy, a fact that should be communicated to women of childbearing age.

In most patients, there is little or no appreciable change in glomerular filtration rate following the administration of ACE inhibitors. However, in renovascular hypertension, the glomerular filtration rate is generally maintained as the result of increased resistance in the postglomerular arteriole caused by AngII. Accordingly, in patients with bilateral renal artery stenosis or stenosis in a sole kidney, the administration of an ACE inhibitor will reduce the filtration fraction and cause a substantial reduction in glomerular filtration rate. The same mechanism of lowering the pressure in the filtrating vessels of the glomerulus probably participates in ACE inhibitor-induced slowing of the progression of chronic kidney disease. Serum creatinine levels and K^+ should be monitored in the first weeks after establishing therapy with an ACE inhibitor. Increases in serum creatinine greater than 20% predict the presence of renal artery stenosis (van de Ven et al., 1998) and are a reason to discontinue the treatment with ACE inhibitors.

ACE inhibitors lower blood pressure to some extent in most patients with hypertension. Following the initial dose of an ACE inhibitor, there may be a considerable fall in blood pressure in some patients; this response to the initial dose is a function of plasma renin activity prior to treatment. The potential for a large initial drop in blood pressure is the reason for using a low dose to initiate therapy, especially in patients who may have a very active RAS supporting blood pressure, such as patients with diuretic-induced volume contraction or congestive heart failure, both associated with an activated RAS. It should also be realized that, generally, no reason exists for normalizing blood pressure in a few days in patients with a lifelong disease. Attempts to do so increase the frequency of side effects and decrease compliance. With continuing treatment, there usually is a progressive fall in blood pressure that in most patients does not reach a maximum for several weeks. The blood pressure seen during chronic treatment is not strongly correlated with the pretreatment plasma renin activity. Young and middle-aged Caucasian patients have a higher probability of responding to ACE inhibitors; elderly African American patients as a group are more resistant to the hypotensive effect of these drugs. While most ACE inhibitors are approved for once-daily dosing for hypertension, a significant fraction of patients have a response that lasts less than 24 h and may require twice-daily dosing for adequate control of blood pressure (e.g., *enalapril, ramipril*). *Captopril*, with its very short duration of action, is not a good choice in the treatment of hypertension.

AT_1 Receptor Blockers

The importance of AngII in regulating cardiovascular function has led to the development of nonpeptide antagonists of the AT_1 (type 1 receptor for angiotensin II) subtype of AngII receptor. *Losartan, candesartan, irbesartan, valsartan, telmisartan, olmesartan, azilsartan*, and *eprosartan* (no longer marketed in the U.S.) have been approved for the treatment of hypertension. The pharmacology of ARBs is presented in detail in Chapter 30. By antagonizing the effects of AngII as competitive receptor antagonists, these agents relax smooth muscle and thereby promote vasodilation, increase renal salt and water excretion, reduce plasma volume, and decrease cellular hypertrophy. Given the central role of AT_1 receptors for the action of AngII, it is not surprising that ARBs have the same pharmacological profile as ACE inhibitors with one notable exception. ARBs do not inhibit the ACE-mediated degradation of bradykinin and substance P and thereby cause no cough.

Initial hopes for superiority of ARBs over ACE inhibitors have not been fulfilled. They were based on the idea that the AT_2 subtype elicits beneficial effects of AngII (e.g., antigrowth and antiproliferative responses). Because the AT_1 receptor mediates feedback inhibition of renin release, renin and AngII concentrations are increased during AT_1 receptor antagonism, leading to increased stimulation of uninhibited AT_2 receptors. Despite considerable interest, not much evidence supports any extra benefit from AT_1 blockade versus ACE inhibition, and attempts to show greater reductions in cardiovascular events by ARBs or by the combination of an ARB plus an ACE inhibitor over ACE inhibitor alone failed. ON-TARGET, one of the largest studies to date in patients with high vascular risk (70% hypertension), showed that *telmisartan* caused less cough and angioedema than *ramipril* (1.1% vs. 4.2%, and 0.1% vs. 0.3%, respectively) but had identical efficacy. The combination, although not more efficacious, was associated with greater worsening of renal function (13.5% vs. 10.2%), hypotension, and syncope (Yusuf et al., 2008).

Therapeutic Uses

The ARBs have a sufficient 24-h effect at once-daily dosing (except *losartan*). The full effect of ARBs on blood pressure typically is not observed until about 4 weeks after the initiation of therapy. If blood pressure is not controlled by an ARB alone, a second drug acting by a different mechanism (e.g., a diuretic or Ca^{2+} channel blocker) may be added. The combination of an ACE inhibitor and an ARB is not recommended for the treatment of hypertension.

Adverse Effects and Precautions

Adverse effects of ACE inhibitors that result from inhibiting AngII-related functions (see previous discussion and Chapter 30) also occur with ARBs. These include hypotension, hyperkalemia, and reduced glomerular filtration, including that associated with bilateral renal artery stenosis and stenosis in the artery of a solitary kidney. Hypotension is most likely to occur in patients whose blood pressure is highly dependent on AngII, including those with volume depletion (e.g., with diuretics), renovascular hypertension, cardiac failure, and cirrhosis; in such patients, initiation of treatment with low doses and attention to blood volume are essential. Hyperkalemia may occur in conjunction with other factors that alter K^+ homeostasis, such as renal insufficiency, ingestion of excess K^+, and the use of drugs that promote K^+ retention. Cough and angioedema occur rarely. ACE inhibitors and ARBs should not be administered during pregnancy and should be discontinued as soon as pregnancy is detected.

Direct Renin Inhibitors

Aliskiren, an orally effective direct renin inhibitor, is FDA approved for the treatment of hypertension. The detailed pharmacology of *aliskiren* is covered in Chapter 30. *Aliskiren* is an effective antihypertensive drug but has not been studied sufficiently in monotherapy of hypertension. A large study comparing a placebo or *aliskiren* added to a background of an ARB or an ACE inhibitor was stopped prematurely due to a trend toward increased cardiovascular events in the *aliskiren* treatment group (McMurray et al., 2012). The combination also induced more renal worsening, hypotension, and hyperkalemia. This mirrors previous studies with ARB/ACE inhibitor combinations and indicates that complete blockade of the RAS system results in more harm than benefit.

Pharmacology

The initial renin inhibitors were peptide analogues of sequences of renin itself or included the renin cleavage site in angiotensinogen. While effective in inhibiting renin and lowering blood pressure, these peptide analogues were effective only parenterally. However, *aliskiren* is effective following oral administration; it directly and competitively inhibits the catalytic activity of renin, leading to diminished production of AngI, AngII, and aldosterone—with a resulting fall in blood pressure. *Aliskiren*, along with ACE inhibitors and ARBs, leads to an adaptive increase in the plasma concentrations of renin; however, because *aliskiren* inhibits renin activity, plasma renin activity does not increase as occurs with these other classes of drugs (Table 30–2). Nevertheless, the aldosterone escape known from ACE inhibitors and ARBs has also been observed under continuous treatment with *aliskiren* (Bomback et al., 2012).

ADME

Aliskiren is poorly absorbed, with an oral bioavailability of less than 3%. Taking the drug with a high-fat meal may substantially decrease plasma concentrations. *Aliskiren* has an elimination $t_{1/2}$ of at least 24 h. Elimination of the drug may be primarily through hepatobiliary excretion with limited metabolism via CYP3A4.

Therapeutic Uses

Given the unclear effectiveness and safety of *aliskiren* monotherapy, the place of this drug in the treatment of hypertension remains clouded. The combination of *aliskiren* with other RAS inhibitors is contraindicated.

Toxicity and Precautions

Aliskiren is generally well tolerated. Diarrhea may occur, especially at higher-than-recommended doses. The incidence of cough may be higher than for placebo but substantially less than found with ACE inhibitors. *Aliskiren* has been associated with several cases of angioedema in clinical trials (Frampton and Curran, 2007). Drugs acting on the RAS may damage the fetus and should not be used in pregnant women.

Ca^{2+} Channel Blockers

The Ca^{2+} channel–blocking agents are an important group of drugs for the treatment of hypertension. The general pharmacology of these drugs is presented in Chapter 31. The basis for their use in hypertension comes from the understanding that hypertension generally is the result of increased peripheral vascular resistance. Because contraction of vascular smooth muscle is dependent on the free intracellular concentration of Ca^{2+}, inhibition of transmembrane movement of Ca^{2+} through voltage-sensitive Ca^{2+} channels can decrease the total amount of Ca^{2+} that reaches intracellular sites. Indeed, all of the Ca^{2+} channel blockers lower blood pressure by relaxing arteriolar smooth muscle and decreasing peripheral vascular resistance. As a consequence of a decrease in peripheral vascular resistance, the Ca^{2+} channel blockers evoke a baroreceptor reflex–mediated sympathetic discharge. In the case of the dihydropyridines, tachycardia may occur from the adrenergic stimulation of the sinoatrial (SA) node; this response is generally quite modest except when the drug is administered rapidly. Tachycardia is typically minimal or absent with *verapamil* and *diltiazem* because of the direct negative chronotropic effect of these two drugs. Indeed, the concurrent use of a β blocker may magnify negative chronotropic effects of these drugs or cause heart block in susceptible patients. Consequently, the concurrent use of β blockers with either *verapamil* or *diltiazem* should be avoided.

The Ca^{2+} channel blockers are among the preferred drugs for the treatment of hypertension, both as monotherapy and in combination with other antihypertensives, because they have a well-documented effect on cardiovascular end points and total mortality. The combination of *amlodipine* and the ACE inhibitor *perindopril* proved superior to the combination of the β blocker *atenolol* and *hydrochlorothiazide* (Dahlof et al., 2005). *Amlodipine* was superior to *hydrochlorothiazide* as the combination partner for the ACE inhibitor *benazepril* (Jamerson et al., 2008).

The Ca^{2+} channel blockers most studied and used for the treatment of hypertension are long-acting dihydropyridines with sufficient 24-h efficacy at once-daily dosing (e.g., *amlodipine*, *felodipine*, *lercanidipine* [no longer available in the U.S.], and sustained-release formulations of others). Peripheral edema (ankle edema) is the main unwanted effect. Fewer patients appear to experience this harmless, but possibly distracting, side effect with newer compounds such as *lercanidipine* (Makarounas-Kirchmann et al., 2009), but the commonly used combination with RAS inhibitors has the same effect (Messerli et al., 2000). Immediate-release *nifedipine* and other short-acting dihydropyridines have no place in the treatment of hypertension. *Verapamil* and *diltiazem* also have short half-lives, more cardiac side effects, and a high drug interaction potential (*verapamil* > *diltiazem*) and are therefore not first-line antihypertensives.

Compared with other classes of antihypertensive agents, there may be a greater frequency of achieving blood pressure control with Ca^{2+} channel blockers as monotherapy in elderly subjects and in African Americans, population groups in which the low renin status is more prevalent. However, intrasubject variability is more important than relatively small differences between population groups. Ca^{2+} channel blockers are effective in lowering blood pressure and decreasing cardiovascular events in the elderly with isolated systolic hypertension (Staessen et al., 1997) and may be a preferred treatment in these patients.

Diuretics

An early strategy for the management of hypertension was to alter Na^+ balance by restriction of salt in the diet. Pharmacological alteration of Na^+ balance became practical with the development of the orally active thiazide diuretics (see Chapter 29). These and related diuretic agents have antihypertensive effects when used alone, and they enhance the efficacy of virtually all other antihypertensive drugs. Thus, this class of drugs remains important in the treatment of hypertension.

The exact mechanism for reduction of arterial blood pressure by diuretics is not certain. The initial action of thiazide diuretics decreases extracellular volume by interacting with a thiazide-sensitive NaCl cotransporter (*SLC12A3*) expressed in the distal convoluted tubule in the kidney, enhancing Na^+ and water excretion in the urine, which leads to a decrease in cardiac output. However, cardiac output returns to pretreatment values, and extracellular volume returns to almost normal due to compensatory responses such as activation of the RAS, while the hypotensive effect is maintained during long-term therapy due to decreased vascular resistance. The long-term vasodilation induced by thiazide diuretics must be secondary to their effect on the kidney, because no blood pressure–lowering effect of *chlorothiazide* was seen in nephrectomized dogs (Orbison, 1962). The fact that SLC12A3, the major drug target of thiazides, is expressed predominantly in the distal convoluted tubules and not in vascular smooth muscle or the heart further suggests that these drugs decrease peripheral resistance as an indirect effect of negative Na^+ balance. Accordingly, the hypotensive effect of a chronic treatment with thiazides has been reversed by a high-salt diet (Winer, 1961). That thiazides lose efficacy in treating hypertension in patients with coexisting renal insufficiency is compatible with this hypothesis. Moreover, carriers of rare functional mutations in SLC12A3 that decrease renal Na^+ reabsorption have lower blood pressure than appropriate controls (Ji et al., 2008); in a sense, this is an experiment of nature that may mimic the therapeutic effect of thiazides. Newer data suggest that large amounts of Na^+ can be stored in the body independently of changes in water content (challenging the dogma of strict coupling between Na^+ and water). High concentrations of Na^+ have been visualized in human skeletal muscle and skin of patients with Conn syndrome and were sensitive to adrenalectomy or *spironolactone* (Titze, 2015). Not all details are clear, but Na^+ accumulation in T cells and macrophages goes along with a proinflammatory state likely important in hypertension. It is well established that even small increases in intracellular Na^+ concentrations in cardiomyocytes have major effects on contractility (see Chapter 33; positive inotropic mechanism of digoxin). Similar mechanisms in smooth muscle cells could explain why chronic treatment with a thiazide markedly lowered the sensitivity to vasoconstrictors such as *phenylephrine* and AngII (Noveck, 1983).

Benzothiadiazines and Related Compounds

Benzothiadiazines ("thiazides") and related diuretics are the most frequently used class of antihypertensive agents in the U.S. Following the discovery of *chlorothiazide*, a number of oral diuretics were developed that have an arylsulfonamide structure and block the NaCl cotransporter. Some of these are not benzothiadiazines but have structural features and molecular functions that are similar to the original benzothiadiazine compounds; consequently, they are designated as members of the thiazide class of diuretics. For example, *chlorthalidone* (also written as *chlortalidone*), one of the nonbenzothiadiazines, is widely used in the treatment of hypertension, as is *indapamide*.

Regimen for Administration of the Thiazide-Class Diuretics in Hypertension

Because members of the thiazide class have similar pharmacological effects, they generally have been viewed as interchangeable with appropriate adjustment of dosage (see Chapter 29). However, the pharmacokinetics and pharmacodynamics of these drugs differ, so they may not necessarily have the same clinical efficacy in treating hypertension. In a direct comparison, the antihypertensive efficacy of *chlorthalidone* was greater than that of *hydrochlorothiazide*, particularly during the night (Ernst et al., 2006), suggesting the much longer $t_{1/2}$ of *chlorthalidone* (>24 h) compared to *hydrochlorothiazide* (several hours) gave more stable blood pressure reductions. Meta-analyses confirmed the superiority of *chlorthalidone* over *hydrochlorothiazide* in long-term studies (Roush and Messerli, 2021). *Chlorthalidone* appears to be an underutilized drug in hypertensive patients requiring a diuretic.

Antihypertensive effects can be achieved in many patients with as little as 12.5 mg daily of *chlorthalidone* or *hydrochlorothiazide*. Furthermore, when used as monotherapy, the maximal daily dose of thiazide-class diuretics usually should not exceed 25 mg of *hydrochlorothiazide* or *chlorthalidone* (or equivalent). Even though more diuresis can be achieved with higher doses, some evidence suggests that doses higher than this are not generally more efficacious in lowering blood pressure in patients with normal renal function. Low doses of either thiazide reduce the risk of adverse effects such as K^+ wasting and inhibition of uric acid excretion, indicating an improved risk-to-benefit ratio at low doses of a thiazide. However, other studies suggested that low doses of *hydrochlorothiazide* have inadequate effects on blood pressure when monitored in a detailed manner (Lacourciere et al., 1995).

Clinical trials of antihypertensive therapy in the elderly demonstrated the best outcomes for cardiovascular morbidity and mortality when 25 mg of *hydrochlorothiazide* or *chlorthalidone* was the maximum dose given; if this dose did not achieve the target blood pressure reduction, a second drug was initiated (Dahlof et al., 1991). A case-control study found a dose-dependent increase in the occurrence of sudden death at doses of *hydrochlorothiazide* greater than 25 mg daily (Siscovick et al., 1994), supporting the hypothesis that higher diuretic doses are associated with increased cardiovascular mortality as long as hypokalemia is not corrected. Thus, if adequate blood pressure reduction is not achieved with the 25 mg daily dose of *hydrochlorothiazide* or *chlorthalidone*, the addition of a second drug is indicated rather than an increase in the dose of diuretic.

Urinary K^+ loss can be a problem with thiazides. ACE inhibitors and ARBs will attenuate diuretic-induced loss of K^+ to some degree, and this is a consideration if a second drug is required to achieve further blood pressure reduction beyond that attained with the diuretic alone. Because the diuretic and hypotensive effects of these drugs are greatly enhanced when they are given in combination, care should be taken to initiate combination therapy with low doses of each of these drugs (Vlasses et al., 1983). Administration of ACE inhibitors or ARBs together with other K^+-sparing agents or with K^+ supplements requires great caution; combining K^+-sparing agents with each other or with K^+ supplementation can cause potentially dangerous hyperkalemia in some patients.

In contrast to the limitation on the dose of thiazide-class diuretics used as monotherapy, the treatment of severe hypertension that is unresponsive to three or more drugs may require larger doses of the thiazide-class diuretics. Indeed, hypertensive patients may become refractory to drugs that block the sympathetic nervous system or to vasodilator drugs, because these drugs engender a state in which the blood pressure is very volume dependent. Therefore, it is appropriate to consider the use of thiazide-class diuretics in doses of 50 mg of daily *hydrochlorothiazide* equivalent when treatment with appropriate combinations and doses of three or more drugs fails to yield adequate control of the blood pressure. Alternatively, there may be a need to use higher-capacity diuretics such as *furosemide*, especially if renal function is not normal.

The effectiveness of thiazides as diuretics or antihypertensive agents is progressively diminished when the glomerular filtration rate falls below 30 mL/min. Exceptions are the thiazide-like diuretics *metolazone* and *xipamide*, which retain efficacy in patients with this degree of renal insufficiency. In the case of *xipamide*, this is explained by its access to the NaCl cotransporter from the blood side, which contrasts with classical thiazides reaching it via the tubular side.

Most patients will respond to thiazide diuretics with a reduction in blood pressure within about 4 to 6 weeks. Therefore, doses should not be increased more often than every 4 to 6 weeks. There is no way to predict the antihypertensive response from the duration or severity of the hypertension in a given patient, although diuretics are unlikely to be effective as sole therapy in patients with stage 2 hypertension (Table 32–1). Because the effect of thiazide diuretics is additive with that of other antihypertensive drugs, combination regimens that include these diuretics are common and rational. A wide range of fixed-dose combination products containing a thiazide are marketed for this purpose. Diuretics also have the advantage of minimizing the retention of salt and water that is commonly caused by vasodilators and some sympatholytic drugs. Omitting or underutilizing a diuretic is a frequent cause of "resistant hypertension."

Adverse Effects and Precautions

The adverse effects of diuretics are discussed in Chapter 29. Some of these determine whether a patient can tolerate and adhere to diuretic treatment.

The K^+ depletion produced by thiazide-class diuretics is dose dependent and variable among individuals, such that a subset of patients may become substantially K^+ depleted on diuretic drugs. Given chronically, even small doses lead to some K^+ depletion, which is a well-known risk factor for ventricular arrhythmias by reducing cardiac repolarization reserve. A reduction in the cardiac repolarization reserve has recently been used to explain that insults in a particular repolarization current do not necessarily result in QT interval prolongation, the principal clinical measure of repolarization (see Chapter 34). Hypokalemia directly reduces repolarization reserve by decreasing several K^+ conductances (inward rectifier I_{K1}, delayed rectifier I_{Kr}, and the transient outward current I_{to}) and increasing the binding activity of I_{Kr}-inhibiting drugs such as *dofetilide* (Yang and Roden, 1996). Hypokalemia also reduces the activity of the Na^+,K^+-ATPase (adenosine triphosphatase) (the Na^+ pump), causing intracellular accumulation of Na^+ and Ca^{2+}, further increasing the risk of afterdepolarizations (Pezhouman et al., 2015). Consequently, hypokalemia increases the risk of drug-induced polymorphic ventricular tachycardia (torsade de pointes; see Chapter 34) and the risk for ischemic ventricular fibrillation, the leading cause of sudden cardiac death and a major contributor to cardiovascular mortality in treated hypertensive patients. There is a positive correlation between diuretic dose and sudden cardiac death and an inverse correlation between the use of adjunctive K^+-sparing agents and sudden cardiac death (Siscovick et al., 1994). Thus, hypokalemia needs to be avoided by, for example, combining a thiazide with inhibitors of the RAS or with a K^+-sparing diuretic.

Thiazides have residual carbonic anhydrase–inhibiting activity, thereby reducing Na^+ reabsorption in the proximal tubule. The increased presentation of Na^+ at the macula densa leads to a reduced glomerular filtration rate via tubuloglomerular feedback. While this effect is clinically not meaningful in patients with normal renal function, it reduces diuretic effectiveness and may gain importance in patients with reduced kidney function. RAS inhibitors and Ca^{2+} channel blockers interfere with tubuloglomerular feedback, providing one explanation for the synergistic effect on blood pressure.

Erectile dysfunction is a troublesome adverse effect of the thiazide-class diuretics, and physicians should inquire specifically regarding its occurrence in conjunction with treatment with these drugs. Gout may be a consequence of the hyperuricemia induced by these diuretics. The occurrence of either of these adverse effects is a reason for considering alternative approaches to therapy. However, precipitation of acute gout is relatively uncommon with low doses of diuretics. *Hydrochlorothiazide* may cause rapidly developing, severe hyponatremia in some patients. Thiazides inhibit renal Ca^{2+} excretion (in contrast to loop diuretics increasing it), occasionally leading to hypercalcemia; although generally mild, this can be more severe in patients subject to hypercalcemia, such as those with primary hyperparathyroidism. The thiazide-induced decreased Ca^{2+} excretion may be used therapeutically in patients with osteoporosis or hypercalciuria.

Thiazide diuretics have also been associated with changes in plasma lipids and glucose tolerance that have led to some concern. The clinical significance of the changes has been disputed because the clinical studies demonstrated comparable efficacy of the thiazide diuretic *chlortalidone* in reducing cardiovascular risk (ALLHAT Officers, 2002).

All thiazide-like drugs cross the placenta. While they have no direct adverse effects on the fetus, administration of a thiazide during pregnancy increases a risk of transient volume depletion that may result in placental hypoperfusion. Because the thiazides appear in breast milk, they should be avoided by nursing mothers.

Recent registry data from Denmark revealed an increased risk (10%–70%) of certain types of skin cancers (squamous skin cell carcinoma, nonmelanoma basal cell carcinoma) associated with the use of *hydrochlorothiazide* (Pedersen et al., 2018). The effect may be due to a photosensitizing effect and has led the FDA to require inclusion of that information on the label of all *hydrochlorothiazide*-containing drug formulations. It is not clear whether the risk extends to other thiazides.

Other Diuretic Antihypertensive Agents

The thiazide diuretics are more effective antihypertensive agents than are the loop diuretics, such as *furosemide* and *bumetanide*, in patients who have normal renal function. This differential effect is most likely related to the short duration of action of loop diuretics. In fact, a single daily dose of loop diuretics does not cause a significant net loss of Na^+ for an entire 24-h period because the strong initial diuretic effect is followed by a rebound mediated by activation of the RAS. Unfortunately, loop diuretics are frequently and inappropriately prescribed as a once-a-day medication in the treatment not only of hypertension, but also of congestive heart failure and ascites. The high efficacy of loop diuretics to produce a rapid and profound natriuresis can be detrimental for the treatment of hypertension. When a loop diuretic is given twice daily, the acute diuresis can be excessive and lead to more side effects than occur with a slower-acting, milder thiazide diuretic. Loop diuretics may be particularly useful in patients with azotemia or with severe edema associated with a vasodilator such as *minoxidil*.

K^+-Sparing Diuretics

Amiloride and *triamterene* are K^+-sparing diuretics that have little value as antihypertensive monotherapy but are important in combination with thiazides to antagonize urinary K^+ loss and the concomitant risk of ventricular arrhythmias. They act by reversibly inhibiting the epithelial Na^+ channel (ENaC) in the distal tubule membrane, the transporter responsible for the reabsorption of Na^+ in exchange for K^+. The importance of ENaC in hypertension is illustrated by the fact that an inherited form of hypertension, Liddle syndrome, is due to hyperactivity of ENaC. Gene expression of ENaC is mineralocorticoid sensitive, explaining the antihypertensive and K^+-sparing effect of another class of K^+-sparing diuretics, the MRAs *spironolactone* and *eplerenone*. In contrast to the immediate and short-term inhibition of ENaC by *amiloride* and *triamterene*, the action of MRAs is delayed for about 3 days and is long lasting because MRAs regulate the density of the channel protein in the tubule membrane.

The MRAs have a particular role in hypertension and heart failure (see Chapter 23) because small doses of *spironolactone* are often highly effective in patients with "resistant hypertension." First described decades ago (Ramsay et al., 1980), the concept was recently validated in a prospective, placebo-controlled trial comparing *spironolactone* (25–50 mg) with *bisoprolol* or *doxazosin* as add-ons in patients with uncontrolled hypertension despite triple standard antihypertensive therapy (Williams et al., 2015). *Spironolactone* had about a 2-fold larger blood pressure–lowering effect (8.7 vs. 4.8 and 4 mmHg, respectively). The efficacy of the mineralocorticoid receptor antagonist (MRA) *spironolactone* in resistant hypertension supports a primary role of Na^+ retention in this condition. Some of the effect may be related to the so-called aldosterone-escape phenomenon, or a return to pre-RAS-inhibitor plasma aldosterone levels with extended time of treatment, observed under treatment with RAS inhibitors. Primary hyperaldosteronism occurs in a significant fraction of patients with resistant hypertension (Calhoun et al., 2002). The addition of *spironolactone* at low dose is currently recommended as the third step in the ESC treatment algorithms (Figure 32–2).

Spironolactone has some significant adverse effects, especially in men (e.g., erectile dysfunction, gynecomastia, benign prostatic hyperplasia). *Eplerenone* is a more specific, though less-potent, MRA with reduced side effects.

All K^+-sparing diuretics should be used cautiously, with frequent measurements of plasma K^+ concentrations in patients predisposed to hyperkalemia (e.g., type 2 diabetics). Patients should be cautioned regarding the possibility that concurrent use of K^+-containing salt substitutes could produce hyperkalemia. Renal insufficiency is a relative contraindication to the use of K^+-sparing diuretics. Concomitant use of an ACE inhibitor or an ARB magnifies the risk of hyperkalemia with these agents.

Diuretic-Associated Drug Interactions

Because the antihypertensive effects of diuretics are additive with those of other antihypertensive agents, a diuretic commonly is used in combination with other drugs. The K^+- and Mg^{2+}-depleting effects of the thiazides and loop diuretics can potentiate arrhythmias that arise from digitalis toxicity. Corticosteroids can amplify the hypokalemia produced by the diuretics. NSAIDs (see Chapter 43) that inhibit the synthesis of prostaglandins reduce the antihypertensive effects of diuretics and all other antihypertensives. The renal effects of selective cyclooxygenase 2 (COX-2) inhibitors are similar to those of the traditional NSAIDs. NSAIDs and RAS inhibitors reduce plasma concentrations of aldosterone and can potentiate the hyperkalemic effects of a K^+-sparing diuretic. All diuretics can decrease the clearance of Li^+, resulting in increased plasma concentrations of Li^+ and potential toxicity.

Sympatholytic Agents

With the demonstration in 1940 that bilateral excision of the thoracic sympathetic chain could lower blood pressure, there was a search for effective chemical sympatholytic agents. Many of the early sympatholytic drugs were poorly tolerated and had limiting adverse side effects, particularly on mood. A number of sympatholytic agents are currently in use (Table 32–2). Antagonists of α and β adrenergic receptors have been mainstays of antihypertensive therapy but have recently lost their place as first-line therapy.

β Blockers

β Adrenergic receptor antagonists (β blockers) were not expected to have antihypertensive effects when they were first investigated in patients with angina, their primary indication. However, *pronethalol*, a drug that was never marketed, was found to reduce arterial blood pressure in hypertensive patients with angina pectoris. This antihypertensive effect was subsequently demonstrated for *propranolol* and all other β blockers. The basic pharmacology of these drugs is discussed in Chapter 14; characteristics relevant to their use in hypertension are described here.

Locus and Mechanism of Action

Antagonism of β adrenergic receptors affects the regulation of the circulation through a number of mechanisms, including a reduction in myocardial contractility and heart rate (i.e., cardiac output; see Figure 32–3). Antagonism of β_1 receptors of the juxtaglomerular complex reduces renin secretion and RAS activity. This action likely contributes to the antihypertensive action. Some members of this large, heterogeneous class of drugs have additional effects unrelated to their capacity to bind to β adrenergic receptors. For example, *labetalol* and *carvedilol* are also α_1 blockers, and *nebivolol* promotes endothelial cell–dependent vasodilation via activation of nitric oxide (NO) production (Pedersen and Cockcroft, 2006) (see Figure 14–4).

Pharmacodynamic Differences

The β blockers vary in their selectivity for the β_1 receptor subtype, presence of partial agonist or intrinsic sympathomimetic activity, and vasodilating capacity. While all of the β blockers are effective as antihypertensive agents, these differences influence the clinical pharmacology and spectrum of adverse effects of the various drugs. The antihypertensive effect resides in antagonism of the β_1 receptor, while major unwanted effects result from antagonism of β_2 receptors (e.g., peripheral vasoconstriction, bronchoconstriction, hypoglycemia). Standard therapies are β_1 blockers without intrinsic sympathomimetic activity (e.g., *atenolol, bisoprolol, metoprolol*). They produce an initial reduction in cardiac output (mainly β_1) and a reflex-induced rise in peripheral resistance, with little or no acute change in arterial pressure. In patients who respond with a reduction in blood pressure, peripheral resistance gradually returns to pretreatment values or less. Generally, persistently reduced cardiac output and possibly decreased peripheral resistance account for the reduction in arterial pressure. Nonselective β blockers (e.g., *propranolol*) have stronger adverse effects on peripheral vascular resistance by also blocking β_2 receptors that normally mediate vasodilation. Vasodilating β blockers (e.g., *carvedilol, nebivolol*) may be preferred in patients with peripheral artery disease. Drugs with intrinsic sympathomimetic activity (e.g., *pindolol, xamoterol*) are not recommended for the treatment of hypertension or any other cardiovascular disease because they increase nighttime mean heart rate due to their direct partial agonistic activity.

Pharmacokinetic Differences

Lipophilic β blockers (*metoprolol, bisoprolol, carvedilol, propranolol*) appear to have more antiarrhythmic efficacy than the hydrophilic compounds (*atenolol, nadolol, labetalol*), possibly related to a central mode of action. Many β blockers have relatively short plasma half-lives and require more than once-daily dosing (*metoprolol, propranolol, carvedilol*), a significant disadvantage in the treatment of hypertension. They should generally be prescribed in sustained-release forms. *Bisoprolol* and *nebivolol* have $t_{1/2}$ values of 10 to 12 h and thus achieve sufficient trough levels at once-daily dosing. Hepatic metabolism of *metoprolol*, *carvedilol*, and *nebivolol* is CYP2D6 dependent. The relevance is probably greatest in case of *metoprolol*, for which CYP2D6 poor metabolizers (~7% of the Caucasian population) show 5-fold higher drug exposure and 2-fold higher heart rate decreases than the majority of extensive metabolizers (Rau et al., 2009).

Effectiveness in Hypertension

Meta-analyses have suggested that β blockers reduce the incidence of MI similar to other antihypertensives but are only about half as effective in preventing stroke (Lindholm et al., 2005). This has led to downgrading of this class of drugs in important guidelines (Whelton et al., 2018; Williams et al., 2018; Table 32–1, Figure 32–2). It has been argued that many of the studies supporting this conclusion were conducted with *atenolol*, which may not be the ideal β blocker. *Atenolol*, in contrast to *bisoprolol, carvedilol, metoprolol*, or *nebivolol*, has not been positively tested in heart failure trials. *Atenolol* may not lower central (aortic) blood pressure as effectively as it appears when conventionally measured in the brachial artery using a standard arm cuff (Williams et al., 2006), but it is not clear whether other β blockers have more favorable effects on central blood pressure, arterial stiffness, and endothelial dysfunction. Prospective studies of hypertensive agents have not compared different β blockers head-to-head; therefore, the clinical relevance of pharmacological differences in this heterogeneous drug class remains unclear. Regardless, β blockers as a class are associated with more side effects than ACE inhibitors/ARBs or calcium channel blockers, including increased incidence of diabetes and weight gain. Therefore, β blockers are only recommended in patients with a specific indication for their use (e.g., heart failure; Table 32–1) or in the third step of the treatment algorithm (Figure 32–2).

Adverse Effects and Precautions

The adverse effects of β blockers are discussed in Chapter 14. These drugs should be avoided in patients with reactive airway disease (e.g., asthma) or with SA or atrioventricular (AV) nodal dysfunction or in combination with other drugs that inhibit AV conduction, such as *verapamil*. The risk of hypoglycemic reactions may be increased in diabetics taking *insulin*, but type 2 diabetes is not a contraindication. β Blockers increase concentrations of triglycerides in plasma and lower those of high-density lipoprotein (HDL) cholesterol without changing total cholesterol concentrations. The long-term consequences of these effects are unknown. β Blockers may aggravate depression and psoriasis.

Sudden discontinuation of β blockers can produce a withdrawal syndrome, likely due to upregulation of β receptors during blockade, causing enhanced tissue sensitivity to endogenous catecholamines—potentially exacerbating the symptoms of CAD. The result, especially in active patients, can be rebound hypertension. Thus, β blockers should not be discontinued abruptly, except under close observation; dosage should be tapered gradually over 10 to 14 days prior to discontinuation.

Epinephrine can produce severe hypertension and bradycardia when a nonselective β blocker is present. The hypertension is due to the unopposed stimulation of α adrenergic receptors when vascular β_2 receptors are blocked. The bradycardia is the result of reflex vagal stimulation. Such paradoxical hypertensive responses to β blockers have been observed in patients with hypoglycemia or pheochromocytoma, during withdrawal from *clonidine*, following administration of *epinephrine* as a therapeutic agent, or in association with the illicit use of cocaine.

Therapeutic Uses

The β blockers provide effective therapy for all grades of hypertension. Marked differences in their pharmacokinetic properties should be considered; once-daily dosing is preferred for better compliance. Populations that tend to have a lesser antihypertensive response to β blockers include the elderly and African Americans. However, intraindividual differences in antihypertensive efficacy are generally much larger than statistical evidence of differences between racial or age-related groups. Consequently, these observations should not discourage the use of these drugs in individual patients in groups reported to be less responsive.

The β blockers usually do not cause retention of salt and water, and administration of a diuretic is not necessary to avoid edema or the development of tolerance. However, diuretics do have additive antihypertensive effects when combined with β blockers. The addition of a β blocker to first-line treatment with an ACE inhibitor/ARB or calcium channel blocker and diuretic is effective for patients who require a third antihypertensive drug (Figure 32–2). β Blockers (i.e., *bisoprolol, carvedilol, metoprolol*, or *nebivolol*) remain preferred drugs for hypertensive patients with conditions such as MI, ischemic heart disease, atrial fibrillation, or congestive heart failure and may be preferred for younger patients with signs of increased sympathetic drive.

α_1 Blockers

The availability of drugs that selectively block α_1 adrenergic receptors without affecting α_2 adrenergic receptors adds another group of antihypertensive agents. The pharmacology of these drugs is discussed in detail in Chapter 14. *Prazosin*, *terazosin*, and *doxazosin* are the agents available for the treatment of hypertension. *Phenoxybenzamine*, an irreversible α blocker ($\alpha_1 > \alpha_2$), is only used in the bridging treatment of catecholamine-producing tumors (pheochromocytoma).

Pharmacological Effects

Initially, α_1 blockers reduce arteriolar resistance and increase venous capacitance; this causes a sympathetically mediated reflex increase in heart rate and plasma renin activity. During long-term therapy, vasodilation persists, but cardiac output, heart rate, and plasma renin activity return to normal. Renal blood flow is unchanged during therapy with an α_1 blocker. The α_1 blockers cause a variable amount of postural hypotension, depending on the plasma volume. Retention of salt and water occurs in many patients during continued administration, and this attenuates the postural hypotension. The α_1 blockers reduce plasma concentrations of triglycerides and total LDL cholesterol and increase HDL cholesterol. These potentially favorable effects on lipids persist when a thiazide-type diuretic is given concurrently. The long-term consequences of these small, drug-induced changes in lipids are unknown.

Therapeutic Uses

α_1 Blockers are not recommended as monotherapy for hypertensive patients, primarily as a consequence of the ALLHAT study (see further discussion). They are used primarily in conjunction with diuretics, β blockers, and other antihypertensive agents. β Blockers enhance the efficacy of α_1 blockers. α_1 Blockers are not the drugs of choice in patients with pheochromocytoma because a vasoconstrictor response to epinephrine can still result from activation of unblocked vascular α_2 adrenergic receptors. α_1 Blockers are attractive drugs for hypertensive patients with benign prostatic hyperplasia because they also improve urinary symptoms.

Adverse Effects

The use of *doxazosin* as monotherapy for hypertension increases the risk for developing congestive heart failure (ALLHAT Officers, 2002). This may be a class effect that represents an adverse effect of all of the α_1 blockers and has led to recommendations not to use this class of drugs in patients with heart failure. Interpretation of the outcome of the ALLHAT study is controversial, but the commonly held belief that the higher rate of apparent heart failure development in the groups of patients treated with a nondiuretic was caused by withdrawal of prestudy diuretics has not been substantiated (Davis et al., 2006).

A major precaution regarding the use of the α_1 blockers for hypertension is the so-called first-dose phenomenon, in which symptomatic orthostatic hypotension occurs within 30 to 90 min (or longer) of the initial dose of the drug or after a dosage increase. This effect may occur in up to 50% of patients, especially in patients who are already receiving a diuretic. After the first few doses, patients develop a tolerance to this marked hypotensive response.

Combined α_1 and β Blockers

Carvedilol (see Chapter 14) is a nonselective β blocker with α_1-antagonist activity. *Carvedilol* is approved for the treatment of hypertension and symptomatic heart failure. The ratio of α_1- to β-antagonist potency for *carvedilol* is approximately 1:10. The drug dissociates slowly from its receptor, explaining why the duration of action is longer than the short $t_{1/2}$ (2.2 h) and why its effect can hardly be overcome by catecholamines. *Carvedilol* undergoes oxidative metabolism and glucuronidation in the liver; the oxidative metabolism occurs via CYP2D6. As with *labetalol*, the long-term efficacy and side effects of *carvedilol* in hypertension are predictable based on its properties as a β and α_1 blocker. *Carvedilol* reduces mortality in patients with congestive heart failure (see Chapter 33). Due to the vasodilating effect, it is a β blocker of choice in patients with peripheral artery disease.

Labetalol (see Chapter 14) is an equimolar mixture of four stereoisomers. One isomer is an α_1 blocker, another is a nonselective β blocker with partial agonist activity, and the other two isomers are inactive. *Labetalol* has efficacy and adverse effects that would be expected with any combination of an α_1 and a β blocker. It has the disadvantages that are inherent in fixed-dose combination products: The extent of α_1 to β blockade is somewhat unpredictable and varies from patient to patient. *Labetalol* is FDA approved for eclampsia, preeclampsia, hypertension, and hypertensive emergencies. The main indication for *labetalol* is hypertension in pregnancy, for which it is one of the few compounds known to be safe (Magee et al., 2016).

Centrally Acting Sympatholytic Drugs

Methyldopa

Methyldopa, a centrally acting antihypertensive agent, is a prodrug that exerts its antihypertensive action via an active metabolite. Although used frequently as an antihypertensive agent in the past, *methyldopa*'s adverse effect profile limits its current use largely to treatment of hypertension in pregnancy, where it has a record for safety.

Methyldopa (α-methyl-3,4-dihydroxy-L-phenylalanine), an analogue of 3,4-dihydroxyphenylalanine (DOPA), is metabolized by the L-aromatic amino acid decarboxylase in adrenergic neurons to α-methyldopamine, which then is converted to α-methylnorepinephrine, the pharmacologically active metabolite. α-Methylnorepinephrine is stored in the secretory vesicles of adrenergic neurons, substituting for norepinephrine (NE), such that the stimulated adrenergic neuron now discharges α-methylnorepinephrine instead of NE. α-Methylnorepinephrine acts in the CNS to inhibit adrenergic neuronal outflow from the brainstem, probably by acting as an agonist at presynaptic α_2 adrenergic receptors in the brainstem, attenuating NE release and thereby reducing the output of vasoconstrictor adrenergic signals to the peripheral sympathetic nervous system.

ADME. Because *methyldopa* is a prodrug that is metabolized in the brain to the active form, its C_p has less relevance for its effects than that for many other drugs. C_{pmax} occurs 2 to 3 h following an oral dose. The drug is eliminated with a $t_{1/2}$ of about 2 h. *Methyldopa* is excreted in the urine primarily as the sulfate conjugate (50%–70%) and as the parent drug (25%). Other minor metabolites include methyldopamine, methylnorepinephrine, and their *O*-methylated products. The $t_{1/2}$ of *methyldopa* is prolonged to 4 to 6 h in patients with renal failure.

Despite its rapid absorption and short $t_{1/2}$, the peak effect of *methyldopa* is delayed for 6 to 8 h, even after intravenous administration, and the duration of action of a single dose is usually about 24 h; this permits once- or twice-daily dosing. The discrepancy between the effects of *methyldopa* and the measured concentrations of the drug in plasma is most likely related to the time required for transport into the CNS, conversion to the active metabolite storage of α-methyl NE, and its subsequent release in the vicinity of relevant α_2 receptors in the CNS. *Methyldopa* is a good example of a complex relationship between a drug's pharmacokinetics and its pharmacodynamics. Patients with renal failure are more sensitive to the antihypertensive effect of *methyldopa*, but it is not known if this is due to alteration in excretion of the drug or to an increase in transport into the CNS.

Therapeutic Uses. *Methyldopa* is a preferred drug for treatment of hypertension during pregnancy based on its effectiveness and safety for both mother and fetus (Magee et al., 2016). The usual initial dose of *methyldopa* is 250 mg twice daily; there is little additional effect with doses greater than 2 g/d. Administration of a single daily dose of *methyldopa* at bedtime minimizes sedative effects, but administration twice daily is required for some patients.

Adverse Effects and Precautions. *Methyldopa* produces sedation that is largely transient. A diminution in psychic energy may persist in some patients, and depression occurs occasionally. *Methyldopa* may produce dryness of the mouth. Other adverse effects include diminished libido, parkinsonian signs, and hyperprolactinemia that may become sufficiently pronounced to cause gynecomastia and galactorrhea. *Methyldopa* may precipitate severe bradycardia and sinus arrest.

Methyldopa also produces some adverse effects that are not related to its therapeutic action in the CNS. Hepatotoxicity, sometimes associated with fever, is an uncommon but potentially serious toxic effect of *methyldopa*. At least 20% of patients who receive *methyldopa* for a year develop a positive Coombs test (antiglobulin test) that is due to autoantibodies directed against the Rh antigen on erythrocytes. The development of a positive Coombs test is not necessarily an indication to stop treatment with *methyldopa*; however, 1% to 5% of these patients will develop a hemolytic anemia that requires prompt discontinuation of the drug. The Coombs test may remain positive for as long as a year after discontinuation of *methyldopa*, but the hemolytic anemia usually resolves within a matter of weeks. Severe hemolysis may be attenuated by treatment with glucocorticoids. Adverse effects that are even rarer include leukopenia, thrombocytopenia, red cell aplasia, lupus erythematosus–like syndrome, lichenoid and granulomatous skin eruptions, myocarditis, retroperitoneal fibrosis, pancreatitis, diarrhea, and malabsorption.

Clonidine and Moxonidine

The detailed pharmacology of the α_2 adrenergic agonists *clonidine* and *moxonidine* (no longer available in the U.S.) is discussed in Chapter 14. These drugs stimulate α_{2A} adrenergic receptors in the brainstem, resulting in a reduction in sympathetic outflow from the CNS (MacMillan et al., 1996). The hypotensive effect correlates directly with the decrease in plasma concentrations of NE. Patients who have had a spinal cord transection above the level of the sympathetic outflow tracts do not display a hypotensive response to *clonidine*. At doses higher than those required to stimulate central α_{2A} receptors, these drugs can activate α_{2B} receptors on vascular smooth muscle cells (MacMillan et al., 1996). This effect accounts for the initial vasoconstriction that is seen when given intravenously or overdoses of these drugs are taken. It may also be responsible for the loss of therapeutic effect that is observed with high doses. A major limitation in the use of these drugs is the paucity of information about their efficacy in reducing the risk of cardiovascular consequences of hypertension.

Pharmacological Effects. The α_2 adrenergic agonists lower arterial pressure by effects on both cardiac output and peripheral resistance. In the supine position, when the sympathetic tone to the vasculature is low, the major effect is a reduction in heart rate and stroke volume; however, in the upright position, when sympathetic outflow to the vasculature is normally increased, these drugs reduce vascular resistance. This action may lead to postural hypotension. The decrease in cardiac sympathetic tone leads to a reduction in myocardial contractility and heart rate that could promote congestive heart failure in susceptible patients.

Therapeutic Uses. The CNS effects are such that this class of drugs is not a leading option for monotherapy of hypertension. Indeed, there is no fixed place for these drugs in the treatment of hypertension. They effectively lower blood pressure in some patients who have not responded adequately to combinations of other agents. The greater clinical experience exists with *clonidine*. A study with *moxonidine* in patients with hypertension and paroxysmal atrial fibrillation indicated that the drug reduced the incidence of atrial fibrillation (Giannopoulos et al., 2014). *Clonidine* may be effective in reducing early morning hypertension in patients treated with standard antihypertensives. Overall, enthusiasm for α_2 receptor antagonists is diminished by the relative absence of evidence demonstrating reduction in risk of adverse cardiovascular events.

Clonidine has been used in hypertensive patients for the diagnosis of pheochromocytoma. The failure of *clonidine* to suppress the plasma concentration of NE to less than 500 pg/mL 3 h after an oral dose of 0.3 mg of *clonidine* suggests the presence of such a tumor. A modification of this test, wherein overnight urinary excretion of NE and epinephrine is measured after administration of a 0.3-mg dose of *clonidine* at bedtime, may be useful when results based on plasma NE concentrations are equivocal.

Adverse Effects and Precautions. Many patients experience persistent and sometimes intolerable adverse effects with these drugs. Sedation and xerostomia are prominent adverse effects. The xerostomia may be accompanied by dry nasal mucosa, dry eyes, and swelling and pain of the parotid gland. Postural hypotension and erectile dysfunction may be prominent in some patients. *Clonidine* may produce a lower incidence of dry mouth and sedation when given transdermally, perhaps because high peak concentrations are avoided. *Moxonidine* has additional activity at central imidazoline receptors and may produce less sedation than *clonidine*, but direct comparisons are lacking. Less-common CNS side effects include sleep disturbances with vivid dreams or nightmares, restlessness, and depression (note the use of α_2 adrenergic antagonists such as *mirtazapine* to treat depression). Cardiac effects related to the sympatholytic action of these drugs include symptomatic bradycardia and sinus arrest in patients with dysfunction of the SA node and AV block in patients with AV nodal disease or in patients taking other drugs that depress AV conduction. Some 15% to 20% of patients who receive transdermal *clonidine* may develop contact dermatitis.

Sudden discontinuation of *clonidine* and related α_2 adrenergic agonists may cause a withdrawal syndrome consisting of headache, apprehension, tremors, abdominal pain, sweating, and tachycardia. Arterial blood pressure may rise to levels above those present prior to treatment, but the withdrawal syndrome may occur in the absence of an overshoot in pressure. Symptoms typically occur 18 to 36 h after the drug is stopped and are associated with increased sympathetic discharge, as evidenced by elevated plasma and urine concentrations of catecholamines and metabolites. The frequency of occurrence of the withdrawal syndrome is not known, but withdrawal symptoms are likely dose related and more dangerous in patients with poorly controlled hypertension. Rebound hypertension also has been seen after discontinuation of transdermal administration of *clonidine* (Metz et al., 1987).

Treatment of the withdrawal syndrome depends on the urgency of reducing the arterial blood pressure. In the absence of life-threatening target organ damage, patients can be treated by restoring the use of *clonidine*. If a more rapid effect is required, *sodium nitroprusside* or a combination of an α and β blocker is appropriate. β Blockers should not be used alone in this setting because they may accentuate the hypertension by allowing unopposed α adrenergic vasoconstriction caused by activation of the sympathetic nervous system and elevated circulating catecholamines.

Because perioperative hypertension has been described in patients in whom *clonidine* was withdrawn the night before surgery, surgical patients who are being treated with an α_2 adrenergic agonist either should be switched to another drug prior to elective surgery or should receive their morning dose or transdermal *clonidine* prior to the procedure. All patients who receive one of these drugs should be warned of the potential danger of discontinuing the drug abruptly, and patients suspected of being noncompliant with medications should not be given α_2 adrenergic agonists for hypertension.

Adverse drug interactions with α_2 adrenergic agonists are rare. Diuretics predictably potentiate the hypotensive effect of these drugs. Tricyclic antidepressants may inhibit the antihypertensive effect of *clonidine*, but the mechanism of this interaction is not known.

Reserpine

Reserpine is an alkaloid extracted from the root of *Rauwolfia serpentina*, a climbing shrub indigenous to India. Ancient Hindu Ayurvedic writings describe medicinal uses of the plant; Sen and Bose described its use in the Indian biomedical literature. However, rauwolfia alkaloids were not used in Western medicine until the mid-1950s. *Reserpine* was the first drug found to interfere with the function of the sympathetic nervous system in humans, and its use began the modern era of effective pharmacotherapy of hypertension. *Reserpine* is no longer marketed in the U.S.

Mechanism of Action. *Reserpine* binds tightly to adrenergic storage vesicles in central and peripheral adrenergic neurons and remains bound for prolonged periods of time. The interaction inhibits the vesicular catecholamine transporter VMAT2, so that nerve endings lose their capacity to concentrate and store NE and dopamine. Catecholamines leak into the cytoplasm, where they are metabolized. Consequently, little or no active transmitter is released from nerve endings, resulting in a pharmacological sympathectomy. Recovery of sympathetic function requires synthesis of new storage vesicles, which takes days to weeks after discontinuation of the drug. Because *reserpine* depletes amines in the CNS as well as in the peripheral adrenergic neuron, it is probable that its antihypertensive effects are related to both central and peripheral actions.

Pharmacological Effects. Both cardiac output and peripheral vascular resistance are reduced during long-term therapy with *reserpine*.

ADME. Few data are available on the pharmacokinetic properties of *reserpine* because of the lack of an assay capable of detecting low concentrations of the drug or its metabolites. *Reserpine* that is bound to isolated storage vesicles cannot be removed by dialysis, indicating that the binding is not in equilibrium with the surrounding medium. Because of the irreversible nature of *reserpine* binding, the amount of drug in plasma is unlikely to bear any consistent relationship to drug concentration at the site of action. Free *reserpine* is entirely metabolized; therefore, none of the parent drug is excreted unchanged.

Toxicity and Precautions. Most adverse effects of *reserpine* are due to its effect on the CNS. Sedation and inability to concentrate or perform complex tasks are the most common adverse effects. More serious is the occasional psychotic depression that can lead to suicide. Depression usually appears insidiously over many weeks or months and may not be attributed to the drug because of the delayed and gradual onset of symptoms. *Reserpine* must be discontinued at the first sign of depression. *Reserpine*-induced depression may last several months after the drug is discontinued. The risk of depression is likely dose related. Depression is uncommon, but not unknown, with doses of 0.25 mg/d or less. The drug should never be given to patients with a history of depression. Other adverse effects include nasal stuffiness and exacerbation of peptic ulcer disease, which is uncommon with small oral doses.

Therapeutic Uses. *Reserpine* at low doses, in combination with diuretics, is effective in the treatment of hypertension, especially in the elderly. Several weeks are necessary to achieve maximum effect. In elderly patients with isolated systolic hypertension, *reserpine* (at 0.05 mg/d) was used as an alternative to *atenolol* together with a diuretic (Perry et al., 2000; SHEP Cooperative Research Group, 1991). However, with the availability of newer drugs that have proven life-prolonging effects and are well tolerated, the use of *reserpine* has largely diminished, and it is no longer recommended for the treatment of hypertension.

Vasodilators

Hydralazine

Hydralazine (1-hydrazinophthalazine) was one of the first orally active antihypertensive drugs to be marketed in the U.S.; however, the drug initially was used infrequently because of tachycardia and tachyphylaxis. With a better understanding of the compensatory cardiovascular responses that accompany use of arteriolar vasodilators, *hydralazine* was combined with sympatholytic agents and diuretics with greater therapeutic success. Nonetheless, its role in the treatment of hypertension has markedly diminished with the introduction of new classes of antihypertensive agents.

Mechanism of Action

Hydralazine directly relaxes arteriolar smooth muscle with little effect on venous smooth muscle. The molecular mechanisms mediating this action are not clear but may ultimately involve a reduction in intracellular Ca^{2+} concentrations. While a variety of changes in cellular signaling pathways are influenced by *hydralazine*, precise molecular targets that explain its capacity to dilate arteries remain uncertain. Potential mechanisms include inhibition of inositol trisphosphate–induced release of Ca^{2+} from intracellular storage sites, opening of high-conductance Ca^{2+}-activated K^+ channels in smooth muscle cells, and activation of an arachidonic acid, COX, and prostacyclin pathway that would explain sensitivity to NSAIDs (Maille et al., 2016).

Hydralazine-induced vasodilation is associated with powerful stimulation of the sympathetic nervous system, likely due to baroreceptor-mediated reflexes, resulting in increased heart rate and contractility, increased plasma renin activity, and fluid retention. These effects tend to counteract the antihypertensive effect of *hydralazine*.

Pharmacological Effects

Most of the effects of *hydralazine* are confined to the cardiovascular system. The decrease in blood pressure after administration of *hydralazine* is associated with a selective decrease in vascular resistance in the coronary, cerebral, and renal circulations, with a smaller effect in skin and muscle. Because of preferential dilation of arterioles over veins, postural hypotension is not a common problem; *hydralazine* lowers blood pressure similarly in the supine and upright positions.

ADME

Following oral administration, *hydralazine* is well absorbed via the gastrointestinal (GI) tract. *Hydralazine* is *N*-acetylated in the bowel and the liver, contributing to the drug's low bioavailability (16% in fast acetylators and 35% in slow acetylators). The rate of acetylation is genetically determined; about half of the U.S. population acetylates rapidly, and half does so slowly. The acetylated compound is inactive; thus, the dose necessary to produce a systemic effect is larger in fast acetylators. Because the systemic clearance exceeds hepatic blood flow, extrahepatic metabolism must occur. Indeed, *hydralazine* rapidly combines with circulating α-keto acids to form hydrazones, and the major metabolite recovered from the plasma is hydralazine pyruvic acid hydrazone. This metabolite has a longer $t_{1/2}$ than *hydralazine* but appears to be relatively inactive. Although the rate of acetylation is an important determinant of the bioavailability of *hydralazine*, it does not play a role in the systemic elimination of the drug, probably because hepatic clearance is so high that systemic elimination is principally a function of hepatic blood flow. The peak concentration of *hydralazine* in plasma and the peak hypotensive effect of the drug occurs within 30 to 120 min of ingestion. Although its $t_{1/2}$ in plasma is about 1 h, the hypotensive effect of *hydralazine* can last as long as 12 h. There is no clear explanation for this discrepancy.

Therapeutic Uses

Hydralazine is no longer a first-line drug in the treatment of hypertension on account of its relatively unfavorable adverse effect profile. The drug has a role as a combination pill containing *isosorbide dinitrate* (BiDil) in the treatment of heart failure (see Chapter 33). *Hydralazine* may have utility in the treatment of some patients with severe hypertension, can be part of evidence-based therapy in patients with congestive heart failure (in combination with nitrates for patients who cannot tolerate ACE inhibitors or ARBs), and may be useful in the treatment of hypertensive emergencies, especially preeclampsia, in pregnant women. *Hydralazine* should be used with great caution in elderly patients and in hypertensive patients with CAD because of the possibility of precipitating myocardial ischemia due to reflex tachycardia. The usual oral dosage of *hydralazine* is 25 to 100 mg twice daily. Off-label twice-daily administration is as effective as administration four times a day for control of blood pressure, regardless of acetylator phenotype. The maximum recommended dose of *hydralazine* is 200 mg/d to minimize the risk of drug-induced lupus syndrome.

Toxicity and Precautions

Two types of adverse effects occur after the use of *hydralazine*. The first, which are extensions of the pharmacological effects of the drug, include headache, nausea, flushing, hypotension, palpitations, tachycardia, dizziness, and angina pectoris. Myocardial ischemia can occur on account of increased O_2 demand induced by the baroreceptor reflex–induced stimulation of the sympathetic nervous system. Following parenteral administration to patients with CAD, the myocardial ischemia may be sufficiently severe and protracted to cause frank MI. For this reason, parenteral administration of *hydralazine* is not advisable in hypertensive patients with CAD, hypertensive patients with multiple cardiovascular risk factors, or older patients. In addition, if the drug is used alone, there may be salt retention with development of high-output congestive heart failure. When combined with a β blocker and a diuretic, *hydralazine* is better tolerated, although adverse effects such as headache are still commonly described and may necessitate discontinuation of the drug.

The second type of adverse effect is caused by immunological reactions, of which the drug-induced lupus syndrome is the most common. Administration of *hydralazine* also can result in an illness that resembles serum sickness, hemolytic anemia, vasculitis, and rapidly progressive glomerulonephritis. The mechanism of these autoimmune reactions is unknown, although it may involve the drug's capacity to inhibit DNA methylation (Arce et al., 2006). The drug-induced lupus syndrome usually occurs after at least 6 months of continuous treatment with *hydralazine*, and its incidence is related to dose, gender, acetylator phenotype, and race. In one study, after 3 years of treatment with *hydralazine*, drug-induced lupus occurred in 10% of patients who received 200 mg daily, 5% who received 100 mg daily, and none who received 50 mg daily (Cameron and Ramsay, 1984). The incidence is four times higher in women than in men, and the syndrome is seen more commonly in Caucasians than in African Americans. The rate of conversion to a positive antinuclear antibody test is faster in slow acetylators than in rapid acetylators, suggesting that the native drug or a nonacetylated metabolite is responsible. However, the majority of patients with positive antinuclear antibody tests do not develop the drug-induced lupus syndrome, and *hydralazine* need not be discontinued unless clinical features (arthralgia, arthritis, and fever) of the syndrome appear. Discontinuation of the drug is all that is necessary for most patients with the *hydralazine*-induced lupus syndrome, but symptoms may persist in a few patients, and administration of corticosteroids may be necessary.

Hydralazine also can produce a pyridoxine-responsive polyneuropathy. The mechanism appears to be related to the ability of *hydralazine* to combine with *pyridoxine* to form a hydrazone. This side effect is unusual with doses of 200 mg/d or less.

K_{ATP} Channel Openers: Minoxidil

The 1965 discovery of the hypotensive action of *minoxidil* was a significant advance in the treatment of hypertension; the drug has proven to be efficacious in patients with the most severe and drug-resistant forms of hypertension.

Mechanism of Action

Minoxidil is not active *in vitro* but must be metabolized by hepatic sulfotransferase to the active molecule, minoxidil *N-O* sulfate; the formation of this active metabolite is a minor pathway in the metabolic disposition of *minoxidil*. Minoxidil sulfate relaxes vascular smooth muscle in isolated systems where the parent drug is inactive. Minoxidil sulfate activates the ATP-modulated K^+ channel permitting K^+ efflux and causes hyperpolarization and relaxation of smooth muscle.

Pharmacological Effects

Minoxidil produces arteriolar vasodilation with essentially no effect on the capacitance vessels; the drug resembles *hydralazine* and *diazoxide* in this regard. *Minoxidil* increases blood flow to skin, skeletal muscle, GI tract, and heart more than to the CNS. The disproportionate increase in blood flow to the heart may have a metabolic basis in that administration of *minoxidil* is associated with a reflex increase in myocardial contractility and in cardiac output. The cardiac output can increase markedly, as much as 3- to 4-fold. The principal determinant of the elevation in cardiac output is the action of *minoxidil* on peripheral vascular resistance to enhance venous return to the heart; by inference from studies with other drugs, the increased venous return probably results from enhancement of flow in the regional vascular beds, with a fast time constant for venous return to the heart (Ogilvie, 1985). The adrenergically mediated increase in myocardial contractility contributes to the increased cardiac output but is not the predominant causal factor.

The effects of *minoxidil* on the kidney are complex. *Minoxidil* is a renal artery vasodilator, but systemic hypotension produced by the drug occasionally can decrease renal blood flow. Renal function usually improves in patients who take *minoxidil* for the treatment of hypertension, especially if renal dysfunction is secondary to hypertension. *Minoxidil* is a potent stimulator of renin secretion. This effect is mediated by a combination of renal sympathetic stimulation and activation of the intrinsic renal mechanisms for regulation of renin release.

Discovery of K^+_{ATP} channels in a variety of cell types and in mitochondria is prompting consideration of K^+_{ATP} channel modulators as therapeutic agents in many cardiovascular diseases (Pollesello and Mebazaa, 2004). *Minoxidil*, similar to other K^+_{ATP} channel openers such as *diazoxide*, *pinacidil*, and *nicorandil*, may have protective effects on the heart during ischemia/reperfusion (Sato et al., 2004). *Pinacidil* and *nicorandil* are not available in the U.S. It also promotes the synthesis of vascular elastin in rats (Slove et al., 2013), a potentially interesting therapeutic effect.

ADME

Minoxidil is well absorbed from the GI tract. Although peak concentrations of *minoxidil* in blood occur 1 h after oral administration, the maximal hypotensive effect of the drug occurs later, possibly because formation of the active metabolite is delayed.

The bulk of the absorbed drug is eliminated as a glucuronide; about 20% is excreted unchanged in the urine. The extent of biotransformation of *minoxidil* to its active metabolite, minoxidil *N-O* sulfate, has not been evaluated in humans. *Minoxidil* has a plasma $t_{1/2}$ of 3 to 4 h, but its duration of action is 24 h and occasionally even longer. It has been proposed that persistence of *minoxidil* in vascular smooth muscle is responsible for this discrepancy, but without knowledge of the pharmacokinetic properties of the active metabolite, an explanation for the prolonged duration of action cannot be given.

Therapeutic Uses

Systemic *minoxidil* is best reserved for the treatment of severe hypertension that responds poorly to other antihypertensive medications, especially in male patients with renal insufficiency. *Minoxidil* has been used successfully in the treatment of hypertension in both adults and children. *Minoxidil* should never be used alone; it must be given concurrently with a diuretic to avoid fluid retention, with a sympatholytic drug (e.g., β blocker) to control reflex cardiovascular effects, and with an inhibitor of the RAS to prevent remodeling effects on the heart. The drug usually is administered either once or twice a day, but some patients may require more frequent dosing for adequate control of blood pressure. The initial daily dose of *minoxidil* may be as little as 1.25 mg, which can be increased gradually to 40 mg in one or two daily doses.

Adverse Effects and Precautions

The adverse effects of *minoxidil*, which can be severe, fall into three major categories: fluid and salt retention, cardiovascular effects, and hypertrichosis. Retention of salt and water results from increased proximal renal tubular reabsorption, which is secondary to reduced renal perfusion pressure and to reflex stimulation of renal tubular α adrenergic receptors. Similar antinatriuretic effects can be observed with the other arteriolar dilators (e.g., *diazoxide* and *hydralazine*). Although administration of *minoxidil* causes increased secretion of renin and aldosterone, this is not an important mechanism for retention of salt and water in this case. Fluid retention usually can be controlled by the administration of a diuretic. However, thiazides may not be sufficiently efficacious, and it may be necessary to use a loop diuretic, especially if the patient has any degree of renal dysfunction.

The cardiac consequences of the baroreceptor-mediated activation of the sympathetic nervous system during *minoxidil* therapy are similar to those seen with *hydralazine*; there is an increase in heart rate, myocardial contractility, and myocardial O_2 consumption. Thus, myocardial ischemia can be induced by *minoxidil* in patients with CAD. The cardiac sympathetic responses are attenuated by concurrent administration of a β blocker. The adrenergically induced increase in renin secretion also can be ameliorated by a β blocker or an ACE inhibitor, with enhancement of blood pressure control.

The increased cardiac output evoked by *minoxidil* has particularly adverse consequences in those hypertensive patients who have left ventricular hypertrophy and diastolic dysfunction. Such poorly compliant ventricles respond suboptimally to increased volume loads, with a resulting increase in left ventricular filling pressure. This probably is a major contributor to the increased pulmonary artery pressure seen with *minoxidil* (and *hydralazine*) therapy in hypertensive patients and is compounded by the retention of salt and water caused by *minoxidil*. Cardiac failure can result from *minoxidil* therapy in such patients; the potential for this complication can be reduced but not prevented by effective diuretic therapy. Pericardial effusion is an uncommon but serious complication of *minoxidil*. Mild and asymptomatic pericardial effusion is not an indication for discontinuing *minoxidil*, but the situation should be monitored closely to avoid progression to tamponade. Effusions usually clear when the drug is discontinued but can recur if treatment with *minoxidil* is resumed.

Flattened and inverted T waves frequently are observed in the electrocardiogram following the initiation of *minoxidil* treatment. These are not ischemic in origin and are seen with other drugs that activate

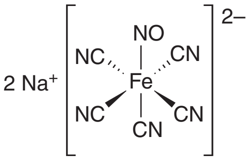
K^+ channels. *Pinacidil* is associated with a lowered ventricular fibrillation threshold and increased spontaneous ventricular fibrillation in the ischemic canine heart, while *minoxidil* had antiarrhythmic effects in the rabbit under ischemia/reperfusion. Whether such findings translate to events in humans is unknown.

Excess hair growth occurs in patients who receive *minoxidil* for an extended period and is probably a consequence of K^+ channel activation. Growth of hair occurs on the face, back, arms, and legs and is particularly offensive to women. Frequent shaving or depilatory agents can be used to manage this problem. Topical *minoxidil* is marketed over the counter for the treatment of male pattern baldness and hair thinning and loss on the top of the head in women. The topical use of *minoxidil* also can cause measurable cardiovascular effects in some individuals.

Other side effects of the drug are rare and include rashes, Stevens-Johnson syndrome, glucose intolerance, serosanguineous bullae, formation of antinuclear antibodies, and thrombocytopenia. *Minoxidil* is contraindicated in patients diagnosed with pheochromocytoma.

Sodium Nitroprusside

Although *sodium nitroprusside* has been known since 1850 and its hypotensive effect in humans was described in 1929, its safety and usefulness for the short-term control of severe hypertension were not demonstrated until the mid-1950s. Several investigators subsequently demonstrated that *sodium nitroprusside* also was effective in improving cardiac function in patients with left ventricular failure (see Chapter 33).



Sodium nitroprusside

Mechanism of Action

Nitroprusside is a nitrovasodilator that acts by releasing NO. NO activates the guanylyl cyclase–cyclic guanosine monophosphate–protein kinase G pathway, leading to vasodilation, mimicking the production of NO by vascular endothelial cells, which is impaired in many hypertensive patients. The mechanism of release of NO from *nitroprusside* is not clear and likely involves both enzymatic and nonenzymatic pathways. Tolerance develops to *nitroglycerin* but not to *nitroprusside*. The pharmacology of the organic nitrates, including *nitroglycerin*, is presented in Chapter 31.

Pharmacological Effects

Nitroprusside dilates both arterioles and venules, and the hemodynamic response to its administration results from a combination of venous pooling and reduced arterial impedance. In subjects with normal left ventricular function, venous pooling affects cardiac output more than does the reduction of afterload; cardiac output tends to fall. In contrast, in patients with severely impaired left ventricular function and diastolic ventricular distention, the reduction of arterial impedance is the predominant effect, leading to a rise in cardiac output (see Chapter 33).

Sodium nitroprusside is a nonselective vasodilator, and regional distribution of blood flow is little affected by the drug. In general, renal blood flow and glomerular filtration are maintained, and plasma renin activity increases. Unlike *minoxidil*, *hydralazine*, *diazoxide*, and other arteriolar vasodilators, *sodium nitroprusside* usually causes only a modest increase in heart rate and an overall reduction in myocardial O_2 demand.

ADME

Sodium nitroprusside is an unstable molecule that decomposes under strongly alkaline conditions or when exposed to light. The drug must be protected from light and given by continuous intravenous infusion to be effective. Its onset of action is within 30 sec; the peak hypotensive effect occurs within 2 min, and when the infusion of the drug is stopped, the effect disappears within 3 min.

Sodium nitroprusside is available in vials that contain 50 mg. The contents of the vial should be dissolved in 2 to 3 mL of 5% dextrose in water. Because the compound decomposes in light, only fresh solutions should be used, and the bottle should be covered with an opaque wrapping. The drug must be administered as a controlled continuous infusion, and the patient must be closely observed. Most hypertensive patients respond to an infusion of 0.25 to 1.5 μg/kg/min. Higher infusion rates are necessary to produce controlled hypotension in normotensive patients under surgical anesthesia. Patients who are receiving other antihypertensive medications usually require less *nitroprusside* to lower blood pressure. If infusion rates of 10 μg/kg/min do not produce adequate reduction of blood pressure within 10 min, the rate of administration of *nitroprusside* should be reduced to minimize potential toxicity.

The metabolism of *nitroprusside* by smooth muscle is initiated by its reduction, which is followed by the release of cyanide and then NO. Cyanide is further metabolized by hepatic rhodanase to form thiocyanate, which is eliminated almost entirely in the urine. The mean elimination $t_{1/2}$ for thiocyanate is 3 days in patients with normal renal function and much longer in patients with renal insufficiency.

Therapeutic Uses

Sodium nitroprusside is used primarily to treat hypertensive emergencies but also can be used in situations when short-term reduction of cardiac preload or afterload is desired. *Nitroprusside* has been used to lower blood pressure during acute aortic dissection; to improve cardiac output in congestive heart failure, especially in hypertensive patients with pulmonary edema that does not respond to other treatment (see Chapter 33); and to decrease myocardial O_2 demand after acute MI. In addition, *nitroprusside* is used to induce controlled hypotension during anesthesia to reduce bleeding in surgical procedures. In the treatment of acute aortic dissection, it is important to administer a β blocker with *nitroprusside* because reduction of blood pressure with *nitroprusside* alone can increase the rate of rise in pressure in the aorta because of increased myocardial contractility, thereby enhancing propagation of the dissection.

Toxicity and Precautions

The short-term adverse effects of *nitroprusside* are due to excessive vasodilation, with hypotension and its consequences. Close monitoring of blood pressure and the use of a continuous variable-rate infusion pump will prevent an excessive hemodynamic response to the drug in most cases.

Less commonly, toxicity may result from conversion of *nitroprusside* to cyanide and thiocyanate. Toxic accumulation of cyanide leading to severe lactic acidosis usually occurs when *sodium nitroprusside* is infused at a rate greater than 5 μg/kg/min but also can occur in some patients receiving doses on the order of 2 μg/kg/min for a prolonged period. The limiting factor in the metabolism of cyanide appears to be the availability of sulfur-containing substrates in the body (i.e., mainly thiosulfate). The concomitant administration of sodium thiosulfate can prevent accumulation of cyanide in patients who are receiving higher-than-usual doses of *sodium nitroprusside*; the efficacy of the drug is unchanged. The risk of thiocyanate toxicity increases when *sodium nitroprusside* is infused for more than 24 to 48 h, especially if renal function is impaired. Signs and symptoms of thiocyanate toxicity include anorexia, nausea, fatigue, disorientation, and toxic psychosis. The plasma concentration of thiocyanate should be monitored during prolonged infusions of *nitroprusside* and should not be allowed to exceed 0.1 mg/mL. Rarely, excessive concentrations of thiocyanate may cause hypothyroidism by inhibiting iodine uptake by the thyroid gland. In patients with renal failure, thiocyanate can be removed readily by hemodialysis. Patients with congenital (Leber) optic atrophy or with tobacco amblyopia have unusually high cyanide/thiocyanate ratios. These rare conditions are probably associated with defective or absent rhodanase, and *sodium nitroprusside* should be avoided in these patients.

Nitroprusside can worsen arterial hypoxemia in patients with chronic obstructive pulmonary disease because the drug interferes with hypoxic pulmonary vasoconstriction and therefore promotes mismatching of ventilation with perfusion.

Diazoxide

Diazoxide was used in the treatment of hypertensive emergencies but fell out of favor at least in part due to the risk of marked falls in blood pressure when large bolus doses of the drug were used. Other drugs are now preferred for parenteral administration in the control of hypertension. *Diazoxide* also is administered orally to treat patients with various forms of hypoglycemia (see Chapter 51).

Nonpharmacological Therapy of Hypertension

Nonpharmacological approaches to the treatment of hypertension may suffice in patients with modestly elevated blood pressure. Such approaches also can augment the effects of antihypertensive drugs in patients with more marked initial elevations in blood pressure. The indications and efficacy of various lifestyle modifications in hypertension were reviewed in recent guidelines (Whelton et al., 2018; Williams et al., 2018).

- Reduction in body weight for people who are modestly overweight or frankly obese may be useful.
- Restricting sodium consumption lowers blood pressure in some patients.
- Restriction of ethanol intake to modest levels (daily consumption <20 g in women, <40 g in men) may lower blood pressure.
- Increased physical activity improves control of hypertension.
- Renal denervation may be effective in patients with well-defined resistant hypertension (Sardar et al., 2019).
- Bariatric surgery in grossly overweight individuals may normalize blood pressure and increase life expectancy (Schiavon et al., 2020).

Selection of Antihypertensive Drugs in Individual Patients

Choice of an antihypertensive drug should be driven by the likely benefit in an individual patient, considering concomitant diseases such as diabetes mellitus or CAD and problematic adverse effects of specific drugs. Costs have lost relevance because the most important antihypertensive drug classes (ACE inhibitors/ARBs, Ca^{2+} channel blockers, diuretics, and β blockers) are out of patent protection and available as low-cost generics.

After a long debate about blood pressure–independent effects of certain antihypertensive drug classes, there is a consensus that blood pressure lowering *per se* is the most important goal of antihypertensive treatment. This conclusion is based on a number of large comparative prospective trials that, overall, did not show major differences in outcome depending on drug class. Nevertheless, the current evidence for an overall better benefit/risk ratio of ACE inhibitors/ARBs, Ca^{2+} channel blockers, and diuretics (Table 32–4) has led to the recommendation in both American and European guidelines that these four classes of drugs should be preferred as first-line agents in patients with essential hypertension devoid of compelling indications for other drugs (Whelton et al., 2018; Williams et al., 2018; Figure 32–2). The guidelines and evidence for specific therapeutic benefit in special patient groups are the basis of recommendations for a compilation of drug choices in Table 32–4.

TABLE 32–4 ■ ANTIHYPERTENSIVE AGENTS PREFERRED IN SPECIFIC PATIENT POPULATIONS

MEDICAL CONDITION	PREFERRED ANTIHYPERTENSIVE AGENTS
Left ventricular hypertrophy	ACEI, ARB, CCB
Asymptomatic atherosclerosis	CCB
Microalbuminuria	ACEI, ARB
Renal dysfunction	ACEI, ARB
Previous stroke	ACEI, ARB, diuretics
Previous myocardial infarction	ACEI, ARB, BB
Coronary artery disease	ACEI, ARB, BB
Angina pectoris	BB, CCB
Heart failure	ACEI, ARB, BB, diuretics, MRA
Aortic aneurysm	BB
Atrial fibrillation, prevention	ACEI, ARB, BB
Atrial fibrillation, rate control	BB, CCB (nondihydropyridines)
End-stage renal disease, proteinuria	ACEI
Peripheral artery disease	ACEI, CCB
Isolated systolic hypertension	ACEI, ARB, CCB, diuretics
Metabolic syndrome	ACEI, ARB, CCB
Diabetes mellitus	ACEI, ARB, CCB, diuretics
Diabetes mellitus with proteinuria	ACEI, ARB
Hyperaldosteronism	MRA
Pregnancy	BB, CCB, α-methyldopa
Black ethnicity	CCB, diuretics

ACEI, ACE inhibitor; CCB, calcium channel blocker. The drug choices depicted represent a combined view from nine guidelines that differ; thus, the table is, necessarily, a didactic simplification (for details, consult Kjeldsen et al., 2014).

Several pharmacological principles should be considered for optimizing the antihypertensive drug regimen.

1. **Pharmacokinetics:** Hypertension is a chronic, often lifelong disease without major symptoms but with serious complications, making compliance to antihypertensive drugs a factor of utmost prognostic importance. Antihypertensives should be chosen that exhibit relatively even plasma concentrations at once-daily dosing, achieving sufficient 24-h control of blood pressure and trough-peak effect ratios greater than 50%. The longer the half-life, the less is the variation of plasma concentrations (e.g., *chlorthalidone* vs. *hydrochlorothiazide*). Drugs with stable pharmacokinetics, that is, low drug interaction potential and no pharmacogenetic influence, are preferred (e.g., *bisoprolol* vs. *metoprolol*).
2. **Drug combinations:** Two-thirds of patients with hypertension require two or more antihypertensives for sufficient blood pressure control. It is therefore recommended to start combining drugs at low-to-medium doses instead of increasing the dose of a single drug (Figure 32–2). Prescribing fixed drug combinations (e.g., a Ca^{2+} channel blocker + ACE inhibitor or an ACE inhibitor + diuretic) improves compliance.
3. **Strength of scientific evidence:** Data from large prospective trials provide a high level of confidence for a beneficial risk-benefit ratio and are a reason to use one drug over another.
4. **Pharmacodynamic considerations:** Although not formally tested in prospective trials, certain drug combinations make more sense than others. Thiazide diuretics increase the antihypertensive actions of all other classes, but their combination with RAS inhibitors makes particular sense as their K$^+$-sparing effect and thus their main risk are reduced by members of this class.
5. **Adverse drug effects and contraindications:** The major classes of antihypertensives are generally well tolerated and, in placebo-controlled trials, showed rates of adverse effects in the range of placebo with some notable exceptions that need to be taken into consideration when choosing a specific drug for a specific patient (Table 32–5). The rate of adverse effects such as hypotension or bradycardia can be largely reduced by starting antihypertensives at low doses and employing a slow dose-escalation strategy.

TABLE 32–5 ■ COMPELLING AND POSSIBLE CONTRAINDICATIONS[a] TO ANTIHYPERTENSIVE DRUGS

DRUG CLASS	COMPELLING CONTRAINDICATION	POSSIBLE CONTRAINDICATION/PRECAUTION
ACE inhibitors	Pregnancy Angioneurotic edema Hyperkalemia Bilateral renal artery stenosis	Women with childbearing potential
Angiotensin receptor blockers	Pregnancy Hyperkalemia Bilateral renal artery stenosis	Women with childbearing potential
Ca^{2+} channel blockers (dihydropyridines)		Tachycardia/arrhythmia Heart failure
Ca^{2+} channel blockers (verapamil, diltiazem)	AV block (grade 2–3) Severe left ventricular dysfunction Heart failure	Co-medication with CYP3A4- or P-glycoprotein–dependent drugs (e.g., statins, digoxin)
Diuretics (thiazides)	Gout	Metabolic syndrome Glucose intolerance Pregnancy Hypercalcemia Hypokalemia Erectile dysfunction
Mineralocorticoid receptor antagonists (MRA)	Hyperkalemia Serum creatinine >2.5 mg/dL in men, >2.0 mg/dL in women	Situations associated with higher risk of hyperkalemia (ACE inhibitor, ARB, diabetes)
β Blockers	Asthma AV block (grade 2–3)	Metabolic syndrome Glucose intolerance Athletes and physically active patients Chronic obstructive lung disease Psoriasis Depression
α Blockers	Heart failure	
Central sympatholytic drugs	Depression AV block (grade 2–3)	Erectile dysfunction Xerostomia

[a] *Possible contraindications and precautions* noted in column 3 are not formal contraindications, but rather patient characteristics that should be considered on an individual basis and that may mitigate against use of a class of drugs (e.g., metabolic syndrome and glucose intolerance for diuretics and β blockers). Similarly, some patients with chronic obstructive lung disease can be treated with β_1 blockers without deterioration of lung function, whereas other patients may experience significant bronchoconstriction with β blockers.

6. **Compelling indications:** Several compelling indications exist for specific antihypertensive agents on account of other serious, underlying cardiovascular disease (Table 32–4). These include heart failure, CAD, post-MI, chronic kidney disease, or diabetes. For example, a hypertensive patient with congestive heart failure ideally should be treated with a β blocker, ACE inhibitor/AT_1 receptor blocker, diuretic, and, in selected patients, *spironolactone* because of the benefit of these drugs in congestive heart failure, even in the absence of hypertension (see Chapter 33). Similarly, ACE inhibitors/ARBs should be first-line drugs in the treatment of diabetics with hypertension in view of these drugs' well-established benefits in diabetic nephropathy.
7. **Comorbidities:** Some patients have other diseases that could influence the choice of antihypertensive drugs. For example, a hypertensive patient with symptomatic benign prostatic hyperplasia might benefit from having an α_1 blocker as part of his therapeutic program because α_1 blockers are efficacious in both diseases. Similarly, a patient with recurrent migraine attacks might particularly benefit from use of a β blocker because a number of drugs in this class are efficacious in preventing migraine attacks. Women with a high risk of osteoporosis may benefit from the Ca^{2+}-increasing effect of thiazide diuretics. On the other hand, in pregnant hypertensives, some drugs that are otherwise little used (e.g., *labetalol, methyldopa*) may be preferred and popular drugs (e.g., ACE inhibitors) need to be avoided on account of concerns about safety.
8. **Second- and third-line hypertensives:** In most cases, hypertension can be well controlled by antihypertensives of the four major classes with or without *spironolactone* at low doses. However, patients with chronic kidney disease often require the additional use of drugs such as *hydralazine* or *minoxidil*. The place of *clonidine*/*moxonidine* or α_1 blockers in the treatment of hypertension is not well defined.

Acute Antihypertensive Treatment

The considerations mentioned apply to patients with hypertension who need treatment to reduce long-term risk, not patients in immediately life-threatening settings due to hypertension. While there are limited clinical trial data, clinical judgment favors rapidly lowering blood pressure in patients with life-threatening complications of hypertension, such as encephalopathy or pulmonary edema due to severe hypertension. However, rapid reduction in blood pressure has considerable risks for the patients; if blood pressure is decreased too quickly or extensively, cerebral blood flow may diminish due to adaptations in the cerebral circulation that protect the brain from the sequelae of very high blood pressures.

The temptation to treat patients merely on the basis of increased blood pressure should be resisted. Appropriate therapeutic decisions need to encompass how well a patient's major organs are reacting to the very high blood pressures. While many drugs have been used parenterally to rapidly decrease blood pressure in emergencies (including *nitroprusside, enalaprilat, esmolol, fenoldopam, labetalol, clevidipine* and *nicardipine*, *hydralazine*, and *phentolamine*), the clinical significance of differing actions of many of these drugs in this setting is largely unknown (Perez et al., 2009).

Resistant Hypertension

Some patients with hypertension fail to respond to recommended antihypertensive treatments. There are many potential explanations. To achieve stringent control of hypertension, many patients require two, three, or four appropriately selected drugs used at optimal doses. Exhibiting *an abundance of caution* and *therapeutic inertia*, clinicians may be reluctant to prescribe enough medications that exploit the drugs' full dose-response curves; conversely, patients may not adhere to the recommended pharmacological regimen. Sometimes, multiple drugs in the same therapeutic class that act by the same mechanism are combined; that is generally not a rational approach. Excess salt intake and the tendency of some antihypertensive drugs, especially vasodilators, to promote salt retention may mitigate falls in blood pressure; consequently, inadequate diuretic treatment commonly is found in patients with resistant hypertension. A relevant fraction of patients with resistant hypertension have primary hyperaldosteronism and benefit from the addition of daily *spironolactone* at 25 to 50 mg (Williams et al., 2015). Patients may take prescription drugs, over-the-counter drugs, or herbal preparations that oppose the actions of antihypertensive drugs (e.g., NSAIDs, sympathomimetic decongestants, *cyclosporine*, *erythropoietin*, *ephedra* [also called ma huang], or licorice). Illicit drugs such as cocaine and amphetamines may raise blood pressure. The physician must inquire about a patient's other medications and supplements and individualize the antihypertensive regimen.

Drug Facts for Your Personal Formulary: *Antihypertensives*

Antihypertensive Drug	Therapeutic Uses	Major Toxicity and Clinical Pearls
Diuretics		
Thiazide type Chlorothiazide Hydrochlorothiazide ***Thiazide-like*** Chlorthalidone Indapamide Metolazone Xipamide	• Hypertension (HTN) • Edema associated with heart failure (HF), liver cirrhosis, chronic kidney disease, nephrotic syndrome • Nephrogenic diabetes insipidus • Kidney stones caused by Ca^{2+} crystals • Metolazone and xipamide (not approved in the U.S.) are also effective at glomerular filtration rate (GFR) <30 mL/min	• First choice for treating HTN • Chlorthalidone may be superior to hydrochlorothiazide in HTN • Lose efficacy at GFR <30–40 mL/min (exceptions: indapamide, metolazone, xipamide) • Potentiate effect of loop diuretics in HF (sequential tubular blockade) • Risk of hypokalemia and arrhythmia when combined with QT-prolonging drugs • Combine with ACE inhibitor (ACEI)/ARB or K^+-sparing diuretic/MRA to prevent hypokalemia
Loop diuretics Bumetanide Furosemide Torsemide	• Acute pulmonary edema • Edema associated with HF, liver cirrhosis, chronic kidney disease, nephrotic syndrome • Hyponatremia • Hypercalcemia • HTN	• Not first choice for treating HTN with normal renal function: action too short and followed by rebound • Indicated acutely in malignant HTN and GFR <30–40 mL/min • Torsemide may be superior to furosemide in HF • Risk of hypokalemia and arrhythmia when combined with QT-prolonging drugs
Sympatholytic Drugs		
β_1 Blockers Atenolol Bisoprolol Metoprolol Nebivolol Many others	• HTN • Heart failure (bisoprolol, metoprolol, nebivolol) • Widely used for other indications (angina, prevention of arrhythmias, rate control in atrial fibrillation, migraine, etc.)	• Not first choice in uncomplicated HTN; clear indication for angina, HF, atrial fibrillation, etc. • Bradycardia and AV block • Bronchospasm, peripheral vasoconstriction • Worsening of *acute* heart failure • Depression • Worsening of psoriasis • Polymorphic CYP2D6 metabolism (metoprolol) • Nebivolol NO-mediated vasodilation
Nonselective β blocker Propranolol	• HTN • Migraine	• Not first choice for treating HTN • Unwanted effects via blockade of β_2 receptors
α_1 Blockers Alfuzosin Doxazosin Prazosin Terazosin	• Benign prostate hyperplasia • HTN	• Not first choice for treating HTN • Higher rate of HF development (?) • Tachyphylaxis • Phenoxybenzamine (irreversible α_1/α_2 blockade) used in pheochromocytoma • Silodosin and tamsulosin are approved for the treatment of prostate hyperplasia and blood pressure–lowering effects need to be considered
α_1 and β blockers Carvedilol Labetalol	• HTN • Heart failure (carvedilol)	• β Blocker of choice in patients with peripheral artery disease • Among first choices for treating HF • Labetalol first choice for HTN in pregnancy
Central sympatholytic drugs Methyldopa Clonidine/moxonidine	• HTN	• Not first choice in treating HTN • Fatigue, depression • Nasal congestion

Drug Facts for Your Personal Formulary: *Antihypertensives* (*continued*)

Antihypertensive Drug	Therapeutic Uses	Major Toxicity and Clinical Pearls
Ca^{2+} Channel Blockers		
Dihydropyridines Amlodipine Felodipine Isradipine Lercanidipine Nifedipine (extended release) Nitrendipine ***Others*** Diltiazem, verapamil	• HTN • Angina • Rate control in atrial fibrillation (verapamil, diltiazem)	• Long-acting dihydropyridines among first choice in HTN • Diltiazem and verapamil: only if effects on heart rate and AV conduction are wanted, not in combination with β blockers; beware CYP3A4-mediated drug interactions
Inhibitors of the Renin-Angiotensin System		
ACE inhibitors Benazepril Captopril Enalapril Lisinopril Quinapril Ramipril Moexipril Fosinopril Trandolapril Perindopril	• HTN • Heart failure • Diabetic nephropathy	• Among first choice for treating HTN • Short-acting captopril only for initiation of therapy; enalapril and ramipril twice daily • Cough in 5%–10% of patients, angioedema • Hypotension, hyperkalemia, skin rash, neutropenia, anemia, fetopathic syndrome • Contraindications: pregnancy, renal artery stenosis; caution in patients with impaired renal function or hypovolemia • Fosinopril: hepatic and renal elimination, thus eliminated in patients with HF and low renal perfusion
Angiotensin receptor blockers Candesartan Eprosartan Irbesartan Losartan Olmesartan Telmisartan Valsartan Azilsartan	• HTN • Heart failure • Diabetic nephropathy	• Same as ACEI, less cough or angioedema • No evidence for superiority over ACEI • In combination with ACEI, more harm than benefit • Contraindicated in pregnancy
Direct renin inhibitors Aliskiren	• HTN	• Therapeutic value unclear; no evidence for superiority over ACEIs or ARBs • Combination with RAS inhibitors contraindicated
Vasodilators		
Hydralazine	• HTN • Heart failure in African Americans (fixed combination with isosorbide dinitrate)	• Not first choice in treating HTN • Adverse effects: headache, nausea, flushing, hypotension, palpitations, tachycardia, dizziness, and angina pectoris; generally combined with β blocker to reduce baroreceptor reflex effects • Use cautiously in patients with CAD • Lupus syndrome at high doses • Polymorphic metabolism (slow/fast acetylation)
Minoxidil	• HTN • Alopecia	• Reserve antihypertensive in patients with renal insufficiency • Water retention, tachycardia, angina, pericardial effusion • Use in combination with diuretic, β blocker, and RAS inhibitor • Hypertrichosis • Contraindicated in pheochromocytoma
Sodium nitroprusside	• Hypertensive emergencies	• Only short-term intravenously • Adverse effect: hypotension • Cyanide intoxication

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Chapter 33

Therapy of Heart Failure

Thomas Eschenhagen

PATHOPHYSIOLOGY OF HEART FAILURE
- Definitions
- Common Final Pathway of Multiple Cardiac Diseases
- Pathophysiological Mechanisms
- Heart Failure With Preserved Ejection Fraction
- Heart Failure Staging
- Prevention and Treatment

DRUG TREATMENT OF CHRONIC SYSTOLIC HEART FAILURE
- Treatment Principle I: Neurohumoral Modulation
- Treatment Principle II: Preload Reduction
- Treatment Principle III: Afterload Reduction
- Treatment Principle IV: Increasing Cardiac Contractility
- Treatment Principle V: Heart Rate Reduction
- Treatment Principle VI: SGLT2 Inhibition

DRUG TREATMENT OF ACUTELY DECOMPENSATED HEART FAILURE
- Diuretics
- Vasodilators
- Positive Inotropic Agents
- Myofilament Calcium Sensitizers (Levosimendan, Pimobendan)
- Other Drugs Used in Heart Failure
- Role of Standard Combination Therapy

LESSONS FROM HEART FAILURE DRUG DEVELOPMENT
- Lessons From Failed Drugs
- Lessons From Treating Acute Heart Failure
- Recent Developments; Novel Approaches
- Myosin Modulators

Heart failure is responsible for more than half a million deaths annually in the U.S. Its prevalence is stable in developed countries but increasing worldwide, mainly due to an adoption of western lifestyle and an aging population. Median survival rates after the first hospitalization associated with heart failure are worse than in most cancers but have improved over the past 30 years (1.3 to 2.3 years in men and 1.3 to 1.7 years in women) (Jhund et al., 2009). This positive survival trend was associated with a 2- to 3-fold higher prescription rate of angiotensin-converting enzyme (ACE) inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), β receptor antagonists (β blockers), and mineralocorticoid receptor antagonists (MRAs), suggesting that improved drug therapy has contributed to enhanced survival of patients with heart failure. However, a more complex picture evolved over the past decade with an increasing incidence in people younger than 55 years of age, a decrease in heart failure with reduced ejection fraction (HFrEF); and an increase in heart failure with preserved ejection fraction (HFpEF; Chan et al., 2021; Tsao et al., 2018).

Pathophysiology of Heart Failure

Definitions

Heart failure is a state in which the heart is unable to pump blood at a rate commensurate with the requirements of the body's tissues or can do so only at elevated filling pressure. This leads to symptoms that define the heart failure syndrome clinically. Low output (forward failure) causes fatigue, dizziness, muscle weakness, and shortness of breath, which is aggravated by physical exercise. Increased filling pressure leads to congestion of the organs upstream of the heart (backward failure), clinically apparent as peripheral or pulmonary edema, maldigestion, and ascites.

Most patients with heart failure are diagnosed exclusively on the basis of symptoms; that is, their heart function has never been directly measured (e.g., by echocardiography). Under these circumstances, it is not possible to differentiate between HFrEF (or systolic heart failure) and HFpEF (or diastolic heart failure, see discussion that follows). Other diseases associated with similar symptoms can therefore be wrongly categorized as heart failure (e.g., chronic obstructive pulmonary disease).

Common Final Pathway of Multiple Cardiac Diseases

Heart failure is not a single disease entity but a clinical syndrome that represents the final pathway of multiple cardiac diseases. The most common reason for systolic heart failure today is ischemic heart disease causing either acute (myocardial infarction) or chronic loss of viable heart muscle mass. Other reasons include chronic arterial hypertension and valvular diseases (both are decreasing in incidence due to improved therapy), genetically determined primary heart muscle defects (cardiomyopathies), viral infections (cytomegalovirus and possibly parvovirus), and cardiotoxic agents. The last encompass excessive alcohol, cocaine, amphetamines, and cancer drugs such as *doxorubicin*, *trastuzumab* (the monoclonal antibody directed against the growth factor receptor Her-2/Erb-B2), and immune checkpoint inhibitors (see Section VII).

Pathophysiological Mechanisms

Systolic heart failure (i.e., HFrEF) is relatively well understood, whereas mechanisms underlying HFpEF are much less clear. The pathophysiology of heart failure involves four major interrelated systems (Figure 33–1):

- The heart itself
- The vasculature
- The kidney
- Neurohumoral regulatory circuits

The Heart Itself: Cardiomyopathy of the Overload

Any overload of the myocardium—loss of relevant muscle mass, which overloads the remaining healthy myocardium; chronic hypertension; or valvular defects—will eventually lead to the organ's failure to produce sufficient cardiac output. This concept can be extended to the genetically determined cardiomyopathies in which essentially any defect in an organelle of cardiac myocytes can lead to primary myocyte contractile dysfunction and then, secondarily, to the picture commonly seen in the

Abbreviations

ACC: American College of Cardiology
ACE: angiotensin-converting enzyme
ACEI: angiotensin-converting enzyme inhibitor
ADR: adverse drug reaction
AHA: American Heart Association
AngII: angiotensin II
ANP: atrial natriuretic peptide
ARB: AT$_1$ angiotensin receptor antagonist (blocker)
ARNI: angiotensin receptor–neprilysin inhibitor
AV: atrioventricular
AVP: arginine vasopressin
BNP: brain-type natriuretic peptide
CG: cardiac glycoside
CHF: congestive heart failure
CNP: C-type natriuretic peptide
CYP: cytochrome P450
DA: dopamine
ECG: electrocardiogram
EF: ejection fraction
eNOS: endothelial nitric oxide synthase
EPI: epinephrine
ESC: European Society of Cardiology
ET: endothelin
GC: guanylyl cyclase
GFR: glomerular filtration rate
GI: gastrointestinal
GPCR: G protein-coupled receptor
HCN: hyperpolarization-activated, cyclic nucleotide–gated cation channel
HFpEF: heart failure with preserved ejection fraction (diastolic heart failure)
HFrEF: heart failure with reduced ejection fraction (systolic heart failure)
iNOS: inducible nitric oxide synthase
ISDN: isosorbide 2,5′-dinitrate
ISMN: isosorbide 5′-mononitrate
MRA: mineralocorticoid receptor antagonist
NCX: Na$^+$/Ca^{2+} exchanger
NE: norepinephrine
NO: nitric oxide
NOS: nitric oxide synthase
NSAID: nonsteroidal anti-inflammatory drug
NYHA: New York Heart Association
PKA: protein kinase A
RAAS: renin-angiotensin-aldosterone system
RAS: renin-angiotensin system
ROS: reactive oxygen species
SERCA: sarco/endoplasmic reticulum Ca^{2+} ATPase
sGC: soluble guanylyl cyclase
SGLT2: sodium glucose co-transporter 2
SNS: sympathetic nervous system
SR: sarcoplasmic reticulum
TnC: troponin C
TNF: tumor necrosis factor

cardiomyopathy of the overload. Not surprisingly, the most common cardiomyopathies (dilated cardiomyopathy, hypertrophic cardiomyopathy) are due to mutations in genes encoding proteins of the contractile machinery, the sarcomere, proteins anchoring the sarcomere to the plasma membrane, or proteins mediating and maintaining cell-cell contact.

The overload (or the primary contractile defect) leads to alterations of the heart that can partially compensate but that come at a price. Because cardiac myocytes essentially stop replicating in the early postnatal period, the usual response to overload is not myocyte division but rather hypertrophy, growing in size and assembling more sarcomeres that can contribute to contractile force development. Whereas hypertrophy is principally a normal response to physiological needs such as body growth, pregnancy, and physical exercise ("physiological hypertrophy"), hypertrophy in response to chronic overload comes with features that make it a major risk factor for the development of heart failure ("pathological hypertrophy"). A direct consequence of cardiac myocyte hypertrophy is a reduced capillary/myocyte ratio (i.e., less O$_2$ and nutrient supply per myocyte), causing an energy deficit and metabolic reprogramming. Altered gene expression of ion channels, Ca^{2+}-regulating proteins, and contractile proteins can be interpreted as partially beneficial, energy-saving adaptations; on the other hand, the adaptations also aggravate contractile failure and favor arrhythmias. Concurrently, fibroblasts proliferate and deposit increased amounts of extracellular matrix (e.g., collagen). This fibrosis in heart failure also favors arrhythmias, increases the stiffness of the heart, and interrupts myocyte-to-myocyte communication (coordinated conduction and force transmission). Finally, overload leads to cardiac myocyte death by apoptosis or necrosis. Collectively, these adverse adaptations are called *pathological remodeling*.

Some of these alterations are direct, heart-intrinsic consequences of overload (e.g., hypertrophy, altered gene expression); others are secondary to neurohumoral activation and thereby susceptible to neurohumoral blocking agents (see discussion that follows and Figure 33–1).

The Vasculature

A critical parameter of cardiac function is the stiffness of the vasculature. It determines the resistance against which the heart must expel the blood. Vascular stiffness increases with aging. Heart failure may be the consequence of premature aging of the vasculature (Strait and Lakatta, 2012). Aging-induced loss of elasticity of the great blood vessels reduces their compliance, that is, the elasticity that permits vessels to extend in systole and contract in diastole. Good compliance reduces peak systolic pressure and increases diastolic pressure, which favors perfusion in diastole. It is negatively correlated with pulse pressure, that is, the difference between systolic and diastolic blood pressure, which is low in children and high in the elderly. Arterial hypertension and diabetes mellitus are the major reasons for premature stiffening of blood vessels, which imposes increased afterload to the heart and contributes to heart failure. Theoretically, stiffening and loss of compliance could be directly tackled by drugs (see section Recent Developments; Novel Approaches).

Another critical aspect of vascular function is the ability to adapt the vessel diameter to hemodynamic and neurohumoral stimuli, a function that is governed by cross talk between luminal endothelial and underlying smooth muscle cells (see Chapter 32). The main signaling pathway involves receptors that increase intracellular Ca^{2+} levels in endothelial cells, which activates endothelial nitric oxide synthase (eNOS) to produce nitric oxide (NO). This gaseous transmitter diffuses into smooth muscle cells and activates soluble guanylyl cyclase (sGC) to produce cGMP, which causes relaxation of vascular smooth muscle. Heart failure is always accompanied by endothelial dysfunction, which is a disturbed balance between vasodilating NO and proconstrictor reactive oxygen species (ROS). ROS, by inactivating the two critical enzymes eNOS and sGC and converting NO in peroxynitrite, a strong ROS, favor vasoconstriction. Several common cardiovascular drugs (ACEIs/ARBs, MRAs, statins) improve endothelial function by reducing ROS production. Cyclic nucleotide phosphodiesterase (PDE)5 inhibitors have similar consequences by inhibiting cGMP degradation in smooth muscle cells and thereby promoting relaxation. Stimulators of sGC like the recently approved new heart failure drug *vericiguat* dilate blood vessels by direct stimulation of the enzyme and sensitization to endogenous NO.

The Kidney

The kidney regulates Na$^+$ and H$_2$O excretion and thereby intravascular volume. Under normal conditions, autoregulatory and neurohumoral

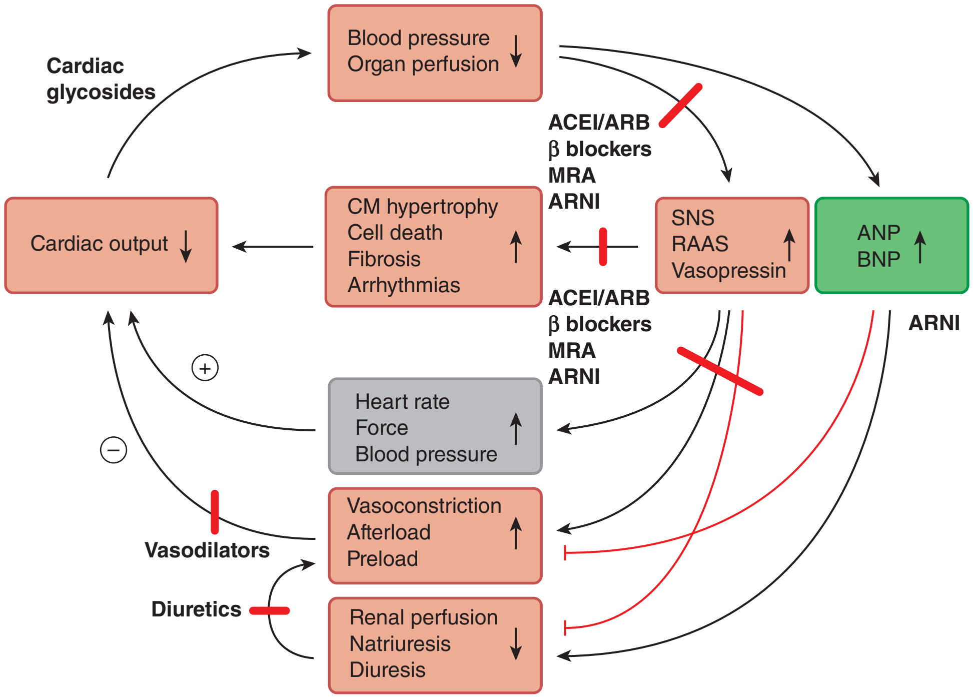

Figure 33–1 *Pathophysiologic mechanisms of systolic heart failure (HFrEF) and therapeutic interventions.* Any major decrease in cardiac contractile function leads to activation of neurohumoral systems, including the SNS, the RAAS, and vasopressin (antidiuretic hormone) secretion, which acutely stabilize blood pressure and organ perfusion by stimulating cardiac output, constricting resistance vessels, decreasing kidney perfusion, and increasing Na^+ and H_2O retention. Unfortunately, these responses are maladaptive, causing chronic overloading and overstimulation of the failing heart. Direct hypertrophic, proapoptotic, fibrotic, and arrhythmogenic effects of NE and AngII further accelerate the deleterious process. Note that the concomitant activation of the ANP/BNP system is the consequence of stretch and increased wall stress in the heart and has opposite and beneficial effects. See Abbreviations list at the beginning of the chapter.

mechanisms ensure an adequate glomerular filtration rate (GFR) and diuresis over a wide range of renal perfusion pressures. Prominent regulatory mechanisms with relevance for heart failure are (1) the angiotensin II (AngII)-mediated regulation of filtration rate by regulating the diameter of the efferent glomerular arteriole; (2) the regulation of kidney perfusion by a balance between constrictor-promoting effects of AngII (via AT_1 receptors) and vasopressin (arginine vasopressin [AVP], via V_1 receptors) and the vasodilating influence of prostaglandins (hence the deleterious effects of nonsteroidal anti-inflammatory drugs [NSAIDs]); (3) the aldosterone-mediated regulation of Na^+ reabsorption in the distal tubule; and (4) AVP-regulated water transport in the collecting ducts (via V_2 receptors). In heart failure, all mechanisms are dysregulated and constitute therapeutic targets of ACEIs/ARBs, MRAs, and diuretics. Newer agents, such as adenosine A_1 receptor antagonists and AVP receptor antagonists, have failed to exert therapeutic benefit in clinical studies.

Neurohumoral Regulation and HFrEF

The decrease in cardiac output in heart failure leads to the activation of the sympathetic nervous system (SNS) and the renin-angiotensin-aldosterone system (RAAS) and increases in plasma levels of AVP and endothelin (ET) (Figure 33–1). This concerted response ensures the perfusion of centrally important organs such as the brain and the heart (at the expense of kidney, liver, and skeletal muscle perfusion) in situations of acute blood loss. These responses are components of the "fight-or-flight response" and provide useful short-term physiological responses to alarm and danger. Chronically, however, neurohumoral activation exerts deleterious effects that constitute a vicious cycle in heart failure. Vasoconstriction initially not only stabilizes blood pressure but also increases afterload, which is the resistance against which the heart works to expel blood (see Figures 33–4 and 31–1). Because of the decreased contractile reserve, the failing heart is particularly sensitive to increases in afterload (see Figure 33–4); such increases further decrease cardiac output. Decreased kidney perfusion and increased aldosterone production reduce diuresis and promote volume overload, which increases cardiac preload, dilation, and ventricular wall stress, a major determinant of cardiac O_2 consumption. Tachycardic and positive inotropic actions of catecholamines not only acutely increase cardiac output but also promote arrhythmias and increase O_2 consumption in a failing, energy-depleted heart. AngII, norepinephrine (NE), and ET accelerate pathological cardiac remodeling (hypertrophy, fibrosis, and cell death). Aldosterone has prominent profibrotic actions. This spectrum of adverse consequences of chronic neurohumoral activation explains why inhibitors of these systems (ACEIs/ARBs, β blockers, and MRAs) exert long-term, life-prolonging effects in heart failure and are the cornerstones of current therapy.

Unexpectedly, ET and AVP receptor antagonists provide no beneficial effect in patients with heart failure, despite promising results in preclinical studies. Clinical trials suggested that neurohumoral activation in response to altered cardiac function may be sufficiently inhibited by the standard combination therapy, leaving no room for improvement from the addition of ET and AVP antagonists; however, recent data indicate that additional benefit may accrue via another therapeutic route: a drug combination called angiotensin receptor–neprilysin inhibitors (ARNIs). The FDA has approved a fixed-dose combination of the ARB *valsartan* with the neprilysin inhibitor *sacubitril*. *Valsartan* blocks AT_1 receptors, reducing the deleterious effects of AngII. *Sacubitril* inhibits the degradation of the natriuretic peptides atrial natriuretic peptide (ANP) and brain-type natriuretic peptide (BNP). The *valsartan/sacubitril* combination appears superior to the ACEI *enalapril*, reducing the rates of hospitalization and death from all cardiovascular causes in patients with HFrEF (Hubers and Brown, 2016).

This finding reflects the fact that neurohumoral activation in heart failure includes one system that exerts beneficial effects: the natriuretic peptides. Normally, ANP and BNP are expressed in the atria and released upon increased preload (stretch). During heart failure, ANP and BNP are also produced by the ventricles, such that plasma levels are elevated. Indeed, BNP is used as a biomarker of heart failure. ANP and BNP stimulate the plasma membrane guanylyl cyclase. In the kidney, elevated cGMP has diuretic effects. Elevated cellular cGMP mediates vasodilation in the vasculature and, in the heart, antihypertrophic, antifibrotic, and compliance-increasing effects related to phosphorylation of titin.

Enhancing these effects by inhibiting the degradation of ANP/BNP likely explains the clinical benefits of *sacubitril/valsartan*.

Heart Failure With Preserved Ejection Fraction

Systematic echocardiographic determination of left ventricular ejection fraction (EF) in thousands of patients with heart failure revealed that about 50% had no reduction; that is, they exhibited EF values greater than 50%. Still, patients had typical heart failure symptoms, including acute decompensation with pulmonary edema and a survival prognosis not much better or even identical to patients with reduced EF (HFrEF). These data point to a different pathophysiology in which abnormalities of the diastolic and not the systolic component of cardiac function prevail. Due to difficulties in defining diastolic function by standard techniques, the term *HFpEF* has been introduced and applies to patients with typical heart failure symptoms and "normal" (>50%) or only mildly reduced EF.

Even more than HFrEF, HFpEF is a multifactorial disease (Figure 33–2). HFpEF is typically associated with arterial hypertension, ischemic heart disease, diabetes mellitus, and obesity (metabolic syndrome); it is more frequent in women than men and shows a strong increase in prevalence with age (Shah et al., 2020). Hearts of patients with HFpEF are generally not dilated, wall thickness is enlarged (hypertrophy), and left atrial size often is enlarged as a sign of chronically elevated end-diastolic pressures. Central to the pathophysiology of HFpEF is, presumably, compromised diastolic relaxation of the left ventricle, which causes congestion of the lung, shortness of breath, or pulmonary edema. Clinical decompensation is often associated with strongly elevated blood pressure.

Molecular alterations include increased myocardial fibrosis (causing a permanent relaxation deficit) as well as more dynamic changes, such as reduced phosphorylation of titin, the sarcomeric protein that spans the large region from the Z to the M band. Titin contains several molecular spring domains whose elastic modulus determines the passive tension of cardiomyocytes, particularly at low-to-medium levels of stretch. Titin stiffness is determined by its isoforms and by cGMP-dependent phosphorylation, suggesting that agents that increase cellular cGMP might be beneficial in HFpEF. However, the PDE5 inhibitor *sildenafil*, which preserves and elevates cellular cGMP in some cells (see Chapters 3, 35, and 49), failed to show benefit (Redfield et al., 2013). This lack of efficacy is, unfortunately, also true for all other pharmacological interventions in HFpEF, including ACEIs, ARBs, and *spironolactone*. A new facet of HFpEF may be upregulation of inducible nitric oxide synthase (iNOS), the inducible isoform of nitric oxide synthase (NOS), in the myocardium, leading to increased nitrosylation and disturbance of the endoplasmic reticulum stress response (Schiattarella et al., 2019). The hypothesis is attractive as it is based on a mouse model integrating both hypertensive and metabolic stress, classical risk factors of HFpEF in humans. This hypothesis offers potential new therapy targets. Presently, exercise training is the only intervention that significantly increases exercise capacity (maximal oxygen consumption, peak VO_2) in HFpEF patients. In the absence of evidence-based clinical trial data, current therapy recommendations concentrate on optimal treatment of the underlying diseases, such as hypertension, diabetes, and obesity.

Heart Failure Staging

Heart failure was one of the first diseases for which guidelines described specific therapies for each stage of the disease. An early classification of the stages of heart failure was that of the New York Heart Association (NYHA), a classification still in use: class I (left ventricular dysfunction, no symptoms); class II (symptoms at medium-to-high levels of physical exercise); class III (symptoms at low levels of physical exercise); and class IV (symptoms at rest or daily life physical activities such as brushing teeth). The more recent guidelines of the American Heart Association (AHA) and American College of Cardiology (ACC) extended this classification by considering the following:

- Heart failure is part of the cardiovascular continuum with preventable risk factors (stage A).
- An asymptomatic stage exists that requires treatment to delay transition to symptomatic heart failure (stage B).
- Patients oscillate between different degrees of symptoms and therefore between class II and III (class C, which generally includes NYHA class II/III patients).

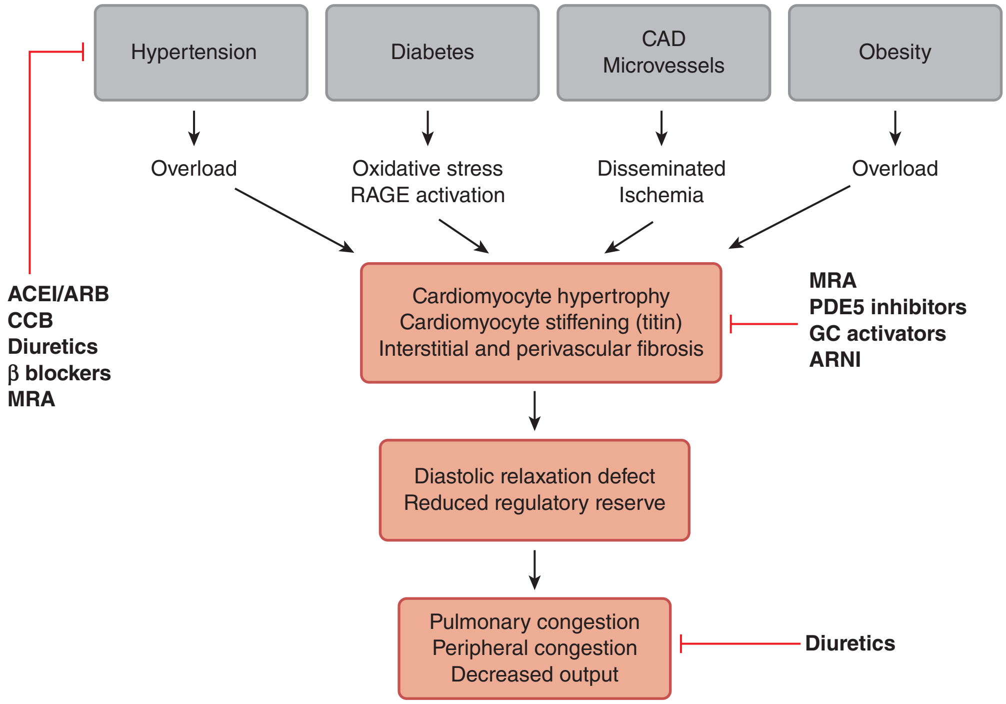

Figure 33–2 *Pathophysiological mechanisms of diastolic heart failure HFpEF and possible therapeutic interventions.* Unlike the case with HFrEF, the pharmacological agents shown have not been proven to have clinical efficacy toward HFpEF, although these agents can help to control underlying diseases, such as hypertension, diabetes, and obesity. Only exercise training has proven effective in increasing maximal exercise capacity. RAGE, receptor for advanced glycosylation end-products. CAD, coronary artery disease; CCB, calcium channel blocker.

- A final stage of the disease requires different treatment and special considerations, such as heart transplantation and left ventricular assist device implantation (stage D).

This chapter uses the AHA/ACC classification and considers the recent guidelines of the European Society of Cardiology (ESC) (Ponikowski et al., 2016), which provide more specific treatment algorithms, and the 2017 AHA/ACC update (Yancy et al., 2017). Treatment guidelines are summarized in Figure 33–3.

Prevention and Treatment

Ischemic heart disease, hypertension, and valvular diseases are the most prevalent causes of heart failure. People at high risk of heart failure (stage A) should be treated with drugs that mitigate the harmful effects of these diseases, in conjunction with appropriate lifestyle changes. Studies in thousands of patients have reproducibly shown that blood pressure lowering in hypertensive patients and lipid lowering with statins in dyslipidemic patients reduce not only the incidence of myocardial infarction and death but also the incidence of heart failure. The data are weaker for antidiabetic drugs, but consensus exists that blood glucose should be controlled with a hemoglobin A_{1C} goal of 7% to 7.5%.

Treatment of heart failure has seen a dramatic change over the past decades. Until the late 1980s, choice of drugs and dosing was symptom oriented and based on pathophysiological considerations of *acute* systolic heart failure. Treatment was mainly directed toward symptom relief and short-term improvement of hemodynamic function. Subsequent randomized clinical trials, which mainly tested effects of drugs on long-term morbidity (hospitalizations) and mortality, disproved many former beliefs. For example, positive inotropic drugs (sympathomimetics and PDE inhibitors) that exert acute symptomatic benefit reduce life expectancy when chronically administered. In contrast, β blockers decrease cardiac output acutely and may make people feel weak at the start of therapy but prolong life expectancy when given in increasing doses for extended periods. Vasodilators once seemed a logical choice for heart failure, but pure vasodilators such as the α_1 receptor antagonist *prazosin* or the nitrate isosorbide 2,5′-dinitrate (ISDN), in combination with the vasodilator *hydralazine*, do not positively affect the prognosis in Caucasians (see further discussion). Finally, successful trials can occur unexpectedly and before the mechanism of action is fully understood. A recent example is the beneficial effect of sodium glucose co-transporter 2 (SGLT2) inhibitors on outcome in patients with heart failure and without diabetes. Thus, clinical trials have established important principles for assessing efficacy of therapies for heart failure:

1. Drugs treating chronic heart failure should reduce the patient morbidity and mortality.
2. Short-term drug effects poorly predict the outcome of randomized clinical trials and optimal therapies for heart failure.
3. Considerations for stage of disease are critical.
4. New drugs for heart failure should be compared to the most effective current combination therapy, a principle often ignored in preclinical animal work.
5. Clinical trials often provide unexpected results, which then initiate research into advanced understanding of mechanisms.
6. Nonpharmacological treatment options such as cardiac resynchronization devices and intracardiac defibrillator/cardioverters are important for their documented lifesaving effect in selected patient populations.

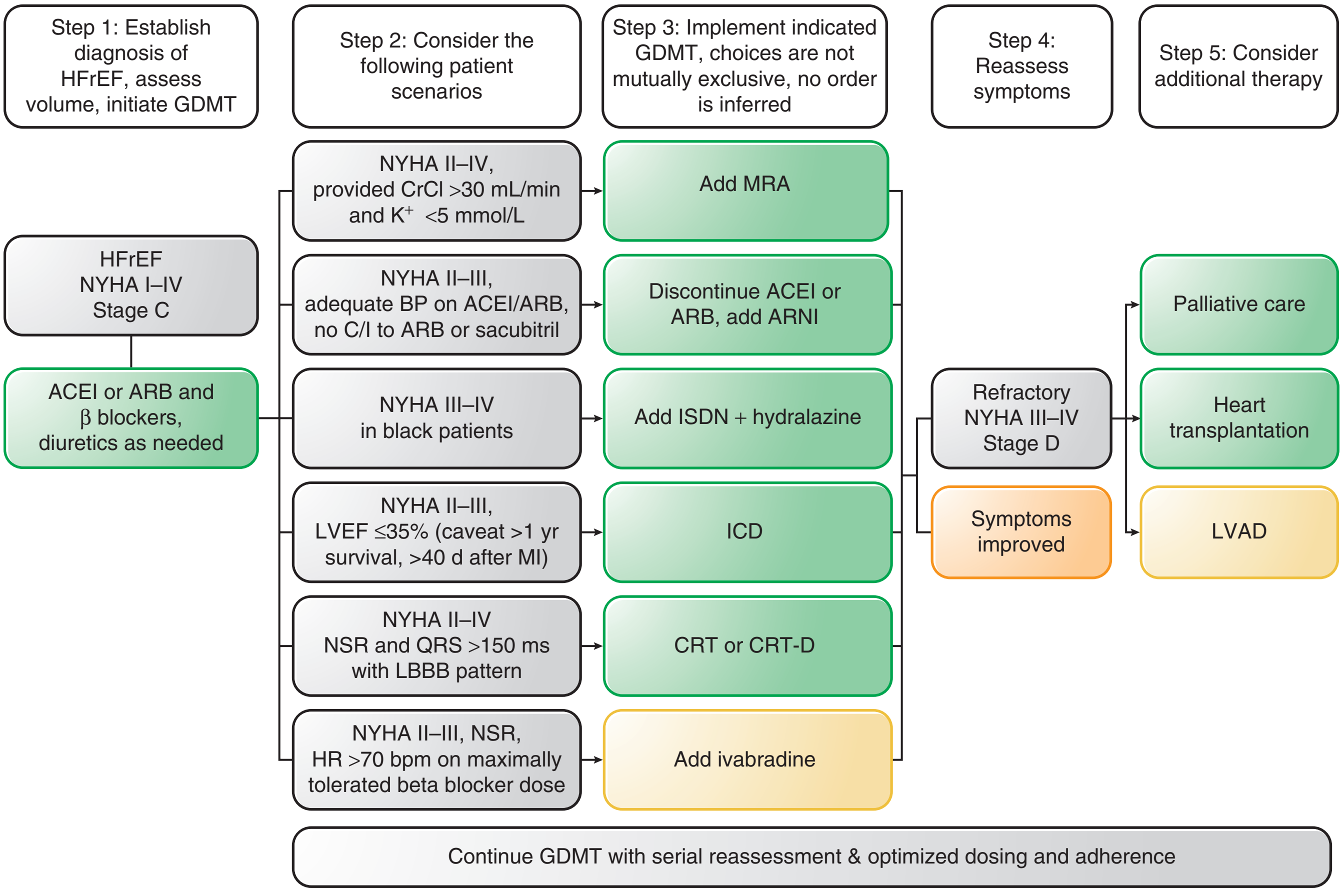
Step 1: Establish diagnosis of HFrEF, assess volume, initiate GDMT
Step 2: Consider the following patient scenarios
Step 3: Implement indicated GDMT, choices are not mutually exclusive, no order is inferred
Step 4: Reassess symptoms
Step 5: Consider additional therapy
HFrEF NYHA I–IV Stage C
ACEI or ARB and β blockers, diuretics as needed
NYHA II–IV, provided CrCl >30 mL/min and K+ <5 mmol/L
Add MRA
NYHA II–III, adequate BP on ACEI/ARB, no C/I to ARB or sacubitril
Discontinue ACEI or ARB, add ARNI
NYHA III–IV in black patients
Add ISDN + hydralazine
NYHA II–III, LVEF ≤35% (caveat >1 yr survival, >40 d after MI)
ICD
NYHA II–IV NSR and QRS >150 ms with LBBB pattern
CRT or CRT-D
NYHA II–III, NSR, HR >70 bpm on maximally tolerated beta blocker dose
Add ivabradine
Refractory NYHA III–IV Stage D
Symptoms improved
Palliative care
Heart transplantation
LVAD
Continue GDMT with serial reassessment & optimized dosing and adherence

Figure 33–3 *AHA/ACC 2017 Heart Failure Treatment Guidelines (adapted from Yancy et al., 2017).* Colors indicate the guideline class of recommendation (green Ia—clinical efficacy established, yellow IIa—clinical efficacy likely). C/I, contraindication; CrCl, estimated creatinine clearance; CRT-D, cardiac resynchronization therapy device; GDMT, guideline-directed medical therapy; ICD, implantable cardioverter-defibrillator; LBBB, left bundle branch block; LVAD, left ventricular assist device; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NSR, normal sinus rhythm.

TABLE 33–1 ■ LANDMARK STUDIES IN THE TREATMENT OF PATIENTS WITH CHRONIC HFrEF

STUDY ACRONYM AND/OR AUTHOR	YEAR	STUDY POPULATION	NO. OF PATIENTS	BASELINE DRUGS (% of patients on each drug)	EFFECT ON ALL-CAUSE MORTALITY (vs. placebo or another drug)
Cohn et al.	1986	Men, impaired cardiac function and exercise capacity	642	CG, diuretics	ISDN/hydralazine, –34% Prazosin +/– vs. placebo (absolute mortality 19%/year)
CONSENSUS Trial Study Group	1987	Severe HF, NYHA class IV	253	Diuretics 100%, spironolactone 52%, CG 93%, vasodilators ~50%, BB 2%	Enalapril, –40% vs. placebo (absolute mortality 54%/year)
SOLVD Treatment	1991	NYHA II–III, EF <35%	2569	Diuretics 86%, CG 67%, vasodilators 51%, BB 7.5%	Enalapril, –16% vs. placebo (absolute mortality 13%/year)
SOLVD Prevention	1992	NYHA I, EF <35%	4228	Vasodilators 46%, diuretics 17%, CG 13%	Enalapril, –8% (n.s.) vs. placebo (absolute mortality 5%/year); HF development, –20%
DIG	1997	NYHA II–III	6800	Diuretics 81%, ACE 95%, nitrates 43%	Digoxin +/– vs. placebo (absolute mortality 11%/year); HF hospitalizations, –27%
RALES, Pitt et al.	1999	Severe HF, EF <35%	1663	Diuretics 100%, ACEI 94%, CG 72%, BB 10%	Spironolactone, –30% vs. placebo (absolute mortality 18%)
MERIT-HF	1999	NYHA II–IV	3991	Diuretics 90%, ACEI/ARB 95%, CG 63%	Metoprolol CR/XL, –34% vs. placebo (absolute mortality 11%/year)
PARADIGM, McMurray et al.	2014	NYHA II–IV	8442	Diuretics 80%, BB 93%, MRA 56%, CG 30%, ICD 15%, CRT 7%	Sacubitril/valsartan, –16% vs. enalapril (absolute mortality ~8%/year)
DAPA-HF	2019	NYHA II–IV	4744	Diuretics 93%, ACEI/ARB 85%, BB 96%, MRA 71%, CG 19%, ICD 26%, CRT 7%	Dapagliflozin, –17% vs. placebo (absolute mortality 9%/year)

BB, β blocker; CRT, cardiac resynchronization therapy; HF, heart failure; ICD, implantable cardioverter-defibrillator; n.s., nonsignificant.

Attention to these principles for assessing long-term efficacy of heart failure therapies has provided evidence-based principles of treatment.

HISTORICAL PERSPECTIVE

A series of landmark studies over three decades has established the current thinking on the treatment of patients with chronic HFrEF. These studies are not reviewed here, but interested readers may wish to consult the evidence that supports current therapies. These studies, often indicated by an acronym, are summarized in Table 33–1.

Drug Treatment of Chronic Systolic Heart Failure

Treatment Principle I: Neurohumoral Modulation

Dampening neurohumoral activation and its deleterious consequences on the heart, blood vessels, and kidney is the cornerstone of heart failure therapy. Therapy consists of ACEIs/ARBs, β blockers, MRAs, and neprilysin inhibitors (Figure 33–1). A systematic discussion of the drugs is found in Chapters 14, 29, 30, 31, and 32.

Angiotensin-Converting Enzyme Inhibitors

Angiotensin II, the most active of the many angiotensin peptides, is largely derived from angiotensinogen in two proteolytic steps. First, *renin*, an enzyme released from the macula densa of the kidneys, cleaves the decapeptide AngI from the amino terminus of angiotensinogen (renin substrate) made by the liver. Then, ACE removes a carboxy-terminal dipeptide (His^9-Leu^{10}) from AngI, yielding the active octapeptide, AngII (see Chapter 30). Thus, ACEIs reduce circulating levels of AngII. All patients with heart failure (stages B and C; NYHA I–IV) should receive an ACEI.

Mechanism of Action. AngII interacts with two heptahelical G protein-coupled receptors (GPCRs), AT_1 and AT_2, and has four major cardiovascular actions that are all mediated by the AT_1 receptor:

- Vasoconstriction
- Stimulation of aldosterone release from the adrenal glands
- Direct hypertrophic and proliferative effects on cardiomyocytes and fibroblasts, respectively
- Stimulation of NE release from sympathetic nerve endings and the adrenal medulla

Physiological Effects. The ACEIs decrease AngII concentrations and thereby reduce its deleterious effects. Thus, ACEIs not only act as vasodilators but also reduce aldosterone levels and thereby act as an indirect diuretic, have direct antiremodeling effects on the heart, and produce sympatholytic effects (thus moderating the reflex tachycardia that accompanies vasodilation and the lowering of blood pressure).

The ACEIs have important renal effects. When renal perfusion pressure is reduced, AngII constricts renal efferent arterioles, and this serves to maintain glomerular filtration pressure and GFR. Thus, when renal perfusion pressure is compromised, inhibition of the RAAS may induce a sudden and marked decrease in GFR. For this reason, ACEIs are contraindicated in bilateral renal artery stenosis. Likewise, because patients with heart failure often have low renal perfusion pressures, aggressive treatment with ACEIs may induce acute renal failure. To avoid this, for patients with heart failure, ACEIs should be initiated at very low doses; blood pressure, blood creatinine, and K^+ levels should be monitored; and the ACEI dose slowly increased over weeks toward target levels (for agents that have been carefully evaluated in clinical trials; Table 33–2). The potentially dangerous acute effects become beneficial with long-term use of ACEIs because the (small) chronic lowering of glomerular pressures protects the glomerulus from fibrotic degeneration.

The ACEI-induced lowering of aldosterone levels causes reduced expression of the aldosterone-dependent epithelial Na^+ channel (ENaC)

TABLE 33–2 ■ ACEIs AND ARBs APPROVED AND CLINICALLY EVALUATED FOR THE THERAPY OF HFrEF[a]

DRUG	HALF-LIFE (h)	START DOSE (mg)	TARGET DOSE (mg)	IMPORTANT ADVERSE EFFECTS, INTERACTIONS, AND CONTRAINDICATIONS
ACEIs				
Captopril	1.7	3 × 6.25	3 × 50	**Adverse effects:** Cough (~5%), increase in serum creatinine (<25% normal, if >50%, think about renal artery stenosis), hyperkalemia, hypotension, angioedema; rare cholestatic jaundice, hepatic failure, agranulocytosis **Interactions:** Increased rate of hyperkalemia in combination with potassium-sparing diuretics, potassium supplements, cyclosporine, NSAIDs (PD), reduced efficacy in combination with NSAIDs (PD), increased lithium serum concentrations (PK) and hypoglycemic risk in combination with insulin or oral antidiabetics; increased effect in renal insufficiency (PK) **Contraindications:** Bilateral renal artery stenosis, therapy with neprilysin inhibitor (sacubitril)
Enalapril	11	2 × 2.5	2 × 20	
Lisinopril	13	1 × 2.5–5	1 × 20–35	
Ramipril	13–17	1 × 2.5	1 × 10	
Trandolapril	15–23	1 × 0.5	1 × 4	
ARBs				
Candesartan	9	1 × 4-8	1 × 32	**Adverse effects:** Similar to ACE, but less cough **Interactions and contraindications:** Same as for ACEI
Losartan	6-9	1 × 50	1 × 150	
Valsartan	6	2 × 40	2 × 160	

PD, pharmacodynamics; PK, pharmacokinetic interaction.

[a] Plasma half-lives partially apply to active metabolites (e.g., losartan).

in the distal tubule (see Figure 29–6). This target of K$^+$-sparing diuretics (see discussion that follows) normally mediates Na$^+$ reabsorption and K$^+$ excretion. Lower levels of ENaC lead to less absorption of Na$^+$ and less excretion of K$^+$. Thus, ACEIs favor hyperkalemia, which can be detrimental in patients with renal insufficiency but is normally beneficial for patients with heart failure who more often present with hypokalemia, a condition that promotes cardiac arrhythmias. ACEIs shift the balance of vascular smooth muscle tone toward vasodilation and thereby increase renal blood flow, another reason for their chronic protective effects on the kidney. This effect also explains why NSAIDs, which reduce the production of vasodilating prostaglandins, antagonize effects of ACEIs and should be avoided in patients with heart failure.

Other Actions, Good and Adverse. Angiotensin-converting enzyme has other actions, including the inactivation of bradykinin and substance P. ACEIs increase bradykinin and substance P levels, with two prominent consequences: cough, the most frequent adverse drug reaction (ADR) (~5%); and angioedema, a rare (~0.7%) but life-threatening condition presenting with swelling of the skin and mucous membranes of the throat and asphyxia (three times more common among people of African origin; McDowell et al., 2006). Experimental evidence suggests that increases in bradykinin contribute to the therapeutic efficacy of ACEIs and may explain why ARBs, which do not increase bradykinin (and therefore rarely cause cough), have not been consistently associated with improved survival in patients with HFrEF (Ponikowski et al., 2016).

The ACEIs are generally well tolerated in most patients. Important ADRs are the following:

- Dry cough, necessitating a change to ARBs
- Creatinine plasma concentration increase (<20%, normal; 20%–50%: careful observation and reduction of ACEI dosage; >50%, stop ACEI and consult specialist for evaluation of renal artery stenosis)
- Hyperkalemia (small increase normal, but requires careful observation in patients with diabetes, renal insufficiency, or comedication with MRAs, K$^+$-sparing diuretics, or NSAIDs)
- Angioedema (stop drug immediately and treat with antihistamines, corticosteroids, or, in severe case, epinephrine [EPI])
- Allergic skin reactions

Angiotensin Receptor Antagonists

The ARBs are systematically discussed in Chapter 30. They are highly selective, competitive receptor antagonists at the AT$_1$ receptor, which mediates the major effects of AngII. They are therapeutic alternatives to ACEIs and second choice in all stages of heart failure in patients who do not tolerate ACEIs. Given the central role of the AT$_1$ receptor for the actions of AngII, it is not surprising that ARBs show the same pharmacological profile as ACEIs with the exception of not inducing cough. The unopposed activity of AT$_2$ receptor pathways in the presence of AT$_1$ blockade by an ARB seems to confer no therapeutic advantage to ARBs over ACEIs. Moreover, the addition of an ARB to therapy with an ACEI does not affect the prognosis of patients with heart failure but does increase hypotension, hyperkalemia, and renal dysfunction. A negative interaction between ACEIs and ARBs appears to extend to patients with higher renal risk. There is, therefore, no routine indication for this combination.

β Adrenergic Receptor Antagonists

Major Effects of β Adrenergic Antagonists. The sympathetic neurotransmitters NE (released at adrenergic nerve varicosities) and EPI (secreted by the adrenal medulla) are strong stimuli of heart function. They increase heart rate (positive chronotropic effect) and force of contraction (positive inotropic effect) and thereby augment cardiac output. They hasten the rate of force development (increased +dP/dt, positive clinotropy) and accelerate cardiac muscle relaxation (greater −dP/dt, positive lusitropic effect), which aids ventricular filling during diastole. Acceleration of the atrial-ventricular conduction rate (positive dromotropic effect) shortens the heart cycle and allows higher beating rates. Catecholamines enhance cardiac myocyte automaticity and lower the threshold for arrhythmias (positive bathmotropic effect). All these acute effects are mediated by β_1 receptors and, to a smaller extent, β_2 receptors. Extracardiac effects include bronchodilation (β_2), vasodilation (β_2) as well as vasoconstriction (α_1 receptors, which dominate at higher concentrations of catecholamines), stimulation of hepatic glycogen metabolism and gluconeogenesis (β_2), and, importantly, stimulation of renin release from the macula densa (β_1). Thus, activation of the SNS coactivates the RAAS, and, as outlined previously, activation of the RAAS activates the SNS by stimulation of NE release (see Chapters 14 and 30).

The β blockers competitively reduce β receptor–mediated actions of catecholamines and thus, depending on the activation level of the SNS, reduce heart rate and force, slow relaxation, slow atrioventricular (AV) conduction, suppress arrhythmias, lower renin levels, and, depending on their selectivity for the β_1 receptor, permit more or less bronchoconstriction, vasoconstriction, and lowering of hepatic glucose production.

Why Use β Blockers in Heart Failure? In light of the above actions, the efficacy of β blockers in heart failure came as a surprise and had to overcome resistance in the medical community. How can a drug with cardiodepressant actions on heart function be beneficial in a clinical situation in which the heart is already dysfunctional and depending on catecholamines to maintain cardiac output? The first therapeutic application of β blockers at low doses was to a Swedish cohort of patients with heart failure with cardiac decompensation and heart rate greater than 120 beats/min; the goal was to reduce heart rate and cardiac energy consumption (Waagstein et al., 1975). The success of the experiment led to large clinical trials (i.e., MERIT-HF) that showed an impressive 35% prolongation of life expectancy in patients treated with β blockers (Table 33–1), on top of effects of ACEIs, diuretics, and *digoxin*.

Key to the understanding of the success of β blockers in heart failure were two lessons. *First*, therapy must be initiated in a clinically stable condition and at very low doses (one-eighth of target), and dose escalation requires time (e.g., doubling every 4 weeks in ambulatory settings; “start low, go slow”). Under these conditions, the heart has time to adapt to decreasing stimulation by catecholamines and to find a new equilibrium at a lower adrenergic drive. Importantly, β blockers do not fully block the receptors; rather, they are competitive antagonists that shift the concentration-response curve of catecholamines to the right (see Chapter 3).

Second, although the acute effects of catecholamines can be lifesaving, that level of β adrenergic stimulation applied chronically, as the SNS does in response to heart failure, is deleterious. Positive chronotropic, inotropic, and lusitropic effects culminate in overproportional increase in energy consumption. This is irrelevant in situations of acute blood loss or other stresses, but critical if persistent. The heart reacts to chronic sympathetic stimulation by a heart failure–specific gene program (e.g., downregulation of β adrenergic receptor density; upregulation of inhibitory G proteins; and decreases of sarcoplasmic reticulum [SR] Ca^{2+}-ATPase, the fast isoform of myosin heavy chain, and repolarizing K^+ currents), changes that come at the price of decreased dynamic range and increased propensity for arrhythmias. Reversal of the heart failure gene program by β blockers (Lowes et al., 2002) likely contributes to the paradoxical increase in left ventricular EF after 3 to 6 months of therapy and to the reduced rate of arrhythmogenic sudden cardiac death noted in large studies. In a simple view, β blockers protect the heart from the adverse long-term consequences of adrenergic overstimulation, for example, increased energy consumption, fibrosis, arrhythmias, and cell death. Lower heart rates not only save energy but also improve contractile function because the failing heart, in contrast to the healthy human heart, has a negative force-frequency relation (Pieske et al., 1995). In addition, β blockers improve perfusion of the myocardium by prolonging diastole, thereby reducing ischemia.

Available Agents. Four β blockers have been successfully tested in randomized clinical trials (Table 33–1): the β_1-selective agents *metoprolol* (MERIT-HF Investigators, 1999) and *bisoprolol* (CIBIS-II Investigators, 1999) and the third-generation agents with additional actions, *carvedilol* and *nebivolol*. *Carvedilol* is a nonselective β blocker and an α_1 receptor antagonist. *Nebivolol* (Flather et al., 2005) is β_1 selective and has additional vasodilatory actions that may be NO mediated (Chapter 14). The COMET trial (Poole-Wilson et al., 2003), the only randomized, prospective, head-to-head comparison of two β blockers, suggested a more favorable effect of *carvedilol* over *metoprolol* but used *metoprolol* in an unsuitable formulation (*metoprolol* tartrate instead of succinate in zero-order kinetics formulation) and at an insufficient dose. Subsequent observational studies and meta-analyses collectively did not confirm a clinically meaningful difference.

Pharmacokinetic Considerations. There are important pharmacokinetic differences among these β blockers (Table 33–3), distinctions that are relevant because successful therapy of heart failure (and most other chronic cardiovascular diseases) requires stable plasma concentrations over the entire day (trough levels before next dose application >50% of maximum).

Metoprolol has a short $t_{1/2}$ (3–5 h) and is only approved for the treatment of heart failure as the zero-order prolonged-release formulation that has been used in the successful outcome trials (*metoprolol succinate CR/XL*; Ponikowski et al., 2016; Yancy et al., 2017). Standard extended-release formulations (*metoprolol tartrate*) exhibit much larger daily fluctuations of plasma concentrations and an insufficient 24-h trough-peak ratio when given twice daily. A principal disadvantage of *metoprolol* is its dependency on the polymorphic cytochrome P450 (CYP) 2D6 for its metabolism. CYP2D6 “poor metabolizers,” about 8% of the Caucasian population, exhibit C_{Pmax} levels of *metoprolol* 5-fold higher than those of standard metabolizers; in a prospective longitudinal study, that difference correlated with 2-fold differences in heart rate responses (Rau et al., 2009). *Bisoprolol* has a sufficiently long plasma $t_{1/2}$ (10–12 h) for once-daily dosing and is not metabolized by CYP2D6. *Carvedilol* has a shorter $t_{1/2}$ (6–10 h) and requires twice-daily dosing. An advantageous peculiarity of *carvedilol* is that it dissociates only slowly from β receptors and therefore acts longer than its plasma $t_{1/2}$ suggests. *Carvedilol* metabolism depends on CYP2D6, but less so than *metoprolol*. *Nebivolol* plasma concentrations are 10- to 15-fold higher in CYP2D6 poor metabolizers, but this is without clinical consequence, likely because the first metabolite is similarly active as the parent compound. *Nebivolol* is not approved in the U.S. for the treatment of heart failure (only for hypertension), but it is approved in 71 countries worldwide, including Europe (patients >70 years of age).

Clinical Use. All patients with symptomatic heart failure (stage C, NYHA II–IV) and all patients with left ventricular dysfunction (stage B, NYHA I) after myocardial infarction should be treated with a β blocker. The therapy with β blockers should be initiated only in clinically stable patients at very low doses, generally one-eighth of the final target dose, and titrated upward every 4 weeks. Even when initiated properly, a tendency to retain fluid exists that may require diuretic dose adjustment. The improvement of left ventricular function generally takes 3 to 6 months, and in this period, patients should be carefully monitored.

The β blockers should not be administered in new-onset or acutely decompensated heart failure. If patients are hospitalized with acute decompensation under current therapy with β blockers, doses may need to be reduced or the drug discontinued until clinical stabilization, after which therapy should be restarted.

Precautions. Formally, β blockers have long lists of adverse drug responses and contraindications. Practically, however, they are generally well tolerated if properly initiated. If doses are increased too rapidly, fall of blood pressure, fluid retention, and dizziness are common and require dose reduction.

TABLE 33–3 ■ β BLOCKERS APPROVED AND CLINICALLY EVALUATED FOR THE THERAPY OF HFrEF

β BLOCKER	β_1 SELECTIVE	VASODILATION	HALF-LIFE (h)	START DOSE (mg)	TARGET DOSE (mg)	CYP2D6 DEPENDENCE[a]
Bisoprolol	Yes	No	10–12	1 × 1.25	1 × 10	No
Carvedilol	No	Yes	6–10	2 × 3.125	2 × 25	Yes
Metoprolol succinate[b]	Yes	No	>12[b]	1 × 12.5[b]	1 × 200	Yes
Nebivolol	Yes	Yes	10	1 × 1.25	1 × 10	Yes

[a] CYP2D6 indicates dependence on polymorphic cytochrome P450 metabolism; likely less relevant for nebivolol as first metabolite is active.

[b] Clinical studies in heart failure have mainly used metoprolol succinate in a slow-release formulation (zero order of kinetics); metoprolol itself has a half-life of 3–5 h.

The major cardiovascular responses associated with use of β blockers are the following:

- *Heart rate lowering,* a desirable effect that indicates proper dosing (no decrease indicates insufficient dosing). A reasonable target resting heart rate is 60 to 70 beats/min.
- *AV block* (beware preexisting conduction disturbance; consider pacemaker implantation).
- *Bronchoconstriction.* Asthma is a contraindication for all β blocker use; however, chronic obstructive lung disease is not, because the β_2 receptor–dependent dynamic range is low in these patients, and studies have documented safety. Nonetheless, only β_1-selective compounds should be used in patients with chronic obstructive pulmonary disease.
- *Peripheral vasoconstriction (cold extremities).* Initial vasoconstriction turns into vasodilation under chronic therapy with β blockers. Cold extremities are generally not a problem in patients with heart failure. Yet, patients with peripheral artery disease or symptoms of claudication or Raynaud disease should be carefully monitored and treated with *carvedilol* if a β blocker is employed.

Mineralocorticoid Receptor Antagonists

The third group of drugs with a documented life-prolonging effect in patients with heart failure are MRAs. They should be given in low doses to all patients in stage C (NYHA class II–IV), that is, with symptomatic HFrEF, even though the combination of ACEIs/ARBs and MRA is formally contraindicated due to the risk of hyperkalemia. The safety of a low-dose MRA (25 mg vs. the standard 100 mg of *spironolactone*) was demonstrated in a large, randomized trial in a patient cohort with severe heart failure (NYHA III–IV), with the MRA added to ACEIs, diuretics, and *digoxin* (Pitt, 2004). Later studies with *eplerenone* in less-severe heart failure essentially confirmed the efficacy of this class of drugs.

Mechanism of Action. The MRAs act as antagonists of nuclear receptors of aldosterone (see Figure 29–6). They are K^+-sparing diuretics (see discussion that follows) but gained more importance in the treatment of heart failure for their additional efficacy in suppressing the consequences of neurohumoral activation. Aldosterone, as the second major actor of the RAAS, promotes Na^+ and fluid retention, loss of K^+ and Mg^{2+}, sympathetic activation, parasympathetic inhibition, myocardial and vascular fibrosis, baroreceptor dysfunction, and vascular damage, all adverse effects in the setting of heart failure. Aldosterone plasma levels decrease under therapy with ACEIs or ARBs, but quickly increase again, a phenomenon called *aldosterone escape*. It is likely explained by incomplete blockade of the RAAS (e.g., AngI can be converted to AngII by chymase, in addition to ACE; see Chapter 30) and by the fact that aldosterone secretion is regulated not only by AngII but also by plasma concentrations of Na^+ and K^+. MRAs inhibit all the effects of aldosterone, of which reduction in fibrosis may be of particular importance.

Clinical Use; Adverse Responses. Currently, two steroidal MRAs are available, *spironolactone* and *eplerenone*. *Spironolactone* is a nonspecific steroid hormone receptor antagonist with similar affinity for progesterone and androgen receptors; it causes gynecomastia (painful breast swelling, 10% of patients) in men and dysmenorrhea in women. *Eplerenone* is selective for the mineralocorticoid receptor and therefore does not cause gynecomastia. A nonsteroidal MRA (*finerenone*) received FDA approval in 2021. It has a higher selectivity for mineralocorticoid over other steroid receptors and may cause relatively less hyperkalemia.

The most important ADR of MRAs is hyperkalemia. Under the well-controlled conditions of clinical trials, serious hyperkalemia (>5.5 mmol/L) occurred in 12% in the eplerenone group versus 7% in the placebo group (Zannad et al., 2011). Rates of hyperkalemia may be higher in clinical practice when risk conditions, comedication, and dose restrictions are not well controlled (Juurlink et al., 2004). Guidelines for the use of MRAs in patients with heart failure are as follows:

- Administer no more than 50 mg/d.
- Do not use if the GFR is less than 30 mL/min (creatinine ~2 mg/dL or higher).
- Be careful with elderly patients, in whom improvement in prognosis may be less relevant than prevention of serious side effects.
- Be careful with diabetics, who carry a higher risk of hyperkalemia.
- Do not combine with NSAIDs, which are contraindicated in heart failure but are frequently prescribed for chronic degenerative diseases of the musculoskeletal system.
- Do not combine with other K^+-sparing diuretics.

Angiotensin Receptor and Neprilysin Inhibitors

An addition to standard combination therapy of heart failure is *sacubitril/valsartan*. It is made by co-crystallizing the well-known ARB *valsartan* with *sacubitril*, a prodrug that, after deesterization, inhibits neprilysin, a peptidase mediating the enzymatic degradation and inactivation of natriuretic peptides (ANP, BNP, C-type natriuretic peptide [CNP]), bradykinin, and substance P. Thus, the drug combines inhibition of the RAAS with activation of a beneficial axis of neurohumoral activation, the natriuretic peptides. Consequently, the ARNI is expected to promote the beneficial effects of natriuresis, diuresis, and vasodilation of arterial and venous blood vessels and to inhibit thrombosis, fibrosis, cardiac myocyte hypertrophy, and renin release. Augmentation of ANP/BNP levels by inhibiting degradation is a better pharmacological principle than giving the agonist BNP (*nesiritide*; see under acute heart failure) directly because it enhances *endogenous* regulation of plasma and tissue levels. *Sacubitril/valsartan* causes smaller increases in bradykinin and substance P than *omapatrilat*, an earlier drug combining a neprilysin inhibitor and an ACEI (which itself and additionally inhibits degradation of these peptides). This difference may explain why *sacubitril/valsartan* is not associated with an increased rate of angioedema, the adverse effect that stopped the development of *omapatrilat*. A large head-to-head comparison study in patients with stable heart failure showed superiority of *sacubitril/valsartan* over *enalapril* (McMurray et al., 2014). It is currently recommended as a replacement for an ACEI or ARB in all patients with NYHA class II–III (U.S.; Yancy et al., 2017) or, in the ESC guidelines, for all patients still symptomatic under triple therapy including MRA (Ponikowski et al., 2016).

Treatment Principle II: Preload Reduction

Fluid overload with increased filling pressures (increased preload) and dilation of the ventricles in heart failure is the consequence of decreased kidney perfusion and activation of the RAAS. Normally, increased preload and stretch of the myofilaments increase contractile force in an autoregulatory manner, the positive force-length relationship or Frank-Starling mechanism. However, the failing heart in congestion operates at the flat portion of this relationship (Figure 33–4) and cannot generate sufficient force with increasing preload, leading to edema in the lungs and the periphery.

Diuretics increase Na^+ and water excretion by inhibiting transporters in the kidney and thereby improve symptoms of congestive heart failure (CHF) by moving patients to lower cardiac filling pressures along the same ventricular function curve. Diuretics are an integral part of the combination therapy of symptomatic forms of heart failure. Prognostic efficacy of diuretics in heart failure will remain an academic question, simply because randomization for a trial of diuretics would be ethically impermissible. Diuretics should *not* be given to patients without congestion because they activate the RAAS and may accelerate a vicious downward spiral. On the other hand, in severe heart failure, diuretic resistance may occur for various reasons and cause clinical deterioration (Table 33–4).

Loop Diuretics

Loop diuretics (*furosemide, torsemide, bumetanide*; Table 33–5) inhibit the Na^+-K^+-2Cl symporter in the ascending limb of the loop of Henle, where up to 15% of the primary filtrate (~150 L/d) is reabsorbed, explaining their strong diuretic action. The increase in Na^+ and fluid delivery to distal nephron segments has two consequences:

- It is sensed in the macula densa and normally activates tubuloglomerular feedback to decrease GFR. This autoregulation explains the quick

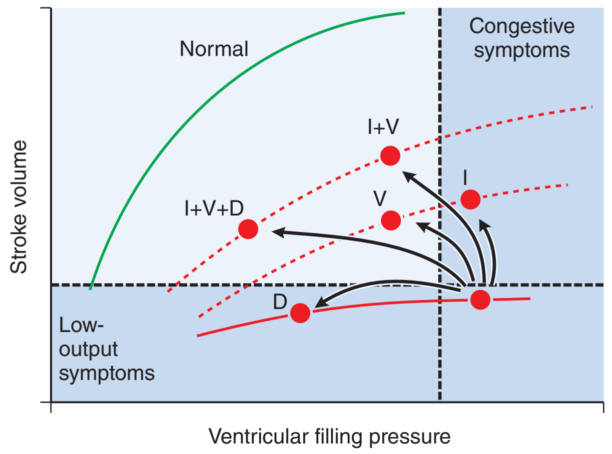

Figure 33–4 *Hemodynamic responses to pharmacologic interventions in heart failure.* The relationships between diastolic filling pressure (preload) and stroke volume (ventricular performance) are illustrated for a normal heart (green line; the Frank-Starling relationship) and for a patient with heart failure with systolic dysfunction (red line). Note that positive inotropic agents (I), such as CGs or *dobutamine*, move patients to a higher ventricular function curve (lower dashed line), resulting in greater cardiac work for a given level of ventricular filling pressure. Vasodilators (V), such as ACEIs or *nitroprusside*, also move patients to improved ventricular function curves while reducing cardiac filling pressures. Diuretics (D) improve symptoms of CHF by moving patients to lower cardiac filling pressures along the same ventricular function curve.

loss of efficacy of older diuretics of the carbonic anhydrase inhibitor class (e.g., *acetazolamide*), acting in the proximal tubule. Thiazides (see discussion that follows) are derived from this class and cause a small decrease in the GFR. Loop diuretics inhibit the feedback mechanism because it is mediated by the Na^+-K^+-2Cl symporter; they exhibit stable action and do not affect the GFR.

- It leads to increased ENaC-mediated reabsorption of Na^+ and, in exchange, to more K^+ excretion in the distal tubule, explaining the main side effect, hypokalemia.

The bioavailability of orally administered *furosemide* ranges from 40% to 70%. High drug doses are often required to initiate diuresis in patients with worsening symptoms or in those with impaired gastrointestinal (GI) absorption, as may occur in severely hypervolemic patients with CHF-induced GI edema. Oral bioavailabilities of *bumetanide* and *torsemide* are greater than 80%, and as a result, these agents are more consistently absorbed than *furosemide*. *Furosemide* and *bumetanide* are short-acting drugs. The $t_{1/2}$ of *furosemide* in normal kidney function is about 1 h (increases in terminal kidney failure to >24 h). Rebound Na^+ retention normally requires dosing twice a day or more. *Bumetanide* reaches maximal plasma concentrations in 0.5 to 2 h and has a $t_{1/2}$ of 1 to 1.5 h. *Torsemide* has a slower onset of action (maximal effect 1–2 h after ingestion) and a plasma $t_{1/2}$ of 3 to 4 h. Kidney failure does not critically affect the elimination of *bumetanide* or *torsemide*. *Ethacrynic acid*, in contrast to other loop diuretics, thiazides, and thiazide-like diuretics, is not a sulfonamide and is generally reserved for patients with sulfa allergy. It acts like *furosemide* and causes ototoxicity at high doses.

TABLE 33–4 ■ CAUSES OF DIURETIC RESISTANCE IN HF

Noncompliance with medical regimen; excess dietary Na^+ intake
Decreased renal perfusion and glomerular filtration rate due to: *Excessive vascular volume depletion and hypotension due to aggressive diuretic or vasodilator therapy* *Decline in cardiac output due to worsening heart failure, arrhythmias, or other primary cardiac causes* *Selective reduction in glomerular perfusion pressure following initiation (or dose increase) of ACEI therapy*
Nonsteroidal anti-inflammatory drugs
Primary renal pathology (e.g., cholesterol emboli, renal artery stenosis, drug-induced interstitial nephritis, obstructive uropathy)
Reduced or impaired diuretic absorption due to gut wall edema and reduced splanchnic blood flow

Thiazide Diuretics

Thiazide diuretics (*hydrochlorothiazide, chlorthalidone*; Table 33–5) have a limited role in heart failure for their low maximal diuretic effect and loss of efficacy at a GFR below 30 mL/min. Thiazide-like diuretics such as *metolazone* and *xipamide* (not available in the U.S.) retain their diuretic effect at low GFR and are therefore somewhat positioned in between loop diuretics and classical thiazides. Combination therapy of thiazides with loop diuretics is often effective in those refractory to loop diuretics alone ("sequential tubulus blockade"), as refractoriness is often caused by upregulation of the Na^+-Cl cotransporter in the distal convoluted tubule, the main target of thiazide diuretics (see Chapter 29). Thiazides are associated with a greater degree of K^+ wasting per fluid volume reduction than loop diuretics, and combination therapy requires particularly careful monitoring of K^+ loss.

K^+-Sparing Diuretics

K^+-Sparing diuretics (see Chapter 29) inhibit apical Na^+ channels in distal segments of the tubulus directly (ENaC; e.g., *amiloride*, *triamterene*) or reduce its gene expression (MRAs *spironolactone* and *eplerenone*). These agents are weak diuretics, but they are often used in the treatment of hypertension in combination with thiazides or loop diuretics to reduce K^+ and Mg^{2+} wasting. The prognostic efficacy of MRAs, which is at least partially independent of its K^+-sparing activity, makes *amiloride* and *triamterene* largely dispensable in the therapy of heart failure. They should not be combined with ACEIs and MRAs.

Treatment Principle III: Afterload Reduction

The failing heart is exquisitely sensitive to increased arterial resistance (i.e., afterload; Figure 33–5). Vasodilators, therefore, should have beneficial effects on patients with heart failure by reducing afterload and allowing the heart to expel blood against lower resistance. However, clinical trials with pure vasodilators were mainly disappointing, whereas inhibitors of the RAAS, vasodilators with a broader mode of action, were successful. Likely reasons include reflex tachycardia and tachyphylaxis (*prazosin*, ISDN) and negative inotropic effects (dihydropyridine calcium channel antagonists).

Hydralazine–Isosorbide Dinitrate

A remarkable exception is the therapeutic effect of a fixed combination of *hydralazine* and ISDN. In a pioneering trial, Cohn and colleagues showed moderate efficacy of this combination in patients with heart failure (Cohn et al., 1986). The benefit was restricted to improvement in the cohort of African Americans. In a second trial in African Americans only, the combination conferred a 43% survival benefit (Taylor et al., 2004). It was FDA approved in 2006, the first ethnically restricted approval.

As an orally available organic nitrate, ISDN, like *nitroglycerine* and isosorbide 5′-mononitrate (ISMN), preferentially dilates large blood vessels, for instance, venous capacitance and arterial conductance vessels (see Chapter 31). The main effect is "venous pooling" and reduction of diastolic filling pressure (preload) with little effect on systemic vascular resistance (which is regulated by small-to-medium arterioles). Sustained monotherapy is compromised by nitrate tolerance (i.e., loss of effect and induction of a pro-constrictory state with high levels of ROS). *Hydralazine* is a direct vasodilator whose mechanism of action remains unresolved (see Chapter 32). It was suggested that *hydralazine* prevents nitrate tolerance by reducing ROS-mediated inactivation of NO (Munzel et al., 2005), an action that could explain the efficacy of this drug combination in heart failure among African Americans. A test of this hypothesis in patients with NYHA class II–III heart failure (Chirkov et al., 2010)

TABLE 33–5 ■ PROPERTIES AND THERAPEUTIC DOSES OF DIURETICS FOR THE THERAPY OF HFrEF[a]

DIURETIC	START DOSE (mg)		COMMON DAILY DOSE (mg)		TIME TO START OF EFFECT (h)	HALF-LIFE (h)	ADVERSE EFFECTS AND INTERACTIONS
Loop diuretics							
Bumetanide	0.5–1		1–5		0.5	1–1.5	**Adverse effects:** Hypokalemia, hyponatremia, hypomagnesemia, hyperuricemia, hypocalcemia, nephrotoxicity, ototoxicity (loop diuretics), hypercalcemia (thiazides), glucose intolerance, sulfonamide hypersensitivity **Interactions:** Can increase lithium levels (PK) and cardiac glycoside toxicity (PD, hypokalemia), anion exchanger resins (PK), NSAIDs and glucocorticoids (PD) can decrease effect of diuretics
Furosemide	20–40		40–240		0.5	1	
Torsemide	5–10		10–20		1	3–4	
Thiazides							
Chlorthalidone	50		50–100		2	50	
Hydrochlorothiazide	25		12.5–100		1–2	6–8	
Potassium-sparing diuretics							
	+RAS blocker	−RAS blocker	+RAS blocker	−RAS blocker			**Adverse effects:** Hyperkalemia (all), gynecomastia, erectile dysfunction, and menstrual bleeding disorders (spironolactone) **Interactions:** Increased risk of hyperkalemia when given with ACE or ARB (note different dosing!), but also cyclosporine, NSAIDs **Contraindication:** Renal insufficiency with creatinine clearance <30 mL/min
Eplerenone, spironolactone	12.5–25	50	50	100–200	2–6	24–36	
Amiloride	2.5	5	5–10	10–20	2	10–24	
Triamterene	25	50	100	200	2	8–16	

PD, pharmacodynamics; PK, pharmacokinetic interaction.

[a]Dosing recommendations were adapted from ESC guidelines (Ponikowski et al., 2016).

failed to confirm the hypothesis. The relevant differences in responsiveness between African American and Caucasian patients with heart failure have not been explained.

The fixed-combination formulation contains 37.5 mg *hydralazine* and 20 mg ISDN and is uptitrated to a target dose of two tablets, thrice daily. Patients will also generally be taking a β blocker. Therefore, hypotension may be dose limiting. Frequent adverse effects include dizziness and headache. Adherence to the thrice-daily dosing regimen may impose practical problems (Cohn et al., 1986), and *hydralazine* doses greater than 200 mg have been associated with lupus erythematosus.

Vericiguat

A new addition to heart failure therapeutics is *vericiguat*, which was FDA approved in early 2021. It directly stimulates soluble guanylyl cyclase (GC) and sensitizes the enzyme to endogenous NO, like *riociguat* (approved for the treatment of pulmonary arterial hypertension; see Chapter 35), but with a longer half-life. GCs are established targets for natriuretic peptides (the membrane form, mGC) and NO and organic nitrates (the soluble form, sGC). A recent trial in patients with advanced heart failure and recent hospitalization showed that the addition of *vericiguat* (10 mg once daily) to a guideline-directed therapy (including 60% receiving triple therapy [renin-angiotensin system (RAS) inhibitor, β blocker, MRA] and 15% ARNI) reduced a composite endpoint of death from cardiovascular causes or first hospitalization for heart failure by 10% (Armstrong et al., 2020). As expected from an sGC stimulator, the rate of symptomatic hypotension was slightly higher in the *vericiguat* group.

Treatment Principle IV: Increasing Cardiac Contractility

The failing heart is unable to generate force sufficient to meet the needs of the body for perfusion of oxygenated blood (Figure 33–1). Historically, physicians attempted to stimulate force generation with positive inotropic drugs. Unfortunately, when used chronically, these agents do not improve life expectancy or cardiac performance. Rather, chronic use of positive inotropes is associated with excess mortality. Of the available inotropic agents, only cardiac glycosides are used in the treatment of chronic heart failure; this is for two reasons: long history of use (see historical perspective) and one large trial in patients with NYHA class II–III heart failure showing that *digoxin* reduced the rate of heart failure–associated hospitalizations without increasing mortality (Table 33–1).

Inotropic Agents and the Regulation of Cardiac Contractility

Cardiac myocytes contract and develop force in response to membrane depolarization and subsequent increases in intracellular Ca^{2+} concentrations (Figure 33–6). The mechanisms of this *excitation-contraction coupling* are the basis for understanding the mode of action of positive inotropic drugs and cardiac myocyte function in general. Positive inotropes and novel compounds in development act by increasing the concentration of free intracellular Ca^{2+} ($[Ca^{2+}]_i$). Ca^{2+} “sensitizers” (e.g., *levosimendan*) sensitize myofilaments to Ca^{2+}; that is, they shift the relationship between free Ca^{2+} concentration and force to the left.

Na^+/K^+ ATPase Inhibitors. Cardiac glycosides inhibit the plasma membrane Na^+/K^+ ATPase, a key enzyme that actively pumps Na^+ out and K^+ into the cell and thereby maintains the steep concentration gradients of Na^+ and K^+ across the plasma membrane. Inhibition of this enzyme slightly reduces the Na^+ gradient across the myocyte membrane, reducing the driving force for Ca^{2+} extrusion by the Na^+/Ca^{2+} exchanger (NCX), thereby providing more Ca^{2+} for storage in the SR to activate contraction. The details are explained by Figure 33–6 and its legend.

cAMP-Dependent Inotropes. The strongest stimulation of the heart is achieved by receptor-mediated stimulation of adenylyl cyclase. This explains the use of *dobutamine*, EPI, and NE in cardiogenic shock (see discussion that follows). Inhibition of cAMP degradation by PDE inhibitors such as *milrinone* or *enoximone* elevates cellular cAMP

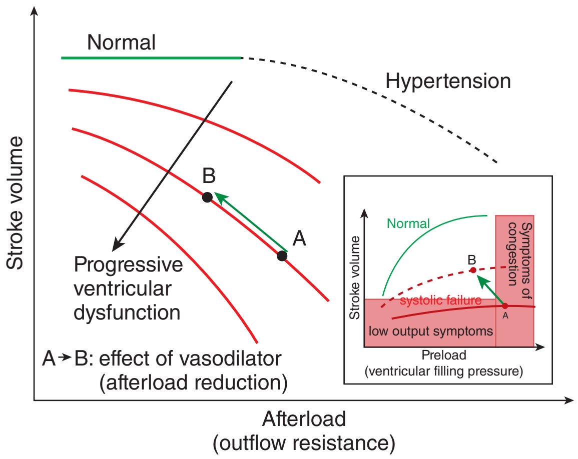

Figure 33–5 *Stroke volume versus afterload (outflow resistance): effects of heart failure. Increasing the resistance to ventricular outflow, a basic determinant of afterload, has little effect on stroke volume in normal hearts until high levels of outflow resistance (top curve).* However, in patients with systolic ventricular dysfunction (lower curves), an increase in outflow resistance elicits a noticeable decrease in cardiac performance (= stroke volume) that is progressive with increasing failure. Such an increase in outflow resistance can occur as a compensatory response by the SNS and RAAS to decreased cardiac function and depressed arterial pressure as a result of heart failure. A higher resistance to ventricular outflow increases peak pressure development in the left ventricle in opening the aortic valve, thereby increasing ventricular wall stress and end-systolic volume. This can cause end-diastolic volume to increase. In the normal heart, increasing ventricular stretch enhances cardiac contractile performance (stroke volume); this is the Frank-Starling effect (inset). However, in the failing heart, the positive contractile response embodied in the Frank-Starling effect is poor and provides only a small increase in stroke volume. Reducing outflow resistance with agents that reduce systemic vascular resistance, such as arterial vasodilators, can shift cardiac performance to a larger stroke volume in patients with myocardial dysfunction (from A to B). Such an increase in stroke volume may provide sufficient output and compensate for the decrease in systemic vascular resistance and moderate the fall in systemic arterial pressure due to the vasodilator. For details, see Figure 33–4 and the work of Klabunde (2015).

concentrations and activates the cAMP-PKA (protein kinase A) pathway and other cAMP-responsive systems (see Chapter 3). This concerted action results in higher peak Ca^{2+} concentrations in systole and thereby peak force (Figure 33–6). All cAMP-dependent inotropes hasten contraction (positive clinotropic effect) and relaxation (positive lusitropic effect), allowing sufficient perfusion of the ventricles in diastole under catecholamine stimulation and with the concomitant tachycardia. On the downside, acceleration of contraction during catecholamine stimulation, by promoting net Ca^{2+} entry per unit of time, increases the utilization of ATP (i.e., increases energy consumption) for Ca^{2+} reuptake into the SR via the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) and to restore the membrane potential by the activity of the Na^+/K^+ ATPase.

Myofilament Ca^{2+} Sensitizers. Calcium sensitizers increase the affinity of the myofilaments for Ca^{2+}, for example, by inducing a conformational change in troponin C (TnC). They enhance force for a given $[Ca^{2+}]_i$ and do not elevate $[Ca^{2+}]_i$ with its potentially deleterious proarrhythmic and energy-increasing consequences. On the other hand, increased myofilament Ca^{2+} sensitivity causes reduced dissociation of Ca^{2+} from the myofilaments in diastole and prolongation of relaxation ("negative lusitropic effect"). This effect can aggravate the already-compromised diastolic function in heart failure. It could also lead to delayed Ca^{2+} release from myofilaments in diastole and arrhythmias (Schober et al., 2012). Calcium sensitizers failed to improve prognosis in clinical trials of patients with chronic heart failure. However, *levosimendan* is approved in some countries for the treatment of acute heart failure. It has additional selective and potent inhibitory effects on PDE3, whose positive lusitropic consequence appears to antagonize the negative lusitropic effect of Ca^{2+} sensitization. Agonists of G_q-coupled receptors (α_1, AT_1, ET_A) also increase myofilament Ca^{2+} sensitivity, likely due to increased myosin light chain phosphorylation. The positive inotropic effect is smaller than that of β receptor stimulation, develops more slowly, and is independent of cAMP.

Cardiac Glycosides

Actions and Therapeutic Use of Digoxin. ***Positive Inotropic Effect.*** Cardiac glycosides (CGs) at therapeutic concentrations mildly inhibit the cardiac Na^+/K^+ ATPase, causing an increase in intracellular $[Na^+]$. Increased $[Na^+]_i$ inhibits Ca^{2+} extrusion via the NCX, resulting in higher intracellular $[Ca^{2+}]$ and enhanced contractility (Figure 33–6). The increased contractility and hence cardiac output provides symptomatic relief in patients with heart failure (Figure 33–1). With the main trigger for neurohumoral activation removed, sympathetic nerve tone and, consequently, heart rate and peripheral vascular resistance drop. These decreases in preload and afterload reduce chamber dilation and thereby wall stress, a strong determinant of myocardial O_2 consumption. Increased renal perfusion lowers renin production and increases diuresis, further decreasing preload. It is not clear whether these potentially beneficial effects occur on top of current guideline-directed medical therapy (e.g., β blockers). Current guidelines list CGs as a third-line choice (Ponikowski et al., 2016) or not at all (Yancy et al., 2017; Figure 33–3).

Electrophysiological Actions. CGs at therapeutic concentrations shorten action potentials by accelerating the inactivation of L-type Ca^{2+} channels due to higher $[Ca^{2+}]_i$. Shorter action potentials (= refractory period) favor reentry arrhythmias, a reason that CGs promote atrial fibrillation. With the loss of intracellular K^+ and increase in intracellular Na^+, the resting membrane potential (determined largely by the K^+ current, now diminished) moves to less-negative values with two consequences. Diastolic depolarization and automaticity are enhanced, and, due to partial inactivation of Na^+ channels, impulse propagation is strongly reduced. Both phenomena promote reentry arrhythmias. At even higher CG concentrations, SR Ca^{2+} overload reaches a point at which Ca^{2+} is spontaneously released at amounts large enough to initiate Ca^{2+} waves and, via the NCX, depolarization of the cell (Figure 33–6). The typical ECG signs at this stage of CG intoxication are extrasystoles and bigeminies with a high risk of ventricular fibrillation.

Extracardiac Effects. CGs also inhibit Na^+/K^+ ATPase in other excitable tissues. (1) At low plasma concentrations, CGs stimulate vagal efferents and sensitize baroreceptor reflex mechanisms, causing increased parasympathetic and decreased sympathetic tone. The beneficial effect of *digoxin* at low plasma concentrations (Rathore et al., 2003), at which positive inotropic effects are minor, suggests that the neurohumoral actions of CGs may be therapeutically more relevant than the direct positive

HISTORICAL PERSPECTIVE

The British botanist William Withering (1741–1799) systematically described the actions of *Digitalis purpurea* in patients with heart failure ("dropsy") and gave exact dosing recommendations (Skou, 1986). Oswald Schmiedeberg (1833–1921), working in Strasbourg, France, isolated the first chemical entities from foxglove leaves; one of these entities was digitoxin. Until diuretics became available, CGs were the only heart failure drugs. CGs encompass many chemical entities, but only *digoxin* and its derivatives *β-acetyl digoxin, methyldigoxin*, and *digitoxin* are in clinical use in most countries. Until the 1980s, CGs were dosed according to therapeutic effects (e.g., improved diuresis [Withering considered CGs as diuretics], reduction of heart size [verifiable by X-ray], or alterations of the surface electrocardiogram [ECG]) and to symptoms of overdosing, such as nausea and altered color perception (yellow-green). Now, serum *digoxin* concentrations can be measured by radioimmunoassay. *Digoxin* has therapeutic efficacy (including a small survival benefit) only at serum concentrations between 0.5 and 0.8 ng/mL (Rathore et al., 2003). Concentrations greater than 1.2 ng/mL are associated with increased mortality. Serum *digoxin* concentrations greater than 0.8 ng/mL should be avoided.

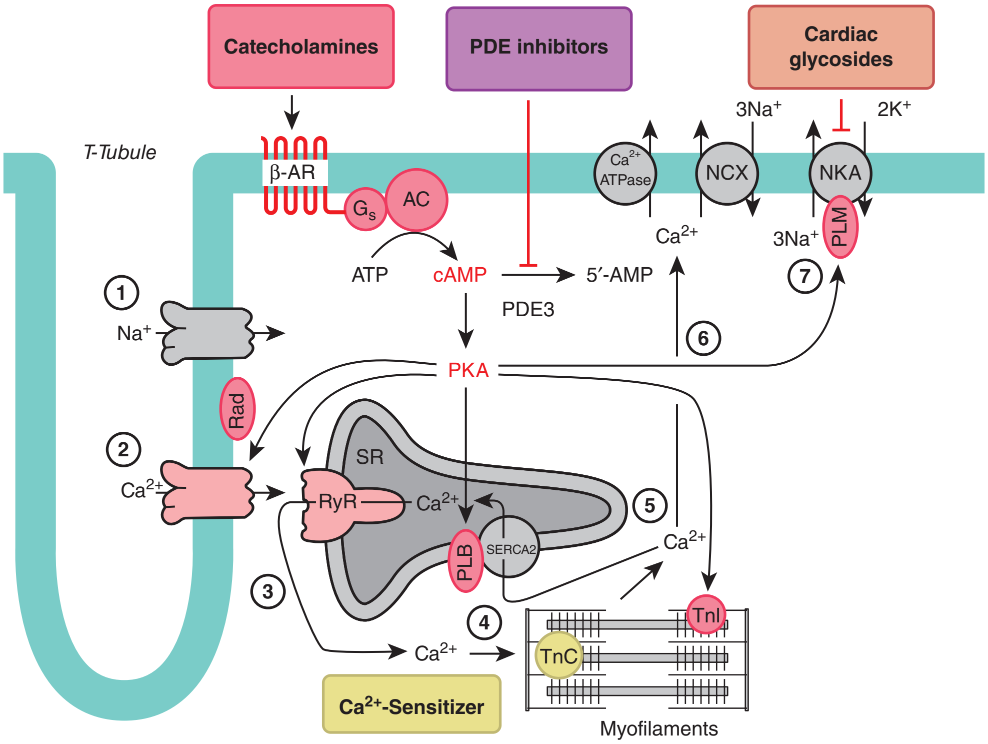

Figure 33–6 *Cardiac excitation-contraction coupling and its regulation by positive inotropic drugs.* The cardiac cycle is initiated by membrane depolarization, which causes the opening of voltage-dependent Na$^+$ (**1**) and L-type Ca^{2+} channels (**2**), permitting Na$^+$ and Ca^{2+} flow down their electrochemical gradients into the myocyte. Thus, Na$^+$ and Ca^{2+} enter the cardiac myocyte during each cycle of membrane depolarization, triggering the release, through the ryanodine receptor (RyR), of larger amounts of Ca^{2+} from internal stores in the SR (**3**). The resulting increase in intracellular Ca^{2+} interacts with troponin C (**4**) and activates interactions between actin and myosin that result in sarcomere shortening. The bulk of cytosolic Ca^{2+} (70%) is pumped back into the SR by a Ca^{2+}-ATPase, SERCA2 (**5**). The remainder is removed from the cell by a high-capacity NCX and, to a smaller extent, a sarcolemmal Ca^{2+}-ATPase (**6**). The NCX exchanges three Na$^+$ for a Ca^{2+}, using the electrochemical potential of Na$^+$ to drive Ca^{2+} extrusion. The electrochemical gradient for Na$^+$ across the sarcolemma is maintained by active transport of Na$^+$ out of the cell by the sarcolemmal Na$^+$/K$^+$ ATPase (NKA; **7**). The β adrenergic agonists (acting at β-AR, the β adrenergic receptor) and PDE inhibitors, by increasing intracellular cAMP levels, activate PKA, which phosphorylates the small GTPase Rad binding to and inhibiting the L-type Ca^{2+} channel (Liu et al., 2020), and regulatory components of the RyR, as well as phospholamban (PLB) on the SR and TnI (inhibitory subunit of troponin) in the sarcomere. As a result, Rad-mediated inhibition of L-type Ca^{2+} channels is released and the RyR2 Ca^{2+} channel open probability is increased; SERCA2 inhibition by PLB is released, with the result that SERCA2 accumulates Ca^{2+} into the SR faster, more avidly, and to a higher concentration; and relaxation occurs at slightly higher [Ca^{2+}]$_i$ due to slightly reduced sensitivity of the troponin complex to Ca^{2+}. The net effect of these phosphorylations is a positive inotropic effect: a faster rate of tension development to a higher level of tension, followed by a faster rate of relaxation (positive lusitropic effect). CGs, by inhibiting the NKA, reduce Na$^+$ extrusion from the cell, thereby permitting [Na$^+$]$_{in}$ to rise, reducing the inward gradient for Na$^+$ that drives Ca^{2+} extrusion by NCX. As a consequence, Ca^{2+} accumulates in the SR, and a positive inotropic effect follows, but without faster relaxation. See the text for details of additional effects of CGs. Note that, under steady-state conditions, the amount of Ca^{2+} leaving the cell exactly matches the amount entering it. As NCX exchanges three Na$^+$ for every Ca^{2+}, it creates a depolarizing current. This makes not only the direction of transport dependent on the chemical gradients of Na$^+$ and Ca^{2+} across the membrane but also on the membrane potential. Thus, the direction of Na$^+$-Ca^{2+} exchange may briefly reverse during depolarization, when the electrical gradient across the sarcolemma is transiently reversed. phospholemman (PLM) is a tonic inhibitor of the NKA, which supplies the driving force (an appropriately low [Na$^+$]$_{in}$) for maintaining low diastolic Ca^{2+}. Phosphorylation of PLM by PKA removes this inhibitory influence, thereby stimulating the activity of the NKA and limiting [Na$^+$]$_{in}$ and [Ca^{2+}]$_{in}$. This may reduce the tendency toward arrhythmias during adrenergic stimulation (see Pavlovic et al., 2013). Note that L-type Ca^{2+} channels, SERCA2, and NKA are all regulated indirectly by phosphorylation of inhibitory regulator proteins.

inotropic effects. (2) CGs at higher plasma concentrations increase Ca^{2+} concentrations in vascular smooth muscle cells and cause vasoconstriction. In patients with heart failure, vasodilation normally prevails due to the decrease in sympathetic nervous tone, but the direct vascular effect explains mesenteric artery ischemia or occlusion, a rare but severe adverse effect of CGs.

Indirect Actions. The vagotonic and sympatholytic effects of CGs cause bradycardia and AV prolongation (negative dromotropic effect) and can promote atrial flutter and fibrillation. Fibrillation is explained by the acetylcholine-induced shortening of atrial action potentials, which is further enhanced by the direct CG effect described previously. On the other hand, CGs are therapeutically used for frequency control of permanent atrial fibrillation because of their negative dromotropic effects.

Interactions with K$^+$, Ca^{2+}, and Mg^{2+}. Hyperkalemia reduces and hypokalemia increases the binding affinity of CG to the Na$^+$/K$^+$ ATPase. In addition, hypokalemia reduces repolarizing K$^+$ currents, with the consequence of increased spontaneous diastolic depolarization and automaticity. Hypokalemia is therefore a major risk factor for arrhythmogenic effects of CGs. Hypercalcemia as well as hypomagnesemia favor SR Ca^{2+} overload and spontaneous Ca^{2+} release events. Control of serum electrolytes is therefore mandatory.

Adverse Effects. The therapeutic index of CG is extremely narrow, as documented in the DIG trial: plasma concentrations between 0.5 and 0.8 ng/mL are associated with beneficial effects, and concentrations of 1.2 ng/mL and greater are associated with a tendency toward increased mortality (Rathore et al., 2003). The most frequent and most serious adverse effects are arrhythmias. In CG overdosing, patients exhibit arrhythmias (90%), GI symptoms (~55%), and neurotoxic symptoms (~12%). The most frequent causes of toxicity are renal insufficiency and overdosing.

Cardiac toxicity in healthy persons presents as extreme bradycardia, atrial fibrillation, and AV block, whereas ventricular arrhythmias are rare. In patients with structural heart disease, frequent signs of CG toxicity are ventricular extrasystoles, bigeminy, ventricular tachycardia, and fibrillation. In principle, however, every type of arrhythmia can be CG induced. GI adverse effects are anorexia, nausea, and vomiting, mainly because of CG effects on chemosensors in the area postrema. Spastic contraction of the mesenteric artery can rarely lead to severe diarrhea and life-threatening necrosis of the intestine. Headache, fatigue, and sleeplessness can be early symptoms of CG toxicity.

Typical, albeit not too common (10%), are visual effects: altered color perception and coronas (halos). Some have speculated that the visual effects of digitalis intoxication contributed to the qualities of late paintings by Vincent van Gogh, who may have been treated for neurological complaints with foxglove by Dr. Paul Gachet, whose portraits by van Gogh (painted in June 1890) show the doctor seated next to a sprig of the plant, a natural source of CGs and used widely in the 19th century (Lee, 1981).

Therapy of CG Toxicity. Cessation of CG medication normally suffices as therapy of CG toxicity. However, severe arrhythmias, such as extreme bradycardia or complex ventricular arrhythmias, require active therapy.

- Extreme sinus bradycardia, sinoatrial block, or AV block grade II or III: Atropine (0.5–1 mg) IV. If not successful, a temporary pacemaker may be necessary.
- Tachycardic ventricular arrhythmias and hypokalemia: K^+ infusion (40–60 mmol/d). Consider that high K^+ can aggravate AV conduction defects.
- An effective antidote for *digoxin* toxicity is antidigoxin immunotherapy. Purified Fab fragments from ovine antidigoxin antisera are usually dosed by the estimated total dose of *digoxin* ingested to achieve a fully neutralizing effect.

Treatment Principle V: Heart Rate Reduction

Heart rate is a strong determinant of cardiac energy consumption, and higher heart rates in patients with heart failure are associated with poor prognosis (Bohm et al., 2010). Partial agonists at β receptors such as *xamoterol* increase nocturnal heart rate (i.e., they prevent the physiological dip) and are associated with excess mortality in patients with heart failure (Xamoterol Study Group, 1990). Conversely, β blockers lower heart rate and improve survival prognosis.

Ivabradine

The circumstantial evidence for beneficial effects of heart rate lowering led to the development of *ivabradine*, a selective inhibitor of cardiac pacemaker channels (hyperpolarization-activated, cyclic nucleotide–gated cation channels [HCNs]). *Ivabradine* is used for the treatment of heart failure and stable angina pectoris in patients not tolerating β blockers or in whom β blockers did not sufficiently lower heart rate (<70 beats/min; guideline class IIa recommendation, Figure 33–3). Approval was based on a study showing a decrease in hospitalization and heart failure mortality, but not total or cardiovascular mortality (Swedberg et al., 2010). Of note, the effect of *ivabradine* was not superior to that of *digoxin* in an earlier study (Digitalis Investigation Group, 1997). In a study in patients with stable angina (85% on β blockers), *ivabradine* conferred no benefit but led to phosphenes (typical transient enhanced brightness in a restricted area of the visual field) and increased the rate of bradycardia, atrial fibrillation, and QT prolongation (Fox et al., 2014), casting doubts about the role of the compound in ischemic heart disease.

Treatment Principle VI: SGLT2 Inhibition

The most recent addition to the combination drug treatment of patients with heart failure came unexpectedly. Studies in patients with diabetes mellitus showed that SGLT2 inhibitors (*empagliflozin, canagliflozin*), developed to increase the excretion of glucose by the kidneys, improved overall and particularly heart failure–related outcomes (Zinman et al., 2015). A trial in patients with heart failure and reduced EF confirmed the beneficial effect of this class and showed that it occurred independently of the presence of diabetes (McMurray et al., 2019). *Dapagliflozin* was the first SGLT2 inhibitor approved for the treatment of heart failure in the U.S. in 2020. The mechanism of the salutary effect on the prognosis of patient with heart failure is not well understood. SGLT2 is not present in the heart, and therefore, it is likely that the primary effect of inhibition of glucose reuptake in the kidney tubulus causes improved diuresis and preload reduction ("a better diuretic effect"). Alternatively, evidence suggests that the class of SGLT2 inhibitors exerts beneficial off-target effects such as inhibition of the sodium hydrogen exchanger (Packer, 2017) or the later sodium influx (Philippaert et al., 2021). This new finding with SGLT2 inhibitors may open new angles for drug development to treat heart failure. The exact place of SGLT2 inhibitors in guideline-directed medical therapy (Figure 33–3) has not yet been defined.

Drug Treatment of Acutely Decompensated Heart Failure

Acutely decompensated heart failure is a leading cause of hospitalization in patients older than 65 and represents a sentinel prognostic event in the natural course of the disease, with a high recurrence rate and a 1-year mortality rate of about 30%. Even in decompensated heart failure, about 50% of patients exhibit preserved left ventricular function (HFpEF). The HFpEF cohort is older, is more likely to be female and hypertensive, and has less coronary artery disease than the HFrEF cohort. Therapeutically, it is important to quickly identify and treat specific reasons for decompensation. Besides acute myocardial ischemia, these include uncorrected high blood pressure, atrial fibrillation and other arrhythmias, pulmonary embolism, and kidney failure. Pharmacological reasons for acute decompensation include nonadherence to heart failure medication and Na^+/fluid restriction, negative inotropic drugs (e.g., *verapamil, diltiazem, nifedipine*), and NSAIDs, especially cyclooxygenase 2–specific inhibitors.

The therapy of acutely decompensated heart failure aims at fast symptom relief, short-term survival, fast recompensation, and reduction of readmission rates. Treatment is less evidence-based than therapy for chronic heart failure, and no single drug given to patients experiencing acute decompensation has shown improvement in the long-term prognosis. The main principles (besides nonpharmacological treatment modalities such as O_2 and noninvasive or [rarely] invasive ventilatory support) are diuretics and vasodilators, with positive inotropes in selected cases and mechanical support systems as an ultimate step.

Diuretics

Patients with dyspnea and signs of fluid overload/congestion should be promptly treated with an intravenous loop diuretic such as *furosemide* that exerts an acute vasodilator and slightly delayed but still fast diuretic effect. Optimal doses and regimens need to be adapted to the clinical picture. An intravenous bolus of 40 to 80 mg *furosemide* is a common starting dose, often continued by an infusion of *furosemide* at a daily dose equal to the (oral) daily dose prescribed before hospitalization. Doses may need to be escalated according to symptoms and diuresis. Additional use of a thiazide diuretic in small doses can break a relative resistance to loop diuretics but requires careful monitoring of K^+ losses. Excessive doses of *furosemide* must be avoided because they can cause hypotension, a reduction in GFR, electrolyte disturbance, and further neurohumoral activation.

Vasodilators

Vasodilators such as *nitroglycerin* and *nitroprusside* reduce preload and afterload. The reduction in preload (= diastolic filling pressure) moves the patient to the left on the stroke volume-preload relationship, like the effect of diuretic-induced volume reduction (Figure 33–4). The accompanying reduction in chamber dimension reduces wall stress and thus O_2 consumption. The additional reduction in afterload allows the heart to expel blood against a lower output resistance (Figure 33–4). These mechanisms explain why vasodilators (which have no inotropic efficacy and lower blood pressure) increase stroke volume. Yet, robust evidence for symptomatic benefit or improved clinical outcome is lacking. They are probably best suited for patients with hypertension and should be avoided in patients with systolic blood pressure less than 110 mmHg (Ponikowski et al., 2016). The main risk is hypotension, which is negatively associated with favorable outcomes in patients with acutely decompensated heart failure (Patel et al., 2014).

Nesiritide, recombinant human BNP, dilates arterial and venous blood vessels by stimulating the membrane-bound GC to produce more cGMP.

By this mechanism, it decreases preload and afterload and reduces pulmonary capillary wedge pressure. It was initially approved for the treatment of acutely decompensated heart failure in the U.S., but not in several European countries. Early clinical studies and a meta-analysis raised concerns that the use of *nesiritide* was associated with an increased risk for renal failure and death when compared to a noninotrope control therapy (Sackner-Bernstein et al., 2005). This risk was not confirmed in a more recent study (O'Connor et al., 2011), but beneficial effects (dyspnea relief) were modest. Based on this study, *nesiritide* is not recommended for routine use in patients with acute heart failure. In 2018, the manufacture of *nesiritide* was discontinued.

Positive Inotropic Agents

Stimulating the heart's force of contraction in a situation of critically diminished cardiac output may appear to be the most intuitive intervention. Yet, inotropes in acutely decompensated heart failure are associated with worse outcome and should therefore be restricted to patients with critically low cardiac output and perfusion of vital organs. Hypotension with a systolic pressure less than 85 mm Hg has been suggested as a practical limit (Ponikowski et al., 2016). Reasons for the adverse consequences of positive inotropes are probably complex. All inotropic agents increase cardiac energy expenditure (greater and faster force development ⇒ more ATP consumption ⇒ greater O_2 demand), which carries the risk of diffuse cardiac myocyte death. In acutely decompensated heart failure, the risk is exaggerated by the low perfusion pressure, any preexisting coronary artery disease, and the likely presence of cardiac myocyte hypertrophy and myocyte–endothelial cell mismatch. Tachycardia, aggravated by many inotropes, adds to the problem by strongly increasing energy expenditure and reducing the time for coronary perfusion in diastole. All positive inotropes increase the risk of arrhythmias.

Dobutamine

Dobutamine is the β adrenergic agonist of choice for the management of patients with acute CHF with systolic dysfunction. *Dobutamine* has relatively well-balanced cardiac and vascular actions: stimulation of cardiac output with less tachycardia than EPI and with a concomitant decrease in pulmonary artery wedge pressure. *Dobutamine* is a racemic mixture of (–) and (+) enantiomers. The (–) enantiomer is a potent agonist at α_1 adrenergic receptors and a weak agonist at β_1 and β_2 receptors. The (+) enantiomer is a potent β_1 and β_2 agonist without much activity at α_1 adrenergic receptors. *Dobutamine* has no activity at dopamine receptors. At infusion rates that result in a positive inotropic effect in humans, the β_1 adrenergic effect in the myocardium predominates. In the vasculature, the α_1 adrenergic agonist effect of the (–) enantiomer appears to be offset by the vasodilating effects of the (+) enantiomer at β_2 receptors. Thus, the principal hemodynamic effect of *dobutamine* is an increase in stroke volume from positive inotropy, augmented by a small decrease in systemic vascular resistance and, therefore, afterload. Lowering of pulmonary artery capillary pressure is considered an advantage compared to other catecholamines, as is the smaller chronotropic effect (reasons for which are not clear).

Continuous *dobutamine* infusions are typically initiated at 2 to 3 μg/kg/min and uptitrated until the desired hemodynamic response is achieved. Pharmacologic tolerance may limit infusion efficacy beyond 4 days; therefore, addition or substitution of a PDE3 inhibitor may be necessary to maintain adequate circulatory support. The major side effects of *dobutamine* are tachycardia and supraventricular/ventricular arrhythmias, which may require a reduction in dosage. The concurrent use of β blockers is a common cause of blunted clinical responsiveness to *dobutamine*. It can be overcome by higher doses in case of *bisoprolol* and *metoprolol*, but not as easily for *carvedilol*, which has a very slow dissociation rate.

Epinephrine

The natural sympathetic agonist is mainly produced by the adrenal gland and systemically released. It is a balanced β_1, β_2, and α_1 adrenergic agonist and has a similar net hemodynamic effect as *dobutamine*, but with a stronger tachycardic effect, which makes it a second-choice inotrope in acutely decompensated heart failure.

Norepinephrine

The main sympathetic neurotransmitter released from sympathetic nerve endings is a potent β_1 and α_1 agonist and weak β_2 receptor agonist. This profile causes the positive inotropism accompanied by prominent vasoconstriction and increased afterload. Vasoconstriction of coronary blood vessels promotes ischemia; increased afterload may impede cardiac output (Figure 33–4). However, the stronger blood pressure–increasing effect of NE may be needed in persistent hypotension despite adequate cardiac filling pressures. Moreover, the increase in mean blood pressure leads to a reflex increase in parasympathetic nervous tone that can antagonize the direct tachycardic effect of NE and cause bradycardia.

Dopamine

The pharmacologic and hemodynamic effects of dopamine (DA) vary with concentration. Low doses (≤2 μg/kg lean body mass/min) induce cAMP-dependent vascular smooth muscle vasodilation by direct stimulation of D2 receptors. Activation of D2 receptors on sympathetic nerves in the peripheral circulation also inhibits NE release and reduces α adrenergic stimulation of vascular smooth muscle, particularly in splanchnic and renal arterial beds. This is the pharmacological basis for the "low-dose DA infusion" historically used to increase renal blood flow and maintain an adequate GFR and diuresis in hospitalized patients with CHF with impaired renal function refractory to diuretics. However, mainly negative clinical studies argue against the validity of this concept (Chen et al., 2013; Vargo et al., 1996). At intermediate infusion rates (2–5 μg/kg/min), DA directly stimulates cardiac β receptors to enhance myocardial contractility. At higher infusion rates (5–15 μg/kg/min), α adrenergic receptor stimulation–mediated peripheral arterial and venous constriction occurs. The complex profile and negative clinical data on low-dose infusion make DA a second or third choice in the treatment of heart failure.

Phosphodiesterase Inhibitors

The cAMP-PDE inhibitors decrease cellular cAMP degradation, resulting in elevated levels of cAMP. This results in positive inotropic and chronotropic effects in the heart and dilation of resistance and capacitance vessels, effectively decreasing preload and afterload (thus the term *inodilator*). PDE inhibitors may be more advantageous than catecholamines in patients on β blockers and in patients with high systemic or pulmonary artery resistance. Hypotension is often dose limiting; tachycardic and arrhythmogenic effects are like those of catecholamines.

Milrinone and Enoximone. Parenteral formulations of *milrinone* and *enoximone* are used for short-term circulation support in advanced CHF. *Enoximone* (not available in the U.S.) is a relative selective inhibitor of PDE3, the cGMP-inhibited cAMP PDE and main isoform involved in inotropic control in human heart. *Milrinone* inhibits human heart PDE3 and PDE4 with similar potency (Bethke et al., 1992). By increasing intracellular cAMP concentrations, they have similar actions as the β receptor agonists *dobutamine* and EPI but tend to lower systemic and pulmonary vascular resistance more than do the catecholamines. It should be kept in mind that PDE inhibitors potentiate the actions of β receptor agonists, both beneficial and detrimental. The loading dose of *milrinone* is ordinarily 25 to 75 μg/kg, and the continuous infusion rate ranges from 0.375 to 0.75 μg/kg/min. Bolus doses of *enoximone* at 0.5 to 1.0 mg/kg over 5 to 10 min are followed by an infusion of 5 to 20 μg/kg/min. The elimination half-lives of *milrinone* and *enoximone* in normal individuals are 0.5 to 1 h and 2 to 3 h, respectively, but can be increased in patients with severe CHF.

Myofilament Calcium Sensitizers (Levosimendan, Pimobendan)

In some countries but not in the U.S., calcium sensitizers are approved for the short-term treatment of acutely decompensated heart failure (e.g., *levosimendan* in Sweden, *pimobendan* in Japan). Calcium sensitizers increase the sensitivity of contractile myofilaments to Ca^{2+} by binding to and inducing a conformational change in the thin-filament regulatory protein troponin C. This causes an increased force for a given cytosolic Ca^{2+} concentration, theoretically without raising the $[Ca^{2+}]_{cytosol}$. However, a variety of other effects have been ascribed to *pimobendan* and *levosimendan*, including inhibition of PDE3, inhibition of the production of proinflammatory cytokines, and opening of ATP-dependent potassium channels (Maack et al., 2019). Clinical data provide evidence for symptomatic benefit and reductions in the length of stay in the hospital but do not support a better safety profile of *levosimendan* compared to catecholamines or classical PDE inhibitors (Mebazaa et al., 2007). Increased rates of arrhythmia and death are likely related to the PDE3 inhibitor activity of these compounds.

Other Drugs Used in Heart Failure

The vasopressin receptor antagonist *tolvaptan* is FDA approved for the treatment of therapy-resistant hyponatremia, a common and difficult-to-treat complication in decompensated heart failure. *Tolvaptan* is also approved to slow kidney function decline in adults at risk of rapidly progressing autosomal dominant polycystic kidney disease. Unfortunately, studies in an unselected cohort of patients with heart failure failed to show convincing beneficial effects of *tolvaptan*. Severe thirst and dehydration are common side effects. *Heparin* or other anticoagulants are routinely used in hospitalized patients with heart failure to prevent thromboembolism.

Role of Standard Combination Therapy

Most patients hospitalized with acutely decompensated heart failure have preexisting heart failure and respective maintenance therapy. Guidelines suggest reviewing a patient's existing therapy on admission to determine whether recent changes in the medication could be causally related to an exacerbation of cardiac disease. If not, the standard heart failure medication (ACEI/ARB, β blocker, MRA, diuretic) should be continued in the absence of hemodynamic instability or contraindications (Yancey et al., 2017; Figure 33–3).

Lessons From Heart Failure Drug Development

Heart failure is an attractive but difficult indication for drug development. The number of drug development failures over the past two decades largely exceeded that of successes, indicating our incomplete understanding of the pathophysiology of heart failure, but sometimes also signaling problematic trial design. Even negative trials have helped to better understand the disease. Examples of drugs that have been tested in large prospective trials and failed are listed in Table 33–6.

Lessons From Failed Drugs

The failure of *positive inotropic agents* (PDE inhibitors, catecholamines, calcium sensitizers, mixed-acting compounds such as *flosequinan* or *vesnarinone*; Cohn et al., 1998) to improve long-term outcome of patients with heart failure has induced a paradigm shift toward drugs that unload the heart and reduce neurohumoral activation, the current standard. It demonstrated that further stimulating the failing heart may transiently improve symptoms but increase mortality. Unfortunately, reducing the hemodynamic load without protecting the heart from the adverse consequences of the activated SNS and RAAS also seems inefficient, as exemplified by the neutral effect of the α_1 receptor antagonist *prazosin* in the VeHeFT-I trial (Cohn et al., 1986). *Moxonidine*, a centrally acting α_2/imidazole agonist with similar sympatholytic actions as *clonidine*, should theoretically have similar efficacy to β blockers, but a larger prospective trial showed *moxonidine* increased mortality (Cohn et al., 2003). It is unclear whether doses and dose titration were too aggressive or whether the principle of central sympatholysis is unsafe in heart failure. Multiple lines of laboratory and clinical evidence suggested that heart failure has an important inflammatory component; yet, two blockers of tumor necrosis factor (TNF), *infliximab* and *etanercept,* induced harm rather than benefit in patients with chronic heart failure (Chung et al., 2003; Mann et al., 2004).

Endothelin 1, a potent vasoconstrictor, is upregulated in heart failure and could play an adverse role in heart failure, like that of AngII. Nonselective ET receptor antagonists such as *bosentan* had striking efficacy in postinfarct rodent models and are successfully used in pulmonary hypertension (see Chapter 35). However, *bosentan* showed no efficacy in patients with chronic heart failure (Packer et al., 2005). *Omapatrilat*, a dual inhibitor of ACE and neprilysin, can decrease AngII and increase ANP/BNP, conditions promoting vasodilation, diuresis, and antihypertrophic effects; however, expectations that *omapatrilat* would be more efficacious than an ACEI in heart failure were not confirmed in a prospective study (Packer et al., 2002).

TABLE 33–6 ■ AGENTS FOR HF THAT FAILED IN CLINICAL TRIALS

DRUG (type)	YEAR OF PUBLICATION	REASON FOR FAILURE
Milrinone (PDE inhibitor)	1991	Increased mortality
Pimobendan (PDE inhibitor)	1996	Trend toward increased mortality
Flosequinan (unclear)	1993	Increased mortality
Vesnarinone (unclear)	1998	Increased mortality, arrhythmias
Moxonidine (central antisympathetic)	1998	Increased mortality
Infliximab (TNFα blocker)	2003	Increased mortality
Etanercept (TNFα blocker)	2004	Trend toward increased mortality
Bosentan (ET receptor blocker)	2005	Liver toxicity, trend toward benefit over time (?)
Etomoxir (CPT1 blocker)	2007	Liver toxicity
Omapatrilat (dual ACEI and neprilysin inhibitor)	2002	Angioedema, no clear benefit
ARB + ACEI	2003 and 2008	No benefit, more angioedema and renal side effects
Rosuvastatin (HMG-CoA reductase inhibitor)	2007	No benefit
Tolvaptan (vasopressin V2 receptor blocker)	2009	No benefit
Rolofylline (adenosine A_1 receptor blocker)	2009	No benefit, seizures

CPT1, carnitine palmitoyltransferase 1; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A.

Several clinical trials have tested the idea that adding an ARB or the renin inhibitor *aliskiren* to standard therapy that includes an ACEI would be beneficial by more completely inhibiting the RAAS. Except for one trial (McMurray et al., 2003), studies of these combinations consistently showed a lack of benefit and an increase in adverse effects, particularly decreased renal function and hyperkalemia. The premise was that if some inhibition of the RAAS is good, more would be better; perhaps the premise was wrong.

Statins were proposed to have anti-inflammatory, antihypertrophic, and proangiogenic effects independent of their cholesterol-lowering effect (Liao and Laufs, 2005). Trials testing this hypothesis by adding statins to standard treatment of chronic heart failure demonstrated that the combination was safe but had no added beneficial effect on mortality (Kjekshus et al., 2007). An antagonist of the V_2 vasopressin receptor *tolvaptan* was ineffective in patients with chronic stable heart failure (Udelson et al., 2007). The discrepancy to several positive preclinical and early clinical studies suggests that the vasopressin axis of the neurohumoral activation program in heart failure may be sufficiently addressed by standard combination therapy, leaving no room for further improvement.

Lessons From Treating Acute Heart Failure

The drugs currently recommended (*furosemide, nitroglycerin, dobutamine*) for the treatment of acutely decompensated heart failure have never been tested in adequately powered prospective clinical trials. All novel drugs tested either in comparison to standard inotropes or noninotropes or as an add-on have failed to show convincing superiority or benefit in terms of symptoms, duration of hospitalization, and 30-day mortality. The A_1 adenosine receptor antagonist *rolophylline* should produce several beneficial effects on the kidney, including inhibition of tubular reabsorption of Na^+ and water, dilation of the afferent arteriole, and inhibition of tubular-glomerular feedback, but its addition to standard therapy in patients with acute heart failure with impaired kidney function produced no salutary renal or cardiac effects and caused unacceptable adverse effects such as seizures, a typical side effect of central A_1 adenosine antagonism known also from theophylline (Massie et al., 2010).

Recent Developments; Novel Approaches

Numerous pharmacological and nonpharmacological treatment options are being tested in preclinical and clinical studies (https://www.clinicaltrials.gov). They range from cell and gene therapies to food supplements (vitamins, polyunsaturated fatty acid) and intravenous iron to classical small molecules. In the CUPID2 trial, gene therapy, in the form of an intracoronary infusion of adeno-associated virus 1/SERCA2 to improve the defective diastolic uptake of Ca^{2+} into the sarcoplasmic reticulum, did not provide any benefit in HFrEF (Greenberg et al., 2016). *Serelaxin*, recombinant human *relaxin 2*, is a naturally occurring peptide with 53 amino acids discovered in 1926 as an ovarian hormone inducing relaxation of the uterus during pregnancy. Its actions on the cardiovascular system include increased arterial compliance, cardiac output, and renal blood flow, characteristics of a promising drug for the treatment of acutely decompensated heart failure. However, the promising results of an earlier study (Teerlink et al., 2013) were not confirmed in a larger phase III trial (Metra et al., 2019).

Heart failure is often associated with anemia, a predictor of a poor prognosis. Yet, correction of anemia by an erythropoietin derivative, *darbepoetin alpha*, did not affect any clinical end point but increased the rate of thromboembolic events and ischemic strokes in patients with heart failure and mild-to-moderate anemia (Swedberg et al., 2013). However, intravenous iron, added to standard therapy in patients with NYHA class II–III heart failure, iron deficiency, and hemoglobin levels of 9.5 to 13.5 g/dL, improved quality of life, NYHA class, and physical exercise capacity (Anker et al., 2009). The beneficial effects seemed to be independent of the presence of anemia and may be related to other roles of iron in the body. In patients hospitalized for acute heart failure and signs of iron deficiency, intravenous iron reduced the rate of heart failure hospitalizations but had no effect on cardiovascular death (Ponikowski et al., 2020).

Myosin Modulators

There are two new small molecule drugs entering clinical use that alter the rate or extent of myosin's interaction with actin to produce force: *mavacamten* and *omacamtiv mecarbil*.

Mavacamten was FDA-approved in 2022 for treating patients with symptomatic obstructive hypertrophic cardiomyopathy (HCM). HCM is often caused by mutations in genes encoding sarcomeric proteins (e.g., cardiac myosin) and the mutations commonly result in sarcomeric hypercontractility. *Mavacamten* is an allosteric and reversible inhibitor selective for cardiac myosin and hence directly targets the underlying pathophysiology of HCM. Specifically, *mavacamten* reduces the number of myosin heads that can enter "on actin" (power-generating) states, thus reducing the probability of force-producing (systolic) and residual (diastolic) cross-bridge formation. Excess myosin actin cross-bridge formation and decreased fraction of myosin in the super-relaxed state are mechanistic hallmarks of HCM. *Mavacamten* shifts the overall myosin population towards an energy-sparing, recruitable, super-relaxed state. Due to its inherent negative inotropic effects and hence the risk for causing heart failure, *mavacamten* is available only through the manufacturers Risk Evaluation and Mitigation Strategy (REMS) Program, and patients must be closely monitored by echocardiography. Use of *mavacamten* is restricted to HCM patients with a left ventricular ejection fraction above 55%, and the drug should be stopped if left ventricular ejection fraction falls below 50% or if the patient experiences heart failure symptoms. *Mavacamten* is extensively metabolized, primarily through CYP2C19, which is inhibited by drugs such as fluconazole and several antidepressants. Concomitant use of such drugs will increase *mavacamten* serum concentrations. In CYP2C19 poor metabolizers (~5% of Caucasians, 15-20% of Asians), its half-life increases from 6-9 days to 23 days. Given its narrow therapeutic indication and likelihood for drug-drug interactions, patients receiving *mavacamten* will have to be closely monitored.

Omacamtiv mecarbil is also an allosteric myosin modulator but has opposite effects to mavacamten in that it promotes force generation. *Omacamtiv mecarbil* is being evaluated as a positive inotropic agent for use in patients with systolic heart failure (HFrEF). Its clinical utility remains to be determined

Drug Facts for Your Personal Formulary: *Heart Failure Drugs*

Drug	Therapeutic Uses	Major Toxicity and Clinical Pearls
Inhibitors of the Renin-Angiotensin System		
ACE Inhibitors Benazepril Captopril Enalapril Lisinopril Quinapril Ramipril	• Heart failure • Hypertension • Diabetic nephropathy	• First choice in treating heart failure • Short-acting captopril only for initiation of therapy; enalapril requires twice-daily dosing • Cough in 5%–10% of patients, angioedema • Hypotension, hyperkalemia, skin rash, neutropenia, anemia, fetopathic syndrome, rare hepatic failure, rare agranulocytosis • Contraindicated in combination with neprilysin inhibitors and in patients with renal artery stenosis; caution in patients with impaired renal function or hypovolemia
Fosinopril Trandolapril Perindopril		• Both hepatic and renal elimination, caution in patients with renal or hepatic impairment
Angiotensin Receptor Blockers Candesartan, Eprosartan Irbesartan, Losartan Olmesartan, Telmisartan Valsartan	• Hypertension • Heart failure • Diabetic nephropathy	• Only in cases of intolerance to ACEI • Unwanted effects as ACEI, but less cough or angioedema • No evidence for superiority over ACEI • In combination with ACEI more harm than benefit

Drug Facts for Your Personal Formulary: *Heart Failure Drugs* (*continued*)

Drug	Therapeutic Uses	Major Toxicity and Clinical Pearls
β Blockers		
Bisoprolol Carvedilol Metoprolol Nebivolol	• Heart failure • Hypertension • Widely used for angina, prevention of arrhythmias, rate control in atrial fibrillation, migraine	• First choice in the treatment of heart failure • Start low (1/10 target dose), go slow (2- to 4-weekly doubling) • Adverse effects: bradycardia, AV block, bronchospasm, peripheral vasoconstriction, worsening of acute heart failure, depression, worsening of psoriasis • Polymorphic CYP2D6 metabolism (metoprolol, carvedilol, nebivolol)
Mineralocorticoid Receptor Antagonists		
Eplerenone Spironolactone Finerenone	• Heart failure • Hypertension • Hyperaldosteronism, hypokalemia, ascites • Chronic kidney disease in diabetic patients (finerenone)	• First choice in treating symptomatic heart failure • Low doses (25–50 mg) • Most serious side effect is hyperkalemia • Spironolactone causes painful breast swelling and impotence in men, dysmenorrhea in women due to binding to sex hormone receptors • Finerenone reduces heart failure events and renal function decline in diabetic patients with chronic kidney disease
Neprilysin Inhibitor/Angiotensin Receptor Blocker		
Sacubitril/valsartan	• Heart failure	• Superior to the ACEI enalapril • May become first choice in treating heart failure • ↓ Degradation of natriuretic peptides, ↑ their beneficial actions • Hypotension, angioedema, hyperkalemia, contraindicated with ACEIs
Diuretics		
Thiazide Type Chlorothiazide Hydrochlorothiazide *Thiazide-like* Chlorthalidone Indapamide Metolazone	• Edema associated with congestive heart failure, liver cirrhosis, chronic kidney disease, and nephrotic syndrome • Hypertension • Nephrogenic diabetes insipidus • Kidney stones caused by Ca^{2+} crystals	• Symptomatic treatment of milder forms of heart failure • Loose efficacy at GFR <30–40 mL/min (exception indapamide and metolazone) • Potentiate effect of loop diuretics in severe heart failure (sequential tubulus blockade) • Risk for hypokalemia and arrhythmia when combined with QT-prolonging drugs • Sulfonamide hypersensitivity, photosensitivity
Loop Diuretics Bumetanide Ethacrynic acid Furosemide Torsemide	• Acute pulmonary edema (intravenous) • Edema associated with congestive heart failure, liver cirrhosis, chronic kidney disease, and nephrotic syndrome • Hyponatremia • Hypercalcemia • Hypertension with renal insufficiency	• Symptomatic treatment of severe heart failure and acute decompensation • Often required in treating severe chronic heart failure, twice-daily dosing or more • Torsemide may be superior to furosemide in heart failure • Risk for hypokalemia and arrhythmia when combined with QT-prolonging drugs • Nephrotoxicity, ototoxicity, sulfonamide hypersensitivity (except ethacrynic acid) • Ethacrynic acid as a reserve for patients with sulfa allergy
Vasodilators		
• ISDN/hydralazine	• Heart failure in African Americans	• Approved only for African Americans • Headache, nausea, flushing, hypotension, palpitations, tachycardia, dizziness, angina pectoris; ⇒ use in combination with β blocker • Contraindicated with PDE5 inhibitors • Drug-induced lupus-like syndrome; other immunological reactions
Positive Inotropes		
• Digoxin • Digitoxin	• Heart failure • Atrial fibrillation	• Not first choice in treating heart failure • Low therapeutic index: proarrhythmic, nausea, diarrhea, visual disturbances • Digoxin kidney dependent, digitoxin not • Half-life 1.5 (digoxin) or 7 days (digitoxin) • Plasma concentration: 0.5–0.8 ng/mL (digoxin) or 10–25 ng/mL (digitoxin)
Heart Rate Reduction		
Ivabradine	• Heart failure	• Not first choice in treating heart failure • May exert benefits in patients not tolerating β blockers or having heart rate >75 under β blockers • Unwanted effects: bradycardia, QT prolongation, atrial fibrillation, phosphenes

Drug Facts for Your Personal Formulary: *Heart Failure Drugs* (*continued*)

Drug	Therapeutic Uses	Major Toxicity and Clinical Pearls
Intravenous Vasodilators: Acute decompensated heart failure		
Nitroglycerin Sodium nitroprusside	• Acute decompensated heart failure	• May ↑ cardiac output in acute congestion (↑ filling pressure and dilation) via ↓ preload and afterload • NO releaser, stimulates soluble guanylyl cyclase • Avoid if systolic blood pressure <110 mmHg • Nitroglycerine: headache, rebound hypertension • Nitroprusside: cyanide toxicity, methemoglobinemia
Intravenous Positive Inotropes: Acutely decompensated heart failure		
Dobutamine Dopamine Epinephrine Norepinephrine	• Acute decompensated heart failure	• β_1 Receptor–mediated stimulation of cardiac output and, depending on drug, complex vascular actions • Last option in patients with systolic blood pressure <85 mmHg • ↑ Cardiac energy consumption and risk of arrhythmia • Use of catecholamines correlates with poor prognosis; use lowest possible doses for shortest possible time • Dobutamine causes less tachycardia than EPI and less afterload increase than NE • Role of low-dose dopamine unclear
Enoximone Milrinone	• Acute decompensated heart failure	• PDE3/4 inhibitors, ↑ cellular cAMP • ↑ Cardiac output and dilate blood vessels (“inodilator”) • May be used in patients on β blockers and with high peripheral and pulmonary arterial resistance • Blood pressure decrease is dose limiting • Risks and prognostic effects: same as catecholamines (above)
Levosimendan (not available in the U.S.)	• Acute decompensated heart failure	• Combined Ca^{2+} sensitizer (troponin C binding) and PDE3 inhibitor • ↑ Cardiac output and ↓ vascular resistance (“inodilator”) • Advantages over catecholamines or simple PDE inhibitors unclear

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Chapter 34

Antiarrhythmic Drugs

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PRINCIPLES OF CARDIAC ELECTROPHYSIOLOGY

- The Cardiac Cell at Rest: A K^+-Permeable Membrane
- The Cardiac Action Potential
- Maintenance of Intracellular Ion Homeostasis
- Genetic Arrhythmia Diseases
- Action Potential Heterogeneity in the Heart
- Impulse Propagation and the Electrocardiogram
- Refractoriness and Conduction Failure

MECHANISMS OF CARDIAC ARRHYTHMIAS

- Enhanced Automaticity
- Afterdepolarizations and Triggered Automaticity
- Reentry
- Common Arrhythmias and Their Mechanisms

MECHANISMS OF ANTIARRHYTHMIC DRUG ACTION

- State-Dependent Ion Channel Block
- Classifying Antiarrhythmic Drugs

PRINCIPLES IN THE CLINICAL USE OF ANTIARRHYTHMIC DRUGS

- 1. Identify and Remove Precipitating Factors
- 2. Establish the Goals of Treatment
- 3. Minimize Risks
- 4. Consider the Electrophysiology of the Heart as a "Moving Target"

ANTIARRHYTHMIC DRUGS

- Adenosine
- Amiodarone
- Bretylium
- Digoxin
- Disopyramide
- Dofetilide
- Dronedarone
- Esmolol
- Flecainide
- Ibutilide
- Lidocaine
- Magnesium
- Mexiletine
- Procainamide
- Propafenone
- Quinidine
- Sotalol

Cardiac cells undergo depolarization and repolarization about 60 times per minute to form and propagate cardiac action potentials. The shape and duration of each action potential are determined by the activity of ion channel protein complexes in the membranes of individual cells, and the genes encoding most of these proteins and their regulators have now been identified. Action potentials in turn provide the primary signals to release Ca^{2+} from intracellular stores (sarcoplasmic reticulum) and to thereby initiate contraction. Thus, each normal heartbeat results from the highly integrated electrophysiological behavior of multiple proteins on the surface and within multiple cardiac cells. Disordered cardiac rhythm can arise from influences such as inherited variation in ion channels or other genes, ischemia, sympathetic stimulation, or myocardial scarring. Available antiarrhythmic drugs suppress arrhythmias by modulating flow through specific ion channels or by altering autonomic function. An increasingly sophisticated understanding of the molecular basis of normal and abnormal cardiac rhythm may lead to identification of new targets for antiarrhythmic drugs and perhaps improved therapies (Al-Khatib et al., 2018).

Arrhythmias can range from incidental, asymptomatic clinical findings to life-threatening abnormalities. Mechanisms underlying cardiac arrhythmias have been identified in cellular and animal experiments. For some human arrhythmias, precise mechanisms are known, and treatment can be targeted specifically to those mechanisms. In other cases, mechanisms can only be inferred, and the choice of drugs is based largely on results of prior experience. Antiarrhythmic drug therapy has two goals: termination of an ongoing arrhythmia or prevention of an arrhythmia. Unfortunately, antiarrhythmic drugs not only may help control arrhythmias but also can cause them, even during long-term therapy. Thus, prescribing antiarrhythmic drugs requires that precipitating factors be excluded or minimized, that a precise diagnosis of the type of arrhythmia (and its possible mechanisms) be made, that the prescriber has reason to believe that drug therapy will be beneficial, and that the risks of drug therapy can be minimized.

Principles of Cardiac Electrophysiology

The flow of ions across cell membranes generates the currents that make up cardiac action potentials. Factors that determine the magnitude of individual currents and their modulation by drugs include transmembrane potential, time since depolarization, and the presence of specific ligands (Priori et al., 1999). Further, because the function of many channels is time and voltage dependent, even a drug that targets a single ion channel may, by altering the trajectory of the action potential, alter the function of other channels. Most antiarrhythmic drugs affect more than one ion current, and many exert ancillary effects, such as modification of cardiac contractility or autonomic nervous system function. Thus, antiarrhythmic drugs usually exert multiple actions and can be beneficial or harmful in individual patients (Priori et al., 1999; Roden, 1994).

The Cardiac Cell at Rest: A K^+-Permeable Membrane

Ions move across cell membranes in response to electrical and concentration gradients, not through the lipid bilayer but through specific ion channels or transporters. The normal cardiac cell at rest maintains a transmembrane potential approximately 80 to 90 mV negative to the exterior; this gradient is established by pumps, especially the Na^+, K^+–ATPase, and fixed anionic charges within cells. There are both electrical and concentration gradients that would move Na^+ ions into resting cells (Figure 34–1). However, Na^+ channels, which allow Na^+ to move along this gradient, are closed in the cardiac cell at rest, so Na^+ does not enter normal resting

Abbreviations

AF: atrial fibrillation
AV: atrioventricular
β blocker: β adrenergic receptor antagonist
CPVT: catecholaminergic polymorphic ventricular tachycardia
DAD: delayed afterdepolarization
DC: direct current
EAD: early afterdepolarization
ECG: electrocardiogram
ERP: effective refractory period
GX: glycine xylidide
ICD: implantable cardioverter-defibrillator
LQTS: long QT syndrome
NCX: Na^+-Ca^{2+} exchanger
PSVT: paroxysmal supraventricular tachycardia
RV: right ventricle
RyR2: ryanodine receptor type 2
SA: sinoatrial
SR: sarcoplasmic reticulum
VF: ventricular fibrillation
VT: ventricular tachycardia
WPW: Wolff-Parkinson-White

cardiac cells. In contrast, a specific type of K^+ channel protein (inward rectifier channels) remains open at negative resting potentials. Hence, K^+ can move through these channels across the cell membrane at negative potentials in response to either electrical or concentration gradients (Figure 34–1). For each individual ion, there is an equilibrium potential E_x at which there is no net driving force for the ion to move across the membrane. E_x can be calculated using the Nernst equation:

$$E_x = -(RT/FZx)\ \ln([x]_i/[x]_o) \qquad \text{(Equation 34–1)}$$

where Zx is the valence of the ion, T is the absolute temperature, R is the gas constant, F is Faraday's constant, $[x]_o$ is the extracellular concentration of the ion, and $[x]_i$ is the intracellular concentration. For typical values for K^+, $[K]_o$ = 4 mM and $[K]_i$ = 150 mM, the calculated K^+ equilibrium potential E_K is –96 mV. There is thus no net force driving K^+ ions into or out of a cell when the transmembrane potential is –96 mV, which is close to the resting potential. If $[K]_o$ is elevated to 10 mM, as might occur in diseases such as renal failure or myocardial ischemia, the calculated E_K rises to –70 mV. In this situation, there is excellent agreement between changes in theoretical E_K owing to changes in $[K]_o$ and the actual measured transmembrane potential, indicating that the normal cardiac cell at rest is permeable to K^+ (because inward rectifier channels are open) and that $[K]_o$ is the major determinant of resting potential.

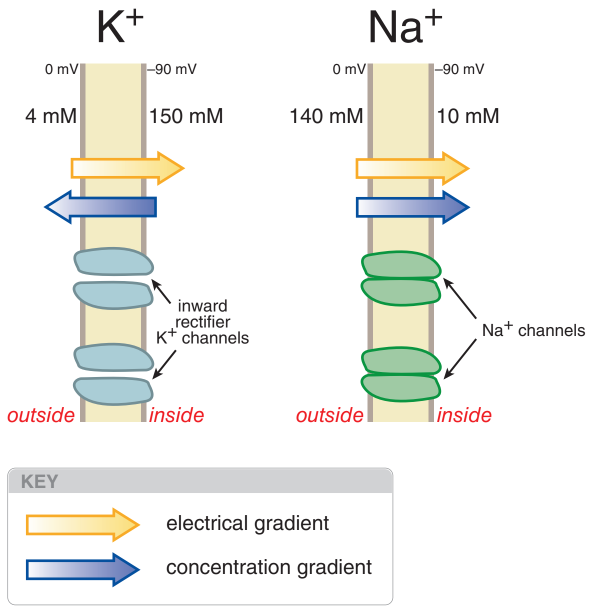


Figure 34–1 *Electrical and chemical gradients for K^+ and for Na^+ in a resting cardiac cell.* Inward rectifier K^+ channels are open (left), allowing K^+ ions to move across the membrane and the transmembrane potential to approach E_K. In contrast, Na^+ does not enter the cell despite a large net driving force because Na^+ channel proteins are in the closed conformation (right) in resting cells.

The Cardiac Action Potential

Transmembrane current through voltage-gated ion channels is the primary determinant of cardiac action potential morphology and duration. Channels are macromolecular complexes consisting of a pore-forming transmembrane structure (which may be a single protein, often termed an α subunit, or a multimer), as well as function-modifying β subunits and other accessory proteins. Common features of the pore-forming structure include a voltage-sensing domain (for voltage-gated channels), a selectivity filter, a conducting pore, and an inactivating particle (Figure 34–2). In response to changes in local transmembrane potential, ion channels undergo conformational changes, allowing for, or preventing, the flow of ions through the conducting pore along their electrochemical gradient, generally in time-, voltage-, and/or ligand-dependent fashion.

To initiate an action potential, a cardiac myocyte at rest is depolarized above a threshold potential, usually via gap junctions by a neighboring myocyte. On membrane depolarization, Na^+ channel proteins, $Na_V1.5$, change conformation from the "closed" (resting) state to the "open" (conducting) state (Figure 34–2), allowing up to 10^7 Na^+ ions/sec to enter each cell and moving the transmembrane potential toward E_{Na} (+65 mV). This surge of Na^+ ions lasts only about a millisecond, after which the Na^+ channel protein rapidly changes conformation from the open state to an "inactivated," nonconducting state (Figure 34–2). The maximum upstroke slope of phase 0 (dV/dt_{max}, or V_{max}) of the action potential (Figure 34–3) is largely governed by Na^+ current and is a major determinant of conduction velocity of a propagating action potential. Under normal conditions, Na^+ channels, once inactivated, cannot reopen until they reassume the closed conformation. However, a small population of Na^+ channels may continue to open during the action potential plateau in some cells (Figure 34–3), providing further inward current, often termed "late" Na^+ current. As the cell membrane repolarizes, the negative membrane potential moves Na^+ channel proteins from inactivated to closed conformations, from which they are again available to open and depolarize the cell. The relationship between Na^+ channel availability and transmembrane potential is an important determinant of conduction and refractoriness in many cells, as discussed in the material that follows.

The changes in transmembrane potential generated by the inward Na^+ current produce, in turn, a series of openings (and in some cases

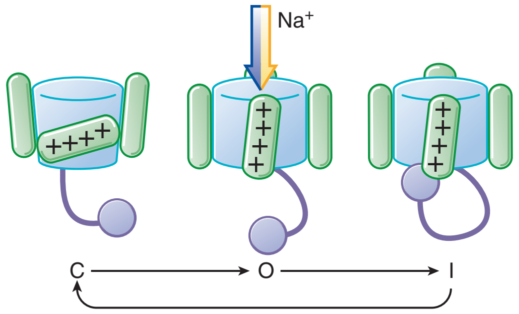


Figure 34–2 *Voltage-dependent conformational changes determine current flow through Na^+ channels.* At hyperpolarized potentials, the channel is in a closed conformation, and no current can flow (left, C). As depolarization begins, the voltage sensor (indicated here as ++++) moves, thus altering channel conformation and opening the pore, allowing conduction (middle, O). As depolarization is maintained, an intracellular particle blocks current flow, making the channel nonconducting in the inactivated state (right, I).

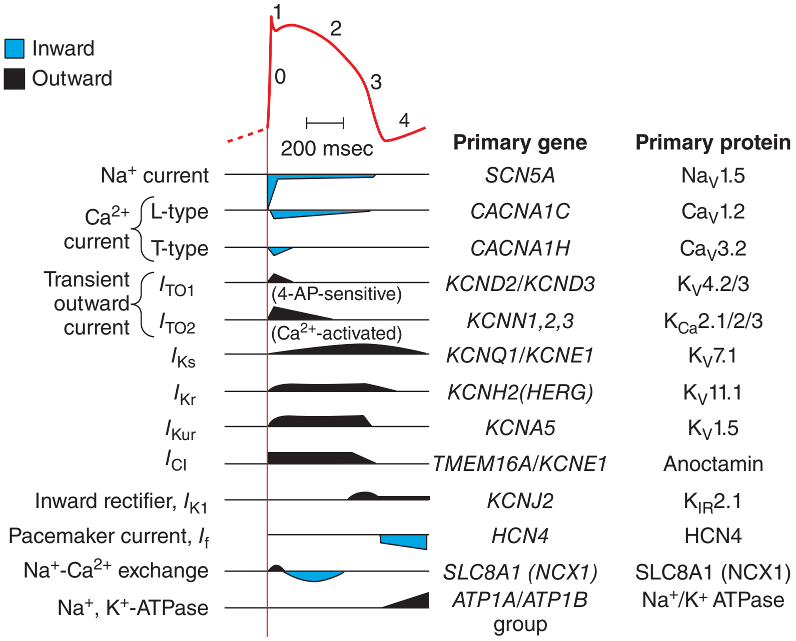


Figure 34–3 *The relationship between an action potential from the ventricular conducting system and the time course of the currents that generate it.* The current magnitudes are not to scale; the peak Na$^+$ current is ordinarily 50 times larger than any other current, although the portion that persists into the plateau (phase 2) is small. Multiple types of Ca^{2+} current, transient outward current I_{TO}, and delayed rectifier I_K have been identified. Each represents a different channel protein, usually associated with ancillary (function-modifying) subunits. 4-Aminopyridine (4-AP) is a widely used *in vitro* blocker of K$^+$ channels. I_{TO2} may be a Cl$^-$ current in some species or a Ca-activated K current generated by apamin-sensitive "small" K channels (K$_{Ca}$2.1/2/3). Components of I_K have been separated on the basis of how rapidly they activate: slowly (I_{Ks}), rapidly (I_{Kr}), or ultrarapidly (I_{Kur}); I_{Kur} is found predominantly in atria. The voltage-activated, time-independent current may be carried by Cl$^-$ (I_{Cl}) or K$^+$ (I_{Kp}, *p* for plateau). The genes encoding the major pore-forming proteins are listed in the right-hand column, followed by the protein name. *KCNE1*, a β subunit of the *KCNQ1* channel required for I_{Ks}, serves also as an auxiliary subunit of the *TMEM16A* channel, a Ca-activated Cl channel, and induces a voltage-dependent Cl current in the absence of intracellular calcium elevation (Ávalos Prado et al., 2021).

subsequent inactivation) of other channels (Figure 34–3). For example, when a cell is depolarized by the Na$^+$ current, "transient outward" K$^+$ channels, K$_V$4.2 and K$_V$4.3, quickly change conformation to enter an open, or conducting, state; because the transmembrane potential at the end of phase 0 is positive to E_K, the opening of transient outward channels results in an outward, or repolarizing, K$^+$ current (termed I_{TO}), which contributes to the phase 1 "notch" seen in some action potentials (e.g., more prominent in epicardium than in endocardium). Transient outward K$^+$ channels, like Na$^+$ channels, inactivate rapidly. During the phase 2 plateau of a normal cardiac action potential, inward, depolarizing currents, primarily through voltage-gated L-type Ca^{2+} channels, Ca$_V$1.2, are balanced by outward, repolarizing currents primarily through K$^+$ ("delayed rectifier") channels. Delayed rectifier currents (collectively termed I_K) increase with time, whereas Ca^{2+} currents inactivate (and so decrease with time); as a result, cardiac cells repolarize (phase 3) several hundred milliseconds after the initial Na$^+$ channel opening. Figure 34–3 summarizes the major inward and outward currents generated during the action potential by ion channels and electrogenic ion transporters in the heart.

A common mechanism whereby drugs prolong cardiac action potentials and provoke arrhythmias is inhibition of a specific delayed rectifier current, I_{Kr}, generated by expression of *KCNH2* (formerly termed *HERG* [human ether-a-go-go related gene]). The ion channel protein, K$_V$7.1, generated by *KCNH2* expression differs from other ion channels in important structural features (i.e., pore-lining aromatic residues) that make it much more susceptible to drug block; understanding these structural constraints is an important first step to designing drugs lacking I_{Kr}-blocking properties. Avoiding I_{Kr}/K$_V$7.1 channel block has become a major issue in drug development (Roden, 2004).

Maintenance of Intracellular Ion Homeostasis

With each action potential, the cell interior gains Na$^+$ and Ca^{2+} ions and loses K$^+$ ions. Figure 34–4 illustrates the major ion channels and membrane transporters regulating ion homeostasis of cardiac myocytes. An ATP-requiring Na$^+$-K$^+$ exchange mechanism, or pump, is activated in most cells to maintain intracellular homeostasis. This Na$^+$, K$^+$–ATPase extrudes three Na$^+$ ions for every two K$^+$ ions shuttled from the exterior of the cell to the interior (Figure 34–4); as a result, the act of pumping itself is electrogenic, generating a net outward (repolarizing) current.

Normally, intracellular Ca^{2+} is maintained at low levels (~100 nM). In cardiac myocytes, the entry of Ca^{2+} during each action potential through L-type Ca^{2+} channels is a signal to the sarcoplasmic reticulum (SR) to release its Ca^{2+} stores and thus initiate Ca^{2+}-dependent contraction, a process termed excitation-contraction coupling. The efflux of Ca^{2+} from the SR occurs through ryanodine receptor Ca^{2+} release channels (RyR2) and subsequent removal of intracellular Ca^{2+} occurs by both the SR Ca^{2+} uptake pump, which moves Ca^{2+} ions back into the SR, and an electrogenic Na$^+$-Ca^{2+} exchanger (NCX) on the cell surface, which exchanges three Na$^+$ ions from the exterior for each Ca^{2+} ion extruded (Figure 34–4).

Genetic Arrhythmia Diseases

Rare congenital arrhythmia diseases such as the long QT syndrome (LQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT) can cause sudden death due to fatal arrhythmias, often in young subjects. The identification of disease genes not only has resulted in improved care of affected patients and their families but also has contributed importantly to our understanding of the normal action potential, arrhythmia mechanisms, and potential antiarrhythmic drug targets (Knollmann and Roden, 2008). Figure 34–4 illustrates which ion channels have been linked to congenital arrhythmia diseases. For example, mutations in the cardiac Na$^+$ channel gene *SCN5A* can cause one form of LQTS by destabilizing fast inactivation, increasing late Na$^+$ current, and thereby prolonging action potentials and thus the QT interval (as discussed in material that follows). Drugs inhibiting this abnormal current may be antiarrhythmic in this form of LQTS (Remme and Wilde, 2013), and drugs increasing late Na$^+$ current may cause arrhythmias (Lu et al.,

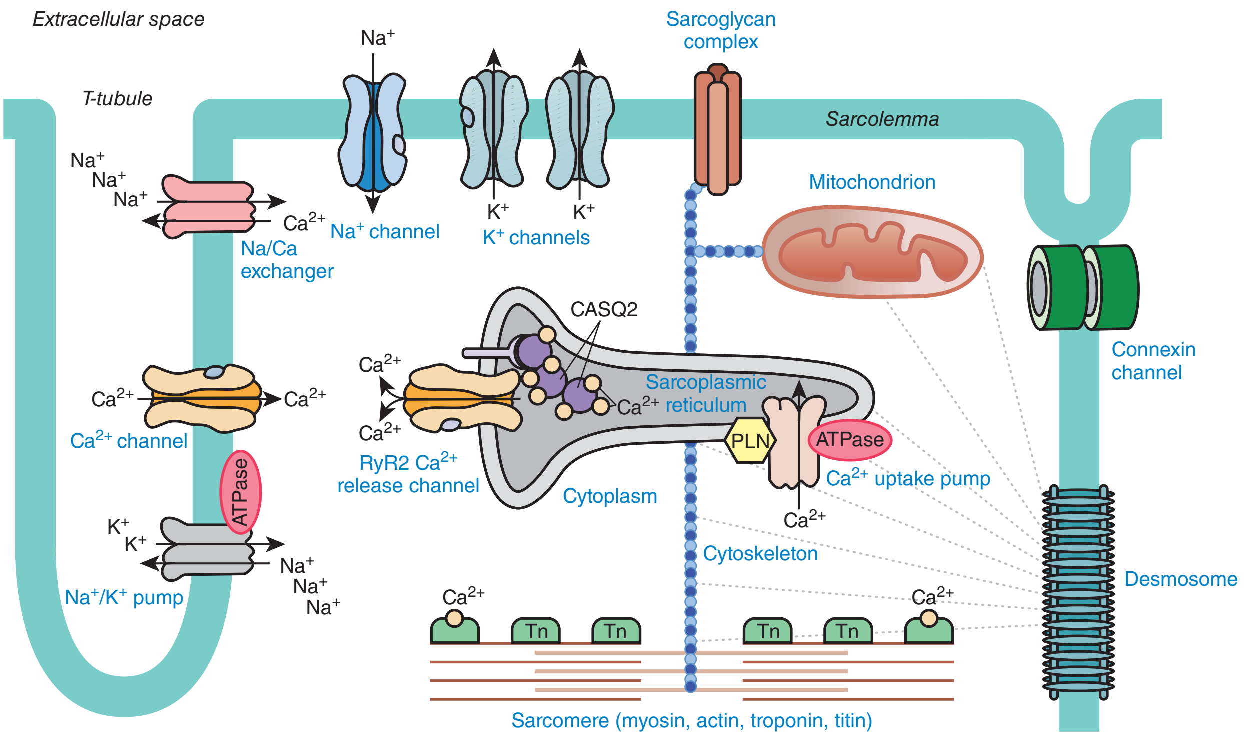


Figure 34–4 *Excitation-contraction coupling and ion homeostasis in a ventricular cardiomyocyte.* Shown are the protein complexes, myocyte architecture, and intracellular organelles involved in cardiac excitation-contraction coupling. The initial event in the cardiac cycle is membrane depolarization, which occurs with ion entry through connexin channels from a neighboring cardiomyocyte (right) followed by opening of voltage-gated Na^+ channels and Na^+ entry (top). The resultant rapid depolarization of the membrane inactivates Na^+ channels and opens both K^+ channels and Ca^{2+} channels. Entry of Ca^{2+} into the cell triggers the release of Ca^{2+} from the sarcoplasmic reticulum through the RyR2 Ca^{2+} release channel. Ca^{2+} then binds to the troponin complex and activates the contractile apparatus (the sarcomere, bottom). Cellular relaxation occurs on removal of Ca^{2+} from the cytosol by the Ca^{2+}-uptake pumps of the sarcoplasmic reticulum and by Na/Ca exchange with the extracellular fluid. Intracellular Na^+ homeostasis is achieved by the Na/K pump. The molecular components that are required for normal electrophysiological activity, contractile function, and cell-cell adhesion (the latter mediated by desmosomes) all need to be positioned correctly within the cell and anchored to each other and the cytoskeleton.

2012; Yang et al., 2014). Inhibitors may include not only antiarrhythmics such as *mexiletine* or *flecainide*, which are discussed in this chapter, but also the antianginal agent *ranolazine* (see Chapter 31), which is a late Na^+ current blocker.

Similarly, mutations in the *RyR2* gene encoding an intracellular Ca^{2+} release channel (or less commonly in other genes regulating RyR2 function) cause CPVT by generating "leaky" RyR2 channels, perturbing intracellular Ca^{2+} homeostasis, and causing delayed afterdepolarization (DAD)-dependent arrhythmias described further in this chapter. Intriguingly, some arrhythmias in acquired heart disease have been attributed to increased late Na^+ current or leaky RyR2 channels. Thus, studies in the rare congenital arrhythmia syndromes may point to new avenues for drug development in more common arrhythmias in acquired heart disease (Knollmann and Roden, 2008; Priori et al., 1999). Drugs such as *flecainide* and *propafenone* that inhibit these abnormal RyR2 channels (Kryshtal et al., 2021) appear to prevent CPVT in mouse models and in humans (Kannankeril et al., 2017; Watanabe et al., 2009). However, as discussed below, these drugs are also sodium channel blockers and are thus not used in patents with many forms of acquired heart disease. In malignant hyperthermia, a disease caused by leaky RyR1 calcium release channels in skeletal muscle, the RyR1 blocker *dantrolene* can be effective, and it has also been investigated in arrhythmias caused by leaky RyR2 channels (Roden and Knollmann, 2014). These precedents have propelled efforts to develop more selective RyR2 blockers (Batiste et al., 2019; Zhou et al., 2011).

Action Potential Heterogeneity in the Heart

The general description of the action potential and the currents that underlie it must be modified for certain cell types (Figure 34–5), primarily due to variability in the expression of ion channels and electrogenic ion transport pumps. The resultant diversity of action potentials in different regions of the heart plays a role in understanding the pharmacological profiles of antiarrhythmic drugs. In the ventricle, action potential duration varies across the wall of each chamber, as well as apicobasally, largely as a consequence of varying densities of repolarizing currents. In the neighboring His-Purkinje conduction system, action potentials are longer, probably due to decreased K^+ currents, increased late Na^+ current, and differences in intercellular Ca^{2+} handling (Dun and Boyden, 2008).

Atrial cells have shorter action potentials than ventricular cells because of larger early repolarization currents such as I_{TO}. Atrial cells also express an additional repolarizing K^+ channel ($I_{K\text{-}Ach}$) that is activated by the neurotransmitter acetylcholine and accounts for action potential shortening with vagal stimulation. Although some cells of the sinus and atrioventricular (AV) nodes likely have small Na^+ current, depolarization is primarily achieved by inward current generated by opening of Ca^{2+} channels. In addition, these cells, as well as cells from the conducting system, normally display the phenomenon of spontaneous diastolic, or phase 4, depolarization and thus spontaneously reach threshold for regeneration of action potentials. The rate of spontaneous firing usually is fastest in sinus node cells, which therefore serve as the natural pacemaker of the heart. The slow diastolic depolarization that underlies pacemaker activity is generated by a nonselective channel () that conducts both Na^+ and K^+ (funny current, I_f) and is activated at hyperpolarized membrane potentials (Cohen and Robinson, 2006). In diseased cells, pacemaker-like activity can arise from spontaneous Ca^{2+} release from the SR, followed by membrane depolarization due to activation of NCX.

Certain ion channels are expressed only in some tissues or become active only under specific pathophysiologic conditions. For example, the T-type Ca^{2+} channel may be important in diseases such as hypertension and play a role in pacemaker activity (Ono and Iijima, 2010). A T-type–selective Ca^{2+} channel antagonist, *mibefradil* was commercially available briefly in the late 1990s but was withdrawn because

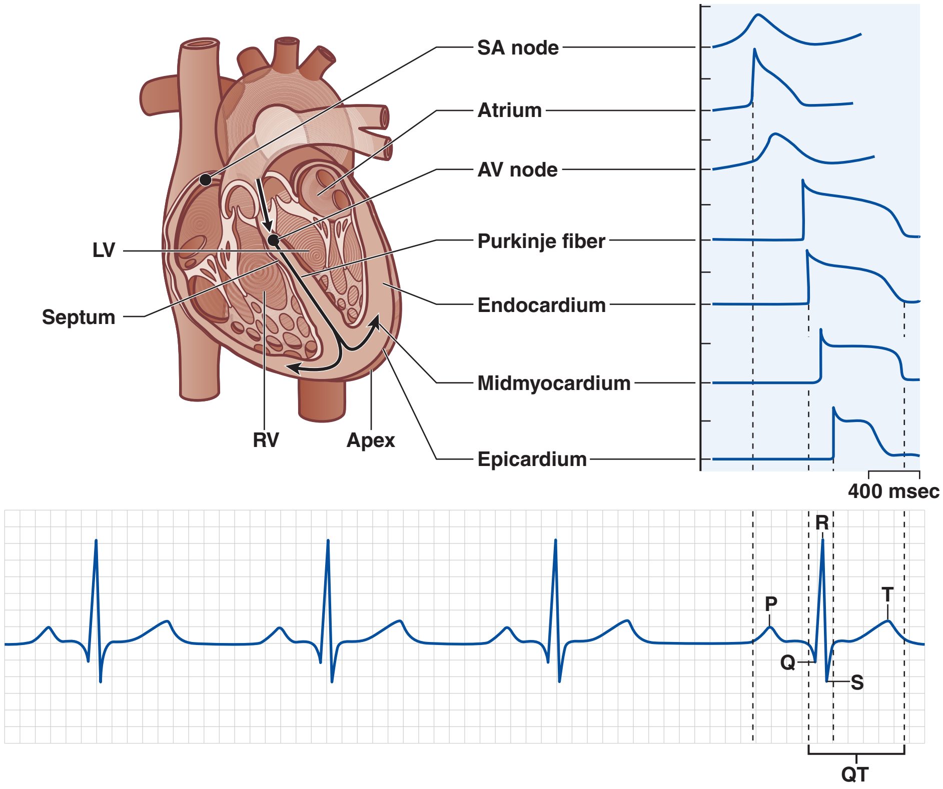


Figure 34–5 *Normal impulse propagation.* A schematic of the human heart with example action potentials from different regions of the heart (top) for a normal beat and their corresponding contributions to the macroscopic ECG (bottom). LV, left ventricle; RV, right ventricle.

of concerns over potentially life-threatening pharmacokinetic interactions with many other drugs. A second example is a small-conductance K^+ channel activated by intracellular Ca^{2+} (SK channel) and blocked by apamin (Zhang et al., 2021). SK channels are prominently expressed in healthy atrial myocytes and pacemaking cells compared to ventricular cells. However, SK channels are upregulated in ventricular myocytes in heart failure and pulmonary veins in atrial fibrillation (AF). Therefore, the SK channel has been implicated mechanistically in atrial and ventricular arrhythmias and may represent a novel therapeutic target. A third example is a channel that transports Cl^- ions and results in repolarizing currents (I_{Cl}) (Duan, 2013); some of these are observed only in association with pathophysiological conditions. A final example is the K^+ channels that are quiescent when intracellular ATP stores are normal and become active when these stores are depleted. Such ATP-inhibited K^+ channels may become particularly important in repolarizing cells during states of metabolic stress such as myocardial ischemia (Tamargo et al., 2004).

Impulse Propagation and the Electrocardiogram

Normal cardiac impulses originate in the sinus node. Impulse propagation in the heart depends on the magnitude of the depolarizing current (usually Na^+ current) and the geometry and density of cell-cell electrical connections (Kleber and Saffitz, 2014). Cardiac cells are relatively long and thin and well coupled through specialized gap junction proteins at their ends (connexins; Figure 34–4), whereas lateral ("transverse") gap junctions are sparser. As a result, impulses spread along cells two to three times faster than across cells. This "anisotropic" (direction-dependent) conduction may be a factor in the genesis of certain arrhythmias described in the material that follows (Priori et al., 1999).

Once impulses leave the sinus node, they propagate rapidly throughout the atria, resulting in atrial systole and the P wave of the surface electrocardiogram (ECG) (Figure 34–5). Propagation slows markedly through the AV node, where the inward current mediating the phase 0 upstroke (through Ca^{2+} channels) is much smaller than the Na^+ current in atria, ventricles, or the subendocardial conducting system. This conduction delay, represented as the PR interval on the ECG, allows the atrial contraction to propel blood into the ventricle, thereby optimizing cardiac output.

Once impulses exit the AV node, they enter the conducting system, where Na^+ currents are larger than in any other tissue, and propagation is correspondingly faster, up to 0.75 m/sec longitudinally. Activation spreads from the His-Purkinje system on the endocardium of the ventricles throughout the rest of the ventricles, stimulating coordinated ventricular contraction. This electrical activation manifests itself as the QRS complex on the ECG. Ventricular repolarization is represented on the surface ECG as the T wave. The time from initial depolarization in the ventricle until the end of repolarization is termed the QT interval. Lengthening of ventricular action potentials prolongs the QT interval and may be associated with arrhythmias in LQTS and other settings. QT interval is rate dependent, and various formulae are commonly used to generate a rate-corrected value (QTc). The most common is the Bazett formula: QTc = QT/(RR^0.5), where RR is the cardiac cycle length expressed in seconds.

Refractoriness and Conduction Failure

In atrial, ventricular, and His-Purkinje cells, if restimulation occurs early during the plateau of an action potential, no Na^+ channels are available to open, so no inward current results, and no new action potential is generated: At this point, the cell is termed *refractory* (Figure 34–6). On the other hand, if such a stimulus occurs after the cell has repolarized completely, Na^+ channels have recovered from inactivation, and a normal Na^+ channel–dependent upstroke results with the same amplitude as the previous upstroke (Figure 34–6A). When a stimulus occurs during phase 3 of the action potential, the upstroke of the premature action potential is slower and of smaller magnitude. The magnitude depends on the number of Na^+ channels that have recovered from inactivation (Figure 34–6A), which in turn is dependent on the membrane potential. Thus, refractoriness is determined by the voltage-dependent recovery of Na^+ channels from inactivation.

Refractoriness frequently is measured by assessing whether premature stimuli applied to tissue preparations (or the whole heart) result

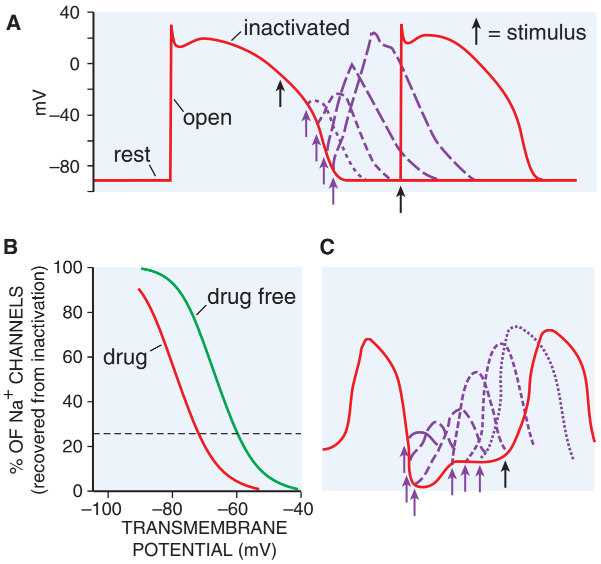


Figure 34–6 *Qualitative differences in responses of slow- and fast-response tissues to premature stimuli.* **A.** With a very early premature stimulus (black arrow) in ventricular myocardium, all Na^+ channels still are in the inactivated state, and no upstroke results. As the action potential repolarizes, Na^+ channels recover from the inactivated to the resting state (see Figure 34–2), from which opening can occur. The phase 0 upstroke slopes of the premature action potentials (purple) are greater with later stimuli because recovery from inactivation is voltage dependent. **B.** The relationship between transmembrane potential and degree of recovery of Na^+ channels from inactivation. The dotted line indicates 25% recovery. Most Na^+ channel–blocking drugs shift this relationship to the left, so in the presence of drug, fewer channels have recovered from inactivation at any potential. **C.** In Ca^{2+}-dependent slow-response tissues such as the AV node, premature stimuli delivered even after full repolarization of the action potential are depressed; recovery from inactivation is time dependent.

in propagated impulses. While the magnitude of the Na^+ current is one major determinant of propagation of premature beats, cellular geometry also is important in multicellular preparations. Propagation from cell to cell requires current flow from the first site of activation and consequently can fail if inward current is insufficient to drive activation in many neighboring cells. The *effective refractory period* (ERP) is the longest interval at which a premature stimulus fails to generate a propagated response and often is used to describe drug effects in intact tissue.

The situation is different in tissue whose depolarization is largely controlled by Ca^{2+} channel current, such as the AV node. Because Ca^{2+} channels have a slower recovery from inactivation, these tissues are often referred to as *slow response* (Figure 34–6C), in contrast to *fast response* in the remaining cardiac tissues. Even after a Ca^{2+} channel–dependent action potential has repolarized to its initial resting potential, not all Ca^{2+} channels are available for reexcitation because recovery from inactivation of these channels depends not only on voltage but also on the time since repolarization. Therefore, an extrastimulus applied shortly after repolarization is complete generates a reduced Ca^{2+} current, which may propagate slowly to adjacent cells prior to extinction. An extrastimulus applied later will result in a larger Ca^{2+} current and faster propagation. Thus, in Ca^{2+} channel–dependent tissues, which include not only the AV node but also tissues whose underlying characteristics have been altered by factors such as myocardial ischemia, refractoriness is prolonged, and propagation occurs slowly. Conduction that exhibits such dependence on the timing of premature stimuli is termed *decremental*. Slow conduction in the heart, a critical factor in the genesis of reentrant arrhythmias (see further discussion), also can occur when Na^+ currents are depressed by disease or membrane depolarization (e.g., elevated $[K]_o$), resulting in decreased steady-state Na^+ channel availability (Figure 34–6B).

Mechanisms of Cardiac Arrhythmias

An arrhythmia is by definition a perturbation of the normal sequence of impulse initiation and propagation. Failure of impulse initiation, in the sinus node, may result in slow heart rates (bradyarrhythmias), whereas failure in the normal propagation of action potentials from atrium to ventricle results in dropped beats (commonly referred to as heart block) and usually reflects an abnormality in either the AV node or the His-Purkinje system. These abnormalities may be caused by drugs (Table 34–1) or by structural heart disease; in the latter case, permanent cardiac pacing may be required.

Abnormally rapid heart rhythms (tachyarrhythmias) are common clinical problems that may be treated with antiarrhythmic drugs. Three major underlying mechanisms have been identified: enhanced automaticity, triggered automaticity, and reentry. These are often interrelated mechanisms as abnormal beats arising from one mechanism can elicit a second; for example, a triggered automatic beat can initiate reentry.

Enhanced Automaticity

Enhanced automaticity may occur in cells that normally display spontaneous diastolic depolarization—the sinus and AV nodes and the His-Purkinje system. β Adrenergic stimulation, hypokalemia, and mechanical stretch of cardiac muscle cells increase phase 4 slope and so accelerate pacemaker rate, whereas *acetylcholine* reduces pacemaker rate both by decreasing phase 4 slope and by hyperpolarization (making the maximum diastolic potential more negative). In addition, automatic behavior may occur in sites that ordinarily lack spontaneous pacemaker activity; for example, depolarization of ventricular cells (e.g., by ischemia) may produce "abnormal" automaticity. When impulses propagate from a region of enhanced normal or abnormal automaticity to excite the rest of the heart, more complex arrhythmias may result from the induction of reentry.

Afterdepolarizations and Triggered Automaticity

Under some pathophysiological conditions, a normal cardiac action potential may be interrupted or followed by an abnormal depolarization (Figure 34–7). If this abnormal depolarization reaches threshold, it may, in turn, give rise to secondary upstrokes that can propagate and create abnormal rhythms. These abnormal secondary upstrokes occur only after an initial normal, or "triggering," upstroke and thus are termed *triggered rhythms*.

Two major forms of triggered rhythms are recognized. In the first case, under conditions of intracellular or SR Ca^{2+} overload (e.g., myocardial ischemia, adrenergic stress, *digitalis* intoxication, or CPVT), a normal action potential may be followed by a *DAD* (Figure 34–7A); as discussed previously, spontaneous SR Ca^{2+} release activating NCX current is a common mechanism underlying DADs (Wit, 2018). If this afterdepolarization reaches threshold, a secondary triggered beat or beats may occur. DAD amplitude is increased *in vitro* by rapid pacing, and clinical arrhythmias corresponding to DAD-mediated triggered beats are more frequent when the underlying sinus heart rate is rapid or in the presence of β adrenergic stimulation (Priori et al., 1999).

In the second type of triggered activity, the key abnormality is marked prolongation of the cardiac action potential. When this occurs, phase 3 repolarization may be interrupted by an early afterdepolarization (EAD) (Figure 34–7B). EAD-mediated triggering *in vitro* and clinical arrhythmias are most common when the underlying heart rate is slow, extracellular K^+ is low, and certain drugs that prolong action potential duration (antiarrhythmics and others) are present. EAD-related triggered upstrokes probably reflect inward current through Ca^{2+} channels or possibly the NCX and/or Na^+ channels (Wit, 2018). Due to their intrinsically longer action potential, EADs are induced more readily in Purkinje cells and in endocardial than in epicardial cells.

When cardiac repolarization is markedly prolonged, polymorphic ventricular tachycardia with a long QT interval, termed *torsades de pointes*, may occur. This arrhythmia is thought to be caused by EADs, which trigger functional reentry (discussed next) owing to heterogeneity of action potential durations across the ventricular wall (Priori et al., 1999). Congenital LQTS, a disease in which torsades de pointes causes syncope or death, is most often caused by mutations in the genes encoding the Na^+

TABLE 34–1 ■ DRUG-INDUCED CARDIAC ARRHYTHMIAS

ARRHYTHMIA	DRUG	LIKELY MECHANISM	TREATMENT*	CLINICAL FEATURES
Sinus bradycardia, AV block	Digoxin	↑Vagal tone	Antidigoxin antibodies, temporary pacing	Atrial tachycardia may also be present
Sinus bradycardia, AV block	Verapamil, diltiazem	Ca^{2+} channel block	Ca^{2+}, temporary pacing	
Sinus bradycardia	β *Blockers*	Sympatholytic	Isoproterenol	
AV block	Clonidine Methyldopa		Temporary pacing	
Sinus tachycardia Any other tachycardia	β Blocker withdrawal	Upregulation of β receptors with chronic therapy; β blocker withdrawal →↑β effects	β Blockade	Hypertension, angina also possible
↑Ventricular rate in atrial flutter	Quinidine Flecainide Propafenone	Conduction slowing in atrium, with enhanced (quinidine) or unaltered AV conduction	AV nodal blockers	QRS complexes often widened at fast rates
↑Ventricular rate in atrial fibrillation in patients with WPW syndrome	Digoxin Verapamil	↓Accessory pathway refractoriness	IV procainamide DC cardioversion	Ventricular rate can exceed 300 beats/min and lead to VF
Multifocal atrial tachycardia	Theophylline	↑Intracellular Ca^{2+} and DADs	Withdraw theophylline ?Verapamil	Often in advanced lung disease
Polymorphic VT with ↑QT interval (torsades de pointes)	Quinidine Sotalol Procainamide Disopyramide Dofetilide Ibutilide “Noncardioactive” drugs (see text) Amiodarone (rare)	EAD-related triggered activity	Magnesium Isoproterenol Cardiac pacing	Hypokalemia, bradycardia frequent Related to ↑ plasma concentrations, except for quinidine
Frequent or difficult to terminate VT (“incessant” VT)	Flecainide Propafenone Quinidine (rarer)	Conduction slowing in reentrant circuits	Na^+ bolus reported effective in some cases	Most often in patients with advanced myocardial scarring
Atrial tachycardia with AV block; ventricular bigeminy, others	Digoxin	DAD-related triggered activity (± ↑ vagal tone)	Antidigoxin antibodies	Coexistence of abnormal impulses with abnormal sinus or AV nodal function
Ventricular fibrillation	Inappropriate use of IV verapamil	Severe hypotension and/or myocardial ischemia	Cardiac resuscitation (DC cardioversion)	Misdiagnosis of VT as PSVT and inappropriate use of verapamil

*In each of these cases, recognition and withdrawal of the offending drug(s) are mandatory; ↑, increase; ↓, decrease; ?, unclear.

channels (10%) or the channels underlying the repolarizing currents I_{Kr} and I_{Ks} (80%–90%) (Figure 34–4; Roden and Knollmann, 2014).

Reentry

Reentry occurs when a cardiac impulse travels in a path such as to return to its original site and reactivate the original site, thus perpetuating rapid reactivation independent of normal sinus node function. Key features enabling reentrant excitation are a pathway; heterogeneity of electrophysiologic properties, notably refractoriness, along the pathway; and slow conduction.

Anatomically Defined Reentry

The prototypical example of reentry is the Wolff-Parkinson-White (WPW) syndrome in which patients have an accessory connection between the atrium and ventricle (Figure 34–8). With each sinus node depolarization, impulses can excite the ventricle via the normal structures (AV node) or the accessory pathway, and this often results in an unusual and characteristic QRS complex in normal sinus rhythm. Importantly, the electrophysiological properties of the AV node and accessory pathways are different: Accessory pathways usually consist of nonnodal tissue with longer refractory periods and without decremental conduction. Thus, with a premature atrial beat (e.g., from abnormal automaticity), conduction may fail in the accessory pathway but continue, albeit slowly, in the AV node and then through the His-Purkinje system; there, the propagating impulse may encounter the ventricular end of the accessory pathway when it is no longer refractory. The likelihood that the accessory pathway is no longer refractory increases as AV nodal conduction slows, demonstrating how slow conduction enables reentry. When the impulse reenters the atrium, it then can reenter the ventricle via the AV node, reenter the atrium via the accessory pathway, and so on (Figure 34–8).

Reentry of this type, referred to as *AV reentrant tachycardia*, is determined by the following:

1. The presence of an anatomically defined circuit
2. Heterogeneity in refractoriness among regions in the circuit
3. Slow conduction in one part of the circuit

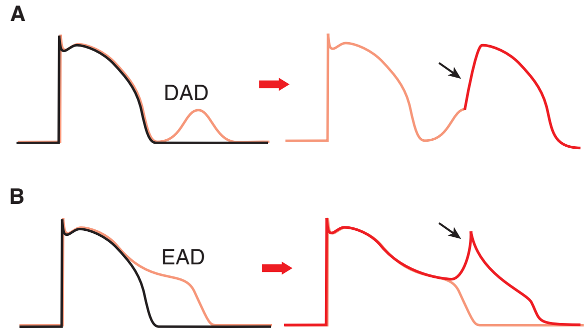


Figure 34–7 *Afterdepolarizations and triggered activity.* **A.** Delayed afterdepolarization (DAD) arising after full repolarization. DADs are typically caused by spontaneous Ca^{2+} release from the SR under conditions of Ca^{2+} overload. This increased cytosolic Ca^{2+} is removed from the cytosol by the electrogenic Na-Ca exchanger (see Figure 34–4), which produces Na^+ influx and causes a cell membrane depolarization in the form of a DAD. A DAD that reaches threshold results in a triggered upstroke (black arrow, right). **B.** Early afterdepolarization (EAD) interrupting phase 3 repolarization. Multiple ion channels and transporters can contribute to EADs (e.g., Na^+ channel, L-type Ca^{2+} channel, Na-Ca exchanger). Under some conditions, triggered beat(s) can arise from an EAD (black arrow, right).

Similar "anatomically defined" reentry commonly occurs in the region of the AV node (*AV nodal reentrant tachycardia*), in the atrium (*atrial flutter*), and in scarred ventricle (*ventricular tachycardia*). The term *paroxysmal supraventricular tachycardia* (PSVT) includes both AV reentry and AV nodal reentry, which share many clinical features.

While antiarrhythmic drugs or electrical cardioversion are used to terminate reentry acutely (discussed further in the chapter and in Table 34–2), anatomically defined reentry is often treated with radio-frequency ablation because its consistent pathway often makes it possible to identify and ablate critical segments of this pathway effectively, curing the patient and obviating the need for long-term drug therapy. Radio-frequency ablation is carried out through a catheter advanced to the interior of the heart and requires minimal convalescence.

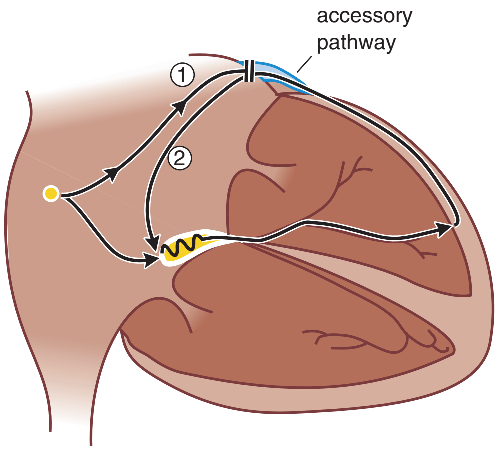


Figure 34–8 *Atrioventricular reentrant tachycardia in the WPW syndrome.* In these patients, an accessory AV connection is present (light blue). A premature atrial impulse blocks in the accessory pathway (1) and propagates slowly through the AV node and conducting system. On reaching the accessory pathway (by now no longer refractory) via the ventricle, the impulse reenters the atrium (2), where it then can reenter the ventricle via the AV node and become self-sustaining (see Figure 34–10C). AV nodal–blocking drugs readily terminate this tachycardia. Recurrences can be prevented by drugs that prevent atrial premature beats, by drugs that alter the electrophysiological characteristics of tissue in the circuit (e.g., they prolong AV nodal refractoriness), and by nonpharmacological ablation techniques that selectively destroy the accessory pathway.

Functionally Defined Reentry

Reentry also may occur in the absence of a distinct, anatomically defined pathway (Figure 34–9). For example, a premature beat from within the ventricular wall may encounter refractory tissue in only one direction, allowing for conduction throughout the rest of the wall until the originally refractory area recovers, reexcites, and then propagates back through the original location of the premature beat. Another example is localized ischemia or other electrophysiological perturbations that result in an area of sufficiently slow conduction in the ventricle that impulses exiting from that area find the rest of the myocardium reexcitable, in which case reentry may ensue. AF and ventricular fibrillation (VF) are extreme examples of "functionally defined" reentry: Cells are reexcited as soon as they are repolarized sufficiently to allow enough Na^+ channels to recover from inactivation. The abnormal activation pathway subsequently provides abnormal spatial heterogeneity of repolarization that can cause other reentrant circuits to form. In AF, these can persist for years, and rotor-like activity can sometimes be recorded, presumably reflecting single or multiple reentrant circuits that can be transiently stable or meander around the atrium.

Common Arrhythmias and Their Mechanisms

The primary tool for diagnosis of arrhythmias is the ECG. More sophisticated approaches sometimes are used, such as recording from specific regions of the heart during artificial induction of arrhythmias by specialized pacing techniques. Table 34–2 lists common arrhythmias, their likely mechanisms, and approaches that should be considered for their acute termination and for long-term therapy to prevent recurrence. Examples of some arrhythmias discussed here are shown in Figure 34–10. Some arrhythmias, notably VF, are treated not pharmacologically but with direct current (DC) cardioversion—the application of a large electric current across the chest. This technique also can be used to immediately restore normal rhythm in less serious cases when the arrhythmia is ongoing (i.e., not starting and stopping spontaneously); if the patient is conscious, a brief period of general anesthesia is required. Implantable cardioverter-defibrillators (ICDs), devices that are capable of detecting ventricular tachycardia (VT) or VF and automatically delivering a defibrillating shock, are used increasingly in patients judged to be at high risk for VF. Often, drugs are used with these devices if defibrillating shocks, which are painful, occur frequently.

Mechanisms of Antiarrhythmic Drug Action

Antiarrhythmic drugs almost invariably have multiple effects in patients, and their effects on arrhythmias can be complex. A drug can modulate other targets in addition to its primary site of action. At the same time, a single arrhythmia may result from multiple underlying mechanisms (e.g., torsades de pointes [Figure 34–10H] can result either from increased late sodium current or decreased repolarizing potassium currents). Thus, antiarrhythmic therapy should be tailored to target the most relevant underlying arrhythmia mechanism, where it is known. Drugs may be antiarrhythmic by suppressing the initiating mechanism or by altering reentrant circuits. In some cases, drugs may suppress an initiator but nonetheless promote reentry (see discussion that follows).

Drugs may slow automatic rhythms by altering any of the four determinants of spontaneous pacemaker discharge (Figure 34–11): (1) increase maximum diastolic potential, (2) decrease phase 4 slope, (3) increase threshold potential, or (4) increase action potential duration. *Adenosine* and acetylcholine may increase maximum diastolic potential, and β blockers (β adrenergic receptor antagonists) (see Chapter 14) may decrease phase 4 slope. Blockade of Na^+ or Ca^{2+} channels usually results in altered threshold, and blockade of cardiac K^+ channels prolongs the action potential.

Antiarrhythmic drugs may suppress arrhythmias owing to DADs or EADs by two major mechanisms:

1. inhibition of the development of afterdepolarizations (usually due to RyR2-mediated SR Ca^{2+} release); and
2. interference with the inward current (usually through Na^+ or Ca^{2+} channels), which is responsible for the upstroke

TABLE 34–2 ■ A MECHANISTIC APPROACH TO ANTIARRHYTHMIC THERAPY

ARRHYTHMIA	COMMON MECHANISM	ACUTE THERAPY[a]	CHRONIC THERAPY[a]
Premature atrial, nodal, or ventricular depolarizations	Unknown	None indicated	None indicated
Atrial fibrillation	Disorganized “functional” reentry Continual AV node stimulation and irregular, often rapid, ventricular rate	1. Control ventricular response: AV node block[b] 2. Restore sinus rhythm: DC cardioversion	1. Control ventricular response: AV nodal block[b] 2. Maintain normal rhythm: K+ channel block, Na+ channel block with $\tau_{recovery}$ >1 sec
Atrial flutter	Stable reentrant circuit in the right atrium Ventricular rate often rapid and irregular	Same as atrial fibrillation	Same as atrial fibrillation AV nodal–blocking drugs especially desirable to avoid ↑ ventricular rate Ablation in selected cases[c]
Atrial tachycardia	Enhanced automaticity, DAD-related automaticity, or reentry within atrium	Same as atrial fibrillation Adenosine sometimes effective	Same as atrial fibrillation Ablation of tachycardia “focus”[c]
AV nodal reentrant tachycardia (PSVT)	Reentrant circuit within or near AV node	*Adenosine AV nodal block[b] Less commonly: ↑ vagal tone (digitalis, edrophonium, phenylephrine)	*AV nodal block Flecainide Propafenone *Ablation[c]
Arrhythmias associated with WPW syndrome: 1. AV reentry (PSVT)	Reentry (Figure 34–8)	Same as AV nodal reentry	K$^+$ channel block Na$^+$ channel block with $\tau_{recovery}$ >1 sec *Ablation[c]
2. Atrial fibrillation with atrioventricular conduction via accessory pathway	Very rapid rate due to nondecremental properties of accessory pathway	*DC cardioversion *Procainamide	*Ablation[c] K$^+$ channel block Na$^+$ channel block with $\tau_{recovery}$ >1 sec (AV nodal blockers can be harmful)
VT in patients with remote myocardial infarction	Reentry near the rim of the healed myocardial infarction	Amiodarone Procainamide Lidocaine Bretylium DC cardioversion	*ICD[d] Amiodarone K$^+$ channel block Na$^+$ channel block
VT in patients without structural heart disease	DADs triggered by ↑ sympathetic tone	Adenosine[e] Verapamil[e] β Blockers[e] *DC cardioversion	Verapamil[e] β Blockers[e]
VF	Disorganized reentry	*Defibrillation Amiodarone Procainamide Lidocaine Bretylium	*ICD[d] Amiodarone K$^+$ channel block Na$^+$ channel block
Torsades de pointes, congenital or acquired (often drug related)	EAD-related triggered activity	Magnesium Isoproterenol Pacing	β Blockade Pacing

*Indicates treatment of choice. [a]Acute drug therapy is administered intravenously; chronic therapy implies long-term oral use. [b]AV nodal block can be achieved clinically by adenosine, Ca^{2+} channel block, β adrenergic receptor blockade, or increased vagal tone (a major antiarrhythmic effect of digitalis glycosides). [c]Ablation is a procedure in which tissue responsible for the maintenance of a tachycardia is identified by specialized recording techniques and then selectively destroyed, usually by high-frequency radio waves delivered through a catheter placed in the heart. [d]ICD, implanted cardioverter-defibrillator: a device that can sense VT or VF and deliver pacing and/or cardioverting shocks to restore normal rhythm. [e]These may be harmful in reentrant VT and so should be used for acute therapy only if the diagnosis is secure.

Thus, arrhythmias owing to DADs in CPVT may be inhibited by β blockers (which block development of DAD by reducing SR Ca^{2+} uptake and thereby decreasing SR Ca^{2+} load and the likelihood of spontaneous Ca^{2+} release from the SR), *verapamil* (which blocks the development of DAD by reducing Ca^{2+} influx into the cell and hence SR Ca^{2+} load), or Na$^+$ channel–blocking drugs which elevate the threshold required to produce the abnormal upstroke. In CPVT, combined RyR2 and Na$^+$ channel block by agents such as *flecainide* or *propafenone* is more effective than *verapamil*. Some manifestations of digital intoxication (see later) are DAD mediated, but other arrhythmia mechanisms could be present in which calcium channel blockers such as *verapamil* are not useful and may even be harmful in this setting. Similarly, two approaches are used in arrhythmias related to EAD-triggered beats (Tables 34–1 and 34–2). EADs can be inhibited by shortening action potential duration; in

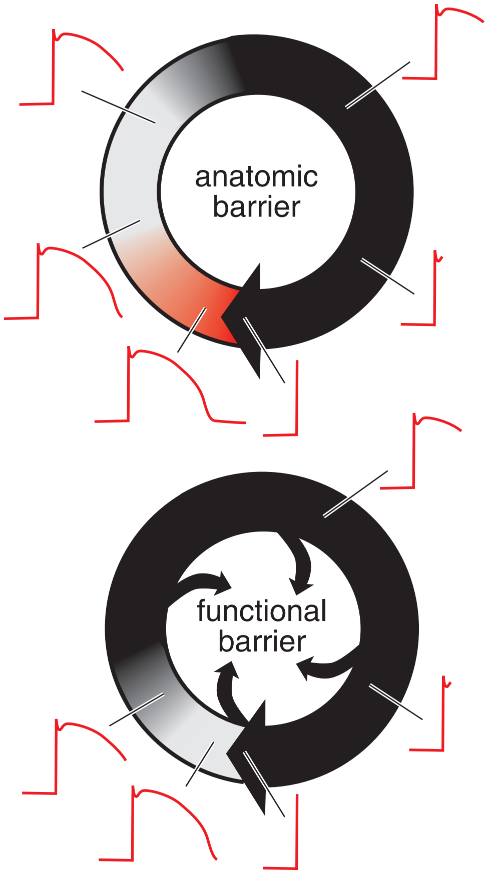


Figure 34–9 *Two types of reentry.* The border of a propagating wavefront is denoted by a heavy black arrowhead. In anatomically defined reentry (top), a fixed pathway is present (e.g., Figure 34–8). The black area denotes tissue in the reentrant circuit that is completely refractory because of the recent passage of the propagating wavefront; the gray area denotes tissue in which depressed upstrokes can be elicited (see Figure 34–6A), and the dark red area represents tissue in which restimulation would result in action potentials with normal upstrokes. The dark red area is termed an *excitable gap*. In functionally defined, or "leading circle," reentry (bottom), there is no anatomic pathway and no excitable gap. Rather, the circulating wavefront creates an area of inexcitable tissue at its core. In this type of reentry, the circuit does not necessarily remain in the same anatomic position during consecutive beats. During mapping of excitation sequences in the heart, this type of activity may be manifest as one or more "rotors."

practice, heart rate is accelerated by *isoproterenol* infusion or by pacing. Triggered beats arising from EADs can be inhibited by Mg^{2+} without normalizing repolarization *in vitro* or clinical QT interval largely by block of Ca^{2+} channels. In most forms of congenital LQTS, torsades de pointes occurs with adrenergic stress; therapy includes β adrenergic blockade (which does not shorten the QT interval but may prevent EADs) as well as pacing to shorten action potentials.

In anatomically determined reentry, drugs may terminate the arrhythmia by blocking propagation of the action potential. Conduction usually fails in a "weak link" in the circuit. In the example of the WPW-related arrhythmia described previously, the weak link is the AV node, and drugs that prolong AV nodal refractoriness and slow AV nodal conduction, such as Ca^{2+} channel blockers, β blockers, or *adenosine*, are likely to be effective. On the other hand, slowing conduction in functionally determined reentrant circuits may change the pathway without extinguishing the circuit. In fact, the effect of conduction slowing in this setting is variable. In some patients, conduction slowing drugs promote the development of reentrant arrhythmias, while in others, conduction slows sufficiently to extinguish propagation. Prolongation of refractoriness is another likely approach for terminating functionally determined reentry (Knollmann and Roden, 2008; Priori et al., 1999; Task Force, 1991). In atrial and ventricular myocytes, refractoriness can be prolonged by delaying the recovery of Na$^+$ channels from inactivation. Drugs that act by blocking Na$^+$ channels generally shift the voltage dependence of recovery from block (Figure 34–6B) and so prolong refractoriness (Figure 34–12).

Drugs that increase action potential duration without direct action on Na$^+$ channels (e.g., by blocking delayed rectifier currents) also prolong refractoriness (Figure 34–12). Particularly in sinoatrial (SA) or AV nodal tissues, Ca^{2+} channel blockade prolongs refractoriness. Drugs that interfere with cell-cell coupling also theoretically should increase refractoriness in multicellular preparations. Arrhythmia-prone hearts often display abnormal anatomy and histology, notably enhanced fibrosis, and some evidence suggests anti-inflammatory or antifibrotic interventions could thus be antiarrhythmic by preventing these changes.

State-Dependent Ion Channel Block

Knowing the structural and molecular determinants of ion channel permeation and drug block has provided key information for analyzing the actions of available and new antiarrhythmic compounds (MacKinnon, 2003). A key concept is that ion channel–blocking drugs bind to specific sites on the ion channel proteins to modify function (e.g., decrease current). The affinity of the ion channel protein for the drug on its target site generally varies as the ion channel protein shuttles among functional conformations (or ion channel "states"; see Figure 34–2). Physicochemical characteristics, such as molecular weight and lipid solubility, are important determinants of this state-dependent binding. State-dependent binding has been studied most extensively in the case of Na$^+$ channel–blocking drugs. Most useful agents of this type block open or inactivated Na$^+$ channels and have little affinity for channels in the resting state. Most Na$^+$ channel blockers bind to a local anesthetic binding site in the pore of the cardiac sodium channel protein Na$_V$1.5 (Fozzard et al., 2005). Thus, during each action potential, drugs bind to Na$^+$ channels and block them, and with each diastolic interval, drugs dissociate, and the block is released. Allosteric mechanisms have also been described whereby drug binding to a site distant from the pore nevertheless alters channel conformation and thus permeation though the pore.

As illustrated in Figure 34–13, the dissociation rate is a key determinant of steady-state block of Na$^+$ channels. When heart rate increases, the time available for dissociation decreases, and steady-state Na$^+$ channel block increases. The rate of recovery from block also slows as cells are depolarized, as in ischemia. This explains the finding that Na$^+$ channel blockers depress Na$^+$ current, and hence conduction, to a greater extent in ischemic tissues than in normal tissues. Open- versus inactivated-state block also may be important in determining the effects of some drugs. Increased action potential duration, which results in a relative increase in time spent in the inactivated state, may increase block by drugs that bind to inactivated channels, such as lidocaine. This likely explains why inactivation state Na$^+$ channels blockers are relatively ineffective in atrial tissue where action potentials are shorter.

The rate of recovery from block often is expressed as a time constant ($\tau_{recovery}$, the time required to complete approximately 63% of an exponentially determined process). In the case of drugs such as *lidocaine*, $\tau_{recovery}$ is so short (<<1 sec) that recovery from block is very rapid, and substantial Na$^+$ channel block occurs only in rapidly driven tissues, particularly in ischemia. Conversely, drugs such as *flecainide* have such long $\tau_{recovery}$ values (>10 sec) that roughly the same numbers of Na$^+$ channels are blocked during systole and diastole. As a result, slowing of conduction occurs at therapeutic concentrations even in normal tissues at normal rates. This is manifest on the ECG as prolongation of PR and QRS durations.

Classifying Antiarrhythmic Drugs

Classifying drugs by common electrophysiological properties emphasizes the connection between basic electrophysiological actions and antiarrhythmic effects (Vaughan Williams, 1970). To the extent that the clinical actions of drugs can be predicted from their basic electrophysiological properties, such classification schemes have merit. However, as each compound is better characterized in a range of *in vitro* and *in vivo* test systems, it becomes apparent that differences in pharmacological effects occur even among drugs that share the same classification, some of which

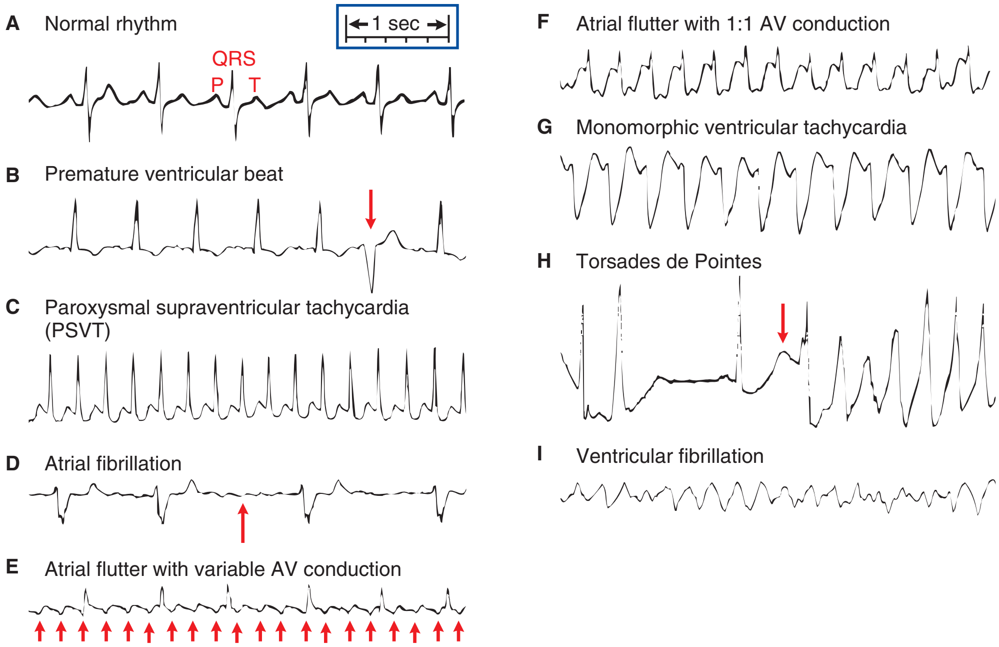

A Normal rhythm
1 sec
QRS
P
T
B Premature ventricular beat
C Paroxysmal supraventricular tachycardia (PSVT)
D Atrial fibrillation
E Atrial flutter with variable AV conduction
F Atrial flutter with 1:1 AV conduction
G Monomorphic ventricular tachycardia
H Torsades de Pointes
I Ventricular fibrillation

Figure 34–10 *Electrocardiograms showing normal and abnormal cardiac rhythms.* The P, QRS, and T waves in normal sinus rhythm are shown in panel **A**. Panel **B** shows a premature beat arising in the ventricle (arrow). PSVT is shown in panel **C**; this is most likely reentry using an accessory pathway (see Figure 34–8) or reentry within or near the AV node. In atrial fibrillation (panel **D**), there are no P waves, and the QRS complexes occur irregularly (and at a slow rate in this example); electrical activity between QRS complexes shows small undulations (arrow) corresponding to fibrillatory activity in the atria. In atrial flutter (panel **E**), the atria beat rapidly, approximately 250 beats per min (arrows) in this example, and the ventricular rate is variable. If a drug that slows the rate of atrial flutter is administered, 1:1 AV conduction (panel **F**) can occur. In monomorphic VT (panel **G**), identical wide QRS complexes occur at a regular rate, 180 beats per min. The electrocardiographic features of the torsades de pointes syndrome (panel **H**) include a very long QT interval (>600 ms in this example, arrow) and VT in which each successive beat has a different morphology (polymorphic VT). Panel **I** shows the disorganized electrical activity characteristic of VF.

may be responsible for the observed clinical differences in responses to drugs of the same broad "class" (Table 34–3).

An alternative way of approaching antiarrhythmic therapy is to attempt to classify arrhythmia mechanisms and then target drug therapy to the electrophysiological mechanism most likely to terminate or prevent the arrhythmia (Priori et al., 1999; Task Force, 1991) (Table 34–2). This approach has been further enhanced by an increasing understanding of arrhythmia mechanisms in genetic diseases such as LQTS and CPVT, so a genetic framework represents a complementary approach for improving antiarrhythmic drug development and therapy (Knollmann and Roden, 2008).

Na^+ Channel Block

The extent of Na^+ channel block depends critically on heart rate and membrane potential, as well as on drug-specific physicochemical characteristics that determine $\tau_{recovery}$ (Figure 34–13). Na^+ channels are blocked at rapid heart rates in diseased tissue with a rapid-recovery drug such as *lidocaine* or even at normal rates in normal tissues with a slow-recovery drug such as *flecainide*. When Na^+ channels are blocked, threshold for excitability is decreased; that is, greater membrane depolarization is required to open enough Na^+ channels to overcome K^+ currents at the resting membrane potential and elicit an action potential. This change in threshold probably contributes to the clinical finding that Na^+ channel blockers tend to increase both pacing threshold and the energy required to defibrillate the fibrillating heart. These deleterious effects may be important if antiarrhythmic drugs are used in patients with pacemakers or implanted defibrillators. Na^+ channel block decreases conduction velocity in fast-response tissue and increases QRS duration. Usual doses of *flecainide* prolong QRS intervals by 25% or more during normal rhythm, whereas *lidocaine* increes QRS intervals only at very fast heart rates. Drugs with $\tau_{recovery}$ values greater than 10 sec (e.g., *flecainide*) also tend to prolong the PR interval; this is likely due to block of fast-response tissue in the region of the AV node, although additional Ca^{2+} channel block is also possible. Drug effects on the PR interval are also highly modified by autonomic effects. For example, *quinidine* tends to shorten the PR interval largely as a result of its vagolytic properties. Action potential duration is either unaffected or shortened by Na^+ channel block; some Na^+ channel–blocking drugs do prolong cardiac action potentials but by other mechanisms, usually K^+ channel block (Table 34–3).

By increasing threshold, Na^+ channel block decreases automaticity (Figure 34–11B) and can potentially inhibit triggered activity arising from DADs or EADs. Many Na^+ channel blockers also decrease phase 4 slope (Figure 34–11A). In anatomically defined reentry, Na^+ channel blockers may decrease conduction sufficiently to extinguish the propagating reentrant wavefront. However, as described previously, conduction slowing owing to Na^+ channel block may exacerbate reentry. Block of Na^+ channels also shifts the voltage dependence of recovery from inactivation (Figure 34–6B) to more negative potentials, thereby tending to increase refractoriness. Thus, whether a given drug exacerbates or suppresses reentrant arrhythmias depends on the balance between its effects on refractoriness and on conduction in a particular reentrant circuit. *Lidocaine* and *mexiletine* are not useful in AF or atrial flutter, likely because they have short $\tau_{recovery}$ values and predominantly block inactivated channels. Conversely, *quinidine*, *flecainide*, *propafenone*, and similar agents are effective in some patients. Many of these agents owe part of their antiarrhythmic activity to blockade of K^+ channels and possibly also RyR2 block (*flecainide*, *propafenone*).

Na^+ Channel Blocker Toxicity

Conduction slowing in potential reentrant circuits can account for toxicity of drugs that block the Na^+ channel (Table 34–1). For example, Na^+ channel block decreases conduction velocity and hence slows atrial flutter rate. However, normal AV nodal function permits a greater number of impulses to penetrate the ventricle, and heart rate may actually increase (Figure 34–10). Thus, with Na^+ channel blocker therapy, atrial flutter rate may drop from 300 per min, with 2:1 or 4:1 AV conduction (i.e., a heart rate of 150 or 75 beats per min, respectively), to 220 per min, but with 1:1 transmission to the ventricle (i.e., a heart rate of 220 beats per min), with potentially disastrous consequences. This form of drug-induced arrhythmia is especially common during treatment with *flecainide*, *propafenone*,

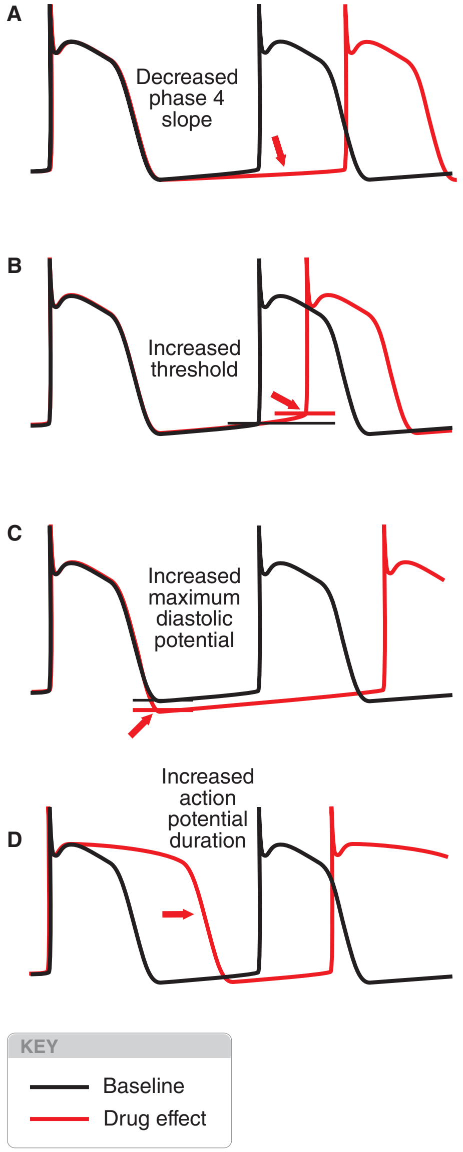

A
Decreased phase 4 slope
B
Increased threshold
C
Increased maximum diastolic potential
D
Increased action potential duration
KEY
Baseline
Drug effect

Figure 34–11 *Four ways to reduce the rate of spontaneous discharge.* The horizontal lines in panels **B** and **C** mark the threshold potentials for triggering an action potential before and after drug application.

and occasionally *amiodarone*; it can also occur with *quinidine*, which also increases AV nodal conduction through its vagolytic properties. Therapy with Na$^+$ channel blockers in patients with reentrant ventricular tachycardia after a myocardial infarction can increase the frequency and severity of arrhythmic episodes. Although the mechanism is unclear, a likely explanation is that slowed conduction allows the reentrant wavefront to persist within the tachycardia circuit. Such drug-exacerbated arrhythmia can be difficult to manage, and deaths owing to intractable drug-induced VT have been reported. In this setting, Na$^+$ infusion may be beneficial. Drug-exacerbated VT or VF also likely accounts for increased mortality with Na$^+$ channel blockers compared to placebo in patients convalescing from acute myocardial infarction in the CAST trial (Echt et al., 1991). Several Na$^+$ channel blockers (e.g., *procainamide* and *quinidine*) have been reported to exacerbate neuromuscular paralysis by D-tubocurarine (see Chapter 13).

Action Potential Prolongation

Most drugs that prolong the action potential do so by blocking I_{Kr} (Roden et al., 1993), although increased late Na$^+$ current also produces this effect (Lu et al., 2012; Yang et al., 2014). Both drug effects increase action potential duration and reduce normal automaticity (Figure 34–11D). Increased action potential duration, seen as an increase in QT interval, increases refractoriness (Figure 34–12) and therefore should be an effective way of treating reentry (Task Force, 1991). Experimentally, K$^+$ channel block produces a series of desirable effects: reduced defibrillation energy requirement, inhibition of VF owing to acute ischemia, and increased contractility (Roden, 1993; Singh, 1993). As shown in Table 34–3, many K$^+$ channel–blocking drugs also interact with β adrenergic receptors (*sotalol*) or other channels (e.g., *amiodarone* and *quinidine*). Amiodarone and sotalol appear to be at least as effective as drugs with predominant Na$^+$ channel–blocking properties in both atrial and ventricular arrhythmias. "Pure" action potential–prolonging drugs (e.g., *dofetilide* and *ibutilide*) are also available (Murray, 1998; Torp-Pedersen et al., 1999).

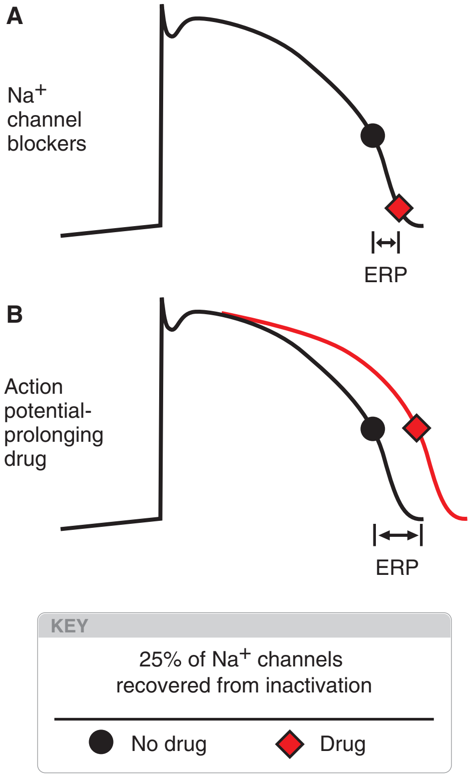
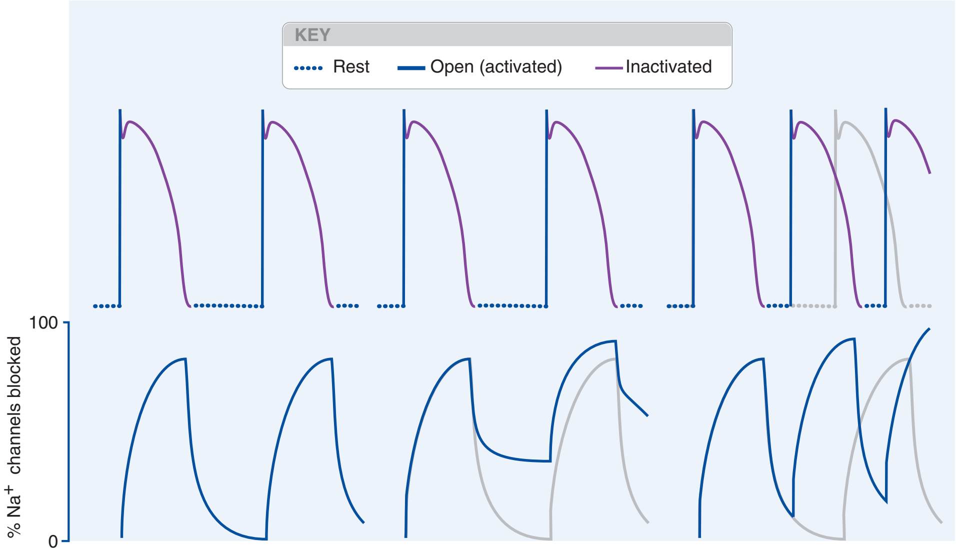

A
Na+ channel blockers
ERP
B
Action potential-prolonging drug
ERP
KEY
25% of Na+ channels recovered from inactivation
No drug
Drug

Figure 34–12 *Two ways to increase refractoriness.* In this figure, the black dot indicates the point at which a sufficient number of Na$^+$ channels (an arbitrary 25%; see Figure 34–6B) have recovered from inactivation to allow a premature stimulus to produce a propagated response in the absence of a drug. Block of Na$^+$ channels (**A**) shifts voltage dependence of recovery (see Figure 34–6B) and so delays the point at which 25% of channels have recovered (red diamond), prolonging the ERP. Note that if the drug also dissociates slowly from the channel (see Figure 34–13), refractoriness in fast-response tissues actually can extend beyond full repolarization ("postrepolarization refractoriness"). Drugs that prolong the action potential (**B**) also will extend the point at which an arbitrary percentage of Na$^+$ channels have recovered from inactivation, even without directly interacting with Na$^+$ channels.

Toxicity of Drugs That Prolong the Action Potential

Most of these agents disproportionately prolong cardiac action potentials and the QT interval when underlying heart rate is slow and can cause torsades de pointes (Table 34–1, Figure 34–10). While this effect is usually seen with QT-prolonging antiarrhythmic drugs, it can occur more rarely with drugs that are used for noncardiac indications. For such agents, the risk of torsades de pointes may not become apparent until after widespread use postmarketing, and recognition of this risk has been a common cause for drug withdrawal (Roden, 2004). Sex hormones modify cardiac ion channels and help account for the clinically observed increased incidence of drug-induced torsades de pointes in women (Tadros et al., 2014). Testosterone deficiency, often in men being treated for prostate cancer, has also been recognized as a cause of torsades de pointes (Salem et al., 2018).



Na^+ channel blocker

Slower dissociation rate increases Na^+ channel block

Tachycardia increases Na^+ channel block

Figure 34–13 *Recovery from block of Na^+ channels during diastole.* This recovery is the critical factor determining extent of steady-state Na^+ channel block. Na^+ channel blockers bind to (and block) Na^+ channels in the open or inactivated states, resulting in phasic changes in the extent of block during the action potential. As shown in the middle panel, a decrease in the rate of recovery from block increases the extent of block. Different drugs have different rates of recovery, and depolarization reduces the rate of recovery. The right panel shows increasing heart rate, which results in relatively less time spent in the rest state and also increases the extent of block. (Reproduced with permission from Roden DM, et al. Clinical pharmacology of antiarrhythmic agents. In: Josephson ME, ed. *Sudden Cardiac Death*. Blackwell Scientific, London, **1993**, 182–185. Permission conveyed through Copyright Clearance Center, Inc.)

Ca^{2+} Channel Block

The major electrophysiological effects resulting from block of cardiac Ca^{2+} channels are in nodal tissues. Dihydropyridines such as *nifedipine*, which are used commonly in angina and hypertension (see Chapters 31 and 32), preferentially block Ca^{2+} channels in vascular smooth muscle; their cardiac electrophysiological effects, such as heart rate acceleration, result principally from reflex sympathetic activation secondary to peripheral vasodilation. Only *verapamil* and *diltiazem* block Ca^{2+} channels in cardiac cells at clinically used doses. These drugs generally slow sinus rate (Figure 34–11A), although hypotension, if marked, can cause reflex sympathetic activation and tachycardia. The velocity of AV nodal conduction decreases, so the PR interval increases. AV nodal block occurs because of decremental conduction, as well as increased AV nodal refractoriness. These effects form the basis of the antiarrhythmic actions of Ca^{2+} channel blockers in reentrant arrhythmias whose circuit involves the AV node, such as AV reentrant tachycardia (Figure 34–8).

Another important indication for antiarrhythmic therapy is to reduce the ventricular rate in atrial flutter or AF. Parenteral *verapamil* and *diltiazem* are approved for temporary control of rapid ventricular rate in atrial flutter or AF and for rapid conversion of PSVT to sinus rhythm (where their use has largely been supplanted by adenosine). Oral *verapamil* or *diltiazem* may be used to control the ventricular rate in chronic atrial flutter or AF and for prophylaxis of repetitive PSVT. Unlike β blockers, Ca^{2+} channel blockers have not been shown to reduce mortality after myocardial infarction (Singh, 1990).

Verapamil and Diltiazem

The major adverse effect of intravenous *verapamil* or, less commonly, *diltiazem* is hypotension, particularly with bolus administration. This was a particular problem when the drugs were used mistakenly in patients with VT (in which Ca^{2+} channel blockers usually are not effective) misdiagnosed as PSVT; the drugs are now rarely used for this indication. Hypotension also is frequent in patients receiving other vasodilators and in patients with underlying left ventricular dysfunction, which the drugs can exacerbate. Severe sinus bradycardia or AV block also occurs, especially in susceptible patients, such as those also receiving β blockers. With oral therapy, these adverse effects tend to be less severe. Constipation is a frequent side effect of oral *verapamil*.

Verapamil is prescribed as a racemate. L-*Verapamil* is the more potent Ca^{2+} channel blocker. However, with oral therapy, the L-enantiomer undergoes more extensive first-pass hepatic metabolism. For this reason, a given concentration of *verapamil* prolongs the PR interval to a greater extent when administered intravenously (where concentrations of the L- and D-enantiomers are equivalent) than when administered orally. *Diltiazem* also undergoes extensive first-pass hepatic metabolism, and both drugs have metabolites that exert Ca^{2+} channel–blocking actions. In clinical practice, adverse effects during therapy with *verapamil* or *diltiazem* are determined largely by *underlying* heart disease and concomitant therapy; plasma concentrations of these agents are not routinely measured. Both drugs can increase serum digoxin concentration, although the magnitude of this effect is variable; excess slowing of ventricular response may occur in patients with AF.

Blockade of β Adrenergic Receptors

β Adrenergic stimulation increases the magnitude of the Ca^{2+} current and slows its inactivation; increases the magnitude of the repolarizing current I_{Ks}; increases pacemaker current (thereby increasing sinus rate; DiFrancesco, 1993); increases the Ca^{2+} stored in the SR by activating the SR Ca^{2+} uptake pump (thereby increasing likelihood of spontaneous Ca^{2+} release and DADs); and under pathophysiological conditions, can increase both DAD- and EAD-mediated arrhythmias. The increases in plasma epinephrine associated with severe stress (e.g., acute myocardial infarction or resuscitation after cardiac arrest) lower serum K^+, especially in patients receiving chronic diuretic therapy. β Blockers inhibit adrenergic effects and can be antiarrhythmic by reducing heart rate, decreasing intracellular Ca^{2+} overload, and inhibiting afterdepolarization-mediated automaticity. Epinephrine-induced hypokalemia appears to be mediated by β_2 adrenergic receptors and is blocked by "noncardioselective" antagonists such as *propranolol* (see Chapter 14). In acutely ischemic tissue, β blockers increase the energy required to fibrillate the heart, an

TABLE 34–3 ■ MAJOR ELECTROPHYSIOLOGIC ACTIONS OF ANTIARRHYTHMIC DRUGS

	Na^+ CHANNEL BLOCK						
DRUG	**$\tau_{recovery}$[1], SECONDS**	**STATE DEPENDENCE[1]**	**K^+ CHANNEL BLOCK, ↑APD**	**Ca^{2+} CHANNEL BLOCK**	**RyR2 CHANNEL BLOCK**	**AUTONOMIC EFFECTS**	**OTHER EFFECTS**
Lidocaine	0.1	I > O					
Phenytoin	0.2	I					
Mexiletine[a]	0.3	I					
Procainamide	1.8	O	✓			Ganglionic blockade (especially IV)	✓: Metabolite prolongs APD
Quinidine	3	O	✓			α Blockade, vagolytic	
Disopyramide[b]	9	O	✓			Anticholinergic	
Propafenone[b]	11	O ≈ I	(x)		✓	β Blockade (variable clinical effect)	
Flecainide[a]	11	O	(x)		✓		
β Blockers: Propanolol[b]						β Blockade	Na^+ channel block *in vitro*
Sotalol[b]			✓			β Blockade	
Bretylium			✓			Adrenergic stimulation followed by ganglionic blockade	↓ Dispersion of repolarization in ischemia
Amiodarone	1.6	I	✓	(x)		Noncompetitive β blockade	Antithyroid action
Dronedarone	Unknown	I	✓	(x)		Noncompetitive β blockade	
Dofetilide			✓				
Ibutilide			✓				
Verapamil[a]				✓			
Diltiazem[a]				✓			
Digoxin						✓: Vagal stimulation	✓: Inhibition of Na^+, K^+–ATPase
Adenosine						✓: Adenosine receptor activation	✓: Activation of K^+ current
Magnesium				✓	(x)		

✓ Indicates an effect that is important in mediating the clinical action of a drug. (x) Indicates a demonstrable effect whose relationship to drug action in patients is less well established. [a]Indicates drugs prescribed as racemates, and the enantiomers are thought to exert similar electrophysiological effects. [b]Indicates racemates for which clinically relevant differences in the electrophysiological properties of individual enantiomers have been reported (see text). One approach to classifying antiarrhythmic drugs is provided in Vaughan Williams (1970):

Class	*Major action*
I	Na^+ channel block
II	β Adrenergic receptor blockade
III	Action potential prolongation (usually by K^+ channel block)
IV	Ca^{2+} channel block

Drugs are listed here according to this scheme. It is important to bear in mind, however, that many drugs exert multiple effects that contribute to their clinical actions. It is occasionally clinically useful to subclassify Na^+ channel blockers by their rates of recovery from drug-induced block ($\tau_{recovery}$) under physiological conditions. Because this is a continuous variable and can be modulated by factors such as depolarization of the resting potential, these distinctions can become blurred: class Ib, $\tau_{recovery}$ <1 sec; class Ia, $\tau_{recovery}$ 1–10 sec; class Ic, $\tau_{recovery}$ > 10 sec. These class and subclass effects are associated with distinctive ECG changes, characteristic "class" toxicities, and efficacy in specific arrhythmia syndromes (see text). [1]These data are dependent on experimental conditions, including species and temperature. The $\tau_{recovery}$ values cited here are from Courtney (1987). APD, action potential duration; O, open-state blocker; I, inactivated-state blocker.

antiarrhythmic action. These effects may contribute to the reduced short-term and long-term mortality observed in trials of chronic therapy with β blockers after myocardial infarction (Singh, 1990).

As with Ca^{2+} channel blockers and *digitalis*, β blockers increase AV nodal conduction time (increased PR interval) and prolong AV nodal refractoriness; hence, they are useful in terminating reentrant arrhythmias that involve the AV node and in controlling ventricular response in AF or atrial flutter. β Blockers may be useful in arrhythmias triggered by physical or emotional stress, such as in many patients with LQTS and all patients with CPVT syndrome (Roden and Spooner, 1999). β Blockers are also reportedly effective in controlling arrhythmias owing to Na^+ channel blockers; this effect may be due in part to slowing of the heart rate, which then decreases the extent of rate-dependent conduction slowing by Na^+ channel block.

Adverse effects of β blockade include fatigue, bronchospasm, hypotension, impotence, depression, aggravation of heart failure, worsening of symptoms owing to peripheral vascular disease, and masking of the symptoms of hypoglycemia in diabetic patients (see Chapter 14). In patients with arrhythmias owing to excess sympathetic stimulation (e.g., pheochromocytoma, *clonidine* withdrawal, or cocaine toxicity), β blockers can result in unopposed α adrenergic stimulation, with resulting severe hypertension or α adrenergic–mediated arrhythmias. In such patients, arrhythmias should be treated with both α and β blockers or with a drug such as *labetalol* that combines α- and β-blocking properties. Abrupt discontinuation of chronic β-blocker therapy can lead to "rebound" symptoms, including hypertension, increased angina, and arrhythmias; thus, β blockers are tapered over 2 weeks prior to discontinuation of chronic therapy (see Chapters 14 and 31–33).

Selected β Blockers

It is likely that most β blockers share antiarrhythmic properties. Some, such as *propranolol*, also exert Na$^+$ channel–blocking effects at high concentrations. Similarly, drugs with intrinsic sympathomimetic activity may be less useful as antiarrhythmics (Singh, 1990). *Acebutolol* is as effective as *quinidine* in suppressing ventricular ectopic beats, an arrhythmia that many clinicians no longer treat. *Sotalol* (see its discussion in a separate section) is more effective for many arrhythmias than other β blockers, probably because of its K$^+$ channel–blocking actions. *Esmolol* (see separate discussion that follows) is a β$_1$-selective agent that has a very short elimination half-life. Intravenous esmolol is useful in clinical situations in which immediate β adrenergic blockade is desired. Some β blockers (e.g., *propranolol*, *metoprolol*) are CYP2D6 substrates; thus, efficacy may vary across individuals (see Chapter 7). *Nadolol* and *propranolol* appear more effective than other β blockers when β blockade is needed in congenital arrhythmia syndromes such as CPVT or LQTS (Ahn et al., 2017; Chockalingam et al., 2012).

Principles in the Clinical Use of Antiarrhythmic Drugs

Drugs that modify cardiac electrophysiology often have a very narrow margin between the doses required to produce a desired effect and those associated with adverse effects. Moreover, antiarrhythmic drugs can induce new arrhythmias with possibly fatal consequences. Nonpharmacological treatments, such as cardiac pacing, electrical defibrillation, or ablation of targeted regions, are indicated for some arrhythmias; in other cases, no therapy is required, even though an arrhythmia is detected. Therefore, the fundamental principles of therapeutics described here must be applied to optimize antiarrhythmic therapy.

1. Identify and Remove Precipitating Factors

Factors that commonly precipitate cardiac arrhythmias include hypoxia, electrolyte disturbances (especially hypokalemia), myocardial ischemia, and certain drugs. Antiarrhythmics, including cardiac glycosides, are not the only drugs that can precipitate arrhythmias (Table 34–1). For example, *theophylline* can cause multifocal atrial tachycardia, which sometimes can be managed simply by reducing the dose of *theophylline*. Torsades de pointes can arise during therapy not only with action potential–prolonging antiarrhythmics but also with other "noncardiovascular" drugs that do not affect ion channels (Roden, 2004). The incidence can vary from 1% to 3% in patients receiving *sotalol* or *dofetilide* and can be very rare (<1/50,000) with some noncardiovascular drugs. Drugs with a very wide range of clinical indications have been implicated: These include some antibiotics (including antibacterials, antiprotozoals, antivirals, and antifungals), antipsychotics, antihistamines, antidepressants, and *methadone*. The website Crediblemeds.org maintains a list of drugs (and levels of evidence) that have been implicated in this adverse effect.

2. Establish the Goals of Treatment

Some Arrhythmias Should Not Be Treated: The CAST Example

Abnormalities of cardiac rhythm are readily detectable by a variety of recording methods. However, the mere detection of an abnormality does not equate with the need for therapy. This was illustrated in CAST. The presence of asymptomatic ventricular ectopic beats is a known marker for increased risk of sudden death owing to VF in patients convalescing from myocardial infarction. In CAST, patients with recent myocardial infarction whose ventricular ectopic beats were suppressed by the potent Na$^+$ channel blocker *encainide* (no longer marketed) or *flecainide* were randomly assigned to receive those drugs or placebo. Unexpectedly, the mortality rate was 2- to 3-fold higher among patients treated with the drugs than in those treated with placebo (Echt et al., 1991). While the explanation for this effect is not known, several lines of evidence suggest that, in the presence of these drugs and myocardial scarring, transient episodes of myocardial ischemia or sinus tachycardia can cause marked conduction slowing (because these drugs have a very long $\tau_{recovery}$), resulting in fatal reentrant ventricular tachyarrhythmias.

One consequence of this pivotal clinical trial was to reemphasize the concept that therapy should be initiated only when a clear benefit to the patient can be identified. When symptoms are obviously attributable to an ongoing arrhythmia, there usually is little doubt that termination of the arrhythmia will be beneficial; when chronic therapy is used to prevent recurrence of an arrhythmia, the risks may be greater (Roden, 1994). *Among the antiarrhythmic drugs discussed here, only β adrenergic blockers* (Connolly, 1999) *demonstrably reduce mortality during long-term therapy*.

Symptoms Due to Arrhythmias

Some patients with an arrhythmia may be asymptomatic; in this case, establishing any benefit for treatment will be difficult. Some patients may present with presyncope, syncope, or even cardiac arrest, which may be due to brady- or tachyarrhythmias. Other patients may present with a sensation of irregular heartbeats (i.e., palpitations) that can be minimally symptomatic in some individuals and incapacitating in others. The irregular heartbeats may be due to intermittent premature contractions or to sustained arrhythmias such as AF (which results in an irregular ventricular rate) (Figure 34–10). Finally, patients may present with symptoms owing to decreased cardiac output attributable to arrhythmias. The most common symptom is breathlessness either at rest or on exertion. Occasionally, sustained or frequent tachycardias may produce no "arrhythmia" symptoms (e.g., palpitations) but will depress contractile function; these patients may present with heart failure due to "tachycardia-induced cardiomyopathy," a condition that can be reversed by treating the arrhythmia.

Choosing Among Therapeutic Approaches

In choosing among available therapeutic options, it is important to establish clear therapeutic goals. For example, three options are available in patients with AF: (1) reduce the ventricular response using AV nodal–blocking agents such as *digitalis*, *verapamil*, *diltiazem*, or β blockers (Table 34–1); (2) restore and maintain normal rhythm using drugs such as *flecainide* or *amiodarone*; or (3) decide not to implement antiarrhythmic therapy, especially if the patient truly is asymptomatic. Most patients with AF also benefit from anticoagulation to reduce stroke incidence regardless of symptoms (Dzeshka and Lip, 2015) (see Chapter 36).

Factors that contribute to the choice of therapy include not only symptoms but also the type and extent of structural heart disease, the QT interval prior to drug therapy, the coexistence of conduction system disease, and the presence of noncardiac diseases (Table 34–4). In the rare patient with the WPW syndrome and AF, the ventricular response can be extremely rapid and can be accelerated paradoxically with *digitalis* or Ca^{2+} channel blockers; deaths owing to drug therapy have been reported under these circumstances.

The frequency and reproducibility of arrhythmia should be established prior to initiating therapy because inherent variability in the occurrence of arrhythmias can be confused with a beneficial or adverse drug effect. Techniques for this assessment include recording cardiac rhythm for prolonged periods or evaluating the response of the heart to artificially induced premature beats. It is important to recognize that drug therapy may be only partially effective. A marked decrease in the duration of paroxysms of AF may be sufficient to render a patient asymptomatic even if an occasional episode still can be detected.

AL GRAWANY

TABLE 34–4 ■ PATIENT-SPECIFIC ANTIARRHYTHMIC DRUG CONTRAINDICATIONS

CONDITION	EXCLUDE/USE WITH CAUTION
Cardiac	
Heart failure	Disopyramide, flecainide
Sinus or AV node dysfunction	Digoxin, verapamil, diltiazem, β blockers, amiodarone
Wolff-Parkinson-White syndrome (risk of extremely rapid rate if atrial fibrillation develops)	Digoxin, verapamil, diltiazem
Infranodal conduction disease	Na^+ channel blockers, amiodarone
Aortic/subaortic stenosis	Bretylium
History of myocardial infarction	Flecainide
Prolonged QT interval	Quinidine, procainamide, disopyramide, sotalol, dofetilide, ibutilide, amiodarone, dronedarone
Cardiac transplant	Adenosine
Noncardiac	
Diarrhea	Quinidine
Prostatism, glaucoma	Disopyramide
Arthritis	Chronic procainamide
Lung disease	Amiodarone
Tremor	Mexiletine
Constipation	Verapamil
Asthma, peripheral vascular disease, hypoglycemia	β Blockers, propafenone

3. Minimize Risks

Antiarrhythmic Drugs Can Cause Arrhythmias

One well-recognized risk of antiarrhythmic therapy is the possibility of provoking new arrhythmias, with potentially life-threatening consequences. Antiarrhythmic drugs can provoke arrhythmias by different mechanisms (Table 34–1). These drug-provoked arrhythmias must be recognized because further treatment with antiarrhythmic drugs often exacerbates the problem, whereas withdrawal of the causative agent is curative. Thus, establishing a precise diagnosis is critical, and targeting therapies at underlying mechanisms of the arrhythmias may be required. For example, treating VT with *verapamil* not only may be ineffective but also can cause catastrophic cardiovascular collapse.

Monitoring of Plasma Concentration

Some adverse effects of antiarrhythmic drugs result from excessive plasma drug concentrations. Measuring plasma concentration and adjusting the dose to maintain the concentration within a prescribed therapeutic range may minimize some adverse effects. In many patients, serious adverse reactions relate to interactions involving antiarrhythmic drugs (often at usual plasma concentrations), transient factors such as electrolyte disturbances or myocardial ischemia, and the type and extent of the underlying heart disease (Roden, 1994). Factors such as generation of unmeasured active metabolites, variability in elimination of enantiomers (which may exert differing pharmacological effects), and disease- or enantiomer-specific abnormalities in drug binding to plasma proteins can complicate the interpretation of plasma drug concentrations.

Patient-Specific Contraindications

Another way to minimize the adverse effects of antiarrhythmic drugs is to avoid certain drugs in certain patient subsets altogether. For example, patients with a history of congestive heart failure are particularly prone to develop heart failure during *flecainide* or *disopyramide* therapy. In other cases, adverse effects of drugs may be difficult to distinguish from exacerbations of underlying disease. *Amiodarone* may cause interstitial lung disease; its use therefore is undesirable in a patient with advanced pulmonary disease in whom the development of this potentially fatal adverse effect would be difficult to detect. Specific diseases that constitute relative or absolute contraindications to specific drugs are listed in Table 34–4.

4. Consider the Electrophysiology of the Heart as a "Moving Target"

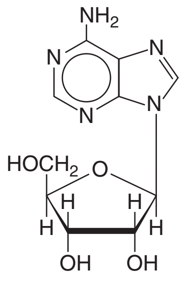
Cardiac electrophysiology varies dynamically in response to external influences such as changing electrolyte levels, autonomic tone, myocardial ischemia, and myocardial stretch (Priori et al., 1999). For example, myocardial ischemia results in changes in extracellular K^+ that make the resting potential less negative, inactivate Na^+ channels, decrease Na^+ current, and slow conduction. In addition, myocardial ischemia may activate channels that otherwise are quiescent, such as the ATP-inhibited K^+ channels. Thus, in response to myocardial ischemia, a normal heart may display changes in resting potential, conduction velocity, intracellular Ca^{2+} concentrations, and repolarization, any one of which then may create arrhythmias or alter response to antiarrhythmic therapy.

Antiarrhythmic Drugs

Summaries of important electrophysiological and pharmacokinetic features of the drugs considered here are presented in Tables 34–3 and 34–5. Ca^{2+} channel blockers and β blockers are discussed in Chapters 14 and 31 to 33. The drugs are presented in alphabetical order. Prescribing patterns have changed over the past several decades in part because fewer suppliers market older drugs, such as *quinidine* or oral *procainamide*, which are therefore increasingly difficult to obtain; this poses a problem for a small number of patients who may still benefit from treatment (Inama et al., 2010; Viskin et al., 2013).

Adenosine

Adenosine is a naturally occurring nucleoside that is administered as a rapid intravenous bolus for the acute termination of reentrant supraventricular arrhythmias (Link, 2012). Rare cases of VT in patients with otherwise-normal hearts are thought to be DAD mediated and can be terminated by *adenosine*. *Adenosine* also has been used to produce controlled hypotension during some surgical procedures and in the diagnosis of coronary artery disease. Intravenous ATP appears to produce effects similar to those of *adenosine*.



ADENOSINE

Pharmacological Effects

The effects of *adenosine* are mediated by its interaction with specific G protein-coupled adenosine receptors. *Adenosine activates* acetylcholine-sensitive K^+ current in the atrium and sinus and AV nodes, resulting in shortening of action potential duration, hyperpolarization, and slowing of normal automaticity (Figure 34–11C). *Adenosine* also inhibits the electrophysiological effects of increased intracellular cyclic AMP that occur with sympathetic stimulation. Because *adenosine* thereby reduces Ca^{2+}

TABLE 34–5 ■ PHARMACOKINETIC CHARACTERISTICS AND DOSES OF ANTIARRHYTHMIC DRUGS

	BIOAVAILABILITY		ELIMINATION						USUAL DOSES[c]	
DRUG	**REDUCED FIRST-PASS METABOLISM**	**PROTEIN BINDING >80%**	**RENAL**	**HEPATIC**	**OTHER**	**ELIMINATION[a] $t_{1/2}$**	**ACTIVE METABOLITE(S)**	**THERAPEUTIC[b] PLASMA CONCENTRATION**	**LOADING DOSES**	**MAINTENANCE DOSES**
Adenosine					✓	<10 sec			6–12 mg (IV)	
Amiodarone	↓ absorption	✓		✓		wks–mos	✓	0.5–2 μg/mL	400–1200 mg/d × 1–3 wk (IV: 1050 mg over 24 h)	100–300 mg/d; IV: 0.25 mg/min
Bretylium	↓ absorption		✓			7–15 h			150–300 mg (IV)	1–4 mg/min (IV)
Digoxin	~80%		✓			36 h		0.5–2.0 ng/mL	0.5–1 mg over 12–24 h	0.0625–0.25 mg q24h
Diltiazem	↓ due to first-pass metabolism			✓		4 h	(x)		0.25–0.35 mg/kg over 10 min (IV)	5–15 mg/h (IV); 120–360 mg/d in 3–4 divided doses; 120–360 mg q24h[d]
Disopyramide	>80%		✓	✓		4–10 h	(x)	2–5 μg/mL		100–200 mg q6h; 200–400 mg q12h[d] (controlled release[e])
Dofetilide	>80%		✓			7–10 h				0.25–0.5 mg q12h
Dronedarone	↓ due to first-pass metabolism	>98%		✓		13–19 h	✓			400 mg q12h
Esmolol					✓	5–10 min			500 μg/kg over 1 min (IV)	50–200 μg/kg/min (IV)
Flecainide	>80%		✓	✓		10–18 h		0.2–1 μg/mL		50–150 mg q12h
Ibutilide	↓ due to first-pass metabolism			✓		6 h			1 mg (IV) over 10 min; may repeat once 10 min later	
Lidocaine	↓ due to first-pass metabolism	✓		✓		120 min	(x)	1.5–5 μg/mL	3 mg/kg in divided doses over 20–30 min (IV)	0.5–3 mg/min (IV)
Mexiletine	>80%			✓		9–15 h		0.5–2 μg/mL	400 mg	100–300 mg q8h[e]

(Continued)

TABLE 34–5 ■ PHARMACOKINETIC CHARACTERISTICS AND DOSES OF ANTIARRHYTHMIC DRUGS (*CONTINUED*)

DRUG	BIOAVAILABILITY		ELIMINATION						USUAL DOSES^{c}	
	REDUCED FIRST-PASS METABOLISM	PROTEIN BINDING >80%	RENAL	HEPATIC	OTHER	ELIMINATION^{a} $t_{1/2}$	ACTIVE METABOLITE(S)	THERAPEUTIC^{b} PLASMA CONCENTRATION	LOADING DOSES	MAINTENANCE DOSES
Procainamide	>80%		✓	✓		3–4 h	✓	4–8 μg/mL	10–17 mg/kg (IV) at a rate of 20 mg/min	1–4 mg/min (IV); 250–750 mg q3h; 500–1000 mg q6h^{d}
(*N*-Acetyl procainamide)	(>80%)		(✓)			(6–10 h)		(10–20 μg/mL)		
Propafenone	↓ due to first-pass metabolism			✓		2–32 h	✓	<1 μg/mL		150–300 mg q8h; 225–425 mg q12h^{d}
Propranolol	↓ due to first-pass metabolism	✓		✓		4 h			1 mg over 1 min, may repeat q2min twice (IV)	10–80 mg q6–8h; 80–240 mg q24h^{d}
Quinidine	>80%	~80%	(x)	✓		4–10 h	✓	2–5 μg/mL		324–648 mg (gluconate) q8h; 200–400 mg q6h (sulfate)
Sotalol	>80%		✓			8 h				80–160 mg q12h
Verapamil	↓ due to first-pass metabolism	✓		✓		3–7 h	✓		5–10 mg over ≥2 min (IV)	80–160 mg q8h; 180–480 mg q24h^{d}

✓ Indicates an effect that affects the clinical action of the drug. (x) Indicates metabolite or route of elimination probably of minor clinical importance. ^{a}The elimination $t_{1/2}$ is one, but not the only, determinant of how frequently a drug must be administered to maintain a therapeutic effect and avoid toxicity (see Chapter 2). For some drugs with short elimination half-lives, infrequent dosing is nevertheless possible, e.g., verapamil. Formulations that allow slow release into the gastrointestinal tract of a rapidly eliminated compound (available for many drugs, including procainamide, disopyramide, verapamil, diltiazem, and propranolol) also allow infrequent dosing. ^{b}The therapeutic range is bounded by a plasma concentration below which no therapeutic effect is likely and an upper concentration above which the risk of adverse effects increases. Many serious adverse reactions to antiarrhythmic drugs can occur at "therapeutic" concentrations in susceptible individuals. When only an upper limit is cited, a lower limit has not been well defined. Variable generation of active metabolites may further complicate the interpretation of plasma concentration data (see Chapter 2). ^{c}Oral doses are presented unless otherwise indicated. Doses are presented as suggested ranges in adults of average build; lower doses are less likely to produce toxicity. Lower maintenance dosages may be required in patients with renal or hepatic disease. Loading doses are only indicated when a therapeutic effect is desired before maintenance therapy would bring drug concentrations into a therapeutic range—that is, for acute therapy (e.g., lidocaine, verapamil, adenosine) or when the elimination $t_{1/2}$ is extremely long (amiodarone). ^{d}Indicates suggested dosage using slow-release formulation. IV, intravenous. ^{e}Can be given as a q12h dosing schedule to improve convenience and compliance.

currents, it can be antiarrhythmic by increasing AV nodal refractoriness and by inhibiting DADs elicited by sympathetic stimulation.

Administration of an intravenous bolus of *adenosine* to humans transiently slows sinus rate and AV nodal conduction velocity and increases AV nodal refractoriness. A bolus of *adenosine* can produce transient sympathetic activation by interacting with carotid baroreceptors; a continuous infusion can cause hypotension.

Adverse Effects

A major advantage of *adenosine* therapy is that adverse effects are short-lived because the drug is transported into cells and deaminated so rapidly. Transient asystole (lack of any cardiac rhythm whatsoever) is common but usually lasts less than 5 sec and is in fact the therapeutic goal. Most patients feel a sense of chest fullness and dyspnea when therapeutic doses (6–12 mg) of *adenosine* are administered. Rarely, an *adenosine* bolus can precipitate AF, presumably by heterogeneously shortening atrial action potentials, or bronchospasm.

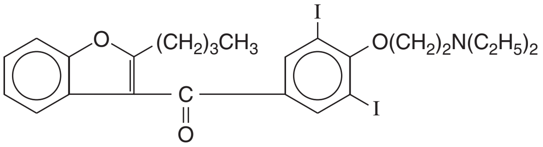
Clinical Pharmacokinetics

Adenosine is eliminated with a half-life of seconds by carrier-mediated uptake, which occurs in most cell types, including the endothelium, followed by metabolism by adenosine deaminase. *Adenosine* is an example of a drug whose efficacy requires a rapid bolus dose, preferably through a large central intravenous line; slow administration results in elimination of the drug prior to its arrival at the heart.

The effects of *adenosine* are potentiated in patients receiving *dipyridamole*, an adenosine-uptake inhibitor, and in patients with cardiac transplants owing to denervation hypersensitivity. Methylxanthines (see Chapters 16 and 44) such as *theophylline* and caffeine block adenosine receptors; therefore, larger-than-usual doses are required to produce an antiarrhythmic effect in patients who have consumed these agents in beverages or as therapy.

Amiodarone

Amiodarone exerts a multiplicity of pharmacological effects, none of which is clearly linked to its arrhythmia-suppressing properties. *Amiodarone* is a structural analogue of thyroid hormone, and some of its antiarrhythmic actions and its toxicity may be attributable to interaction with nuclear thyroid hormone receptors. *Amiodarone* is highly lipophilic, is concentrated in many tissues, and is eliminated extremely slowly; consequently, adverse effects may resolve very slowly. In the U.S., the drug is indicated for oral therapy in patients with recurrent VT or VF resistant to other drugs. In addition, the intravenous form is a first-line drug for management of VT or VF causing cardiac arrest (Dorian et al., 2002). Oral *amiodarone* had a modest beneficial effect on mortality in heart failure patients in a meta-analysis (Amiodarone Trials Meta-Analysis Investigators, 1997) but not in a later randomized clinical trial (Sudden Cardiac Death in Heart Failure Trial [SCD-HeFT] Investigators, 2005). Despite uncertainties about its mechanisms of action and the potential for serious toxicity, *amiodarone* is used widely in the treatment of common arrhythmias such as AF (Roy et al., 2000).



AMIODARONE

Pharmacological Effects

Studies of the acute effects of *amiodarone* in *in vitro* systems are complicated by its insolubility in water, necessitating the use of solvents such as dimethyl sulfoxide, which can have electrophysiological effects on its own. *Amiodarone's* effects may be mediated by perturbation of the lipid environment of the ion channels. *Amiodarone* blocks inactivated Na^+ channels and has a relatively rapid rate of recovery (time constant ≈ .6 sec) from block. It also decreases Ca^{2+} current and transient outward delayed rectifier and inward rectifier K^+ currents and exerts a noncompetitive adrenergic-blocking effect. *Amiodarone* potently inhibits abnormal automaticity and, in most tissues, prolongs action potential duration. *Amiodarone* decreases conduction velocity by Na^+ channel block and by a poorly understood effect on cell-cell coupling that may be especially important in diseased tissue. Prolongations of the PR, QRS, and QT intervals and sinus bradycardia are frequent during chronic therapy. *Amiodarone* prolongs refractoriness in all cardiac tissues; Na^+ channel block and delayed repolarization owing to K^+ channel block, and inhibition of cell-cell coupling all may contribute to this effect.

Adverse Effects

Hypotension owing to vasodilation and depressed myocardial performance are frequent with the intravenous form of *amiodarone* and may be due in part to the solvent. While depressed contractility can occur during long-term oral therapy, it is unusual. Despite administration of high doses that would cause serious toxicity if continued long term, adverse effects are unusual during oral drug-loading regimens, which typically require several weeks. Occasional patients develop nausea during the loading phase, which responds to a decrease in daily dose.

Adverse effects during long-term therapy reflect both the size of daily maintenance doses and the cumulative dose, suggesting that tissue accumulation may be responsible. The most serious adverse effect during chronic *amiodarone* therapy is pulmonary fibrosis, which can be rapidly progressive and fatal. Underlying lung disease, doses of 400 mg/d or more, and recent pulmonary insults such as pneumonia appear to be risk factors. Serial chest X-rays or pulmonary function studies may detect early *amiodarone* toxicity but monitoring plasma concentrations has not been useful. With low doses, such as 200 mg/d or less as used in AF, pulmonary toxicity is less common (Zimetbaum, 2007). Other adverse effects during long-term therapy include corneal microdeposits (which are often asymptomatic), hepatic dysfunction, neuromuscular symptoms (most commonly peripheral neuropathy or proximal muscle weakness), photosensitivity, and hypo- or hyperthyroidism. The multiple effects of *amiodarone* on thyroid function are discussed further in Chapter 47. Treatment consists of withdrawal of the drug and supportive measures, including corticosteroids, for life-threatening pulmonary toxicity; reduction of dosage may be sufficient if the drug is deemed necessary and the adverse effect is not life threatening. While torsades de pointes and other drug-induced tachyarrhythmias can occur, they are unusual despite the marked QT prolongation and bradycardia typical of chronic therapy.

Clinical Pharmacokinetics

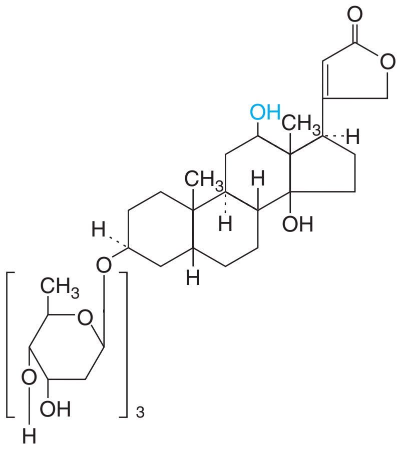
Amiodarone's oral bioavailability is about 30%, presumably due to poor absorption. This incomplete bioavailability is important in calculating equivalent dosing regimens when converting from intravenous to oral therapy. The drug distributes into lipid; heart tissue-to-plasma concentration ratios of greater than 20:1 and lipid-to-plasma ratios of greater than 300:1 have been reported. After the initiation of *amiodarone* therapy, increases in refractoriness, a marker of pharmacological effect, require several weeks to develop. *Amiodarone* undergoes hepatic metabolism by CYP3A4 to desethyl-amiodarone, a metabolite with pharmacological effects similar to those of the parent drug. When *amiodarone* therapy is withdrawn from a patient who has been receiving therapy for several years, plasma concentrations decline with a half-life of weeks to months. The mechanisms of *amiodarone* and desethyl-amiodarone elimination are not well established.

A therapeutic plasma *amiodarone* concentration range of 0.5 to 2 μg/mL has been proposed. However, efficacy apparently depends as much on duration of therapy as on plasma concentration, and elevated plasma concentrations do not predict toxicity. Because of *amiodarone's* slow accumulation in tissue, a high-dose oral loading regimen (e.g., 800–1600 mg/d) usually is administered for several weeks before maintenance therapy is started. If the presenting arrhythmia is life threatening, maintenance dosages of more than 300 mg/d can be used. On the other hand, maintenance doses of 200 mg/d or less are used if recurrence of an arrhythmia would be tolerated, as in patients with AF, because amiodarone slows the ventricular rate during AF.

Dosage adjustments are not required in hepatic, renal, or cardiac dysfunction. *Amiodarone* potently inhibits the hepatic metabolism or renal elimination of many compounds. Mechanisms identified to date include inhibition of CYP3A4, CYP2C9, and P-glycoprotein (see Chapters 5 and 7). Dosages of *warfarin*, other antiarrhythmics (e.g., *flecainide*, *procainamide*, and *quinidine*), or *digoxin* usually require reduction during amiodarone therapy.

Bretylium

Bretylium is a quaternary ammonium compound that prolongs cardiac action potentials and interferes with reuptake of norepinephrine by sympathetic neurons; both actions may be antiarrhythmic (Heissenbuttel and Bigger, 1979). Intravenous *bretylium* to treat VF and prevent its recurrence became unavailable in 2011 and was reintroduced to the U.S. market in 2019 (see Table 34–5 for loading and maintenance doses).



DIGOXIN

Pharmacological Effects

Bretylium prolongs cardiac action potentials likely through block of K$^+$ channels. *Bretylium* has no effect on Na$^+$ channels, except at high concentrations, and no direct effect on automaticity. In animals and humans, administration of *bretylium* initially results in increased norepinephrine release from sympathetic neurons and inhibition of subsequent reuptake.

Adverse Effects

As a result of norepinephrine release, *bretylium* can produce transient hypertension and increased arrhythmias; this effect rarely is observed, since *bretylium* is used in critically ill patients who often are hemodynamically unstable. In theory, *bretylium* should be avoided in patients who are especially prone to increased arrhythmias with norepinephrine release, such as those with *digitalis* intoxication. In contrast, hypotension due to inhibition of norepinephrine reuptake is a common problem during *bretylium* therapy. *Bretylium*-induced hypotension should be managed with judicious fluid replacement if possible. Since *bretylium* effectively results in sympathetic denervation, the administration of normal doses of catecholamines such as dopamine may cause marked hypertension. *Bretylium* should be used only with great caution when the drug's vasodilating effects may be particularly hazardous, as in patients with aortic stenosis, carotid occlusive disease, or hypertrophic cardiomyopathy. The occurrence of torsades de pointes is unusual during *bretylium* therapy.

Clinical Pharmacokinetics

Bretylium is excreted unchanged by the kidneys without undergoing significant hepatic metabolism. Reduction of a maintenance infusion rate has been recommended in patients with renal failure, although adverse effects due to accumulation of *bretylium* in plasma have not been seen. A lag time of approximately 2 h has been reported between peak plasma *bretylium* concentrations and peak prolongation of ventricular refractoriness after an intravenous dose in dogs. This lag time suggests that *bretylium* is distributed to sites in peripheral tissues prior to exerting its pharmacological effect.

The hypotensive effects of *bretylium* are inhibited by coadministration of tricyclic antidepressants, which block *bretylium's* effects on sympathetic norepinephrine release and reuptake, while limited data suggest that its antiarrhythmic effects may be preserved.

Digoxin

Pharmacological Effects

Digitalis glycosides exert positive inotropic effects and have been used in heart failure; now, they are less commonly prescribed (see Chapter 33). Their inotropic action results from increased intracellular Ca^{2+}, which also forms the basis for arrhythmias related to cardiac glycoside intoxication. Cardiac glycosides increase phase 4 slope (i.e., increase the rate of automaticity), especially if $[K]_o$ is low. These drugs (e.g., *digoxin*) also exert prominent vagotonic actions, resulting in inhibition of Ca^{2+} currents in the AV node and activation of acetylcholine-mediated K$^+$ currents in the atrium. Thus, the major "indirect" electrophysiological effects of cardiac glycosides are hyperpolarization, shortening of atrial action potentials, and increases in AV nodal refractoriness. The last action accounts for the utility of *digoxin* in terminating reentrant arrhythmias involving the AV node and in controlling ventricular response in patients with AF. Cardiac glycosides may be especially useful in the last situation because many such patients have heart failure, which can be exacerbated by other AV nodal–blocking drugs such as Ca^{2+} channel blockers or β blockers. However, sympathetic drive is increased markedly in many patients with advanced heart failure, so *digitalis* is not very effective in decreasing the rate; on the other hand, even a modest decrease in rate can ameliorate heart failure.

Similarly, in other conditions in which high sympathetic tone drives rapid AV conduction (e.g., chronic lung disease and thyrotoxicosis), *digitalis* therapy may be only marginally effective in slowing the rate. In heart transplant patients, in whom innervation has been ablated, cardiac glycosides are ineffective for rate control. Increased sympathetic activity and hypoxia can potentiate *digitalis*-induced changes in automaticity and DADs, thus increasing the risk of *digitalis* toxicity. A further complicating feature in thyrotoxicosis is increased *digoxin* clearance.

The major ECG effects of cardiac glycosides are PR prolongation and a nonspecific alteration in ventricular repolarization (manifested by depression of the ST segment), whose underlying mechanism is not well understood.

Adverse Effects

Because of the low therapeutic index of cardiac glycosides, their toxicity is a common clinical problem (see Chapter 33). Arrhythmias, nausea, disturbances of cognitive function, and blurred or yellow vision are the usual manifestations. Elevated serum concentrations of *digitalis*, hypoxia (e.g., owing to chronic lung disease), and electrolyte abnormalities (e.g., hypokalemia, hypomagnesemia, and hypercalcemia) predispose patients to *digitalis*-induced arrhythmias. While *digitalis* intoxication can cause virtually any arrhythmia, certain types of arrhythmias are characteristic. Arrhythmias that should raise a strong suspicion of *digitalis* intoxication are those in which DAD-related tachycardias occur along with impairment of sinus node or AV nodal function. Atrial tachycardia with AV block is classic, but ventricular bigeminy (sinus beats alternating with beats of ventricular origin), "bidirectional" VT (a rare entity), AV junctional tachycardias, and various degrees of AV block also can occur. With severe intoxication (e.g., with suicidal ingestion), severe hyperkalemia owing to poisoning of Na$^+$, K$^+$–ATPase and profound bradyarrhythmias, which may be unresponsive to pacing therapy, are seen. In patients with elevated serum *digitalis* levels, the risk of precipitating VF by DC cardioversion is likely increased; in those with therapeutic blood levels, DC cardioversion can be used safely.

Minor forms of cardiac glycoside intoxication may require no specific therapy beyond monitoring cardiac rhythm until symptoms and signs of toxicity resolve. Sinus bradycardia and AV block often respond to intravenous *atropine*, but the effect is transient. Mg^{2+} has been used successfully in some cases of *digitalis*-induced tachycardia. Any serious arrhythmia should be treated with *antidigoxin Fab fragments* (Digibind, Digifab), which are highly effective in binding *digoxin* and *digitoxin* and greatly enhance their renal excretion (see Chapter 33). Serum glycoside concentrations rise markedly with antidigitalis antibodies, but these represent bound (pharmacologically inactive) drug. Temporary cardiac pacing may be required for advanced sinus node or AV node dysfunction. *Digitalis* exerts direct arterial vasoconstrictor effects, which can be especially deleterious in patients with advanced atherosclerosis who receive intravenous drug; mesenteric and coronary ischemia have been reported.

Clinical Pharmacokinetics

The only digitalis glycoside used in the U.S. is *digoxin*. *Digitoxin* (various generic preparations) also is used for chronic oral therapy outside the U.S. *Digoxin* tablets are incompletely (75%) bioavailable. In some patients, intestinal microflora may metabolize *digoxin*, markedly reducing bioavailability. In these patients, higher-than-usual doses are required for clinical efficacy; toxicity is a serious risk if antibiotics are administered that destroy intestinal microflora. Inhibition of P-glycoprotein (see further discussion) also may play a role in cases of toxicity. *Digoxin* is 20% to 30% protein bound.

The antiarrhythmic effects of *digoxin* can be achieved with intravenous or oral therapy. However, *digoxin* undergoes relatively slow distribution to effector site(s); therefore, even with intravenous therapy, there is a lag of several hours between drug administration and the development of measurable antiarrhythmic effects such as PR interval prolongation or slowing of the ventricular rate in AF. To avoid intoxication, a loading dose of approximately 0.6 to 1 mg *digoxin* is administered over 24 h. Measurement of postdistribution serum *digoxin* concentration and adjustment of the daily dose (0.0625–0.5 mg) to maintain concentrations of 0.5 to 2 ng/mL are useful during chronic *digoxin* therapy (Table 34–5). Some patients may require and tolerate higher concentrations but with an increased risk of adverse effects.

The elimination half-life of *digoxin* ordinarily is about 36 h, so maintenance doses are administered once daily. Renal elimination of unchanged drug accounts for about 80% of *digoxin* elimination. *Digoxin* doses should be reduced (or dosing interval increased) and serum concentrations monitored closely in patients with impaired excretion owing to renal failure or in patients who are hypothyroid. *Digitoxin* undergoes primarily hepatic metabolism and may be useful in patients with fluctuating or advanced renal dysfunction. *Digitoxin* metabolism is accelerated by drugs such as *phenytoin* and *rifampin* that induce hepatic metabolism. *Digitoxin's* elimination half-life is even longer than that of *digoxin* (about 7 days); it is highly protein bound, and its therapeutic range is 10 to 30 ng/mL.

Amiodarone, *dronedarone*, *quinidine*, *verapamil*, *diltiazem*, *cyclosporine*, *itraconazole*, *propafenone*, and *flecainide* decrease *digoxin* clearance by inhibiting P-glycoprotein, the major route of *digoxin* elimination (Fromm et al., 1999). New steady-state *digoxin* concentrations are approached after four to five half-lives (i.e., in about a week). *Digitalis* toxicity results so often with *quinidine* or *amiodarone* that it is routine to decrease the dose of *digoxin* if these drugs are started. In all cases, *digoxin* concentrations should be measured regularly and the dose adjusted if necessary. Hypokalemia, which can be caused by many drugs (e.g., diuretics, *amphotericin B*, and corticosteroids), will potentiate *digitalis*-induced arrhythmias.

Disopyramide

Disopyramide exerts electrophysiological effects very similar to those of *quinidine*, but the drugs have different adverse effect profiles. *Disopyramide* can be used to maintain sinus rhythm in patients with atrial flutter or AF and to prevent recurrence of VT or VF. Because of its negative inotropic effects, it is sometimes used in hypertrophic cardiomyopathy. *Disopyramide* is prescribed as a racemate.

Pharmacological Actions and Adverse Effects

The *in vitro* electrophysiological actions of *S*-(+)-disopyramide are similar to those of quinidine. The *R*-(–)-enantiomer produces similar Na^+ channel block but does not prolong cardiac action potentials. Unlike *quinidine*, racemic *disopyramide* does not antagonize α adrenergic receptors but does exert prominent anticholinergic actions that account for many of its adverse effects. These include precipitation of glaucoma, constipation, dry mouth, and urinary retention; the last is most common in males with prostatism but also occurs in females. *Disopyramide* can cause torsades de pointes and commonly depresses contractility, which can precipitate heart failure. In patients with hypertrophic cardiomyopathy, this depression contractility may be exploited to therapeutic advantage to decrease dynamic outflow tract obstruction (Sherrid and Arabadjian, 2012).

Clinical Pharmacokinetics

Disopyramide is well absorbed. Binding to plasma proteins is concentration dependent, so a small increase in total concentration may represent a disproportionately larger increase in free drug concentration. *Disopyramide* is eliminated by both hepatic metabolism (to a weakly active metabolite) and renal excretion of unchanged drug. The dose should be reduced in patients with renal dysfunction. Higher-than-usual dosages may be required in patients receiving drugs that induce hepatic metabolism, such as *phenytoin*.

Dofetilide

Dofetilide prolongs action potentials and the QT interval by potently blocking the I_{Kr} channel. Increased late Na^+ current, likely due to inhibition of phosphoinositide 3–kinase (Yang et al., 2014), may also contribute. The drug has virtually no extracardiac pharmacological effects. *Dofetilide* is effective in maintaining sinus rhythm in patients with AF. In the DIAMOND studies (Torp-Pedersen et al., 1999), *dofetilide* did not affect mortality in patients with advanced heart failure or in those convalescing from acute myocardial infarction.

Adverse Effects

Torsades de pointes occurred in 1% to 3% of patients in clinical trials where strict exclusion criteria (e.g., hypokalemia) were applied, doses were adjusted based on renal function, and continuous ECG monitoring was used to detect marked QT prolongation in the hospital. Other adverse effects were no more common than with placebo during premarketing clinical trials.

Clinical Pharmacokinetics

Most of a dose of *dofetilide* is excreted unchanged by the kidneys. In patients with mild-to-moderate renal failure, decreases in dosage based on creatinine clearance are required to minimize the risk of torsades de pointes. The drug should not be used in patients with advanced renal failure or with inhibitors of renal cation transport. *Dofetilide* also undergoes minor hepatic metabolism.

Dronedarone

Dronedarone is a noniodinated benzofuran derivative of *amiodarone* that is FDA-approved for the treatment of AF and atrial flutter. In randomized placebo-controlled trials, it was effective in maintaining sinus rhythm and reducing the ventricular response rate during episodes of AF (Patel et al., 2009). Compared to *amiodarone*, *dronedarone* treatment is associated with significantly fewer adverse events but is significantly less effective in maintaining sinus rhythm. *Dronedarone* decreased hospital admissions compared to placebo in patients with a history of AF (Hohnloser et al., 2009). In other studies, however, the drug increased mortality in patients with permanent AF (Connolly et al., 2011) and in those with severe heart failure (Kober et al., 2008).

Pharmacological Effects

Like *amiodarone*, *dronedarone* is a blocker of multiple ion currents, including the rapidly activating delayed rectifier K^+ current (I_{Kr}), the slowly activating delayed rectifier K^+ current (I_{Ks}), the inward rectifier K^+ current (I_{K1}), the acetylcholine-activated K^+ current, the peak Na^+ current, and the L-type Ca^{2+} current. It has stronger antiadrenergic effects than *amiodarone*.

Adverse Effects and Drug Interactions

The most common adverse reactions are diarrhea, nausea, abdominal pain, vomiting, and asthenia. *Dronedarone* causes dose-dependent prolongation of the QTc interval, but torsades de pointes is rare. *Dronedarone* is metabolized by CYP3A and is a moderate inhibitor of CYP3A, CYP2D6, and P-glycoprotein. Potent CYP3A4 inhibitors such as *ketoconazole* may increase *dronedarone* exposure by as much as 25-fold. Consequently, *dronedarone* should not be coadministered with potent CYP3A4 inhibitors (e.g., antifungals, macrolide antibiotics). Coadministration with other drugs metabolized by CYP2D6 (e.g., *metoprolol*) or P-glycoprotein (e.g., *digoxin*) may result in increased drug concentrations. *Dronedarone* may cause severe liver injury; the FDA has suggested monitoring of hepatic enzymes for the first 6 months of therapy.

Esmolol

Esmolol is a β_1-selective agent that is metabolized by erythrocyte esterases and so has a very short elimination half-life (9 min). Intravenous *esmolol* is useful in clinical situations in which immediate β adrenergic blockade is desired (e.g., for rate control of rapidly conducted AF). Because of *esmolol's* very rapid elimination, adverse effects due to β adrenergic blockade—should they occur—dissipate rapidly when the drug is stopped. Although methanol is a metabolite of *esmolol*, methanol intoxication has not been a clinical problem. The pharmacology of *esmolol* is described in further detail in Chapter 13.

Flecainide

The effects of *flecainide* therapy are thought to be attributable to the drug's very long τ_{recovery} from Na^+ channel block. Suppression of DADs triggered by RyR2 Ca^{2+} release also contributes to *flecainide's* antiarrhythmic effect (Kryshtal et al., 2021). In CAST, *flecainide* increased mortality in patients convalescing from myocardial infarction (Echt et al., 1991). However, it continues to be used for arrhythmias in patients in whom structural heart disease is absent (Henthorn et al., 1991); this includes the maintenance of sinus rhythm in patients with supraventricular arrhythmias (i.e., AF) and incessant ventricular ectopy. Clinical case series suggested long-term *flecainide* efficacy in two congenital ventricular arrhythmia syndromes: type 3 LQTS due to mutations that cause late Na^+ currents and CPVT due to mutations that cause "leaky" RyR2 SR Ca^{2+} release channels. As discussed previously and supported by data from a recent randomized clinical trial (Kannankeril et al., 2017), *flecainide* has become the drug of choice for preventing arrhythmias in CPVT patients uncontrolled by β blockers.

Pharmacological Effects

Flecainide blocks Na^+ currents, spontaneous SR Ca^{2+} release, and delayed rectifier K^+ current (I_{Kr}) *in vitro* at similar concentrations. It also blocks Ca^{2+} currents *in vitro*. Action potential duration is shortened in Purkinje cells, probably owing to block of late-opening Na^+ channels, but is prolonged in ventricular cells, probably owing to block of delayed rectifier current. *Flecainide* does not cause EADs *in vitro* but has been associated with rare cases of torsades de pointes. In atrial tissue, *flecainide* disproportionately prolongs action potentials at fast rates, an especially desirable antiarrhythmic drug effect; this effect contrasts with that of *quinidine*, which prolongs atrial action potentials to a greater extent at slower rates. *Flecainide* prolongs the duration of PR, QRS, and QT intervals even at normal heart rates. *Flecainide* is also an open channel blocker of RyR2 Ca^{2+} release channels and prevents arrhythmogenic Ca^{2+} release from the SR and hence DADs in isolated myocytes (Hilliard et al., 2010). The RyR2 channel block by *flecainide* directly targets the underlying molecular defect in patients with mutations in the RyR2 gene and the cardiac calsequestrin gene, which may explain why *flecainide* suppresses ventricular arrhythmias in patients with CPVT refractory to β blocker therapy (Kannankeril et al., 2017; Watanabe et al., 2009). Recent experimental work suggests that RyR2 block is the principal mechanism of *flecainide* action in CPVT (Kryshtal et al., 2021).

Adverse Effects

Flecainide produces few subjective complaints in most patients; dose-related blurred vision is the most common noncardiac adverse effect. It can exacerbate congestive heart failure in patients with depressed left ventricular performance. The most serious adverse effects are provocation or exacerbation of potentially lethal arrhythmias. These include acceleration of ventricular rate in patients with atrial flutter, increased frequency of episodes of reentrant VT, and increased mortality in patients convalescing from myocardial infarction. *Flecainide* also can cause heart block in patients with conduction system disease. Overdose causes very wide QRS complexes and hemodynamic collapse; intravenous sodium bicarbonate has been used in this setting. As discussed previously, it is likely that all these effects can be attributed to Na^+ channel block.

Clinical Pharmacokinetics

Flecainide is well absorbed. The elimination $t_{1/2}$ is shorter with urinary acidification (10 h) than with urinary alkalinization (17 h), but it is nevertheless sufficiently long to allow dosing twice daily (Table 34–5). Elimination occurs by both renal excretion of unchanged drug and hepatic metabolism to inactive metabolites. The latter is mediated by the polymorphically distributed enzyme CYP2D6 (see Pharmacogenetics in Chapter 7). However, even in patients in whom this pathway is absent due to genetic polymorphism or inhibition by other drugs (e.g., *quinidine* or *fluoxetine*), renal excretion ordinarily is sufficient to prevent drug accumulation. In the rare patient with renal dysfunction and lack of active CYP2D6, *flecainide* may accumulate to toxic plasma concentrations. *Flecainide* is a racemate, but there are no differences in the electrophysiological effects or disposition kinetics of its enantiomers. Some reports have suggested that plasma *flecainide* concentrations greater than 1 μg/mL should be avoided to minimize the risk of *flecainide* toxicity; however, in susceptible patients, the adverse electrophysiological effects of *flecainide* therapy can occur at therapeutic plasma concentrations.

Ibutilide

Ibutilide is an I_{Kr} blocker that can also activate an inward Na^+ current (Murray, 1998). The action potential–prolonging effect of the drug may arise from either mechanism. *Ibutilide* is administered as a rapid infusion (1 mg over 10 min) for the immediate conversion of AF or atrial flutter to sinus rhythm. The drug's efficacy rate is higher in patients with atrial flutter (50%–70%) than in those with AF (30%–50%). In AF, the conversion rate is lower in those in whom the arrhythmia has been present for weeks or months compared with those in whom it has been present for days. The major toxicity with *ibutilide* is torsades de pointes, which occurs in up to 6% of patients and requires immediate cardioversion in up to one-third of these patients. The drug undergoes extensive first-pass metabolism, so it is not used orally. It is eliminated by hepatic metabolism and has a $t_{1/2}$ of 2 to 12 h (average 6 h).

Lidocaine

Lidocaine is a local anesthetic that also is useful in the acute intravenous therapy of ventricular arrhythmias. When *lidocaine* was administered to all patients with suspected myocardial infarction, the incidence of VF was reduced. However, survival to hospital discharge tended to be decreased, perhaps because of *lidocaine*-exacerbated heart block or congestive heart failure. Therefore, *lidocaine* no longer is administered routinely to all patients in coronary care units.

Pharmacological Effects

Lidocaine blocks both open and inactivated cardiac Na^+ channels. *In vitro* studies suggested that *lidocaine*-induced block reflects an increased likelihood that the Na^+ channel protein assumes a nonconducting conformation in the presence of drug (Balser et al., 1996). Recovery from block is rapid, so *lidocaine* exerts greater effects in depolarized (e.g., ischemic) or rapidly driven tissues. *Lidocaine* is not useful in atrial arrhythmias, possibly because atrial action potentials are so short that the Na^+ channel is in the inactivated state only briefly compared with diastolic (recovery) times, which are relatively long. In some studies, *lidocaine* increased current through inward rectifier channels, but the clinical significance of this effect is not known. *Lidocaine* can hyperpolarize Purkinje fibers depolarized by low $[K]_o$ or stretch; the resulting increased conduction velocity may be antiarrhythmic in reentry.

Lidocaine decreases automaticity by reducing the slope of phase 4 and altering the threshold for excitability. Action potential duration usually is unaffected or is shortened; such shortening may be due to block of the few Na^+ channels that inactivate late during the cardiac action potential. *Lidocaine* usually exerts no significant effect on PR or QRS duration; QT is unaltered or slightly shortened. The drug exerts little effect on hemodynamic function, although rare cases of *lidocaine*-associated exacerbations of heart failure have been reported, especially in patients with very poor left ventricular function. For additional information on *lidocaine*, see Chapter 25 on local anesthetics.

Adverse Effects

When a large intravenous dose of *lidocaine* is administered rapidly, seizures can occur. When plasma concentrations of the drug rise slowly above the therapeutic range, as may occur during maintenance therapy, tremor, dysarthria, and altered levels of consciousness are more common. Nystagmus is an early sign of *lidocaine* toxicity.

Clinical Pharmacokinetics

Lidocaine is well absorbed but undergoes extensive though variable first-pass hepatic metabolism; thus, oral use of the drug is inappropriate. In theory, therapeutic plasma concentrations of *lidocaine* may be maintained by intermittent intramuscular administration, but the intravenous route is preferred (Table 34–5). *Lidocaine's* metabolites, glycine xylidide (GX) and monoethyl GX, are less potent as Na^+ channel blockers than the parent drug. GX and *lidocaine* appear to compete for access to the Na^+ channel, suggesting that with infusions during which GX accumulates, *lidocaine's* efficacy may be diminished. With infusions lasting longer than 24 h, the clearance of *lidocaine* falls—an effect that may result from competition between parent drug and metabolites for access to hepatic drug-metabolizing enzymes.

Plasma concentrations of *lidocaine* decline biexponentially after a single intravenous dose, indicating that a multicompartment model is necessary to analyze *lidocaine* disposition. The initial drop in plasma *lidocaine* following intravenous administration occurs rapidly, with a $t_{1/2}$ of about 8 min, and represents distribution from the central compartment to peripheral tissues. The terminal elimination $t_{1/2}$ of about 2 h represents drug elimination by hepatic metabolism. *Lidocaine's* efficacy depends on maintenance of therapeutic plasma concentrations in the central compartment. Therefore, the administration of a single bolus dose of *lidocaine* can result in transient arrhythmia suppression that dissipates rapidly as the drug is distributed and concentrations in the central compartment fall. To avoid this distribution-related loss of efficacy, a loading regimen of 3 to 4 mg/kg over 20 to 30 min is used (e.g., an initial 100 mg followed by 50 mg every 8 min for three doses). Subsequently, stable concentrations can be maintained in plasma with an infusion of 1 to 4 mg/min, which replaces drug removed by hepatic metabolism. The time to steady-state *lidocaine* concentrations is approximately 8–10 h. If the maintenance infusion rate is too low, arrhythmias may recur hours after the institution of apparently successful therapy. On the other hand, if the rate is too high, toxicity may result. In either case, routine measurement of plasma *lidocaine* concentration at the time of expected steady state is useful in adjusting maintenance infusion rate.

In heart failure, the central volume of distribution is decreased, so the total loading dose should be decreased. Because *lidocaine* clearance also is decreased, the rate of the maintenance infusion should be decreased. *Lidocaine* clearance also is reduced in hepatic disease, during treatment with *cimetidine* or β blockers, and during prolonged infusions. Frequent measurement of plasma *lidocaine* concentration and dose adjustment to ensure that plasma concentrations remain within the therapeutic range (1.5–5 μg/mL) are necessary to minimize toxicity in these settings. *Lidocaine* is bound to the acute-phase reactant α_1-acid glycoprotein. Diseases such as acute myocardial infarction are associated with increases in α_1-acid glycoprotein and protein binding and hence a decreased proportion of free drug. These findings may explain why some patients require and tolerate higher-than-usual total plasma *lidocaine* concentrations to maintain antiarrhythmic efficacy.

Magnesium

The intravenous administration of 1 to 2 g $MgSO_4$ is effective in preventing recurrent episodes of torsades de pointes, even if the serum Mg^{2+} concentration is normal (Brugada, 2000). However, controlled studies of this effect have not been performed. The mechanism of action is likely related to block of L-type Ca^{2+} currents responsible for the triggered upstroke arising from EADs (black arrow, Figure 34–7B). Following its administration, the QT interval is not shortened. Mg^{2+} is also an inhibitor of RyR2 Ca^{2+} release channels *in vitro*, which may explain why intravenous Mg^{2+} has been used successfully in arrhythmias related to *digitalis* intoxication.

Large placebo-controlled trials of intravenous Mg^{2+} to improve outcome in acute myocardial infarction have yielded conflicting results (ISIS-4 Collaborative Group, 1995; Woods and Fletcher, 1994). While oral Mg^{2+} supplements may be useful in preventing hypomagnesemia, there is no evidence that chronic Mg^{2+} ingestion exerts a direct antiarrhythmic action.

Mexiletine

Mexiletine is an analogue of *lidocaine* that has been modified to reduce first-pass hepatic metabolism and permit chronic oral therapy. The electrophysiological actions are similar to those of *lidocaine*. Tremor and nausea, the major dose-related adverse effects, can be minimized by taking the drug with food.

Mexiletine undergoes hepatic metabolism, which is inducible by drugs such as *phenytoin*. *Mexiletine* is approved for treating ventricular arrhythmias; combinations of *mexiletine* with *quinidine* or *sotalol* may increase efficacy while reducing adverse effects. *In vitro* studies and clinical case series have suggested a role for *mexiletine* (or *flecainide*; see previous discussion) in correcting the aberrant late inward Na^+ current in type 3 congenital LQTS (Roden and Knollmann, 2008).

Procainamide

Procainamide is an analogue of the local anesthetic *procaine* (see Figure 25–1). It exerts electrophysiological effects similar to those of *quinidine* but lacks *quinidine's* vagolytic and α adrenergic–blocking activity. *Procainamide* is better tolerated than *quinidine* when given intravenously. Loading and maintenance intravenous infusions are used in the acute therapy of many supraventricular and ventricular arrhythmias. However, long-term oral treatment is poorly tolerated and often is stopped owing to adverse effects.

Pharmacological Effects

Procainamide is a blocker of open Na^+ channels with an intermediate $\tau_{recovery}$ from block. It also prolongs cardiac action potentials in most tissues, probably by blocking outward K^+ current(s). *Procainamide* decreases automaticity, increases refractory periods, and slows conduction. The major metabolite, *N*-acetyl procainamide, lacks the Na^+ channel–blocking activity of the parent drug but is equipotent in prolonging action potentials. Because the plasma concentrations of *N*-acetyl procainamide often exceed those of *procainamide*, increased refractoriness and QT prolongation during chronic *procainamide* therapy may be partly attributable to the metabolite. However, it is the parent drug that slows conduction and produces QRS interval prolongation. Although hypotension may occur at high plasma concentrations, this effect usually is attributable to ganglionic blockade rather than to any negative inotropic effect, which is minimal.

Adverse Effects

Hypotension and marked slowing of conduction are major adverse effects of high concentrations (>10 μg/mL) of *procainamide*, especially during acute intravenous loading. Dose-related nausea is frequent during oral therapy and may be attributable in part to high plasma concentrations of *N*-acetyl procainamide. Torsades de pointes can occur, particularly when plasma concentrations of *N*-acetyl procainamide rise to greater than 30 μg/mL. *Procainamide* produces potentially fatal bone marrow aplasia in 0.2% of patients; the mechanism is not known, but high plasma drug concentrations are not suspected.

During long-term therapy, most patients will develop biochemical evidence of the drug-induced lupus syndrome, such as circulating antinuclear antibodies. Therapy need not be interrupted merely because of the presence of antinuclear antibodies. However, 25% to 50% of patients eventually develop symptoms of the lupus syndrome; common early symptoms are rash and small-joint arthralgias. Other symptoms of lupus, including pericarditis with tamponade, can occur, although renal involvement is unusual. The lupus-like symptoms resolve on cessation of therapy or during treatment with *N*-acetyl procainamide (see discussion that follows).

Clinical Pharmacokinetics

Procainamide is eliminated rapidly ($t_{1/2}$ ~3–4 h) by both renal excretion of unchanged drug and hepatic metabolism. The major pathway for hepatic metabolism is conjugation by *N*-acetyl transferase, whose activity is determined genetically, to form *N*-acetyl procainamide. *N*-Acetyl procainamide is eliminated by renal excretion ($t_{1/2}$ ~6–10 h) and is not significantly converted back to *procainamide*. Because of the relatively rapid elimination rates of both the parent drug and its major metabolite, oral *procainamide* usually is administered as a slow-release formulation. In patients with renal failure, *procainamide* or *N*-acetyl procainamide can accumulate to potentially toxic plasma concentrations. Reduction of *procainamide* dose and dosing frequency and monitoring of plasma concentrations of both compounds are required in this situation. Because the parent drug and metabolite exert different pharmacological effects, the past practice of using the sum of their concentrations to guide therapy is inappropriate.

In individuals who are "slow acetylators," the *procainamide*-induced lupus syndrome develops more often and earlier during treatment than among rapid acetylators. In addition, the symptoms of *procainamide*-induced lupus resolve during treatment with *N*-acetyl procainamide. Both of these findings support results of *in vitro* studies suggesting that it is chronic exposure to the parent drug (or an oxidative metabolite) that results in the lupus syndrome; these findings also provided one rationale for the further development of *N*-acetyl procainamide and its analogues as antiarrhythmic agents (Roden, 1993).

Propafenone

Propafenone is a Na^+ channel blocker with a relatively slow time constant for recovery from block (Funck-Brentano et al., 1990). Some data suggest that, like *flecainide*, *propafenone* also blocks K^+ channels. Its major electrophysiological effect is to slow conduction in fast-response tissues. The drug is prescribed as a racemate; while the enantiomers do not differ in their Na^+ channel–blocking properties, *S*-(+)-propafenone is a β adrenergic receptor antagonist. *Propafenone* prolongs PR and QRS durations. Chronic therapy with oral *propafenone* is used to maintain sinus rhythm in patients with supraventricular tachycardias, including AF. *R*-(−)propafenone blocks RyR2 channels and may be used as an alternative to *flecainide* in CPVT (Hwang et al., 2011).

Adverse Effects

Adverse effects during *propafenone* therapy include acceleration of ventricular response in patients with atrial flutter, increased frequency or severity of episodes of reentrant VT, exacerbation of heart failure, and the adverse effects of β adrenergic blockade, such as sinus bradycardia and bronchospasm (see previous discussion and Chapter 14).

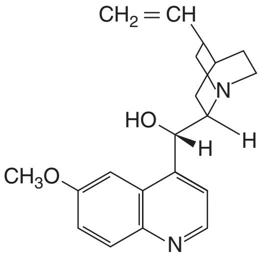
Clinical Pharmacokinetics

Propafenone is well absorbed and is eliminated primarily by CYP2D6-mediated hepatic metabolism (see Chapter 5). In most subjects ("extensive metabolizers"), *propafenone* undergoes extensive first-pass hepatic metabolism to 5-hydroxy propafenone, a metabolite equipotent to *propafenone* as a Na^+ channel blocker but much less potent as a β adrenergic receptor antagonist. A second metabolite, *N*-desalkyl propafenone, is formed by non-CYP2D6–mediated metabolism and is a less-potent blocker of Na^+ channels and β adrenergic receptors. CYP2D6-mediated metabolism of *propafenone* is saturable, so small increases in dose can increase plasma *propafenone* concentration disproportionately. In "poor metabolizer" subjects, in whom CYP2D6 activity is low or absent, first-pass hepatic metabolism is much less than in extensive metabolizers, and plasma *propafenone* concentrations will be much higher after an equal dose. The incidence of adverse effects during *propafenone* therapy is significantly higher in poor metabolizers.

CYP2D6 activity can be inhibited markedly by a number of drugs, including *quinidine* and *fluoxetine*. In extensive metabolizer subjects receiving such drugs or in poor metabolizer subjects, plasma *propafenone* concentrations of more than 1 μg/mL are associated with clinical effects of β adrenergic receptor blockade, such as reduction of exercise heart rate. It is recommended that dosage in patients with moderate-to-severe liver disease should be reduced to approximately 20% to 30% of the usual dose, with careful monitoring. It is not known if *propafenone* doses must be decreased in patients with renal disease. A slow-release formulation allows twice-daily dosing.

Quinidine

As early as the 18th century, the bark of the cinchona plant was used to treat "rebellious palpitations" (Levy and Azoulay, 1994). Studies in the early 20th century identified *quinidine*, a diastereomer of the antimalarial *quinine*, as the most potent of the antiarrhythmic substances extracted from the cinchona plant, and by the 1920s, *quinidine* was used as an antiarrhythmic agent. *Quinidine* is used to maintain sinus rhythm in patients with atrial flutter or AF and to prevent recurrence of VT or VF (Grace and Camm, 1998). *Quinidine* may be especially useful in preventing recurrent VF in unusual congenital arrhythmia syndromes such as Brugada syndrome or short QT syndrome (Inama et al., 2010; Viskin et al., 2013).



QUINIDINE

Pharmacological Effects

Quinidine blocks Na^+ current and multiple cardiac K^+ currents. It is an open-state blocker of Na^+ channels, with a $\tau_{recovery}$ in the intermediate (~3-sec) range. Thus, after therapeutic dosages, QRS duration increases modestly, usually by 10% to 20%. At therapeutic concentrations, *quinidine* commonly prolongs the QT interval up to 25%, but the effect is highly variable. At concentrations as low as 1 μM, *quinidine* blocks Na^+ current and the rapid component of delayed rectifier (I_{Kr}); higher concentrations block the slow component of delayed rectifier, inward rectifier, transient outward current, and L-type Ca^{2+} current.

Quinidine's Na^+ channel–blocking properties result in an increased threshold for excitability and decreased automaticity. As a consequence of its K^+ channel–blocking actions, *quinidine* prolongs action potentials in most cardiac cells, most prominently at slow heart rates. In some cells, such as midmyocardial cells and Purkinje cells, *quinidine* consistently elicits EADs at slow heart rates, particularly when $[K]_o$ is low (Priori et al., 1999). *Quinidine* prolongs refractoriness in most tissues, probably as a result of both prolongation of action potential duration and Na^+ channel blockade.

In intact animals and humans, *quinidine* also produces α adrenergic receptor blockade and vagal inhibition. Thus, the intravenous use of *quinidine* is associated with marked hypotension and sinus tachycardia. *Quinidine's* vagolytic effects tend to inhibit its direct depressant effect on AV nodal conduction, so the effect of drug on the PR interval is variable. Moreover, *quinidine's* vagolytic effect can result in increased AV nodal transmission of atrial tachycardias such as atrial flutter (Table 34–1).

Adverse Effects

Noncardiac. Diarrhea is the most common adverse effect during *quinidine* therapy, occurring in 30% to 50% of patients; the mechanism is not known. Diarrhea usually occurs within the first several days of *quinidine* therapy but can occur later. Diarrhea-induced hypokalemia may potentiate torsades de pointes due to *quinidine*.

Several immunological reactions can occur during *quinidine* therapy. The most common is thrombocytopenia, which can be severe but resolves rapidly with discontinuation of the drug. Hepatitis, bone marrow depression, and lupus syndrome occur rarely. None of these effects are related to elevated plasma *quinidine* concentrations.

Quinidine also can produce cinchonism, a syndrome that includes headache and tinnitus. In contrast to other adverse responses to *quinidine* therapy, cinchonism usually is related to elevated plasma *quinidine* concentrations and can be managed by dose reduction.

Cardiac. Of patients receiving *quinidine* therapy, 2% to 8% will develop marked QT interval prolongation and torsades de pointes. In contrast to effects of *sotalol*, *N*-acetyl procainamide, and many other drugs, *quinidine*-associated torsades de pointes generally occurs at therapeutic or even subtherapeutic plasma concentrations. The reasons for individual susceptibility to this adverse effect are not known.

At high plasma concentrations of *quinidine*, marked Na^+ channel block can occur, with resulting VT. This adverse effect occurs when very high doses of *quinidine* are used to try to convert AF to normal rhythm; this aggressive approach to *quinidine* dosing has been abandoned, and *quinidine*-induced VT is now unusual.

Quinidine can exacerbate heart failure or conduction system disease. However, in most patients with congestive heart failure, *quinidine* is well tolerated, perhaps because of its vasodilating actions.

Clinical Pharmacokinetics

Quinidine is well absorbed and is 80% bound to plasma proteins, including albumin and, like *lidocaine*, the acute-phase reactant α_1-acid glycoprotein. As with *lidocaine*, greater-than-usual doses (and total plasma *quinidine* concentrations) may be required to maintain therapeutic concentrations of free *quinidine* in high-stress states such as acute myocardial infarction. *Quinidine* undergoes extensive hepatic oxidative metabolism, and approximately 20% is excreted unchanged by the kidneys. One metabolite, 3-hydroxyquinidine, is nearly as potent as *quinidine* in blocking cardiac Na^+ channels and prolonging cardiac action potentials. Concentrations of unbound 3-hydroxyquinidine equal to or exceeding those of *quinidine* are tolerated by some patients. Other metabolites are less potent than *quinidine*, and their plasma concentrations are lower; thus, they are unlikely to contribute significantly to the clinical effects of *quinidine*.

There is substantial individual variability in the range of dosages required to achieve therapeutic plasma concentrations of 2 to 5 μg/mL. Some of this variability may be assay dependent because not all assays exclude *quinidine* metabolites. In patients with advanced renal disease or congestive heart failure, *quinidine* clearance is decreased only modestly. Thus, dosage requirements in these patients are similar to those in other patients.

Drug Interactions

Quinidine is a potent inhibitor of CYP2D6. As a result, the administration of *quinidine* to patients receiving drugs that undergo extensive CYP2D6-mediated metabolism may result in altered drug effects owing to accumulation of parent drug and failure of metabolite formation. For example, inhibition of CYP2D6-mediated metabolism of *codeine* to its active metabolite *morphine* results in decreased analgesia. On the other hand, inhibition of CYP2D6-mediated metabolism of *propafenone* results in elevated plasma *propafenone* concentrations and increased β adrenergic receptor blockade. *Quinidine* reduces the clearance of *digoxin* due to inhibition of P-glycoprotein–mediated *digoxin* transport (Fromm et al., 1999). *Dextromethorphan*, a CYP2D6 substrate that undergoes extensive first-pass bioinactivation, has shown promise in treatment of various neurological disorders, notably pseudobulbar affect. A combination of *dextromethorphan* and very-low-dose *quinidine* (30 mg) inhibits the first-pass metabolism, achieves higher systemic concentrations than monotherapy, and is now approved for use in pseudobulbar affect and neuropathic pain (Olney and Rosen, 2010).

Quinidine metabolism is induced by drugs such as *phenobarbital* and *phenytoin*. In patients receiving these agents, very high doses of *quinidine* may be required to achieve therapeutic concentrations. If therapy with the inducing agent is then stopped, *quinidine* concentrations may rise to very high levels, and its dosage must be adjusted downward. *Cimetidine* and *verapamil* also elevate plasma *quinidine* concentrations, but these effects usually are modest.

Drug Facts for Your Personal Formulary: *Antiarrhythmic Agents*

Antiarrhythmic Drug	Therapeutic Uses	Major Toxicity and Clinical Pearls
Class IA: Na^+ Channel Blockers • Slow to intermediate off rate • Concomitant class III action (prolong QT)		
Procainamide	• Acute treatment of AF, VT, and VF • Chronic treatment to prevent AF, VT, and VF	• 40% of patients discontinue within 6 months of therapy due to side effects: hypotension (especially from intravenous use), nausea • QT prolongation and torsades de pointes due to accumulation of active *N*-acetyl metabolite • Lupus-like syndrome (25%–50% with chronic use), especially in genetic slow acetylators • Oral drug no longer available in the U.S.
Quinidine	• Chronic treatment to prevent AF, VT, and VF	• Diarrhea (30%–50% of patients); diarrhea-induced hypokalemia may potentiate torsades de pointes • Marked QT prolongation and high risk (~1%–5%) of torsades de pointes at therapeutic or subtherapeutic concentrations • Immune thrombocytopenia (~1%) • Cinchonism: tinnitus, flushing, blurred vision, dizziness, diarrhea • Potent inhibitor of *CYP2D6* and *ABCB1* (encoding P-glycoprotein): altered effects of digitalis, many antidepressants, and others
Disopyramide	• Chronic treatment to prevent AF, VT, and VF	• Anticholinergic effects (dry eyes, urinary retention, constipation) • Long QT (torsades de pointes) • Depression of contractility can precipitate or worsen heart failure; paradoxically, this can be useful in hypertrophic cardiomyopathy to reduce outflow tract obstruction

Drug Facts for Your Personal Formulary: *Antiarrhythmic Agents* (*continued*)

Antiarrhythmic Drug	Therapeutic Uses	Major Toxicity and Clinical Pearls
Class IB: Na$^+$ Channel Blockers • Fast off rate • Little effect on ECG		
Lidocaine	• Acute treatment of VT and VF	• CNS: seizures and tinnitus • CNS: tremor, hallucinations, drowsiness, coma
Mexiletine	• Chronic treatment to prevent VT and VF	• Tremor and nausea
Class IC: Na$^+$ Channel Blockers • Slow off rate • Prolong PR and broaden QRS intervals		
Flecainide	• Chronic treatment to prevent PSVT, AF, VT, and VF in the absence of structural heart disease • Available in some countries for intravenous use in PSVT, AF • Useful in CPVT uncontrolled by β blockers	• RyR2-blocking effect important for antiarrhythmic efficacy • Much better tolerated than class IA or IB agents • Risk of severe proarrhythmia in patients with structural heart disease; increased mortality in patients with myocardial infarction (CAST) • Blurred vision • Can worsen heart failure
Propafenone	• Chronic treatment to prevent PSVT, AF, VT, and VF in the absence of structural heart disease • Available in some countries for intravenous use in PSVT, AF • Alternative to flecainide for CPVT	• RyR2-blocking effect important for antiarrhythmic efficacy • Also has β adrenergic–blocking effects (worsening of heart failure and bronchospasm), especially prominent in *CYP2D6* poor metabolizers • Risk of severe proarrhythmia in patients with structural heart disease
Class II: β Blockers		
Nadolol Propranolol Metoprolol Many others	• Chronic treatment to prevent arrhythmias in congenital LQTS and CPVT • Rate control in AF • Widely used for other indications (angina, hypertension, migraine, etc.)	• β Adrenergic–blocking effects (worsening of heart failure, bradycardia, bronchospasm) • Nadolol preferred by many for LQTS and CPVT
Esmolol	• Acute treatment to control rate in AF	• Ultrashort $t_{1/2}$, intravenous use only
Class III: K$^+$ Channel Blocker • Increase refractory period (prolong QT)		
Amiodarone	• Drug of choice for acute treatment of VT and VF and to slow ventricular rate and convert AF • Chronic treatment to prevent AF, VT, and VF	• Hypotension, depressed ventricular function and torsades de pointes (*rare*) with intravenous administration • Pulmonary fibrosis with chronic therapy, which can be fatal (requires periodic monitoring of lung function) • Many other adverse effects: corneal microdeposits, hepatotoxicity, neuropathies, photosensitivity, thyroid dysfunction • Note: tissue half-life of several months • Inhibitor of many drug-metabolizing and transport systems, with high potential for drug interactions
Dronedarone	• Chronic treatment to prevent AF	• Amiodarone analogue with lower efficacy than amiodarone • Gastrointestinal disturbances, risk for fatal hepatotoxicity • Increases mortality in patients with severe heart failure
Sotalol	• Chronic treatment to prevent AF, VT, and VF	• Also has β adrenergic–blocking effects • High risk (~1%–5%) of torsades de pointes
Dofetilide	• Chronic treatment to prevent AF	• Few adverse effects except high risk (~1%–5%) of torsades de pointes
Ibutilide	• Acute treatment to convert AF	• High risk (~1%–8%) of torsades de pointes
Class IV: Ca^{2+} Channel Blockers • Nondihydropyridine • Inhibit SA and AV nodes • Prolong PR		
Diltiazem Verapamil	• Acute intravenous use to convert PSVT and for rate control in AF • Chronic treatment to prevent PSVT and control rate in AF	• Hypotension (intravenous) • Sinus bradycardia or AV block especially in combination with β blockers • Constipation • Worsening of heart failure
Antiarrhythmic Drugs with Miscellaneous Mechanisms		
Adenosine (activates A receptors)	• Drug of choice for acute treatment PSVT	• Short $t_{1/2}$ (<5 sec) • Transient asystole • Transient dyspnea • Transient atrial fibrillation (rare)
$MgSO_4$	• Acute treatment of torsades de pointes	• Likely acts by blocking L-type Ca^{2+} channels
Digoxin (Na$^+$, K$^+$–ATPase inhibitor)	• Ventricular rate control in AF • Modest positive inotropic effect	• Adverse effects common and include gastrointestinal symptoms, visual/cognitive dysfunction, and arrhythmias, typically supraventricular arrhythmias with heart block or atrial or ventricular extrasystoles • Severe toxicities (e.g., with overdose) can be treated with antibody • Probably mortality neutral

Sotalol

Sotalol is a nonselective β adrenergic receptor antagonist that also prolongs cardiac action potentials by inhibiting delayed rectifier and possibly other K^+ currents (Hohnloser and Woosley, 1994). *Sotalol* is prescribed as a racemate; the L-enantiomer is a much more potent β adrenergic receptor antagonist than the D-enantiomer, but the two are equipotent as K^+ channel blockers. In the U.S., *sotalol* is approved for use in patients with both ventricular tachyarrhythmias and AF or atrial flutter. Clinical trials suggest that it is as effective as most Na^+ channel blockers in ventricular arrhythmias.

Sotalol prolongs action potential duration throughout the heart and QT interval on the ECG. It decreases automaticity, slows AV nodal conduction, and prolongs AV refractoriness by blocking both K^+ channels and β adrenergic receptors, but it exerts no effect on conduction velocity in fast-response tissue. *Sotalol* causes EADs and triggered activity *in vitro* and can cause torsades de pointes, especially when the serum K^+ concentration is low. Unlike the situation with *quinidine*, the incidence of torsades de pointes (1.5%–2% incidence) seems to depend on the dose of *sotalol*; indeed, torsades de pointes is the major toxicity with *sotalol* overdose. Occasional cases occur at low dosages, often in patients with renal dysfunction, because *sotalol* is eliminated by renal excretion of unchanged drug. The other adverse effects of *sotalol* therapy are those associated with β adrenergic receptor blockade (see previous discussion and Chapter 14).

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Chapter 35

Treatment of Pulmonary Arterial Hypertension

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INTRODUCTION TO PULMONARY HYPERTENSION
- Pulmonary Arterial Hypertension
- Pulmonary Hypertension Associated With Other Disease States
- Routes of Drug Delivery to the Pulmonary Circulation

MECHANISMS OF PULMONARY ARTERIAL HYPERTENSION

CLINICAL USE OF DRUGS FOR PULMONARY HYPERTENSION

PHARMACOTHERAPY FOR PULMONARY HYPERTENSION
- Stimulators of cGMP and PKG Signaling
- Prostacyclin Receptor Agonists
- Endothelin and Endothelin Receptor Antagonists
- Receptor Tyrosine Kinase Inhibitors
- Calcium Channels and Their Blockers

A PHARMACOLOGIST'S VIEW OF SIGNAL INTEGRATION IN PAH

Introduction to Pulmonary Hypertension

The pulmonary circulation plays a unique and essential role in gas exchange and oxygenation of venous blood in the lungs. It is a low-resistance and low-pressure circulatory system; the mean pulmonary arterial pressure (PAP) in healthy control subjects is 14.0 ± 3.3 mmHg. PAP is a function of cardiac output and pulmonary vascular resistance (PVR). Pulmonary hypertension (PH) is defined as a mean PAP greater than 20 mmHg at rest, measured by right heart catheterization (Simonneau et al., 2019). PH can be a primary disorder of the pulmonary vasculature, commonly referred to as pulmonary arterial hypertension (PAH), or can occur as a complication of other cardiopulmonary, vascular, and systemic diseases. Based on shared pathophysiological and pathological characteristics as well as response to therapy, PH is classified into five major groups (Simonneau et al., 2019), as shown in Box 35–1.

Pulmonary Arterial Hypertension

Idiopathic PAH is a rare, progressive, and fatal disease in which vascular changes in the small arteries and arterioles result in progressive increases in PVR, resulting in increased PAP (Kuhr et al., 2012; McLaughlin et al., 2009; Morrell et al., 2009). A heritable form of PAH is caused by heterozygous germline mutations in *BMPR2*, the gene encoding bone morphogenetic protein receptor type 2, a member of the transforming growth factor-β (TGF-β) superfamily (Morrell et al., 2019). Around 70% to 80% of heritable cases of PAH and 10% to 20% of idiopathic PAH cases are caused by mutations in *BMPR2*. Less common PAH genes include *TBX4*, *ACVRL1*, *ENG*, *SMAD9*, and *KCNK3* (0.4%). In patients with PAH, elevated afterload increases stress on the RV, often leading to right heart dysfunction and failure, the major cause of morbidity and mortality in this population (Voelkel et al., 2012). The median survival in untreated disease is 2.8 years, yet with modern therapies, the median survival has been estimated to be about 9 years (Benza et al., 2012). This group of patients is the most well-studied subset of PH and the primary target of available therapeutics.

Pulmonary Hypertension Associated With Other Disease States

Other PH groups represent most cases of PH (see textbox). The presence of PH in these more common diseases portends a much poorer prognosis, often identifying people with multiple comorbidities, late-stage presentations, or more severe disease. While recognition of PH in heart disease carries important prognostic implications, to date, there are no approved targeted therapies for PH in this disease state. Similarly, in lung diseases, the presence of PH is an important risk factor and

BOX 35–1 ■ Classification of Pulmonary Hypertension

Pulmonary arterial hypertension (PAH)
- Idiopathic PAH
- Heritable PAH due to genetic variants
  - *BMPR2* (most common)
  - *TBX4*, *ACVRL1*, *ENG*, *SMAD9*, *KCNK3*
- Drug- and toxin-induced PAH
- PAH associated with:
  - Connective tissue disease
  - HIV infection
  - Portal hypertension
  - Congenital heart disease
  - Schistosomiasis
- PAH long-term responders to calcium channel blockers
- PAH with overt features of venous/capillary involvement
- PAH of the newborn syndrome

PH due to left heart disease
- Heart failure
- Valvular heart disease
- Congenital/acquired cardiovascular conditions leading to postcapillary hypertension

PH due to lung diseases and/or hypoxia
- Obstructive lung disease
- Restrictive lung disease
- Hypoxia without lung disease
- Developmental lung disorders

PH due to pulmonary artery obstructions
- Chronic thromboembolic pulmonary hypertension (CTEPH)
- Other pulmonary artery obstructions

PH with unclear and/or multifactorial mechanisms
- Hematological disorders
- Systemic and metabolic disorders
- Complex congenital heart disease

portends a worse prognosis, but until 2021, there were no approved therapies targeting PH in these populations. Inhaled *treprostinil* is now FDA-approved for treating PH associated with interstitial lung disease, with use and dosing that mirror those in PAH (Waxman et al., 2021). However, in other forms of respiratory disease, there are no currently approved therapies for PH. In patients identified to have chronic thromboembolic pulmonary hypertension (CTEPH), a subtype of PH due to pulmonary artery obstructions, surgical pulmonary thromboendarterectomy is the treatment of choice. Patients determined to be poor surgical candidates or those with persistent PH following surgery respond to pulmonary vasodilator therapy (Fedullo et al., 2011; Ghofrani et al., 2013a).

Abbreviations

BCRP: breast cancer resistance protein
BNP: B-type natriuretic peptide
[Ca^{2+}]$_{cyt}$: cytosolic free Ca^{2+} concentration
CCB: calcium channel blocker
CTEPH: chronic thromboembolic pulmonary hypertension
CYP: cytochrome P450
DAG: diacylglycerol
EC: endothelial cell
ECE: endothelin-converting enzyme
EGF: epidermal growth factor
ER: endoplasmic reticulum
ERA: endothelin receptor antagonist
ET-1: endothelin 1
FDA: U.S. Food and Drug Administration
GPCR: G protein-coupled receptor
IP$_3$: inositol triphosphate
IPR: prostacyclin receptor
mGC: membrane (or particulate) guanylate cyclase
NO: nitric oxide
NO$_2$: nitric dioxide
PA: pulmonary artery
PAH: pulmonary arterial hypertension
PAP: pulmonary arterial pressure
PASMC: pulmonary artery smooth muscle cell
PDE: phosphodiesterase
PDGF: platelet-derived growth factor
PGI$_2$: prostacyclin, prostaglandin I$_2$
Pgp : P-glycoprotein
PH: pulmonary hypertension
PKA: protein kinase A
PKC: protein kinase C
PKG: protein kinase G
PLC: phospholipase C
PVR: pulmonary vascular resistance
REMS: Risk Evaluation and Mitigation Strategy
ROC: receptor-operated Ca^{2+} channel
RV: right ventricle
sGC: soluble guanylate cyclase
SR: sarcoplasmic reticulum
SVR: systemic vascular resistance
TGF-β: transforming growth factor-β
TKR: tyrosine kinase receptor
VDCC: voltage-dependent Ca^{2+} channel
VEGF: vascular endothelial growth factor
VIP: vasoactive intestinal peptide
V/Q: ventilation/perfusion
VSM: vascular smooth muscle

Routes of Drug Delivery to the Pulmonary Circulation

The pulmonary circulation permits delivery of drugs through multiple routes. The pulmonary circulation runs in series with the systemic circulation, receiving the entire cardiac output in each cardiac cycle. Thus, exposure of pulmonary tissue to drugs is excellent and reliable. Continuous intravenous infusion is used to deliver high concentrations of drugs that exhibit short half-lives to the pulmonary circulation while avoiding first-pass metabolism. Alternatively, drug administration by subcutaneous pump may be used to lower the risk of adverse effects. Oral delivery remains a safe, effective, and reliable route for many classes of drugs used to treat PAH. The small pulmonary arteries and precapillary arterioles are also unique in their close proximity to the alveoli and lower airways. Hence, inhalational delivery of therapeutic compounds can directly target the lung vasculature and pulmonary circulation, limit systemic side effects, and preferentially affect well-ventilated parts of the lung to improve ventilation-perfusion matching (see Chapter 44).

Mechanisms of Pulmonary Arterial Hypertension

Pulmonary arterial hypertension is thought to arise from pathophysiological and pathobiological changes in the small pulmonary arteries and arterioles. Regardless of the initial etiological trigger, the putative mechanisms contributing to elevated PVR and PAP include:

- Sustained pulmonary vasoconstriction
- Concentric pulmonary vascular remodeling
- *In situ* thrombosis
- Pulmonary vascular wall stiffening

Each of these mechanisms (Figure 35–1) can contribute to the development and progression of PAH and forms the basis for drug therapy for this disease. Accordingly, an effective therapy for PAH would (1) cause pulmonary vasodilation; (2) exert antiproliferative or proapoptotic effects on highly proliferative cells in the pulmonary vascular wall (e.g., fibroblasts, myofibroblasts, and smooth muscle cells); (3) prevent or resolve *in situ* thrombosis in small arteries and precapillary arterioles; (4) exert antifibrotic effects to attenuate extracellular matrix stiffness; and (5) reduce pulmonary vascular wall stiffness due to myogenic tone and inflammation/cholesterol-associated membrane stiffness (Humbert et al., 2019; Mandegar et al., 2004; Morrell et al., 2009).

Although the cellular and molecular mechanisms leading to these changes in the pulmonary vasculature are complex, an imbalance of vasoactive mediators, mitogenic and angiogenic factors, and pro- and antiapoptotic proteins plays an important role in PAH development. Relative deficiencies of vasodilators such as nitric oxide (NO) and prostacyclin deleteriously accompany an excess of vasoconstrictors such as endothelin 1 (ET-1) and thromboxane A$_2$. NO released by the vascular endothelial cell (EC) normally promotes the production of cyclic GMP in the pulmonary artery smooth muscle cell (PASMC), resulting in PASMC relaxation and pulmonary vasodilation. Prostacyclin, also known as prostaglandin I$_2$ (PGI$_2$), is also released from the EC and promotes the synthesis of cAMP, causing PASMC relaxation and pulmonary vasodilation. In addition, NO and PGI$_2$ both have antiproliferative and anticoagulant effects that inhibit concentric pulmonary vascular wall thickening and *in situ* thrombosis. ET-1 is a potent vasoconstrictor secreted by ECs; it exerts vasoconstrictive and proliferative effects on PASMCs. Other vasoactive mediators such as thromboxane A$_2$, serotonin, and vasoactive intestinal peptide (VIP) appear to play a role in the development of PAH, but the therapeutic potential of targeting these substances has not been well established. Table 35–1 summarizes the changes in these vasoactive mediators and the potential contributions of those changes to the development of PAH.

Sustained vasoconstriction and pulmonary vascular remodeling also result from functional and transcriptional changes in membrane receptors and ion channels on the surface of PASMCs. Several G protein-coupled

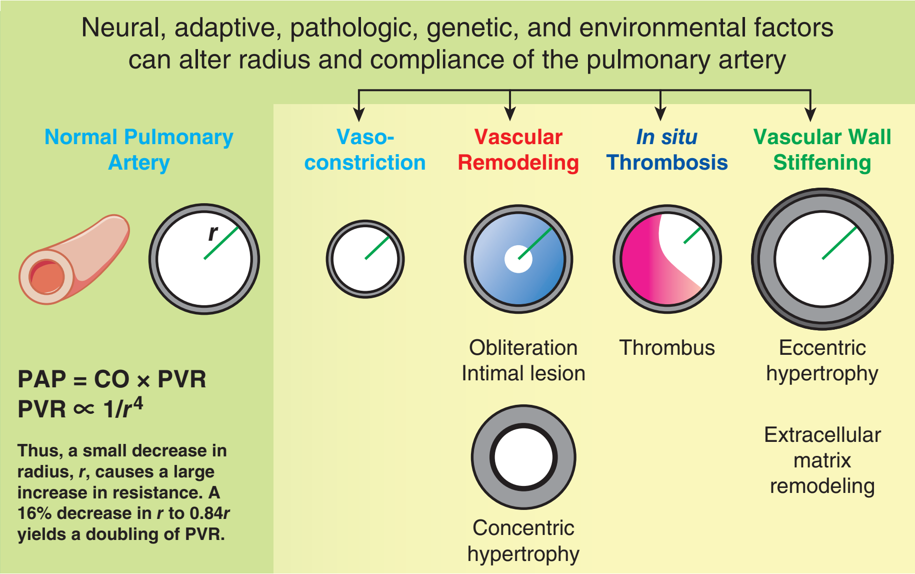

Neural, adaptive, pathologic, genetic, and environmental factors can alter radius and compliance of the pulmonary artery
Normal Pulmonary Artery
r
Vaso-constriction
Vascular Remodeling
In situ Thrombosis
Vascular Wall Stiffening
Obliteration Intimal lesion
Thrombus
Eccentric hypertrophy
Concentric hypertrophy
Extracellular matrix remodeling
PAP = CO × PVR
PVR ∝ 1/r^4
Thus, a small decrease in radius, r, causes a large increase in resistance. A 16% decrease in r to 0.84r yields a doubling of PVR.

Figure 35–1 *Major pathogenic components in the development of PAH.* Vascular remodeling occurs as changes in the intraluminal radius with or without changes in the vascular wall thickness. Changes in intraluminal radius of small pulmonary arteries and arterioles have dramatic effects on the PVR. Pathogenic factors contributing to the development and progression of PAH include sustained vasoconstriction, concentric pulmonary vascular remodeling, *in situ* thrombosis, and vascular wall stiffening.

receptors (GPCRs) and tyrosine kinase receptors (TKRs) are implicated in the development and progression of PAH (Morrell et al., 2009; Schermuly et al., 2011). Increased cytosolic [Ca^{2+}] is an important common pathway by which receptor activation and downstream cellular signaling cascades exert their effects in the pulmonary vasculature. A rise in cytosolic free Ca^{2+} concentration ([Ca^{2+}]$_{cyt}$) in PASMCs is a major trigger for PASMC contraction and an important mediator for PASMC proliferation, migration, and vascular remodeling. Furthermore, ion channels, particularly Ca^{2+}-permeable cation channels and K^+-permeable channels (e.g., KCNA5 and KCNK3) in the plasma membrane of PASMCs, can directly influence [Ca^{2+}]$_{cyt}$ (Mandegar et al., 2004). Changes in activity and expression of ion channels and transporters, such as voltage-dependent Ca^{2+} channel (VDCC), receptor-operated Ca^{2+} channel (ROC), store-operated Ca^{2+} channels, and the Na^+-Ca^{2+} exchanger, are implicated in the development of PAH; all are potential therapeutic targets. Downregulation of K^+-permeable channels in PASMCs leads to membrane depolarization and opening of VDCCs, enhancing Ca^{2+} influx, with a consequent increase in [Ca^{2+}]$_{cyt}$ and further sustained vasoconstriction and vascular remodeling (Kuhr et al., 2012).

TABLE 35–1 ■ ROLES OF VASOACTIVE MEDIATORS IN PAH

EFFECTOR	Δ IN PAP IN PATIENTS WITH PAH	CONSEQUENCE OF ALTERED [EFFECTOR] ON		
		VASCULAR CONTRACTION	THROMBUS FORMATION	CELL PROLIFERATION
NO	↓	↑	↑	–/↑[a]
PGI_2	↓	↑	↑	↑
TxA_2	↑	↑	↑	↑
VIP	↓	↑	↑	↑
5HT	↑	↑	↑	↑
ET	↑	↑	↑[b]	↑

↑, increased, ↓, decreased; –, no change; 5HT, serotonin; TxA_2, thromboxane A_2.

[a],NO also causes vascular smooth muscle cell apoptosis.

↓ in plexiform lesions.

Clinical Use of Drugs for Pulmonary Hypertension

Treatment of PAH must include a proper assessment of symptoms, functional classification, and RV performance for optimal selection of appropriate agents. The most widely used criterion for initiating treatment is the presence of symptoms and impairments in functional capacity, as measured by functional classification. This classification measures the physical limitations imposed on a particular patient from the disease, progressing from class I through class IV (from no impairment through mounting functional limitation to inability to perform physical activity). Clinical trials suggest that class II patients may benefit from therapy, with greater benefit seen in class III. While the overall number of class IV patients is low, these patients with the most severe functional impairments fare far worse. RV dysfunction results from increased PVR, in part, and is the major contributor to morbidity and mortality in this population; therefore, assessment of the RV often is used in conjunction with functional assessment to guide therapy (Figure 35–2).

Goals of treatment include improvement in symptoms, such as dyspnea, fatigue, chest pain, or syncope; improved functional capacity, including 6-min walk distance; and improvement in pulmonary hemodynamics and RV function. Oral formulations, either endothelin receptor antagonists (ERAs), phosphodiesterase (PDE) inhibitors, or soluble guanylate cyclase (sGC) stimulators are usually employed as first-line agents due to ease of use. Treatment of more severe cases generally involves parenteral therapy with *prostacyclin* and prostacyclin analogues (i.e., *epoprostenol* or *treprostinil*), considered the most potent pulmonary vasodilators that also have antiproliferative and antifibrotic effects, yet controversy exists on the initial treatment of patients with moderate-to-severe functional limitations. Sequential combination therapy with the addition of separate classes of medications until treatment goals are achieved is often utilized for severe or progressive disease (Ghofrani and Humbert, 2014). This approach is commonly used in clinical trials and has incremental clinical benefits when agents with different mechanisms of action are combined sequentially. Modest effects with single agents may be enhanced with the use of up-front combinations and could lead to more dramatic improvements in both symptoms and hemodynamics (Galie et al., 2016; Sitbon et al., 2014). Combination therapy is now recommended for initial therapy of all treatment-naïve PAH patients with functional class II or III symptoms (Klinger et al., 2019).

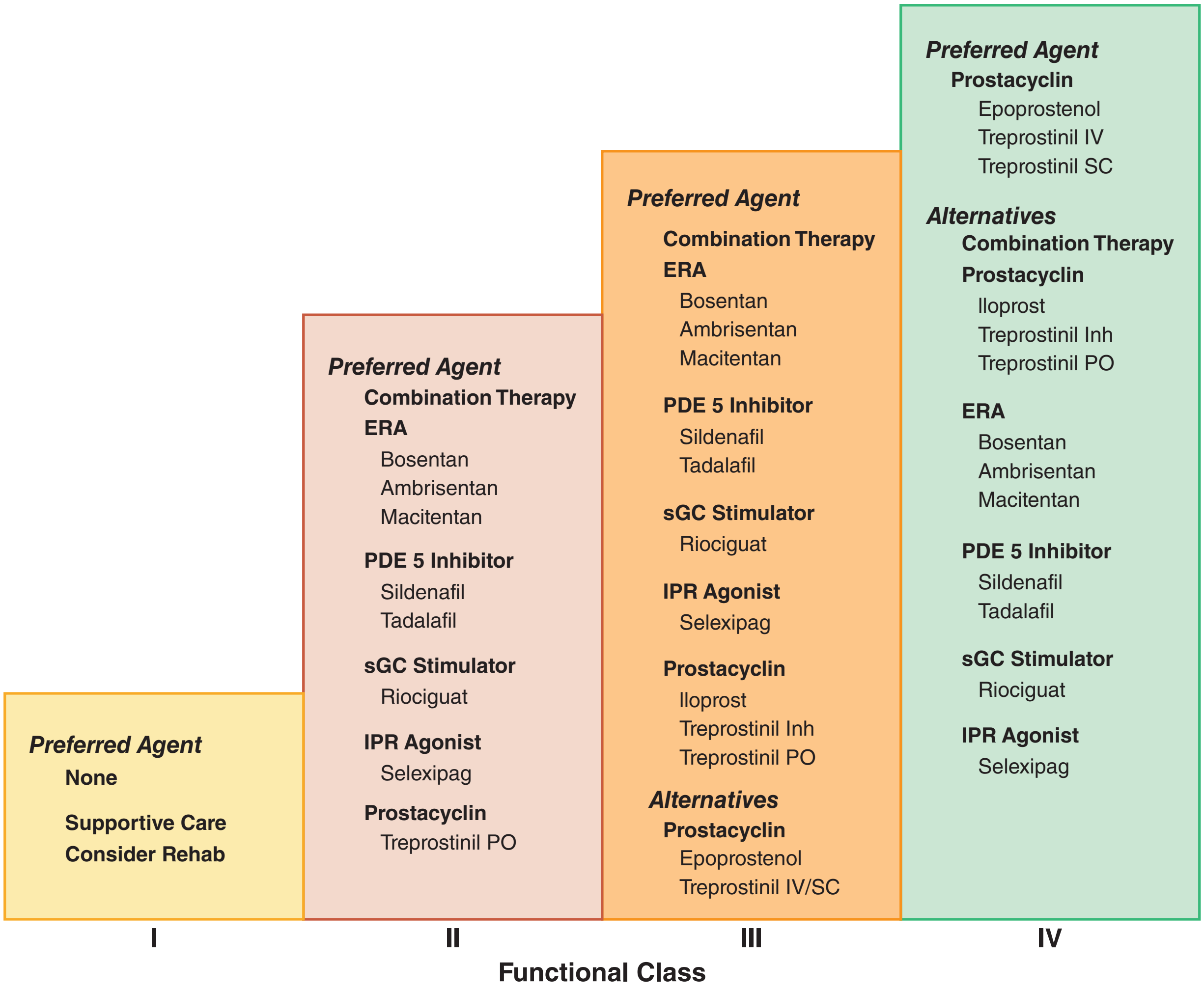

Preferred Agent
None
Supportive Care
Consider Rehab
Preferred Agent
Combination Therapy
ERA
Bosentan
Ambrisentan
Macitentan
PDE 5 Inhibitor
Sildenafil
Tadalafil
sGC Stimulator
Riociguat
IPR Agonist
Selexipag
Prostacyclin
Treprostinil PO
Preferred Agent
Combination Therapy
ERA
Bosentan
Ambrisentan
Macitentan
PDE 5 Inhibitor
Sildenafil
Tadalafil
sGC Stimulator
Riociguat
IPR Agonist
Selexipag
Prostacyclin
Iloprost
Treprostinil Inh
Treprostinil PO
Alternatives
Prostacyclin
Epoprostenol
Treprostinil IV/SC
Preferred Agent
Prostacyclin
Epoprostenol
Treprostinil IV
Treprostinil SC
Alternatives
Combination Therapy
Prostacyclin
Iloprost
Treprostinil Inh
Treprostinil PO
ERA
Bosentan
Ambrisentan
Macitentan
PDE 5 Inhibitor
Sildenafil
Tadalafil
sGC Stimulator
Riociguat
IPR Agonist
Selexipag
I
II
III
IV
Functional Class

Figure 35–2 *Clinical use of PAH drugs based on functional class.* Treatment of PAH is generally based on the patient's functional classification at the time of presentation. Four functional classes have been defined for PAH: (I) no symptoms or functional limitation; (II) slight limitation of physical activity; (III) marked limitation of physical activity; and (IV) symptoms with any activity or at rest. In patients with no functional limitation, there is no specific therapy that has shown benefit in clinical trials. Expert guidelines recommend only supportive care and physical rehabilitation in this group. Patients with symptoms consistent with functional classes II and III have the best evidence for therapeutic benefits. Current guidelines suggest that up-front combination therapy, using *ambrisentan* and *tadalafil* in particular, should be considered in all treatment-naïve patients in these categories unless they are unwilling or unable to tolerate this therapy (Klinger et al., 2019). First-line therapeutics include oral agents such as endothelin receptor antagonists (ERAs), PDE5 inhibitors, sGC stimulators, oral or inhaled PGI_2 analogues, and the IPR agonist *selexipag*. The most severely limited patients, those in functional class IV, or those with evidence of right heart dysfunction should be started on the most potent vasodilators, which include the intravenous and subcutaneous formulations of PGI_2 analogues. Sequential combination therapy using agents from multiple mechanistic classes should be considered if patients are not meeting treatment goals.

Although PH often complicates other diseases of the heart and lungs (see Box 35–1), efficacy of drug therapy had been documented only for primary PAH and CTEPH. In 2021, inhaled *treprostinil* was shown to improve functional capacity in patients with PH associated with interstitial lung disease and was also associated with lower risk of clinical worsening, improved biomarker levels, and fewer exacerbations of underlying lung disease (Waxman et al., 2021). Supportive care therapies described in other chapters include volume management with diuretics (e.g., *furosemide*), anticoagulants (e.g., *warfarin*) for patients at high risk for thrombotic disease, supplemental oxygen therapy for hypoxic patients, and inotropic therapy (e.g., *milrinone*) to improve cardiac contractility in patients with RV dysfunction.

Pharmacotherapy for Pulmonary Arterial Hypertension

Pharmacotherapy for PAH targets the major pathogenic mechanisms of the disease—pulmonary vascular remodeling (e.g., concentric pulmonary vascular thickening and intraluminal obliteration), sustained pulmonary vasoconstriction, *in situ* thrombosis, and pulmonary vascular wall stiffening—with the goals of attenuating the development and progression of PAH and reversing these pathological changes in patients with established PAH. Currently available PAH therapeutics are classified based on their cellular and molecular mechanisms (Humbert and Ghofrani, 2016):

- NO and stimulators of cGMP and PKG signaling
- Membrane receptor agonists
- Membrane receptor antagonists
- Ion channel blockers and openers

Stimulators of cGMP and PKG Signaling

Nitric oxide is synthesized mainly in vascular ECs and diffuses into vascular smooth muscle cells (PASMCs) to activate sGC. Activated sGC generates cGMP, which in turn is hydrolyzed by cyclic nucleotide PDE5 to 5′-GMP (Figure 35–3). cGMP is an intracellular second messenger that signals through (1) cGMP-dependent PKG, the principal downstream mediator of cGMP, and (2) cyclic nucleotide–gated and hyperpolarization-activated cyclic nucleotide–gated channels (Craven and

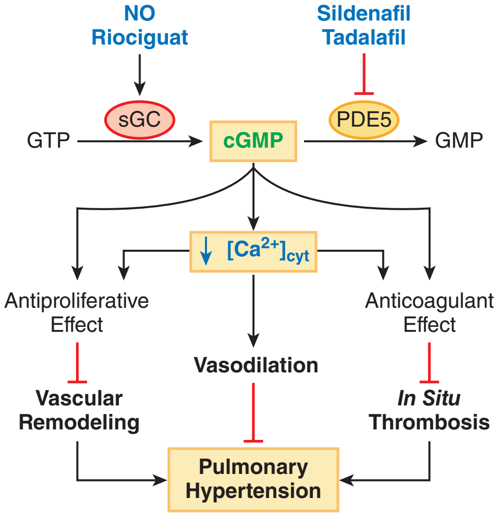


Figure 35–3 *Stimulators of NO/cGMP signaling.* NO activates sGC to produce cGMP, which has vasodilating effects through decreased $[Ca^{2+}]_{cyt}$ as well as anticoagulant and antiproliferative effects that are both dependent and independent of $[Ca^{2+}]_{cyt}$. cGMP is degraded primarily by PDE5 in PASMCs, which is targeted by the PDE5 inhibitors *sildenafil* and *tadalafil*.

Zagotta, 2006). Increased cellular cGMP exerts relaxant and antiproliferative effects on PASMCs and myofibroblasts through activation of cGMP-gated K^+ channels, inhibition of Ca^{2+}-permeable channels (e.g., L-type VDCCs, transient receptor potential cation channels), and attenuation of several specific intracellular signaling cascades that are related to cell proliferation, growth, and migration (the cAMP-PKA pathway exerts similar effects). The drugs currently available for the treatment of PAH in this category include inhaled NO, activators of sGC, and inhibitors of PDE5.

The enzymic catalysts of cGMP formation in tissues are soluble (sGC) and particulate (pGC) or plasma membrane (mGC) GC. NO stimulates sGC, while the natriuretic peptides stimulate mGC (see Chapter 3 for information on the structure and mechanisms of activation of these enzymes). The sGC is the source of cGMP synthesis on which therapeutic agents for PAH rely. In the lung, activation of the cGMP-PKG pathway causes relaxation of smooth muscle, inhibits proliferation of bronchial and vascular smooth muscle cells, and has an antiproliferative effect (and can induce apoptosis) in pulmonary vascular smooth muscle cells and ECs (Figure 35–3).

Nitric Oxide

Nitric oxide is biosynthesized from the terminal nitrogen of L-arginine by nitric oxide synthase (see Chapter 3). Endogenous NO levels are reduced in patients with PAH, PH associated with connective tissue disease, chronic obstructive pulmonary disease, and interstitial lung disease (Girgis et al., 2005; Kawaguchi et al., 2006).

ADME. NO is a soluble gas. *Inhaled NO* is a gaseous blend of NO and N_2. NO must be compressed and stored with an inert gas such as N_2 to minimize the exposure to O_2, decreasing the risk of the accumulation of nitric dioxide (NO_2). Inhaled NO increases the PaO_2 by preferentially vasodilating better-ventilated lung regions from poorly inflated lung areas (i.e., with low ventilation/perfusion [V/Q] ratios). Inhaled NO is generally administered continuously or with a pulsing device that is rapidly triggered with the onset of inspiration; careful monitoring of response is warranted (Griffiths and Evans, 2005). Inhaled NO is a selective pulmonary vasodilator. Its acute and relatively specific effects on PAP and PVR are due to its route of administration and short half-life (2–6 sec), which is primarily a result of the rapid inactivation of NO by hemoglobin binding and oxidation to nitrite; the nitrite interacts with oxyhemoglobin, leading to the formation of nitrate and met-hemoglobin (Bueno et al., 2013). Nitrate has been identified as the predominant NO metabolite excreted in the urine, accounting for more than 70% of the NO dose inhaled (Ichinose et al., 2004).

Clinical Use. NO can acutely lower PAP and PVR without significantly altering systemic arterial pressure. Inhaled NO is used for the treatment of term and near-term neonates with persistent PH of the newborn and acute hypoxemic respiratory failure (Abman, 2013). Acute vasodilator testing is another well-established but off-label use of inhaled NO in adult patients with PAH. Vasodilator testing is performed in the course of deciding whether a patient might derive clinical benefit from Ca^{2+} channel blockade therapy (e.g., *nifedipine*) (Abman, 2013). In the treatment of PAH, a 30% decrease in PVR during the inhalation of NO (10 ppm for 10 min) has been used to identify an association with vascular responsiveness and a favorable response to calcium channel blockers (CCBs) in a small cohort of patients with PAH (McLaughlin et al., 2009).

Adverse Effects and Precautions. High doses of inhaled NO (500–1000 ppm) can be lethal. However, NO doses of less than 40 ppm are well tolerated chronically for up to 6 months and do not cause methemoglobinemia in adults who have normal methemoglobin reductase activity (Griffiths and Evans, 2005). In neonates, methemoglobin accumulation has been investigated during the first 12 h of exposure to 0, 5, 20, and 80 ppm of inhaled NO (Abman, 2013). In the study, methemoglobin concentrations increased during the first 8 h of NO exposure. The mean methemoglobin level remained below 1% in the placebo group and in the 5- and 20-ppm groups but reached approximately 5% in the 80-ppm inhaled NO group.

Drug interactions between properly dosed NO and other medications are not expected, but side effects may include noisy breathing, hematuria, or possibly, atelectasis. Overdosage with inhaled NO manifests as elevations in methemoglobin and pulmonary toxicities associated with inspired NO_2, including acute respiratory distress syndrome. Elevations in methemoglobin reduce the O_2 delivery capacity of the circulation. Based on clinical studies, NO_2 levels greater than 3 ppm or methemoglobin levels greater than 7% are treated by reducing the dose of, or discontinuing, inhaled NO therapy (Abman, 2013). Methemoglobinemia that does not resolve after reduction or discontinuation of inhaled NO therapy can be treated with intravenous vitamin C, intravenous methylene blue, or blood transfusion, based on the clinical situation.

Inhaled NO gas has limitations: Dosing must be individualized and frequently adjusted; delivery is cumbersome and expensive; off-target effects from reactive nitrogen species are possible; and rebound PH may appear when NO administration is interrupted. The oxidative product of NO metabolism, the inorganic anion nitrite, NO_2^-, is relatively stable compared to NO ($t_{1/2}$ = 51 min); NO_2^- can be reduced back to NO under physiological and pathological hypoxia by enzymatic and nonenzymatic processes and thus can serve as an intravascular endocrine reservoir of potential NO bioactivity (Bueno et al., 2013). Other NO donor drugs such as *sodium nitroprusside* and *nitroglycerin* offer protective benefits in PVR or remodeling, but when administered intravenously, these drugs have limited use given their significant systemic vasodilating effects.

Riociguat

In patients with NO deficiency due to dysfunctional endothelial nitric oxide synthase or arginine insufficiency, activation of sGC increases signaling through the cGMP-PKG pathway and exerts a therapeutic effect (Hoeper et al., 2020; Stasch and Evgenov, 2013). *Riociguat*, a direct stimulator of sGC, has been approved to treat patients with PAH and CTEPH (Bishop, 2014; Ghofrani et al., 2013a, 2013b).

Mechanism of Action. *Riociguat* is the first-in-class stimulator of sGC. The agent exhibits a dual mode of action; it sensitizes sGC to endogenous NO, and it also directly stimulates sGC independently of NO.

ADME. The drug has excellent oral absorption, and the plasma concentration peaks approximately 1.5 h after oral intake (Stasch and Evgenov, 2013). Food does not affect the bioavailability of *riociguat*; its volume of distribution is about 30 L. *Riociguat* is metabolized by CYPs 1A1, 3A, 2C8, and 2J2. The action of CYP1A1 forms the major and active metabolite, M1, which is converted to an inactive *N*-glucuronide. The terminal elimination half-life is about 12 h in patients with PAH (7 h in healthy subjects) (Stasch and Evgenov, 2013).

Clinical Use. *Riociguat* at doses up to 2.5 mg given three times daily for 12 weeks increased walking distance and significantly delayed time to clinical worsening for patients with PAH (Ghofrani et al., 2013b). *Riociguat* was also effective in patients with CTEPH, for whom improvements in walking distance were apparent from week 2 onward (Ghofrani et al., 2013a).

Adverse Effects and Precautions. Concurrent use of *riociguat* with *nitroglycerin* or PDE5 inhibitors can cause severe hypotension and syncope. Serious adverse effects include embryo-fetal toxicity, hypotension, and bleeding. Other common adverse reactions include headache, dyspepsia, dizziness, nausea, diarrhea, vomiting, anemia, reflux, constipation, palpitations, nasal congestion, epistaxis, dysphagia, abdominal distension, and peripheral edema (Ghofrani et al., 2013a, 2013b; Stasch and Evgenov, 2013). *Riociguat* is contraindicated in PH associated with idiopathic interstitial pneumonias and may cause pulmonary edema in patients with pulmonary veno-occlusive disease. Drug interactions include antacids, smoking, strong CYP3A inducers, and strong CYP and Pgp/BCRP inhibitors. *Riociguat* is not recommended in patients with creatinine clearance less than 15 mL/min, on dialysis, or with severe hepatic impairment. Due to the potential for teratogenicity, there is an FDA-mandated Risk Evaluation and Mitigation Strategy (REMS) program for female patients.

PDE5 Inhibitors

Cyclic nucleotide PDEs comprise a superfamily of enzymes that hydrolyze 3′-5′ cyclic nucleotides to their cognate 5′ monophosphates (Omori and Kotera, 2007). PDE5, an isoform that is relatively specific for cGMP, is abundant in PASMCs (Kass et al., 2007). The physiological importance of PDE5 in the regulation of vascular tone has been most effectively demonstrated by the successful clinical use of its specific inhibitors in the treatment of erectile dysfunction and PAH (Galie et al., 2005; Ravipati et al., 2007).

Sildenafil. Mechanism of Action. *Sildenafil*, which structurally mimics the purine ring of cGMP, is a competitive and selective inhibitor of PDE5. *Sildenafil* has a relatively high selectivity (>1000-fold) for human PDE5 over other PDEs. By inhibiting cGMP hydrolysis, *sildenafil* elevates cellular levels of cGMP and augments signaling through the cGMP-PKG pathway, *provided guanylyl cyclase is active*.

ADME. The drug is rapidly absorbed and reaches a peak plasma concentration 1 h after oral administration. *Sildenafil* is cleared by the hepatic CYP3A (major route) and CYP2C9 (minor). *Sildenafil* and its major active metabolite, *N*-desmethyl sildenafil, have terminal half-lives of about 4 h. Both the parent compound and the major metabolite are highly bound to plasma proteins (96%) (Cockrill and Waxman, 2013). Metabolites are predominantly excreted into the feces (73%–88%) and to a lesser extent into the urine; unmetabolized drug is not detected in urine or feces (Muirhead et al., 2002). Clearance is reduced in the elderly (>65 years), leading to an increase in area-under-the-curve values for the parent drug and the *N*-desmethyl metabolite.

Clinical Use and Adverse Effects and Precautions. *Sildenafil*, 20 mg three times per day, improves exercise capacity, functional class, and hemodynamics. In addition to improved exercise capacity and hemodynamic parameters, *sildenafil* (initiated at 20 mg three times daily, titrated to 40–80 mg three times daily) plus long-term *epoprostenol* therapy also resulted in delayed time to clinical worsening of PAH in clinical studies.

Dose adjustments for reduced renal and hepatic function are usually not necessary except for severe hepatic and renal impairment (Cockrill and Waxman, 2013). Concomitant administration of potent CYP3A inducers (e.g., *bosentan*) will generally cause substantial decreases in plasma levels of *sildenafil* (Schwartz and Kloner, 2010). The mean reduction in the bioavailability of *sildenafil* (80 mg three times a day) when coadministered with *epoprostenol* is 28% but is not thought to be clinically relevant. CYP3A inhibitors (e.g., protease inhibitors used in human immunodeficiency virus therapy, *erythromycin*, and *cimetidine*) inhibit *sildenafil* metabolism, thereby prolonging the $t_{1/2}$ and elevating blood levels of *sildenafil*. Consistent with its mechanism of action on cGMP signaling, *sildenafil* and other PDE5 inhibitors potentiate the hypotensive effects of nitrate vasodilators, producing dangerously low blood pressure. Thus, the administration of PDE5 inhibitors to patients receiving nitrates in any form is contraindicated. In any event, the patient's underlying cardiovascular status and concurrent use of hypotensive agents (e.g., nitrate vasodilators, α adrenergic antagonists) must be considered prior to use of this class of drugs. Important, but rare, adverse effects include permanent vision loss, hearing impairment, and priapism. *Sildenafil* use is not recommended in children as chronic use has been associated with mortality (Barst et al., 2014). Use of PDE5 inhibitors is not recommended in PH secondary to sickle cell disease as use has been associated with serious vaso-occlusive crises (Machado et al., 2011).

Headache (16%) and flushing (10%) are the most frequently reported side effects. Patients taking *sildenafil* or *vardenafil* may notice a transient blue-green tinting of vision due to inhibition of retinal PDE6, which is involved in phototransduction (see Chapter 74).

Other PDE5 Inhibitors. *Vardenafil* is structurally similar to *sildenafil* and a potent inhibitor of PDE5. Although not FDA-approved for PAH in the U.S., its clinical efficacy in PAH appears similar to that of *sildenafil* (Cockrill and Waxman, 2013). *Tadalafil*, another PDE5 inhibitor used for the treatment of PAH, differs structurally from *sildenafil* and has a longer half-life. It is recommended to reduce the *tadalafil* starting dose or avoid use in patients with renal/hepatic impairment. *Tadalafil* is contraindicated with concurrent guanylate cyclase stimulators and has been associated with toxic skin reactions (Stevens-Johnson syndrome and exfoliative dermatitis). See Table 49–2 for comparative pharmacokinetic data of PDE5 inhibitors.

Prostacyclin Receptor Agonists

Prostacyclin (PGI_2) is synthesized in and released from vascular ECs (and other vascular cells) and exerts relaxant and antiproliferative effects on vascular smooth muscle cells. Similar to NO, endogenous PGI_2 is considered an endothelium-derived relaxing factor. Decreased PGI_2 synthesis occurs in patients with idiopathic PAH, a finding that provided the rationale for using PGI_2 and its analogues for treatment of PAH (Christman et al., 1992).

Mechanism of Action. Prostacyclin binds to the prostacyclin receptor (IPR) in the plasma membrane of PASMCs and activates the G_s-AC-cAMP-PKA pathway (Figure 35–4). PKA continues the signaling cascade by (1) decreasing $[Ca^{2+}]_{cyt}$ via activating K^+ channels (which causes membrane hyperpolarization and repolarization, leading to closure of VDCCs) and (2) inhibiting myosin light chain kinase, thereby causing smooth muscle relaxation and vasodilation (Olschewski et al., 2004; Yuan et al., 1996). Activated PKA can also exert an antiproliferative effect on PASMCs by inhibiting the signaling cascades of hedgehog, ERK/p21, and Akt/mTOR. Inhibition of cyclic nucleotide PDEs, mainly PDE3 and PDE4, enhances the cAMP-PKA–mediated relaxant and antiproliferative effects on vascular smooth muscle cells.

Epoprostenol (Prostacyclin)

Clinical Use, Adverse Effects, and Precautions. The first synthetic PGI_2, *epoprostenol*, has dose-dependent vasodilatory effects on both the systemic and pulmonary vasculature and increases cardiac output in patients with PAH (Rubin et al., 1982). *Epoprostenol*'s short half-life (3–5 min) requires the use of a drug delivery pump system for continuous intravenous infusion to achieve long-term efficacy in the treatment of PAH. In a clinical trial, *epoprostenol* treatment caused significant improvements in pulmonary hemodynamics, patient symptoms, and survival over a 12-week period (Barst et al., 1996).

Epoprostenol is light and temperature sensitive, although a more recent thermostable formulation is now available that permits its use at room temperature (20°C–25°C). This agent remains a mainstay of PAH treatment, particularly in advanced stages of the disease. Adverse effects of *epoprostenol* are similar for the entire class of PGI_2 analogues and

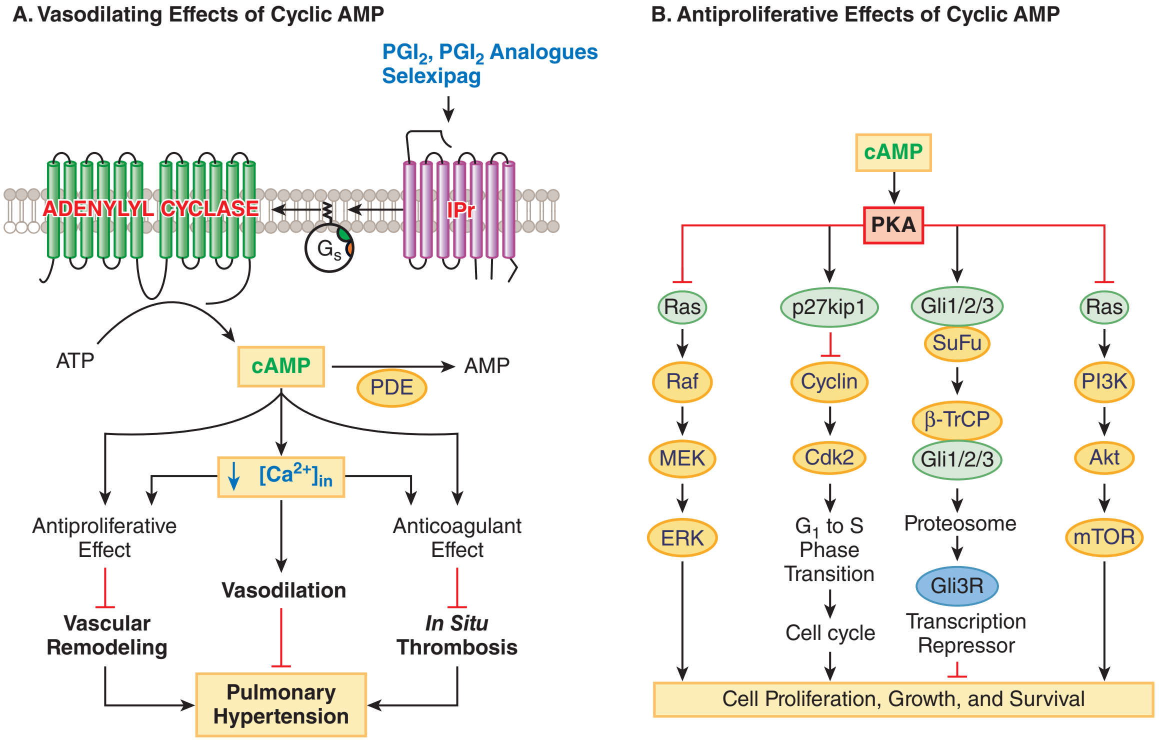


Figure 35–4 *Membrane receptor agonists that increase cAMP.* Therapies targeting the IPr, including PGI_2, PGI_2 analogues, and *selexipag*, increase cAMP through stimulation of its production by adenylyl cyclase. **A.** The vasodilating properties of cAMP are produced through decreased $[Ca^{2+}]_{cyt}$ as well as anticoagulant and antiproliferative effects that are both dependent and independent of $[Ca^{2+}]_{cyt}$. **B.** The antiproliferative effects of cAMP are manifested through numerous distinct pathways, many of which are currently under investigation as therapeutic targets.

include myalgias and pain in the extremities, jaw pain, nausea, headaches, abdominal discomfort, diarrhea, flushing, dizziness, and systemic hypotension. Side effects are generally dose-dependent, and slow titration is required for the drug to be sufficiently tolerated. *Epoprostenol* is contraindicated in heart failure with reduced ejection fraction. There is an increased risk for bleeding and for pulmonary edema.

Treprostinil

Clinical Use. *Treprostinil*, a PGI_2 analogue with longer half-life than that of *epoprostenol*, is available for continuous intravenous infusion, subcutaneous infusion, inhalation, and oral delivery. The risk of bacteremia or other catheter-related complications can be reduced by subcutaneous delivery. Subcutaneous *treprostinil* has similar efficacy to intravenous formulations of *epoprostenol* and *treprostinil* (Simonneau et al., 2002). Adverse effects related to delivery into the subcutaneous tissue of the lower abdomen are common, including pain and erythema in a majority of patients; these effects subside over time. Initial dose reduction is recommended in mild-to-moderate hepatic impairment and with concomitant use of strong CYP2C8 inhibitors. Use of treprostinil in patients with severe hepatic impairment has not been well studied.

Compared to intravenous *treprostinil*, the inhaled formulation has more potent pulmonary vasodilating effects, but patients can find the dosing scheme complex: Multiple breaths are taken through a nebulizer or inhaler four times a day and slowly titrated up to a maximum of 12 breaths four times a day. Inhaled *treprostinil* has comparable hemodynamic effects to inhaled *iloprost* in patients with PAH, though with a longer duration of effect. The most common adverse effect related to inhalation is transient coughing.

Monotherapy with extended-release oral formulations of *treprostinil* is effective in patients with PAH with moderate functional impairments (Jing et al., 2013). The dose is given three times daily, starting at 0.125 or 0.25 mg and titrating up every 3 days to the maximum tolerated dose or to adequate treatment effect. Serum concentrations at a steady dose of 3 mg three times daily are thought to approximate therapeutic levels of intravenous *treprostinil*. Sequential combination therapy with oral *treprostinil* added to existing monotherapy with either an endothelin receptor antagonist or PDE5 inhibitor reduced the risk of clinical worsening and was associated with improved PAH symptoms, improved functional class, and N-terminal pro-BNP levels compared to placebo (White et al., 2020). Note that the oral tablet shell does not dissolve and can lodge in intestinal blind-end pouches and diverticula, possibly causing appendicitis and diverticulitis.

Iloprost

Clinical Use, Adverse Effects, and Precautions. The first PGI_2 analogue available in an inhaled formulation, *iloprost*, was designed to target the pulmonary vasculature and lessen systemic side effects. Inhalation has potent vasodilatory effects on the pulmonary circulation, with less systemic vasodilation than intravenous PGI_2 (Olschewski et al., 1996). The effects of a single inhalation decline to baseline over 60 to 120 min, and current dosing strategies suggest 6 to 9 inhalations daily. The dose is generally titrated from 2.5 mg/inhalation to 5 mg after the first 2 to 4 weeks. *Iloprost* has minor CYP-dependent metabolism. Minor side effects common to the PGI_2 class include headache and jaw pain. Side effects specific to the inhaled formulation are cough, although this appears to resolve over time but may cause bronchospasm. Patients should avoid ingestion as well as contact with the skin and eyes. Drug interactions are limited to drugs with overlapping actions (e.g., vasodilation) and toxicities (e.g., risk for bleeding).

Selexipag

Selexipag is an orally active, selective IPR agonist that is chemically distinct and has different kinetic properties compared to PGI_2 analogues.

ADME. *Selexipag* is rapidly absorbed and hydrolyzed in the liver ($t_{1/2}$ = 1–2 h) to an active metabolite, ACT-333679 (Kaufmann et al., 2015). The active metabolite has a longer half-life, 10 to 14 h, allowing twice-daily dosing. *Selexipag* is a substrate of CYP2C8, CYP3A4, and P-glycoprotein.

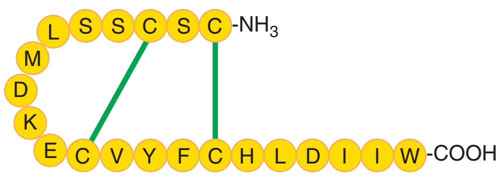
Clinical Use, Adverse Effects, and Precautions. The drug is taken at a starting dose of 200 μg and titrated upward weekly to a maximum dose of 1600 μg twice daily. In phase III clinical trials, *selexipag* reduced the risk of morbidity and mortality in patients with PAH (Simonneau et al., 2012; Sitbon et al., 2015). *Selexipag* is added to existing pulmonary vasodilator therapy in a majority of patients in the clinical trials for this agent. Adverse effects of *selexipag* are similar to those of PGI_2 analogues and include headache, jaw pain, nausea, dizziness, flushing, nasopharyngitis, and vomiting. Adverse effects appear to be more common when the drug is taken while fasting and wane over time. *Selexipag* is contraindicated with strong CYP2C8 inhibitors, and there are drug interactions with CYP2C8 inducers and inhibitors. Precaution should be taken with moderate or severe hepatic impairment, with dose reduction or avoidance of *selexipag*.

Beraprost

The first orally available PGI_2 analogue, *beraprost*, showed promise in early trials, but long-term trials showed no benefit over 12 months of therapy (Barst et al., 2003). As a result, *beraprost* is not approved for use in the U.S. or the European Union.

Endothelin and Endothelin Receptor Antagonists

Endothelin 1



Amino Acid Sequence of Human Endothelin 1

Biosynthesis. Endothelins are a trio of 21 amino acid peptides, each the product of a different gene, produced through a pre-pro and pro-peptide sequence by the endothelin-converting enzyme (ECE) types 1 and 2. ECE-1 is the rate-limiting step in ET-1 synthesis. Each mature ET peptide contains two disulfide bridges. ET-1, the predominant form, is encoded by the *EDN1* gene and produced in vascular ECs, although other cell types can also produce endothelin. A variety of cytokines, angiotensin II, and mechanical stress enhance ET-1 production. NO and PGI_2 reduce *EDN1* gene expression. ETs interact with two GPCRs, the ET_A and ET_B receptors, as described below. ET-1 is cleared by interaction with the ET_B receptor and via proteolytic degradation by neutral endopeptidase NEP24.11. Davenport and colleagues (2016) have reviewed key concepts of the biosynthesis, signaling, and pharmacology of ETs.

Endothelin Signaling. Endothelin 1 was discovered as a potent, endothelium-derived constricting factor (Yanagisawa et al., 1988). The constrictor response is mediated by the ET_A receptor, which is localized on PASMCs. The ET_B receptor is present on both PASMCs and pulmonary arterial endothelial cells. Binding of ET-1 to ET_A receptor on PASMCs activates the G_q-PLC-IP_3-Ca^{2+} and diacylglycerol (DAG) Ca^{2+}-PKC pathways (Figure 35–5 and Chapter 3). IP_3 activates the Ca^{2+} release channel on intracellular Ca^{2+} storage organelles, thereby mobilizing Ca^{2+} and increasing $[Ca^{2+}]_{cyt}$. The elevated cytosolic Ca^{2+} produces vasoconstriction (Figure 35–5). ET-1 is also a mitogenic factor that exerts proliferative effects on many types of cells, including vascular smooth muscle cells and myofibroblasts via intracellular signaling cascades (e.g., PI3K/Akt/mTOR and Ras/ERK/p21 pathways) (Davenport et al., 2016). The activation of ET_B receptors on ECs mediates vasodilation by increasing production of NO and PGI_2 and can inhibit ET-1 production.

Rationale for Antagonizing ET's Effects in PAH. Endothelin 1 is implicated as a contributory factor in idiopathic PAH (Giaid et al., 1993): Plasma ET-1 levels are increased up to 10-fold in patients with PAH and correlate well with severity of disease and the elevation of right atrial pressure. There are no clinically available specific inhibitors of ECE-1, the rate-limiting step in ET-1 synthesis, but several orally effective small-molecule antagonists of ET receptors have been developed. Despite the opposing effects of ET_A and ET_B receptor activation, pharmacological targeting of specific ET_A receptors has not led to significantly altered clinical responses compared to dual antagonism (e.g., antagonism of ET-1 binding to both ET_A and ET_B receptors) in treating PAH.

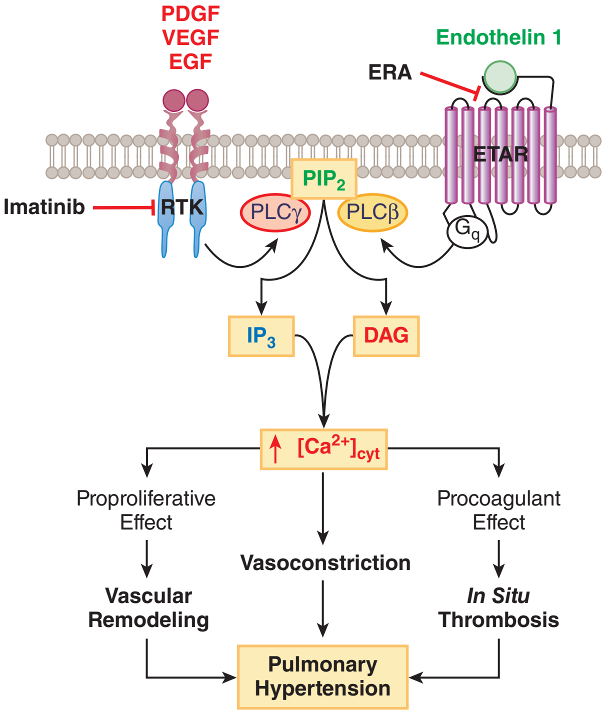


Figure 35–5 *Inhibition of receptor-mediated activation of PLC isoforms on pulmonary artery smooth muscle cells can reduce vasoconstriction.* Endothelin, acting via the ET_A-G_q-PLC_β-IP_3 pathway, can cause vasoconstriction, a response that endothelin receptor antagonists can inhibit. Growth factors such as PDGF, VEGF, and EGF, acting through receptor tyrosine kinases (RTKs), can activate PLC_γ and initiate contraction in a similar fashion; imatinib, a tyrosine kinase inhibitor (see Chapter 71), can inhibit the activation PLC_γ via RTKs, thereby reducing pulmonary artery contraction.

Endothelin Receptor Antagonists

Available ET receptor antagonists are *bosentan*, *macitentan*, and *ambrisentan*.

Commonalities. Endothelin antagonists generally share adverse effects. Common side effects of the class include headache, pulmonary edema, peripheral edema, anemia, and nasal congestion/pharyngitis, with a risk of testicular atrophy and infertility. *Bosentan* may increase liver transaminases, which should be monitored closely, and is contraindicated in patients with moderate-to-severe liver disease; the elevation of liver enzymes generally resolves after discontinuation of treatment. Given the concerns for hepatotoxicity in this class, *ambrisentan* and *macitentan* are not recommended in moderate or severe hepatic impairment.

The three available ET antagonists are metabolized by CYP3A4 and to some extent by CYPs 2C9 and 2C19. Repeated *bosentan* dosing elicits induction of CYPs 3A4 and 2C9, reducing exposure to drugs that are also metabolized by these CYPs (contraceptives, *warfarin*, some statins; coadministration with *cyclosporine* and *glyburide* is contraindicated); likewise, coadministration of *bosentan* or *macitentan* with a CYP inducer such as *rifampin* should be avoided. Inhibitors of these CYPs (e.g., *ketoconazole* and *ritonavir*) can increase *bosentan* and *macitentan* exposure (O'Callaghan et al., 2011).

The ET receptor antagonists are potent teratogens and should be used with caution in women of childbearing age. These agents must not be used in pregnant patients, and the FDA has mandated a REMS program due to hepatic (*bosentan*) and/or fetal toxicities (*bosentan*, *ambrisentan*, and *macitentan*). Documentation of a negative pregnancy test prior to initiation of therapy and a clear contraceptive plan are recommended, and fertile women must use two acceptable methods of birth control while taking ET antagonists.

Bosentan. *Bosentan* is a nonpeptide, orally effective, competitive antagonist of ET_A and ET_B receptors. In patients with PAH with mild-to-severe functional impairment (functional classes II–IV), *bosentan* improves symptoms, functional capacity, and pulmonary hemodynamic parameters (Rubin et al., 2002). *Bosentan* is usually started at 62.5 mg twice daily, increasing to 125 mg twice daily after 4 weeks. *Bosentan* is metabolized by hepatic CYPs 2C9 and 3A4 with a $t_{1/2}$ of about 5 h, with excretion of metabolites in the bile. There is a drug interaction with hormonal contraceptives that can reduce the effectiveness of the contraceptives.

Macitentan. *Macitentan* is an orally active, competitive ET_A and ET_B receptor antagonist. At a dose of 10 mg daily, *macitentan* improves the time to disease progression or death in PAH and improves symptoms, functional capacity, and pulmonary hemodynamic measurements (Pulido et al., 2013). The drug is relatively well tolerated and has thus far not been associated with elevation of hepatic enzymes, but caution is recommended. *Macitentan* is metabolized by CYPs to an active metabolite; the $t_{1/2}$ of the parent compound is about 16 h and that of the active metabolite about 48 h, such that the metabolite contributes about 40% of the total pharmacologic activity over time.

Ambrisentan. Unlike *bosentan* and *macitentan*, *ambrisentan* is a relatively selective ET_A antagonist (~4000 times greater affinity for ET_A than ET_B). *Ambrisentan* is initiated at a dose of 5 mg daily and increased to a maximum of 10 mg daily. The $t_{1/2}$ is 9 h at steady state. Hepatic enzyme abnormalities are much less common than with *bosentan*, and if encountered, current recommendations suggest excluding all other causes of hepatotoxicity before discontinuing *ambrisentan*. Elimination is largely via nonrenal pathways that have not been extensively characterized. There is some metabolism by CYPs 3A4 and 2C19, followed by glucuronidation; thus, drug interactions might be expected, although clinically relevant interactions have not been reported. *Ambrisentan* is contraindicated in patients with idiopathic pulmonary fibrosis. Due to concerns of hepatotoxicity with the class, *ambrisentan* is not recommended in patients with moderate to severe hepatic impairment.

Receptor Tyrosine Kinase Inhibitors

Many growth factors and mitogenic factors are reportedly upregulated in tissues of patients with PAH. Elevations of ET-1, ATP, VIP, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor, and insulin-like growth factor in lung tissue, vascular smooth muscle cells, and peripheral blood have been assessed in PAH (Weiss et al., 2021). These myriad of mitogenic factors can activate TKRs, such as PDGF and EGF receptors. Activation of these receptors can induce cell proliferation, growth, migration, and contraction in PASMCs, pulmonary arterial endothelial cells, and pulmonary vascular fibroblasts. With these actions as a rationale, antagonists of TKRs have been tested as therapeutics for PAH.

Imatinib

Imatinib was initially developed as targeted treatment of chronic myelogenous leukemia by targeting the ABL TKR; the compound is now known to have many other targets, one of which is the PDGF receptor that has been linked to vascular smooth muscle hypertrophy in the development of PAH (Humbert et al., 1998). *Imatinib* as add-on therapy for refractory PAH has shown some efficacy in both case reports and a clinical trial, although serious adverse reactions, particularly subdural hematoma, are of concern (Hoeper et al., 2013). A proposed study of imatinib dosing, of factors affecting the response to imatinib (including patient genotype), and of potential biomarkers of imatinib's effecttiveness in PAH patients may provide definitive information on the tolerability and efficacy of imatinib in treating PAH (Wilkins et al., 2021).

Calcium Channels and Their Blockers

An increase in $[Ca^{2+}]_{cyt}$ in PASMCs causes pulmonary vasoconstriction and is an important stimulant of proliferation, migration, and vascular remodeling. $[Ca^{2+}]_{cyt}$ in PASMCs can be increased by Ca^{2+} influx through membrane Ca^{2+} channels and Ca^{2+} mobilization through the Ca^{2+} release channels in the membranes of the sarcoplasmic reticulum (SR) or endoplasmic reticulum (ER). $[Ca^{2+}]_{cyt}$ can be decreased in three ways: by Ca^{2+} extrusion via the ATP-dependent Ca^{2+} pump in the plasma membrane, export of Ca^{2+} by the Na^+/Ca^{2+} exchanger, and sequestration of cytosolic Ca^{2+} into the SR or ER by SR/ER Ca^{2+}-ATPase. There are three classes of Ca^{2+}-permeable channels functionally expressed in the plasma membrane of PASMCs: (1) VDCCs, (2) ROCs, and (3) store-operated Ca^{2+} channels. These are targets in the current therapy of PAH and putative targets for therapeutics of the future.

Voltage-Dependent Ca^{2+} Channel Blockers

A rare subset of patients (typically less than 5%–15% of all PAH confirmed by right heart catheterization) is considered vasoreactive, which is defined as a significant decrease in mean PAP (>10 mmHg drop to absolute mean PAP <40 mmHg) while preserving cardiac output during the administration of inhaled NO or intravenous injection of PGI_2 or *adenosine* (Rich and Brundage, 1987). Vasoreactive patients can achieve prolonged survival, sustained functional improvement, and hemodynamic improvement with CCB therapy (Hemnes et al., 2015; Rich and Brundage, 1987). The utility of CCB therapy in patients with vasoreactive PAH is supported by a series of well-designed observational studies (Hemnes et al., 2015; Rich and Brundage, 1987; Sitbon et al., 2005).

Clinical Use. Therapy with CCB can be initiated with a low dose of long-acting *nifedipine*, *amlodipine*, *diltiazem*, or *verapamil*. The dose is then increased to the maximal tolerated dose. Systemic blood pressure, heart rate, and oxygen saturation should be carefully monitored during titration. Sustained-release preparations of *nifedipine, verapamil*, and *diltiazem* are available that minimize the adverse effects of therapy, especially systemic hypotension. Patients who respond (defined as asymptomatic or minimal symptoms) to CCB therapy with a dihydropyridine or *diltiazem* are typically reassessed for sustained responses (Figure 35–6).

Adverse Effects and Precautions. Adverse effects are common with CCB therapy. Systemic vasodilation may cause hypotension, while pulmonary vasodilation may reduce hypoxic pulmonary vasoconstriction. Loss or inhibition of hypoxic pulmonary vasoconstriction can worsen

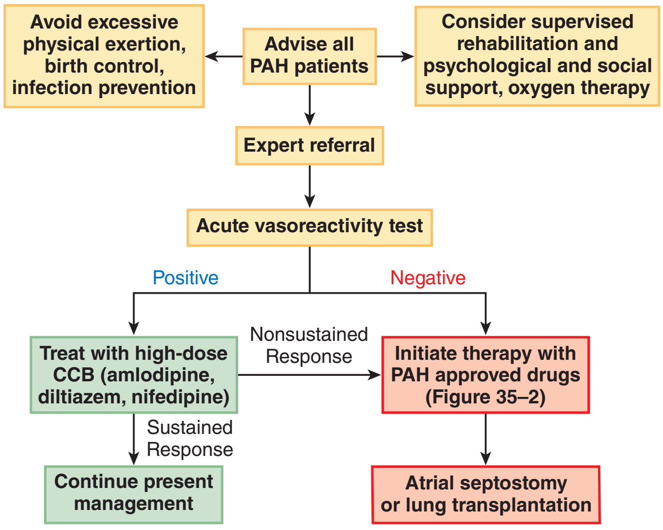

Avoid excessive physical exertion, birth control, infection prevention
Advise all PAH patients
Consider supervised rehabilitation and psychological and social support, oxygen therapy
Expert referral
Acute vasoreactivity test
Positive
Negative
Treat with high-dose CCB (amlodipine, diltiazem, nifedipine)
Nonsustained Response
Initiate therapy with PAH approved drugs (Figure 35–2)
Sustained Response
Continue present management
Atrial septostomy or lung transplantation

Figure 35–6 *Treatment algorithm for use of CCBs in PAH.* Vasoreactivity testing is used to identify the minority of patients who may have a substantial benefit from high-dose CCB therapy. These individuals must be monitored closely to ensure a sustained response. Patients without a positive vasodilator response should potentially be started on therapies approved for PAH based on symptoms at presentation. The patients with the most severe disease who fail to respond to therapy may need referral for surgical intervention to treat their disease.

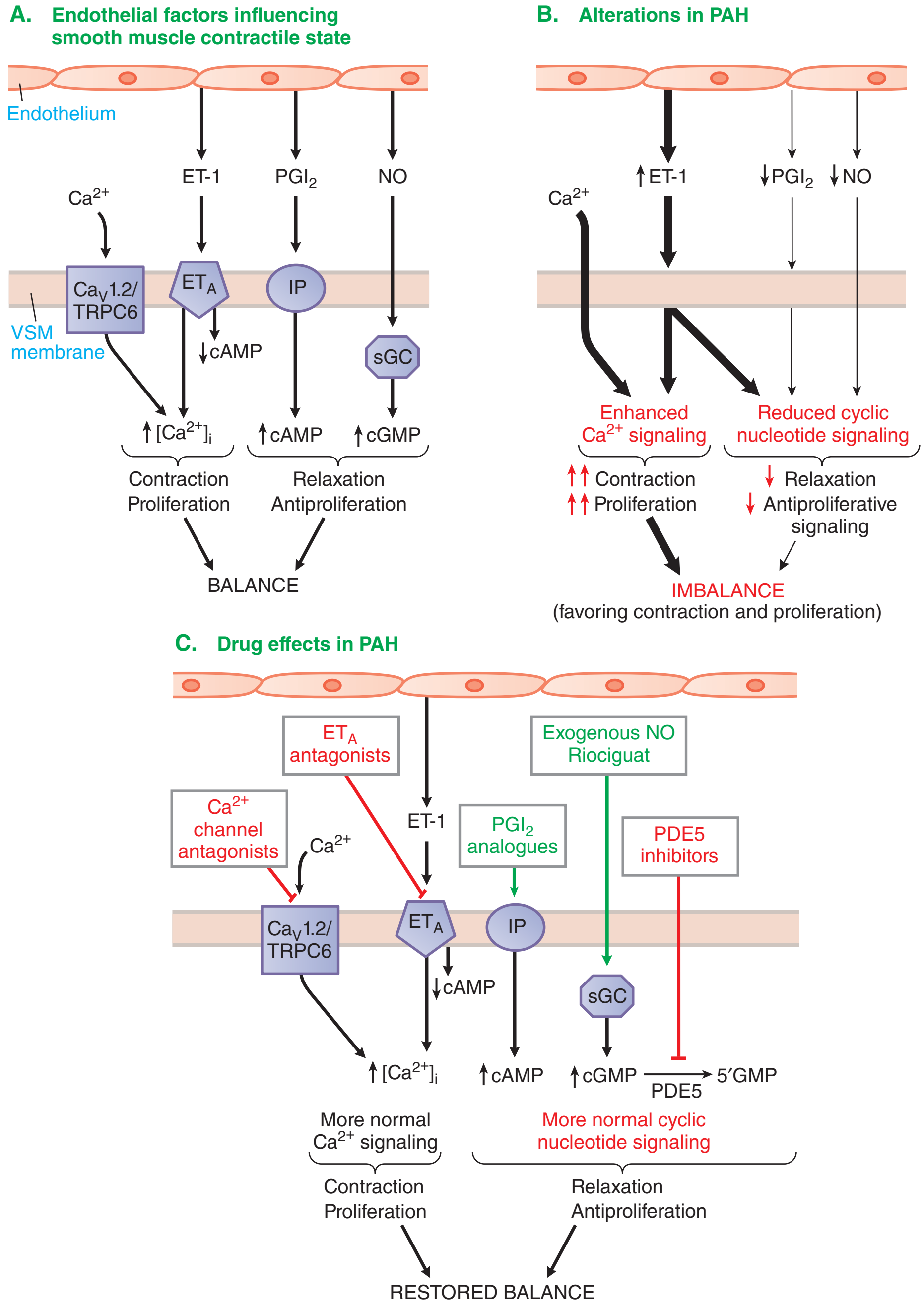

A. Endothelial factors influencing smooth muscle contractile state
Endothelium
ET-1
PGI2
NO
Ca2+
CaV1.2/ TRPC6
ETA
IP
VSM membrane
↓cAMP
sGC
↑[Ca2+]i
↑cAMP
↑cGMP
Contraction
Proliferation
Relaxation
Antiproliferation
BALANCE
B. Alterations in PAH
↑ET-1
↓PGI2
↓NO
Ca2+
Enhanced Ca2+ signaling
Reduced cyclic nucleotide signaling
↑↑ Contraction
↑↑ Proliferation
↓ Relaxation
↓ Antiproliferative signaling
IMBALANCE
(favoring contraction and proliferation)
C. Drug effects in PAH
ETA antagonists
Exogenous NO
Riociguat
Ca2+ channel antagonists
Ca2+
ET-1
PGI2 analogues
PDE5 inhibitors
CaV1.2/ TRPC6
ETA
IP
↓cAMP
sGC
↑[Ca2+]i
↑cAMP
↑cGMP
PDE5
5′GMP
More normal Ca2+ signaling
More normal cyclic nucleotide signaling
Contraction
Proliferation
Relaxation
Antiproliferation
RESTORED BALANCE

Figure 35–7 *Interactions between endothelium and vascular smooth muscle in PAH.* **A. Balance.** In normal pulmonary artery, there is a balance between constrictor and relaxant influences that may be viewed as competition between Ca^{2+} signaling pathways and cyclic nucleotide signaling pathways in vascular smooth muscle (VSM) cells. ET-1 binds to the ET_A receptor on VSM cells and activates the G_q-PLC-IP_3 pathway to increase cytosolic Ca^{2+}; ET-1 may also couple to G_i to inhibit cAMP production. As VSM cells depolarize, Ca^{2+} may enter via the L-type Ca^{2+} channel ($Ca_v1.2$) or transient receptor potential cation channel (TRPC6). ECs also produce relaxant factors, PGI_2 and NO. NO stimulates the sGC, causing accumulation of cGMP in VSM cells; PGI_2 binds to the IPR and stimulates cAMP production; elevation of these cyclic nucleotides promotes VSM relaxation (see Figure 35–3 and Chapters 44 and 49). **B. Imbalance.** In PAH, ET-1 production is enhanced, production of PGI_2 and NO is reduced, and the balance is shifted toward constriction and proliferation of VSM. **C. Restored balance**. In treating PAH, ET_A receptor antagonists can reduce the constrictor effects of ET-1, and Ca^{2+} channel antagonists can further reduce Ca^{2+}-dependent contraction. Exogenous PGI_2 and NO can be supplied to promote vasodilation (relaxation of VSM); the sGC can be activated pharmacologically (*riociguat*); inhibition of PDE5 can enhance the relaxant effect of elevated cGMP by inhibiting the degradation of cGMP. Thus, these drugs can reduce Ca^{2+} signaling and enhance cyclic nucleotide signaling, restoring the balance between the forces of contraction/proliferation and relaxation/antiproliferation. Remodeling and deposition of extracellular matrix by adjacent fibroblasts is influenced positively and negatively by the same contractile and relaxant signaling pathways, respectively. Effects of pharmacological agonists are noted by green arrows, and effects of antagonists by red T-bars.

V/Q mismatch and cause hypoxemia. CCBs may also be associated with deterioration of RV function because of their inhibitory effect on VDCC in cardiomyocytes. The pharmacology of CCBs is discussed in detail in Chapter 31.

PAH Drugs in Development

In addition to the PAH drugs in clinical use, there are many repurposed drugs and newly developed drugs that have therapeutic benefits in experimental models of PH. These agents have potential as future therapies for PAH:

- Long-acting treprostinil prodrug, treprostinil palmitil, for inhalation
- TGF-β/activin ligand trap (e.g., sotatercept)
- Antagonists of serotonin production, serotonin receptors, and transporters (e.g., LY393558, rodatristat ethyl)
- Allosteric antagonists of Ca^{2+}-sensing receptors (e.g., NPS2143 and calhex 231)
- Modulators of Ca^{2+}-activated and voltage-gated K^+ channels and ATP-sensitive K^+ channels (e.g., cromakalim)
- Inhibitors of the PI3K/Akt1/mTOR signaling cascades (e.g., perifosine, ipatasertib, and rapamycin derivatives sirolimus, temsirolimus, everolimus, deforolimus)
- Modulators of 5′ AMP-activated protein kinase (AMPK) (e.g., metformin)
- Inhibitors of pyruvate dehydrogenase kinase (e.g., JTT 251)
- Inhibitors of the Notch signaling pathway (e.g., DAPT and MK-0752)
- VIP (e.g., pemzivaptadil)
- Blockers of transient receptor potential cation channels (e.g., 2-APB, ML204, aniline-thiazoles, BI-749327)
- Extracellular elastase inhibitors (e.g., elafin and sivelestat)
- Rho kinase inhibitors (e.g., fasudil)
- Angiopoietin 1 inhibitors (e.g., trebananib)
- Inhibitors of bromodomain and extraterminal domain proteins (e.g., apabetalone)

Some of these drugs are already in phase III clinical trials; others are still in preclinical development.

A Pharmacologist's View of Signal Integration in PAH

As noted, an imbalance of vasoactive mediators, mitogenic and angiogenic factors, and pro- and antiapoptotic proteins plays an important role in PAH development. The pharmacological agents employed in PAH are focused on restoring the balance between contraction and proliferation on the one hand and relaxation and antiproliferation on the other, as summarized in Figure 35–7.

Drug Facts for Your Personal Formulary: *Pulmonary Hypertension Therapeutics*

Drug	Indication	Clinical Pharmacology and Tips
cGMP Signaling Modulators: PDE5 Inhibitors		
Sildenafil Tadalafil Vardenafil	• First-line therapy for moderate PAH (functional class II–III)	• Oral administration • Avoid nitrates and α adrenergic antagonists due to hypotension • Major side effects: epistaxis, headache, dyspepsia, vision or hearing loss, flushing, insomnia, dyspnea, priapism • Vardenafil, currently not recommended due to limited evidence for efficacy in PAH
cGMP Signaling Modulators: sGC Stimulator, Teratogenic (FDA-mandated REMS program for female patients)		
Riociguat	• First-line therapy for moderate PAH (functional class II–III)	• Oral administration • Efficacy confirmed in PAH patients and CTEPH patients • Side effects: headache, dyspepsia, edema, nausea, dizziness, syncope • Drug interactions with antacids, smoking, strong CYP3A inducers, and strong CYP and Pgp/BCRP inhibitors • Not recommended with severe renal impairment (creatinine clearance <15 mL/min or on dialysis) • Not recommended with severe hepatic impairment
IPr Agonists: Prostacyclin and Prostacyclin Analogues		
Epoprostenol	• First-line therapy for severe PAH (functional class IV)	• Administration by continuous IV infusion • Major side effects: jaw pain, hypotension, myalgia, flushing, nausea, vomiting, dizziness • Short half-life requires immediate medical attention to pump failure, contraindicated in heart failure • Increased risk for bleeding
Treprostinil	• Same as epoprostenol • First-line therapy for PH due to interstitial lung disease	• Available as IV, SC, inhaled, and oral preps • Longer half-life than epoprostenol with similar side effects • Local adverse effects of SC dose may improve over time • Substrate for CYP2C8 and CYP2C9, drug interaction with gemfibrozil and rifampin • Oral treprostinil tablet shell does not dissolve (can be seen in the stool and may lodge in intestinal blind-end pouch or diverticulum) • Initial dose reduction for mild-to-moderate hepatic impairment; limited data in severe hepatic impairment • Initial dose reduction with concomitant strong CYP2C8 inhibitors • Inhibits platelet aggregation and increases the risk of bleeding

Drug Facts for Your Personal Formulary: *Pulmonary Hypertension Therapeutics* (continued)

Drug	Indication	Clinical Pharmacology and Tips
Iloprost	• Alternative for epoprostenol in combination therapy for severe PAH (function class IV)	• Inhaled administration, at least 2 h apart • Side effects include flushing, hypotension, headache, nausea, throat irritation, cough, insomnia • May cause bronchospasm, pulmonary edema • Avoid systemic exposure (ingestion) as well as contact with the skin and eyes • Minor CYP-dependent metabolism
Selexipag	• Alternative for epoprostenol in combination therapy for severe PAH (functional class IV)	• Oral administration • Selective PGI_2 receptor agonist • Side effects include headache, jaw pain, nausea, diarrhea • Substrate of CYP2C8, CYP3A4, and Pgp • Drug interactions with CYP2C8 inducers/inhibitors • Contraindicated with strong CYP2C8 inhibitors • Dose reduction or avoidance recommended in moderate or severe hepatic impairment
Endothelin Receptor Antagonists: Oral Administration, Teratogenic (FDA-mandated REMS program)		
Bosentan	• First-line therapy for moderate PAH (functional class II–III) • Specific FDA indication for pediatric population aged 3 years or older with idiopathic or congenital PAH	• Monitor liver function and hemoglobin levels • Metabolized by CYP2C9, CYP3A4, and CYP2C19 • Drug interaction with hormonal contraceptives (reduced effectiveness of contraceptives) • Side effects: liver impairment, palpitations, itching, edema, anemia, respiratory infections
Ambrisentan	• First-line therapy for moderate PAH (functional class II–III)	• Side effects: edema, nasal congestion, constipation, flushing, palpitations, abdominal pain • Cyclosporin coadministration increases drug levels • Lower risk for liver toxicity
Macitentan	• First-line therapy for moderate PAH (functional class II–III)	• Metabolized by CYP3A4 • Side effects include nasopharyngitis, headache, anemia • Liver function and hemoglobin testing recommended prior to therapy
L-Type Ca^{2+} Channel Blockers		
Nifedipine (long acting) Amlodipine Diltiazem	• Use only in PAH patients with positive vasodilator testing	• Oral administration • Side effects include edema, fatigue, hypotension • Diltiazem: significant negative chronotropic and inotropic effects; avoid in bradycardia

Abbreviations: IV, intravenous; SC, subcutaneous.

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Chapter 36

Blood Coagulation and Anticoagulant, Fibrinolytic, and Antiplatelet Drugs

Jeffrey I. Weitz

OVERVIEW OF HEMOSTASIS: PLATELET FUNCTION, BLOOD COAGULATION, AND FIBRINOLYSIS

- Conversion of Fibrinogen to Fibrin

STRUCTURE OF COAGULATION FACTORS

NONENZYMATIC PROTEIN COFACTORS

- Factor VIII and Factor V Are Procofactors

ACTIVATION OF PROTHROMBIN

- Initiation of Coagulation
- Fibrinolysis
- Coagulation *In Vitro*
- Natural Anticoagulant Mechanisms

PARENTERAL ANTICOAGULANTS

- Heparin, Low-Molecular-Weight Heparin, Fondaparinux
- Other Parenteral Anticoagulants

VITAMIN K ANTAGONIST

- Warfarin

DIRECT ORAL ANTICOAGULANTS

- Direct Oral Thrombin Inhibitor
- Direct Oral Factor Xa Inhibitors
- Reversal Agents for Direct Oral Anticoagulants

FIBRINOLYTIC DRUGS

- Tissue Plasminogen Activator

INHIBITORS OF FIBRINOLYSIS

- ε-Aminocaproic Acid and Tranexamic Acid

ANTIPLATELET DRUGS

- Aspirin
- Dipyridamole
- $P2Y_{12}$ Receptor Antagonists
- Thrombin Receptor Inhibitor
- Glycoprotein IIb/IIIa Inhibitors

THE ROLE OF VITAMIN K

- Physiological Functions and Pharmacological Actions
- Inadequate Intake
- Inadequate Absorption
- Inadequate Utilization

Blood must remain fluid within the vasculature and yet clot quickly when exposed to subendothelial surfaces at sites of vascular injury. Under normal circumstances, a delicate balance between coagulation and fibrinolysis prevents both thrombosis and hemorrhage. Alteration of this balance in favor of coagulation results in thrombosis. Thrombi, composed of platelet aggregates, fibrin, and trapped red blood cells, can form in arteries or veins. Antithrombotic drugs used to treat thrombosis include antiplatelet drugs, which inhibit platelet activation or aggregation; anticoagulants, which attenuate fibrin formation; and fibrinolytic agents, which degrade fibrin. All antithrombotic drugs increase the risk of bleeding.

This chapter reviews the agents commonly used for controlling blood fluidity, including:

- The parenteral anticoagulant *heparin* and its derivatives, which activate antithrombin, a natural inhibitor of coagulant proteases
- The coumarin anticoagulants, which lower the functional levels of multiple coagulation factors
- The direct oral anticoagulants, which inhibit factor Xa or thrombin
- Fibrinolytic agents, which degrade fibrin
- Antiplatelet agents, which attenuate platelet activation (*aspirin, clopidogrel, prasugrel, ticagrelor*, and *vorapaxar*) or aggregation (glycoprotein IIb/IIIa inhibitors)
- Vitamin K, which is required for the biosynthesis of key coagulation factors

Overview of Hemostasis: Platelet Function, Blood Coagulation, and Fibrinolysis

Hemostasis is the cessation of blood loss from a damaged vessel. Platelets first adhere to macromolecules in the subendothelial regions of the injured blood vessel, where they become activated. Adherent platelets release substances that activate nearby platelets, thereby recruiting them to the site of injury. Activated platelets then aggregate to form the primary hemostatic plug.

Vessel wall injury also exposes tissue factor (TF), which initiates the coagulation system. Activated platelets enhance activation of the coagulation system by providing a surface onto which clotting factors assemble and by releasing stored clotting factors. This results in a burst of thrombin (factor IIa) generation. Thrombin converts soluble fibrinogen to fibrin, activates platelets, and feeds back to promote additional thrombin generation. The fibrin strands tie the platelet aggregates together to form a stable clot.

The processes of platelet activation and aggregation and blood coagulation are summarized in Figures 36–1 and 36–2 (see also the animation on the Goodman & Gilman site on *AccessMedicine.com*). Coagulation involves a series of zymogen activation reactions, as shown in Figure 36–2. At each stage, a precursor protein, or zymogen, is converted to an active protease by cleavage of one or more peptide bonds in the precursor molecule. The final protease generated is thrombin. Later, as wound healing occurs, the fibrin clot is degraded. The pathway of clot removal, fibrinolysis, is shown in Figure 36–3, along with sites of action of fibrinolytic agents.

Abbreviations

ACT: activated clotting time
α_2-AP: α_2-antiplasmin
aPTT: activated partial thromboplastin time
COX: cyclooxygenase
CrCL: creatinine clearance
CYP: cytochrome P450
EPCR: endothelial protein C receptor
Gla: γ-carboxyglutamic acid
Glu: glutamic acid
GP: glycoprotein
INR: international normalized ratio
LMWH: low-molecular-weight heparin
PAI: plasminogen activator inhibitor
PAR: protease-activated receptor
PT: prothrombin time
TF: tissue factor
TFPI: tissue factor pathway inhibitor
t-PA: tissue plasminogen activator
TxA_2: thromboxane A_2
u-PA: urokinase plasminogen activator
VKOR: vitamin K epoxide reductase

Conversion of Fibrinogen to Fibrin

Fibrinogen, a 340,000-Da protein, is a dimer, each half of which consists of three pairs of polypeptide chains (designated Aα, Bβ, and γ). Disulfide bonds covalently link the chains and the two halves of the molecule. Thrombin converts fibrinogen to fibrin monomers by releasing fibrinopeptide A (a 16–amino acid fragment) and fibrinopeptide B (a 14–amino acid fragment) from the amino termini of the Aα and Bβ chains, respectively. Removal of the fibrinopeptides creates new amino termini, which form knobs that fit into preformed holes on other fibrin monomers to form a fibrin gel, which is the end point of *in vitro* tests of coagulation (see Coagulation *In Vitro*). Initially, the fibrin monomers are bound to each other noncovalently. Subsequently, factor XIII, a transglutaminase that is activated by thrombin, catalyzes interchain covalent cross-links between adjacent fibrin monomers, which strengthen the clot.

Structure of Coagulation Factors

In addition to factor XIII, the coagulation factors include factors II (prothrombin), VII, IX, X, XI, XII, high-molecular-weight kininogen, and prekallikrein. A stretch of about 200 amino acid residues at the carboxyl termini of each of these zymogens exhibits homology to trypsin and contains the active site of the proteases. In addition, 9 to 12 Glu residues near the amino termini of factors II, VII, IX, and X are converted to Gla (γ-carboxyglutamic acid) residues in a vitamin K–dependent posttranslational step. The Gla residues bind Ca^{2+} and are essential for the coagulant activities of these proteins by enabling their interaction with the anionic phospholipid membrane of activated platelets.

Nonenzymatic Protein Cofactors

TF, factor V, and factor VIII are critical cofactors in coagulation. A nonenzymatic lipoprotein cofactor, TF is not normally present on blood-contacting cells. TF is constitutively expressed on the surface of subendothelial smooth muscle cells and fibroblasts, which are exposed when the vessel wall is damaged. Activated monocytes and leukocyte-derived microvesicles also express TF on their surface. TF binds factor VIIa and enhances its catalytic efficiency. The TF–factor VIIa complex initiates coagulation by activating factors IX and X.

Factor VIII and Factor V Are Procofactors

Factor VIII circulates in plasma bound to von Willebrand factor, which serves to stabilize it. Factor V circulates in plasma, is stored in platelets in a partially activated form, and is released when platelets are activated. Thrombin releases von Willebrand factor from factor VIII and activates factors V and VIII to yield factors Va and VIIIa, respectively. Once activated, the cofactors bind to the surface of activated platelets and serve as receptors; factor VIIIa serves as the receptor for factor IXa, while factor Va serves as the receptor for factor Xa. In addition to binding factors IXa and Xa, factors VIIIa and Va bind their substrates, factors X and prothrombin (factor II), respectively.

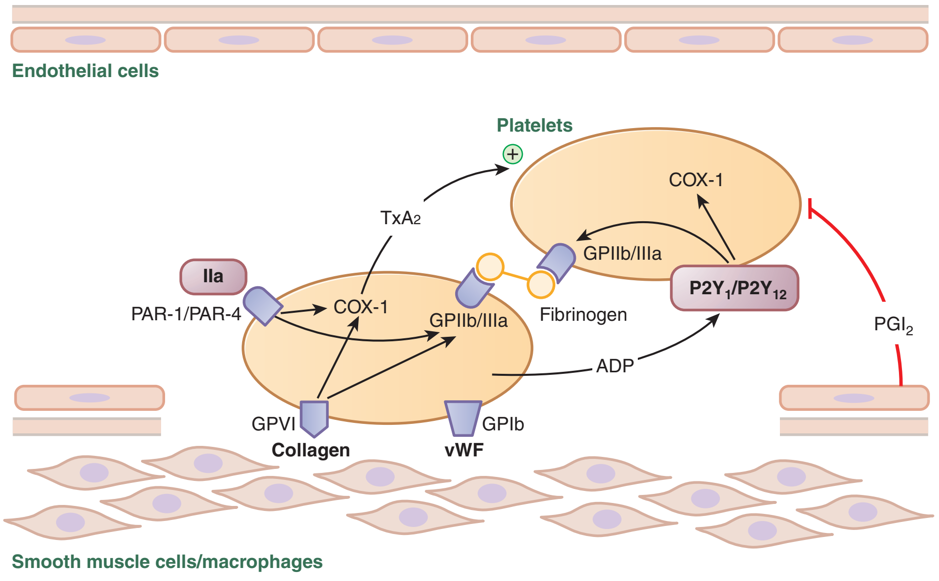


Figure 36–1 *Platelet adhesion and aggregation.* GPVI and GPIb are platelet receptors that bind to collagen and von Willebrand factor (vWF), causing platelets to adhere to the subendothelium of a damaged blood vessel. PAR-1 and PAR-4 are PARs that respond to thrombin (IIa); $P2Y_1$ and $P2Y_{12}$ are receptors for ADP; when stimulated by agonists, these receptors activate the fibrinogen-binding protein GPIIb/IIIa and COX-1 to promote platelet aggregation and secretion. TxA_2 is the major product of COX-1 involved in platelet activation. Prostacyclin (PGI_2), synthesized by endothelial cells, inhibits platelet activation.

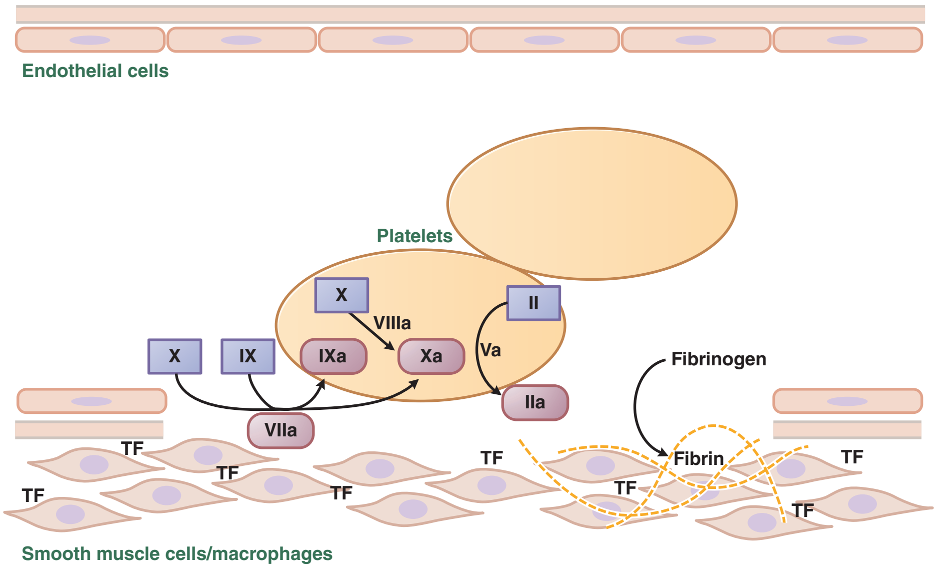


Figure 36–2 *Major reactions of blood coagulation.* Shown are interactions among proteins of the "extrinsic" (TF and factor VII), "intrinsic" (factors IX and VIII), and "common" (factors X, V, and II) coagulation pathways that are important *in vivo*. Blue rectangles enclose the coagulation factor zymogens (indicated by Roman numerals); the rounded boxes represent the active proteases. Activated coagulation factors are followed by the letter *a*: II, prothrombin; IIa, thrombin.

Activation of Prothrombin

By cleaving two peptide bonds on prothrombin, factor Xa converts it to thrombin. In the presence of factor Va, a negatively charged phospholipid surface, and Ca^{2+} (the so-called prothrombinase complex), factor Xa activates prothrombin with 10^9-fold greater efficiency than that of factor Xa alone. This maximal rate of activation only occurs when prothrombin and factor Xa contain Gla residues at their amino termini, which endows them with the capacity to bind calcium and interact with the anionic phospholipid surface.

Initiation of Coagulation

TF exposed at sites of vessel wall injury initiates coagulation via the *extrinsic pathway*. The small amount of factor VIIa circulating in plasma binds subendothelial TF, and the TF–factor VIIa complex then activates factors X and IX (see Figure 36–2). When bound to TF in the presence of anionic phospholipids and Ca^{2+} (extrinsic tenase), factor VIIa activity is increased 30,000-fold over that of factor VIIa alone.

The *intrinsic pathway* is initiated *in vitro* when factor XII, prekallikrein, and high-molecular-weight kininogen interact with kaolin, glass, or another negatively charged surface to generate small amounts of factor XIIa. Factor XII can be activated *in vivo* by contact of the blood with medical devices, such as mechanical heart valves or extracorporeal circuits, or by cell-free DNA, neutrophil extracellular traps (web-like structures composed of DNA and histones extruded from activated neutrophils), or inorganic polyphosphates released from activated platelets. Factor XIIa activates factor XI, and the resultant factor XIa then activates factor IX. Factor IXa activates factor X in a reaction accelerated by factor VIIIa, anionic phospholipids, and Ca^{2+}. Optimal thrombin generation depends on the formation of this factor IXa complex (intrinsic tenase) because it activates factor X more efficiently than the TF–factor VIIa complex.

Activation of factor XII is not essential for hemostasis, as evidenced by the fact that patients deficient in factor XII, prekallikrein, or high-molecular-weight kininogen do not have excessive bleeding. Factor XI deficiency is associated with a variable and usually mild bleeding disorder.

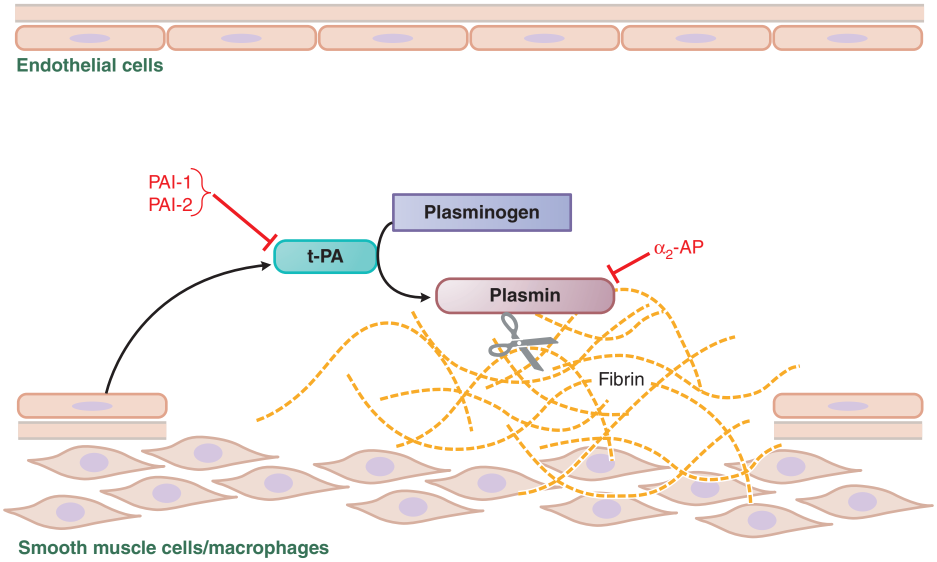


Figure 36–3 *Fibrinolysis.* Endothelial cells secrete t-PA at sites of injury. t-PA binds to fibrin and converts plasminogen to plasmin, which digests fibrin. PAI-1 and PAI-2 inactivate t-PA; α_2-AP inactivates plasmin.

In contrast, congenital deficiency of factor VIII or IX results in hemophilia A or B, respectively, and is associated with spontaneous bleeding, which can be fatal.

Fibrinolysis

The fibrinolysis pathway is summarized in Figure 36–3. The fibrinolytic system dissolves intravascular fibrin through the action of plasmin. To initiate fibrinolysis, plasminogen activators convert single-chain plasminogen, an inactive precursor, into two-chain plasmin by cleavage of a specific peptide bond. There are two distinct plasminogen activators: t-PA and u-PA, which is also known as urokinase. Although both activators are synthesized by endothelial cells, t-PA predominates under most conditions and drives intravascular fibrinolysis, while synthesis of u-PA mainly occurs in response to inflammatory stimuli and promotes extravascular fibrinolysis.

The fibrinolytic system is regulated such that unwanted fibrin thrombi are removed, while fibrin in wounds is preserved to maintain hemostasis. t-PA is released from endothelial cells in response to various stimuli. Released t-PA is rapidly cleared from blood or inhibited by plasminogen activator inhibitor (PAI) type 1 and, to a lesser extent, by PAI-2. Therefore, t-PA exerts little effect on circulating plasminogen in the absence of fibrin, and circulating α_2-antiplasmin rapidly inhibits any plasmin that is generated. The catalytic efficiency of t-PA activation of plasminogen increases more than 300-fold in the presence of fibrin, which promotes plasmin generation on its surface.

Plasminogen and plasmin bind to lysine residues on fibrin via five loop-like regions near their amino termini, which are known as kringle domains. To inactivate plasmin, α_2-antiplasmin binds to the first of these kringle domains and then blocks the active site of plasmin. Because the kringle domains are occupied when plasmin binds to fibrin, plasmin on the fibrin surface is protected from inhibition by α_2-antiplasmin and can digest the fibrin. Once the fibrin clot undergoes degradation, α_2-antiplasmin rapidly inhibits any plasmin that escapes from this local milieu. To prevent premature clot lysis, factor XIIIa mediates covalent cross-linking of small amounts of α_2-antiplasmin onto fibrin.

When thrombi occlude major arteries or veins, therapeutic doses of plasminogen activators are sometimes administered to rapidly degrade the fibrin and restore blood flow. In high doses, these plasminogen activators promote the generation of so much plasmin that the inhibitory controls are overwhelmed. Plasmin is a relatively nonspecific protease; in addition to degrading fibrin, it degrades several coagulation factors. Reduction in the levels of these coagulation proteins impairs the capacity for thrombin generation, which can contribute to bleeding. In addition, unopposed plasmin tends to dissolve fibrin in hemostatic plugs as well as that in pathological thrombi, a phenomenon that also increases the risk of bleeding. Therefore, hemorrhage is the major side effect of fibrinolytic drugs.

Coagulation *In Vitro*

Whole blood normally clots in 4 to 8 min when placed in a glass tube. Under these conditions, contact of the blood with glass activates factor XII, thereby initiating coagulation via the *intrinsic pathway*. Clotting is prevented if a chelating agent such as ethylenediaminetetraacetic acid or citrate is added to bind Ca^{2+}. Recalcified plasma normally clots in 2 to 4 min. The clotting time after recalcification is shortened to 26 to 33 sec by the addition of negatively charged phospholipids and particulate substances, such as silica (silicon dioxide), kaolin (aluminum silicate), or celite (diatomaceous earth), which activate factor XII; the measurement of this is termed the *activated partial thromboplastin time* (aPTT). Alternatively, recalcified plasma clots in 12 to 14 sec after addition of "thromboplastin" (a mixture of TF and phospholipid) and calcium; the measurement of this is termed the *prothrombin time* (PT).

Natural Anticoagulant Mechanisms

Platelet activation and coagulation do not normally occur within an intact blood vessel. Thrombosis is prevented by several regulatory mechanisms that require healthy vascular endothelium. Nitric oxide and prostacyclin synthesized by endothelial cells inhibit platelet activation, as does CD39, an ADP- and ATP-degrading enzyme expressed on the surface of endothelial cells.

Antithrombin is a plasma protein that inhibits coagulation enzymes of the extrinsic, intrinsic, and common pathways. Heparan sulfate proteoglycans synthesized by endothelial cells enhance the activity of antithrombin by about 1000-fold. Another regulatory system involves protein C, a plasma zymogen that is homologous to factors II, VII, IX, and X; its activity depends on the binding of Ca^{2+} to Gla residues within its amino terminal domain. Protein C binds to endothelial protein C receptor (EPCR), which presents it to the thrombin-thrombomodulin complex for activation. Activated protein C then dissociates from EPCR, and, in combination with protein S, its nonenzymatic Gla-containing cofactor, activated protein C degrades factors Va and VIIIa. Without these activated cofactors, the rates of activation of prothrombin and factor X are greatly diminished, and thrombin generation is attenuated. Congenital or acquired deficiency of protein C or protein S is associated with an increased risk of venous thrombosis.

Tissue factor pathway inhibitor (TFPI) is a natural anticoagulant found in the lipoprotein fraction of plasma or bound to endothelial cell surface. TFPI first binds and inhibits factor Xa, and this binary complex then inhibits factor VIIa bound to TF. By this mechanism, factor Xa regulates its own generation.

Parenteral Anticoagulants

Heparin, Low-Molecular-Weight Heparin, Fondaparinux

Heparin and Its Standardization

Heparin, a glycosaminoglycan found in the secretory granules of mast cells, is synthesized from UDP-sugar precursors as a polymer of alternating D-glucuronic acid and *N*-acetyl-D-glucosamine residues. *Heparin* is commonly extracted from porcine intestinal mucosa, which is rich in mast cells, and preparations may contain small amounts of other glycosaminoglycans. Various commercial *heparin* preparations have similar biological activity (~150 USP units/mg). A USP unit reflects the quantity of *heparin* that prevents 1 mL of citrated sheep plasma from clotting for 1 h after calcium addition. European manufacturers measure potency with an anti–factor Xa assay. To determine *heparin* potency, residual factor Xa activity in the sample is compared with that detected in controls containing known concentrations of an international *heparin* standard. When assessed this way, *heparin* potency is expressed in international units per milligram. Effective October 1, 2009, the new USP unit dose was harmonized with the international unit dose. As a result, the new USP unit dose is about 10% less potent than the old one, which results in a requirement for somewhat higher *heparin* doses to achieve the same level of anticoagulation.

Heparin Derivatives

Derivatives of *heparin* in current use include *low-molecular-weight heparin* (LMWH) and *fondaparinux* (see their comparison in Table 36–1).

Mechanism of Action. *Heparin*, LMWH, and *fondaparinux* have no intrinsic anticoagulant activity; rather, these agents bind to antithrombin and accelerate the rate at which it inhibits various coagulation proteases. Synthesized in the liver, antithrombin circulates in plasma at an approximate concentration of 2.5 μM. Antithrombin inhibits activated coagulation factors, particularly thrombin and factor Xa, by serving as a "suicide substrate." Thus, inhibition occurs when the protease attacks a specific Arg–Ser peptide bond in the reactive center loop of antithrombin and becomes trapped as a stable 1:1 complex. *Heparin* binds to antithrombin via a specific pentasaccharide sequence that contains a 3-*O*-sulfated glucosamine residue (Figure 36–4).

Pentasaccharide binding to antithrombin induces a conformational change in antithrombin that renders its reactive site more accessible to the target protease (Figure 36–5). This conformational change accelerates the rate of factor Xa inhibition by at least two orders of magnitude but

TABLE 36–1 ■ COMPARISON OF THE FEATURES OF SUBCUTANEOUS HEPARIN, LOW-MOLECULAR-WEIGHT HEPARIN, AND FONDAPARINUX

FEATURES	HEPARIN	LMWH	FONDAPARINUX
Source	Biological	Biological	Synthetic
Mean molecular weight (Da)	15,000	5000	1500
Target	Xa and IIa	Xa and IIa	Xa
Subcutaneous			
Bioavailability (%)	30 (at low doses)	90	100
$t_{1/2}$ (h)	1–8[a]	4	17
Renal excretion	No	Yes	Yes
Antidote effect	Complete	Partial	None
Thrombocytopenia	<5%	<1%	<0.1%

[a] Half-life *t* is dose dependent; half-life is 1 h with 5000 units given subcutaneously and can extend to 8 h with higher doses.

has no effect on the rate of thrombin inhibition. To enhance the rate of thrombin inhibition by antithrombin, *heparin* serves as a catalytic template to which both the inhibitor and the protease bind. Only *heparin* molecules composed of 18 or more saccharide units (molecular weight >5400) are of sufficient length to bridge antithrombin and thrombin together. Consequently, by definition, *heparin* catalyzes the rates of factor Xa and thrombin inhibition to a similar extent, as expressed by an anti–factor Xa to anti–factor IIa (thrombin) ratio of 1:1 (Figure 36–5A). In contrast, at least half of the LMWH molecules (mean molecular weight of 5000, which corresponds to about 17 saccharide units) are too short to provide this bridging function and have no effect on the rate of thrombin inhibition by antithrombin (Figure 36–5B). Because these shorter molecules still induce the conformational change in antithrombin that accelerates inhibition of factor Xa, LMWH has greater anti–factor Xa activity than anti–factor IIa activity, and the ratio ranges from 3:1 to 2:1 depending on the preparation. *Fondaparinux*, a synthetic analogue of the pentasaccharide sequence in *heparin* or LMWH that mediates their interaction with antithrombin, has only anti–factor Xa activity because it is too short to bridge antithrombin to thrombin (Figure 36–5C).

Heparin, LMWH, and *fondaparinux* act in a catalytic fashion. After binding to antithrombin and promoting the formation of covalent complexes between antithrombin and target proteases, the *heparin*, LMWH, or *fondaparinux* dissociates from the complex and can then catalyze other antithrombin molecules.

Platelet factor 4, a cationic protein released from α-granules during platelet activation, binds *heparin* and prevents it from interacting with antithrombin. This phenomenon may limit the activity of *heparin* in the vicinity of platelet-rich thrombi. Because LMWH and *fondaparinux* have a lower affinity for platelet factor 4, these agents may retain their activity in the vicinity of such thrombi to a greater extent than *heparin*.

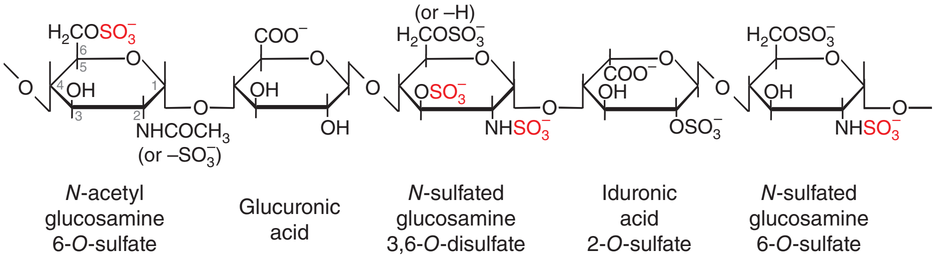
Miscellaneous Pharmacological Effects. High doses of *heparin* can interfere with platelet aggregation and prolong the bleeding time. In contrast, LMWH and *fondaparinux* have little effect on platelets. *Heparin* "clears" lipemic plasma *in vivo* by causing the release of lipoprotein lipase into the circulation. Lipoprotein lipase hydrolyzes triglycerides to glycerol and free fatty acids. The clearing of lipemic plasma may occur at concentrations of *heparin* below those necessary to produce an anticoagulant effect.

Clinical Use. *Heparin*, LMWH, or *fondaparinux* can be used to initiate treatment of deep vein thrombosis and pulmonary embolism (Ortel et al., 2020). They also can be used for the initial management of patients with unstable angina or acute myocardial infarction (Larson et al., 2019). For most of these indications, LMWH or *fondaparinux* has replaced continuous *heparin* infusions because of their pharmacokinetic advantages, which permit subcutaneous administration once or twice daily in fixed or weight-adjusted doses without coagulation monitoring. Thus, LMWH or *fondaparinux* can be used for out-of-hospital management of patients with deep vein thrombosis or pulmonary embolism.

Heparin or LMWH is used during coronary balloon angioplasty with or without stent placement to prevent thrombosis. *Fondaparinux* is not used in this setting because of the risk of catheter thrombosis, a complication caused by catheter-induced activation of factor XII; longer *heparin* molecules are better than shorter ones for blocking this process. Cardiopulmonary bypass circuits also activate factor XII, which can cause clotting of the oxygenator. *Heparin* remains the agent of choice for surgery requiring cardiopulmonary bypass. *Heparin* or LMWH also is used to treat selected patients with disseminated intravascular coagulation. Subcutaneous administration of low-dose *heparin* or LMWH is often used for thromboprophylaxis in immobilized medically ill patients (Schünemann et al., 2018) or in those who have undergone major surgery (Anderson et al., 2019).

Unlike the oral anticoagulants, *heparin*, LMWH, and *fondaparinux* do not cross the placenta and have not been associated with fetal malformations, making them the drugs of choice for anticoagulation during pregnancy. *Heparin*, LMWH, and *fondaparinux* do not appear to increase fetal mortality or prematurity. If possible, the drugs should be discontinued 24 h before delivery to minimize the risk of postpartum bleeding.

ADME. *Heparin*, LMWH, and *fondaparinux* are not absorbed through the GI mucosa and must be given parenterally. *Heparin* is given by continuous intravenous infusion, intermittent infusion every 4 to 6 h, or



H2COSO3−
6
5
O
4
OH
1
3
2
O
NHCOCH3
(or –SO3−)
COO−
O
OH
OH
O
(or –H)
H2COSO3−
O
OSO3−
NHSO3−
O
COO−
O
OH
OSO3−
O
H2COSO3−
O
OH
NHSO3−
O
N-acetyl glucosamine 6-O-sulfate
Glucuronic acid
N-sulfated glucosamine 3,6-O-disulfate
Iduronic acid 2-O-sulfate
N-sulfated glucosamine 6-O-sulfate

Figure 36–[illegible] *The antithrombin-binding pentasaccharide structure of heparin.* Sulfate groups required for binding to antithrombin are indicated in red

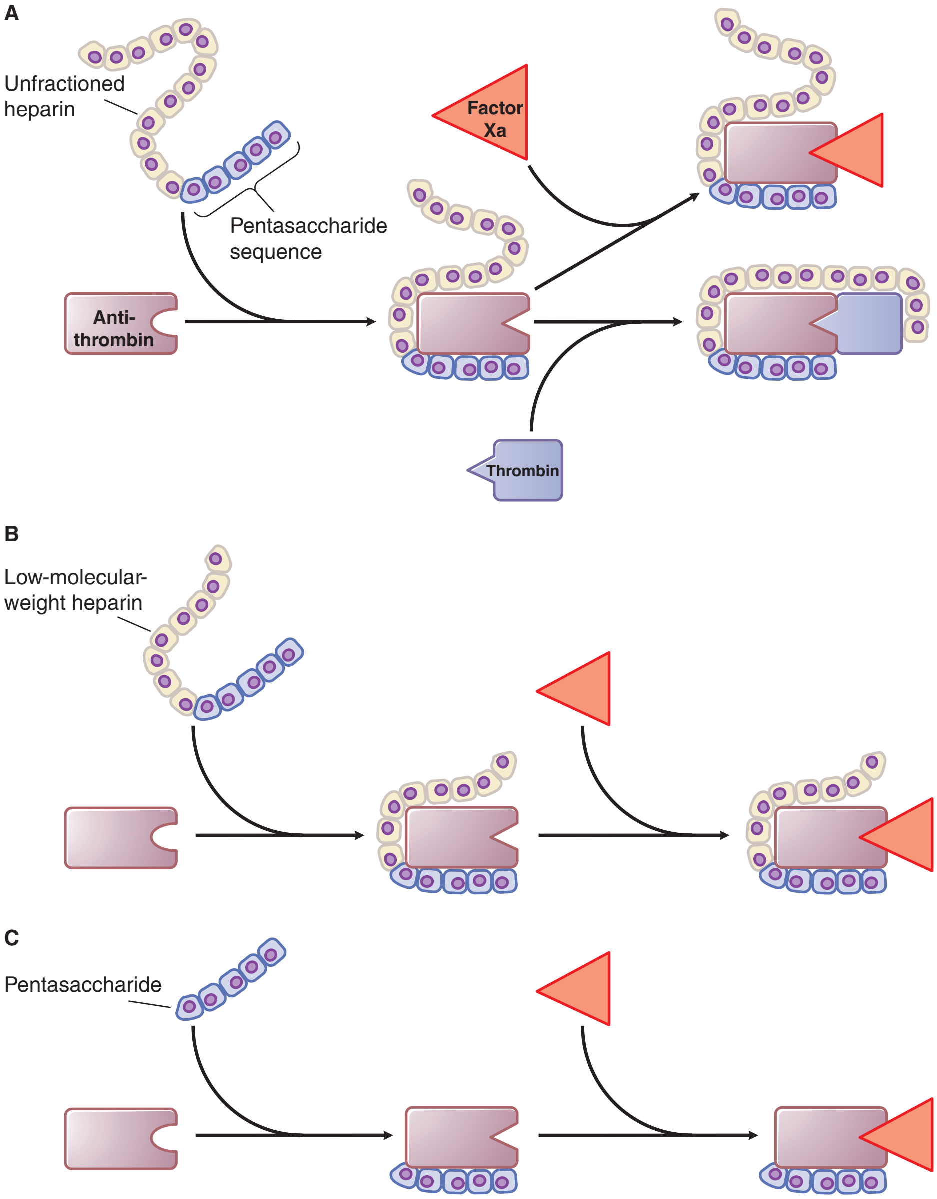


Figure 36–5 *Mechanism of action of heparin, LMWH, and fondaparinux, a synthetic pentasaccharide.* **A.** *Heparin* binds to antithrombin via its pentasaccharide sequence. This induces a conformational change in the reactive center loop of antithrombin that accelerates its interaction with factor Xa. To potentiate thrombin inhibition, *heparin* must simultaneously bind to antithrombin and thrombin. Only *heparin* chains composed of at least 18 saccharide units (molecular weight [MW] ~5400 Da) are of sufficient length to perform this bridging function. With a mean MW of approximately 15,000 Da, virtually all of the *heparin* chains are long enough to do this. **B.** LMWH has greater capacity to potentiate factor Xa inhibition by antithrombin than thrombin because at least half of the LMWH chains (mean MW ~4500–5000 Da) are too short to bridge antithrombin to thrombin. **C.** The pentasaccharide accelerates only factor Xa inhibition by antithrombin; the pentasaccharide is too short to bridge antithrombin to thrombin.

subcutaneous injection every 8 to 12 h. *Heparin* has an immediate onset of action when given intravenously. In contrast, there is considerable variation in the bioavailability of *heparin* given subcutaneously, and the onset of action is delayed by 1 to 2 h. LMWH and *fondaparinux* are absorbed more uniformly after subcutaneous injection. The $t_{1/2}$ of *heparin* in plasma depends on the dose administered. When doses of 100, 400, or 800 units/kg of *heparin* are injected intravenously, the half-lives are about 1, 2.5, and 5 h, respectively. *Heparin* appears to be cleared and degraded primarily by the reticuloendothelial system; a small amount of intact *heparin* appears in the urine.

Both LMWH and *fondaparinux* have longer biological half-lives than *heparin*, 4-6 h and 17 h, respectively. Because these smaller *heparin* fragments are cleared almost exclusively by the kidneys, the drugs can accumulate in patients with renal impairment and lead to bleeding. Both LMWH and *fondaparinux* are contraindicated in patients with a creatinine clearance below 30 mL/min. In addition, *fondaparinux* must be used with caution for thromboprophylaxis in patients undergoing hip fracture, hip replacement, knee replacement, or abdominal surgery for who have a body weight less than 50 kg because of an increased risk for bleeding.

Administration and Monitoring. Full-dose *heparin* usually is administered by continuous intravenous infusion. Treatment of venous thromboembolism is initiated with a fixed-dose bolus injection of 5000 units or with a weight-adjusted bolus, followed by 800 to 1600 units/h delivered by an infusion pump. Therapy is monitored by measuring the aPTT. The therapeutic range for *heparin* is that which is equivalent to a plasma *heparin* level of 0.3 to 0.7 units/mL, as determined with an anti–factor Xa assay. An aPTT that is two or three times the normal mean aPTT value generally is assumed to be therapeutic. The aPTT should be measured initially and the infusion rate adjusted every 6 h. Once a steady dosage schedule has been established, daily aPTT monitoring usually is sufficient. Extremely high doses of *heparin* are required to prevent clotting in patients undergoing percutaneous coronary intervention or cardiac surgery with cardiopulmonary bypass. The aPTT is infinitely prolonged with these high doses of *heparin*, so a less-sensitive coagulation test, the *activated clotting time* (ACT), is employed to monitor therapy in this situation.

For therapeutic purposes, *heparin* also can be administered subcutaneously on a twice-daily basis. A total daily dose of about 35,000 units administered as divided doses every 8 to 12 h usually is sufficient to achieve an aPTT of twice the control value (measured midway between doses). For low-dose *heparin* therapy (to prevent venous thromboembolism in hospitalized medical or surgical patients), a subcutaneous dose of 5000 units is given two or three times daily.

Heparin Resistance. Patients who fail to achieve a therapeutic aPTT with daily doses of *heparin* of 35,000 units or more are considered to have *heparin* resistance, which may reflect pseudo- or true resistance. Concomitant measurement of the aPTT and the anti–factor Xa level distinguishes between these two possibilities. With *heparin* pseudoresistance, the anti–factor Xa level is therapeutic despite the subtherapeutic aPTT, whereas with true *heparin* resistance, both the anti–factor Xa level and the aPTT are subtherapeutic. Pseudoresistance to *heparin* occurs if the aPTT is shorter than the control value prior to initiating *heparin* treatment because of high concentrations of factor VIII and fibrinogen. True *heparin* resistance occurs because of high plasma levels of proteins that compete with antithrombin for *heparin* binding or because of antithrombin deficiency. *Heparin* does not require dose adjustment in patients with pseudoresistance because the anti–factor Xa level is therapeutic. In contrast, with true resistance, *heparin* doses need to be increased until a therapeutic aPTT or anti–factor Xa level is achieved. Patients with severe antithrombin deficiency may require antithrombin concentrate to achieve therapeutic anticoagulation with *heparin*. Alternatively, they may be treated with a direct oral anticoagulant because these agents do not require antithrombin as a cofactor.

LMWH Preparations

Enoxaparin and *dalteparin* are the LMWH preparations marketed in the U.S.; *tinzaparin* is available in other countries. The composition of these agents differs, as do their dosing regimens. Because LMWH produces a relatively predictable anticoagulant response, monitoring is not done routinely. Patients with renal impairment may require monitoring with an anti–factor Xa assay because this condition can prolong the $t_{1/2}$ and slow the elimination of LMWH. Obese patients, pregnant women, and children given LMWH also may require monitoring. Weight-based prophylactic dosing of *enoxaparin* is preferable to fixed dosing for obese patients.

Fondaparinux

Fondaparinux, a synthetic pentasaccharide, is administered by subcutaneous injection, reaches peak plasma levels in 2 h, has a $t_{1/2}$ of 17 h, and is excreted in the urine. Because of the risk of accumulation and subsequent bleeding, *fondaparinux* should not be used in patients with a creatinine clearance less than 30 mL/min. *Fondaparinux* can be given subcutaneously once a day at a fixed or weight-adjusted dose without coagulation monitoring. *Fondaparinux* is much less likely than *heparin* or LMWH to trigger *heparin*-induced thrombocytopenia, and the drug has been used successfully to treat patients with this condition. *Fondaparinux* is approved for thromboprophylaxis in patients undergoing hip or knee surgery or surgery for hip fracture and for initial therapy of patients with deep vein thrombosis or pulmonary embolism. In some countries, but not the U.S., *fondaparinux* also is licensed as an alternative to *heparin* or LMWH in patients with acute coronary syndrome. For this indication and for thromboprophylaxis, *fondaparinux* is administered subcutaneously once daily at a dose of 2.5 mg.

Bleeding. The major untoward effect of *heparin*, LMWH, and *fondaparinux* is bleeding. Major bleeding occurs in 1% to 5% of patients treated with intravenous *heparin* for venous thromboembolism. The incidence of bleeding is somewhat less in patients treated with LMWH for this indication. Often, an underlying cause for bleeding is present, such as recent surgery, trauma, peptic ulcer disease, or platelet dysfunction due to concomitant administration of *aspirin* or other antiplatelet drugs.

The anticoagulant effect of *heparin* disappears within hours of discontinuation of the drug. Mild bleeding due to *heparin* usually can be controlled without administration of an antagonist. If life-threatening hemorrhage occurs, *heparin* can rapidly be reversed by the intravenous infusion of *protamine sulfate* (a mixture of basic polypeptides isolated from salmon sperm), which binds tightly to *heparin* and neutralizes its anticoagulant effect. *Protamine* also interacts with platelets, fibrinogen, and other plasma proteins and may cause an anticoagulant effect of its own. Therefore, one should give the minimal amount of *protamine* required to neutralize the *heparin* present in the plasma. This amount is 1 mg of *protamine* for every 100 units of *heparin* remaining in the patient; *protamine* is given intravenously at a slow rate (up to a maximum of 50 mg over 10 min). *Protamine* binds only long *heparin* molecules. Therefore, *protamine* only partially reverses the anticoagulant activity of LMWH and has no effect on that of *fondaparinux*. *Protamine* can cause severe hypotension, noncardiogenic pulmonary edema, and catastrophic pulmonary vasoconstriction when given rapidly and at high doses. Risk factors include previous administration of *protamine*-containing drugs (e.g., NPH insulin, protamine zinc insulin, and certain β blockers), allergy to fish, severe left ventricular dysfunction, and abnormal pulmonary hemodynamics.

Heparin-Induced Thrombocytopenia. *Heparin*-induced thrombocytopenia (platelet count <150,000/μL or a 50% decrease from the pretreatment value) occurs in about 0.5% of medical patients 5 to 10 days after initiation of therapy with *heparin*. Although the incidence is lower, thrombocytopenia also occurs with LMWH and rarely with *fondaparinux*. Life-threatening thrombotic complications that can lead to limb amputation occur in up to one-half of the affected *heparin*-treated patients and may precede the onset of thrombocytopenia. Women are twice as likely as men to develop this condition, and *heparin*-induced thrombocytopenia is more common in surgical patients than medical patients.

Venous thromboembolism occurs most commonly, but arterial thrombosis causing limb ischemia, myocardial infarction, or stroke also occurs. Bilateral adrenal hemorrhage, skin lesions at the site of subcutaneous *heparin* injection, and a variety of systemic reactions may accompany *heparin*-induced thrombocytopenia. The development of immunoglobulin G antibodies against complexes of *heparin* with platelet factor 4 (or, rarely, other chemokines) causes most of these reactions.

Heparin or LMWH should be discontinued immediately if unexplained thrombocytopenia or any of the clinical manifestations mentioned occur 5 or more days after beginning therapy, regardless of the dose or route of administration (Cuker et al., 2019). The diagnosis of *heparin*-induced thrombocytopenia can be confirmed with a *heparin*-dependent platelet activation assay or an immunoassay for antibodies that react with *heparin*–platelet factor 4 complexes. Because thrombotic complications may occur after cessation of therapy, an alternative anticoagulant such as *bivalirudin*, *argatroban*, or *rivaroxaban* (see below) should be administered to patients with *heparin*-induced thrombocytopenia. *Fondaparinux* is another alternative, although *fondaparinux*-induced thrombocytopenia has been reported in postmarketing studies. LMWH should be avoided because it cross-reacts with *heparin* antibodies. *Warfarin may precipitate venous limb gangrene or skin necrosis in patients with heparin-induced thrombocytopenia and should not be used until the platelet count returns to normal.*

Other Toxicities. Reversible abnormalities of hepatic function tests occur frequently in patients who are receiving *heparin* or LMWH. Osteoporosis occurs occasionally in patients who have received therapeutic doses of *heparin* (>20,000 units/day) for extended periods (e.g., 3–6 months). The risk of osteoporosis is lower with LMWH or *fondaparinux* than it is with *heparin*. *Heparin* can inhibit the synthesis of aldosterone by the adrenal glands and occasionally causes hyperkalemia.

Other Parenteral Anticoagulants

Desirudin and Lepirudin

Desirudin and *lepirudin* (both drugs are no longer available in the U.S.) are recombinant forms of hirudin. *Desirudin* is indicated for thromboprophylaxis in patients undergoing elective hip replacement surgery. Both *desirudin* and *lepirudin* are also used for treating thrombosis in the setting of *heparin*-induced thrombocytopenia (Morgan et al., 2020). *Desirudin* and *lepirudin* are eliminated by the kidneys; the $t_{1/2}$ is about 2 h after subcutaneous administration and about 10 min after intravenous infusion. Both drugs should be used cautiously in patients with decreased renal function, and serum creatinine and aPTT should be monitored daily.

Bivalirudin

Bivalirudin is a synthetic 20–amino acid polypeptide that directly inhibits thrombin. *Bivalirudin* is administered intravenously and is used as an alternative to *heparin* in patients undergoing coronary angioplasty or cardiopulmonary bypass surgery (Barria Perez et al., 2016; Koster et al., 2018). Patients with *heparin*-induced thrombocytopenia or a history of this disorder also can be given *bivalirudin* instead of *heparin* during coronary angioplasty. The $t_{1/2}$ of *bivalirudin* is 25 min; dosage reductions are recommended for patients with renal impairment.

Argatroban

Argatroban, a synthetic compound based on the structure of L-arginine, binds reversibly to the active site of thrombin. *Argatroban* is administered intravenously and has a $t_{1/2}$ of 40 to 50 min. It is metabolized in the liver and excreted in the bile. Therefore, *argatroban* can be used in patients with renal impairment, but dose reduction is required for patients with hepatic insufficiency. *Argatroban* is licensed for the prophylaxis or treatment of patients with, or at risk of developing, *heparin*-induced thrombocytopenia (Morgan et al., 2020). In addition to prolonging the aPTT, *argatroban* prolongs the PT, which can complicate the transitioning of patients from *argatroban* to warfarin. A factor X assay can be used instead of the PT to monitor *warfarin* in these patients.

Vitamin K Antagonist

Warfarin

Warfarin or other vitamin K antagonists are commonly used oral anticoagulants.

Mechanism of Action

Coagulation factors II, VII, IX, and X and proteins C and S are synthesized in the liver and are biologically inactive unless 9 to 13 of the amino-terminal Glu residues are γ-carboxylated to form the Ca^{2+}-binding Gla domain. This carboxylation reaction requires CO_2, O_2, and reduced vitamin K and is catalyzed by γ-glutamyl carboxylase (Figure 36–6). Carboxylation is coupled to the oxidation of vitamin K to its corresponding epoxide form. Reduced vitamin K must be regenerated from the epoxide form for sustained carboxylation and synthesis of functional proteins. The enzyme that catalyzes this reaction, *vitamin K epoxide reductase* (VKOR), is inhibited by therapeutic doses of *warfarin*.

At therapeutic doses, *warfarin* decreases the functional amount of each vitamin K–dependent coagulation factor made by the liver by 30% to 70%. *Warfarin* has no effect on the activity of fully γ-carboxylated factors already in the circulation, and these must be cleared before it can produce an anticoagulant effect. The approximate $t_{1/2}$ values of factors VII, IX, X, and II are 6, 24, 36, and 50 h, respectively, while the $t_{1/2}$ values of protein C and protein S are 8 and 24 h, respectively. Because of the long $t_{1/2}$ of some of the coagulation factors, in particular factor II, the full antithrombotic effect of *warfarin* is not achieved for 4 to 5 days. For this reason, *warfarin* must be overlapped with a rapidly acting parenteral anticoagulant, such as *heparin*, LMWH, or *fondaparinux*, in patients with thrombosis or at high risk for thrombosis.

ADME

The bioavailability of *warfarin* is nearly complete when the drug is administered orally, intravenously, or rectally. Generic *warfarin* tablets may vary

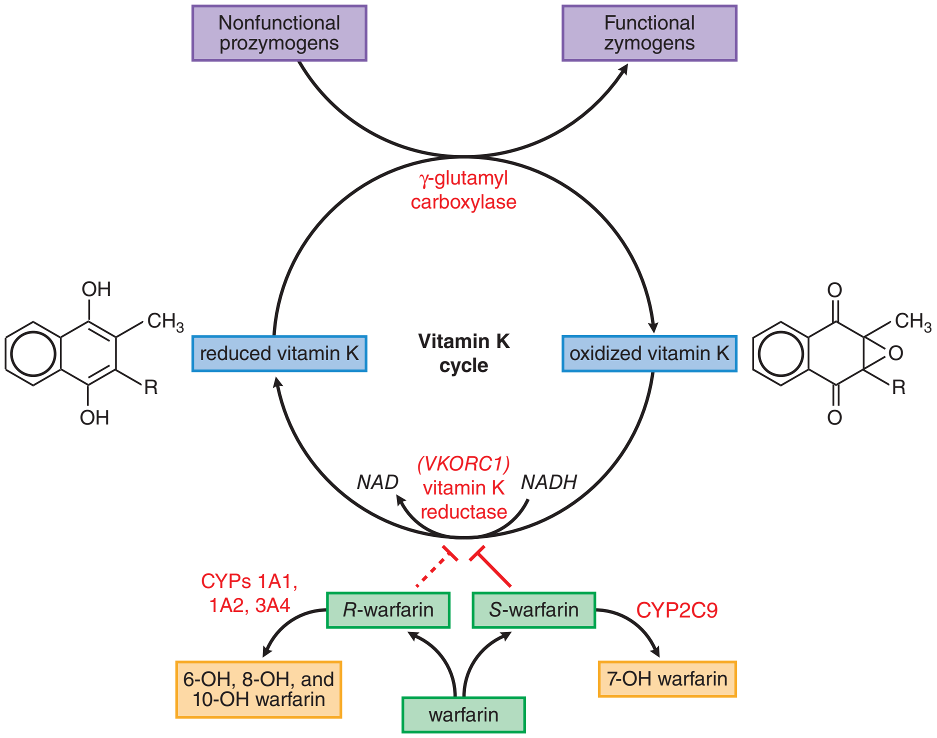


Figure 36–6 *The vitamin K cycle and mechanism of action of warfarin.* In the racemic mixture of *S*- and *R*-enantiomers, *S*-warfarin is more active. By blocking VKOR encoded by the *VKORC1* gene, *warfarin* inhibits the conversion of oxidized vitamin K epoxide to its reduced form, vitamin K hydroquinone. This inhibits vitamin K–dependent γ-carboxylation of factors II, VII, IX, and X because reduced vitamin K serves as a cofactor for a γ-glutamyl carboxylase that catalyzes the γ-carboxylation process whereby prozymogens are converted to zymogens capable of binding Ca^{2+} and interacting with anionic phospholipids. *S*-Warfarin is metabolized by *CYP2C9*; common genetic polymorphisms in this enzyme increase *warfarin* metabolism. Polymorphisms in the VKORC1 increase the susceptibility of the enzyme to *warfarin*-induced inhibition. Thus, patients expressing polymorphisms in these two enzymes require reduction of *warfarin* dosage (see Table 36–2).

TABLE 36–2 ■ EFFECT OF *CYP2C9* GENOTYPES AND *VKORC1* HAPLOTYPES ON WARFARIN DOSING

GENOTYPE or HAPLOTYPE	FREQUENCY (%)			DOSE REDUCTION COMPARED WITH WILD TYPE (%)
	CAUCASIANS	AFRICAN AMERICANS	ASIANS	
CYP2C9				
*1/*1	70	90	95	—
*1/*2	17	2	0	22
*1/*3	9	3	4	34
*2/*2	2	0	0	43
*2/*3	1	0	0	53
*3/*3	0	0	1	76
VKORC1				
Non-A/non-A	37	82	7	—
Non-A/A	45	12	30	26
A/A	18	6	63	50

Polymorphisms in two genes, *CYP2C9* and *VKORC1*, largely account for the genetic contribution to the variability in warfarin response. *CYP2C9* variants affect warfarin pharmacokinetics. *CYP2C9* metabolizes warfarin, and the non-*1/*1 variants are less active than *CYP2C9**1/*1, necessitating a reduction in dose. *VKORC1* variants affect warfarin pharmacodynamics. *VKORC1* is the target of coumarin anticoagulants such as warfarin. The non-A/A and A/A forms have decreased requirements for warfarin.

Source: Ghimire LV, Stein CM. Warfarin pharmacogenetics. Goodman and Gilman Online.

in their rate of dissolution, and this may cause some variation in the rate and extent of absorption. Food in the GI tract also can decrease the rate of absorption. Plasma *warfarin* concentrations peak in 2 to 8 h. *Warfarin* is administered as a racemic mixture of *S*- and *R*-warfarin. *S*-Warfarin is 3- to 5-fold more potent than *R*-warfarin and is mainly metabolized by cytochrome P450 (CYP) type 2C9. Inactive metabolites of *warfarin* are excreted in urine and stool. The $t_{1/2}$ varies (25–60 h), but the duration of action of *warfarin* is 2 to 5 days.

Table 36–2 summarizes the effects of known genetic factors on *warfarin* dose requirements. Polymorphisms in two genes, *CYP2C9* and *VKORC1*, account for most of the genetic contribution to variability in *warfarin* response (Johnson et al., 2017). *CYP2C9* variants affect *warfarin* pharmacokinetics, whereas *VKORC1* variants affect *warfarin* pharmacodynamics. Common variations in the *CYP2C9* gene (designated *CYP2C9**2* and **3*), encode an enzyme with decreased activity and thus are associated with higher drug concentrations and reduced *warfarin* dose requirements. *VKORC1* variants are more prevalent than those of *CYP2C9*, particularly in Asians, followed by European Americans and African Americans. The *warfarin* dose requirement is decreased in patients with these variants. Point-of-care methods for *CYP2C9* and *VKORC1* genotyping and algorithms that incorporate genotype information have been developed to facilitate precision dosing of *warfarin*. It remains uncertain, however, whether precision dosing improves clinical outcome compared with usual *warfarin* management (Li et al., 2019; Tse et al., 2018).

Clinical Use

Vitamin K antagonists are used to prevent the progression or recurrence of acute deep vein thrombosis or pulmonary embolism following an initial course of *heparin*, LMWH, or *fondaparinux*. They also are effective in preventing stroke or systemic embolization in patients with atrial fibrillation, mechanical heart valves, or ventricular assist devices.

Prior to initiation of therapy, laboratory tests are used in conjunction with the history and physical examination to uncover hemostatic defects that might make the use of warfarin more dangerous (e.g., congenital coagulation factor deficiency, thrombocytopenia, hepatic or renal insufficiency, vascular anomalies). Thereafter, the *international normalized ratio* (INR) calculated from the patient's PT is used to monitor the extent of anticoagulation and compliance. The target therapeutic INR ranges for various clinical indications have been established and reflect the extent of anticoagulation that reduces the morbidity from thromboembolic disease while minimally increasing the risk of serious hemorrhage. For most indications, an INR range of 2 to 3 is used. A higher INR range (2.5–3.5) is recommended for patients with mechanical heart valves in the mitral position or for patients with mechanical valves in another position who have concomitant atrial fibrillation or a prior history of stroke.

For treatment of acute venous thromboembolism, *heparin*, LMWH, or *fondaparinux* usually is continued for at least 5 days after *warfarin* therapy is begun. The parenteral agent is stopped when the INR is in the therapeutic range on 2 consecutive days. This overlap allows for adequate depletion of vitamin K–dependent coagulation factors with long half-lives, especially factor II. Frequent INR measurements are indicated at the onset of therapy to ensure that a therapeutic effect is obtained. Once a stable dose of *warfarin* has been identified, the INR can be monitored every 3 to 4 weeks.

Dosage

The usual adult dosage of *warfarin* is 2 to 5 mg/day for 2 to 4 days, followed by 1 to 10 mg/day as indicated by measurements of the INR (see the functional definition of INR in the section on clinical use). A lower initial dose should be given to patients with an increased risk of bleeding, including the elderly.

Interactions

Warfarin interactions can be caused by drugs, foods, or genetic factors that alter (1) uptake or metabolism of *warfarin* or vitamin K; (2) synthesis, function, or clearance of clotting factors; or (3) the integrity of any epithelial surface. Reduced *warfarin* efficacy can occur because of reduced absorption (e.g., binding to cholestyramine in the GI tract) or increased hepatic clearance from induction of hepatic enzymes (e.g., *CYP2C9* induction by barbiturates, *carbamazepine*, or *rifampin*). *Warfarin* has a decreased volume of distribution and a short $t_{1/2}$ with hypoproteinemia, such as occurs with nephrotic syndrome. Relative *warfarin* resistance can also be caused by ingestion of large amounts of vitamin K–rich foods or supplements or by increased levels of coagulation factors during pregnancy.

Drug interactions that enhance the risk of hemorrhage in patients taking *warfarin* include decreased metabolism due to *CYP2C9* inhibition by *amiodarone*, azole antifungals, *cimetidine, clopidogrel, cotrimoxazole, disulfiram, fluoxetine, isoniazid, metronidazole, sulfinpyrazone, tolcapone*, or *zafirlukast*. Relative deficiency of vitamin K may result from inadequate diet (e.g., postoperative patients on parenteral fluids), especially when coupled with the elimination of intestinal flora by antimicrobial agents. Gut bacteria synthesize vitamin K and are an important source of this vitamin. Consequently, antibiotics can cause an increase in the INR in patients on *warfarin*. Low concentrations of coagulation factors may result from impaired hepatic function, congestive heart failure, or hypermetabolic states, such as hyperthyroidism; generally, these conditions enhance the effect of *warfarin* on the INR. Serious interactions that do not alter the INR include inhibition of platelet function by agents such as *aspirin* and gastritis or frank ulceration induced by anti-inflammatory drugs. Agents may have more than one effect (e.g., *clofibrate* increases the rate of turnover of coagulation factors and inhibits platelet function).

Hypersensitivity to Warfarin

About 10% of patients require less than 1.5 mg/day of *warfarin* to achieve an INR of 2 to 3. These patients often possess variants of *CYP2C9* or *VKORC1*; these variants affect the pharmacokinetics or pharmacodynamics of *warfarin*, respectively. Supplementation with low daily doses of vitamin K renders these patients less sensitive to *warfarin* and may result in more stable dosing.

Adverse Effects

Bleeding. The most common side effect of *warfarin* is bleeding. The risk of bleeding increases with the intensity and duration of anticoagulant therapy, the use of other medications that interfere with hemostasis, and the presence of an anatomical source of bleeding. The incidence of major bleeding episodes is generally less than 3% per year in patients treated to a target INR of 2 to 3. The risk of intracranial hemorrhage increases dramatically with an INR greater than 4, although up to two-thirds of intracranial bleeds on *warfarin* occur when the INR is therapeutic.

If the INR is above the therapeutic range and the patient is not bleeding or in need of a surgical procedure, *warfarin* can be held temporarily and restarted at a lower dose once the INR is within the therapeutic range. If the INR is 10 or greater, vitamin K_1 can be given orally at a dose of 2.5 to 5 mg. These doses of oral vitamin K_1 generally cause the INR to decrease substantially within 24 to 48 h without rendering the patient resistant to further *warfarin* therapy. Higher doses of vitamin K_1 or parenteral administration may be required if more rapid correction of the INR is necessary. The effect of vitamin K_1 is delayed for at least several hours because reversal of anticoagulation requires synthesis of fully carboxylated coagulation factors. If immediate hemostatic competence is necessary because of serious bleeding or profound *warfarin* overdosage, adequate concentrations of vitamin K–dependent coagulation factors can be restored by transfusion of four-factor prothrombin complex concentrate, supplemented with 10 mg of vitamin K_1, given by slow intravenous infusion. Vitamin K_1 administered intravenously carries the risk of anaphylactoid reactions. Patients who receive high doses of vitamin K_1 may become unresponsive to *warfarin* for several days, but *heparin* or LMWH can be given if continued anticoagulation is required.

Birth Defects. Administration of *warfarin* during pregnancy causes birth defects and abortion. CNS abnormalities have been reported following exposure during the second and third trimesters. Fetal or neonatal hemorrhage and intrauterine death may occur, even when maternal INR values are within the therapeutic range. Vitamin K antagonists should not be used during pregnancy, but *heparin* or LMWH can be used safely.

Skin Necrosis. *Warfarin*-induced skin necrosis is a rare complication characterized by the appearance of skin lesions 3 to 10 days after treatment is initiated. The lesions typically are on the extremities, but adipose tissue, the penis, and the female breast also may be involved. Skin necrosis occurs in patients with protein C or S deficiency or in those with *heparin*-induced thrombocytopenia.

Other Toxicities. A reversible, sometimes painful, blue-tinged discoloration of the plantar surfaces and sides of the toes that blanches with pressure and fades with elevation of the legs (purple toe syndrome) may develop 3 to 8 weeks after initiation of therapy with *warfarin*; cholesterol emboli released from atheromatous plaques have been implicated as the cause. *Warfarin* can cause fatal calciphylaxis (calcium accumulation in small blood vessels) or calcium uremic arteriolopathy. Other infrequent reactions include alopecia, urticaria, dermatitis, fever, nausea, diarrhea, abdominal cramps, and anorexia.

Direct Oral Anticoagulants

Direct Oral Thrombin Inhibitor

Dabigatran

Dabigatran etexilate is a synthetic prodrug with a molecular weight of 628 Da.

Mechanism of Action. *Dabigatran etexilate* is rapidly converted to *dabigatran* by plasma esterases. *Dabigatran* competitively and reversibly blocks the active site of free and clot-bound thrombin, thereby inhibiting thrombin-mediated conversion of fibrinogen to fibrin, feedback activation of coagulation, and platelet activation.

ADME. *Dabigatran* has oral bioavailability of about 6%, a peak onset of action in 2 h, and a plasma $t_{1/2}$ of 12 to 14 h. *Dabigatran* is given twice a day in capsule form. The bioavailability of the drug is altered if capsules are chewed or broken prior to ingestion. Therefore, the capsules should be swallowed whole. Circulating *dabigatran* is 35% bound to plasma proteins. Around 80% of absorbed *dabigatran* is excreted unchanged by the kidneys. A dosage reduction is required when *dabigatran* is administered to patients with severe renal impairment (creatinine clearance 15–30 mL/min). Dosage recommendations are not available for patients with a creatinine clearance below 15 mL/min.

When given in fixed doses, *dabigatran etexilate* produces such a predictable anticoagulant response that routine coagulation monitoring is unnecessary. Although *dabigatran* prolongs the aPTT, the values plateau with higher drug levels. *Dabigatran* has an unreliable effect on the INR. The thrombin time is too sensitive to use to monitor *dabigatran* therapy because the test is markedly prolonged with even low levels of drug. To circumvent this problem, a diluted thrombin time assay has been developed. By comparing the results with those obtained with *dabigatran* calibrators, this test can be used to quantify plasma *dabigatran* concentrations.

Therapeutic Uses. *Dabigatran* is licensed for treatment of acute venous thromboembolism after at least 5 days of parenteral anticoagulation with *heparin*, LMWH, or *fondaparinux* (Schulman et al., 2014), for secondary prevention of venous thromboembolism, and for stroke prevention in patients with nonvalvular atrial fibrillation (Connolly et al., 2009). It is contraindicated in patients with mechanical heart valves (Eikelboom et al., 2013). In some countries, lower-dose regimens of once-daily *dabigatran* are licensed for thromboprophylaxis after knee or hip arthroplasty.

Adverse Effects. Bleeding is the major side effect of *dabigatran*. In elderly patients with atrial fibrillation, the annual risk of major bleeding with *dabigatran* 150 mg twice daily is like that with *warfarin*, about 3.0%. However, the risk of intracranial bleeding is reduced by 70% with *dabigatran* compared with *warfarin*. In contrast, the risk of GI bleeding is higher with *dabigatran*, particularly in those over 75 years of age. Additional risk factors for bleeding with *dabigatran* include renal impairment and concurrent use of antiplatelet agents or nonsteroidal anti-inflammatory drugs. *Dabigatran* should not be used in patients with mechanical prosthetic heart valves and in patients with triple-positive antiphospholipid antibody syndrome (positive for lupus anticoagulant, anticardiolipin antibodies, and anti–β_2-glycoprotein I antibodies).

Drug Interactions. *Dabigatran* is a substrate for P-glycoprotein, so drugs that inhibit or induce P-glycoprotein have the potential to increase or decrease plasma *dabigatran* concentrations, respectively. *Verapamil, dronedarone, quinidine, ketoconazole*, and *clarithromycin* can increase *dabigatran* concentrations, while *rifampicin* may decrease the concentration.

Direct Oral Factor Xa Inhibitors

Rivaroxaban, Apixaban, Edoxaban, and Betrixaban

Mechanism of Action. *Rivaroxaban*, *apixaban*, *edoxaban*, and *betrixaban* inhibit free and clot-associated factor Xa, which results in reduced thrombin generation. In turn, platelet aggregation and fibrin formation are suppressed. Manufacturing of *betrixaban* was stopped in 2020, and the drug is no longer available.

ADME. *Rivaroxaban* has 80% oral bioavailability, a peak onset of action in 3 h, and a plasma $t_{1/2}$ of 7 to 11 h. Maximum absorption of *rivaroxaban* occurs in the stomach, and when given in therapeutic doses, the drug should be administered with a meal to enhance absorption. *Rivaroxaban* is provided in tablet form; the tablet can be crushed and delivered via nasogastric tube. *Rivaroxaban* is 95% plasma protein bound. About one-third of the drug is excreted unchanged in the urine; the remainder is metabolized by hepatic CYP3A4, and inactive metabolites are excreted equally in the urine and feces. *Rivaroxaban* exposure is increased in patients with renal impairment or severe hepatic dysfunction. The therapeutic dose of *rivaroxaban* is reduced from 20 mg once daily to 15 mg once daily if the creatinine clearance is 15 to 50 mL/min. The drug should not be used in those with a lower creatinine clearance. In patients with acute deep vein thrombosis or pulmonary embolism, *rivaroxaban* is started at a dose of 15 mg twice daily for 21 days, and the dose is then reduced to 20 mg once daily thereafter. For secondary prevention of venous thromboembolism after 6 months of treatment, the *rivaroxaban* dose can be reduced from 20 mg once daily to 10 mg once daily (Weitz et al., 2017).

The bioavailability of *apixaban* is around 50%, and peak concentrations are achieved 1 to 3 h after ingestion. Food does not affect absorption, and the drug can be administered as a whole tablet, or the tablet can be crushed in water and delivered via a nasogastric tube. *Apixaban* is 87% plasma protein bound, and about 27% of the drug is cleared unchanged via the kidneys. *Apixaban* is metabolized by hepatic CYP3A4, and metabolites are excreted in the bile, intestines, and urine. The usual dose of *apixaban* is 5 mg twice daily. The dose is reduced to 2.5 mg twice daily in patients who have two of the following three characteristics: age over 80 years, body weight of 60 kg or less, or serum creatinine concentration of 1.5 mg/dL or higher. In patients with acute venous thromboembolism, patients start *apixaban* at a dose of 10 mg twice daily for 7 days, and the dose is then decreased to 5 mg twice daily thereafter. For those requiring treatment beyond 6 to 12 months, the *apixaban* dose can be decreased to 2.5 mg twice daily.

The bioavailability of *edoxaban* is 62%, and peak drug concentrations are achieved 1 to 2 h after ingestion. Food does not affect absorption. *Edoxaban* is 55% protein bound. Of the absorbed *edoxaban*, about 50% is eliminated as unchanged drug in the urine. There is minimal hepatic metabolism, and liver disease does not affect drug pharmacodynamics. Drug exposure is increased by renal impairment, low body weight, and concomitant intake of potent P-glycoprotein inhibitors. Therefore, the dose of *edoxaban* should be reduced from 60 to 30 mg once daily in patients with a creatinine clearance between 15 and 50 mL/min, in those with a body weight of 60 kg or less, or in those taking *quinidine*, *dronedarone*, *rifampin*, *erythromycin*, *ketoconazole*, or *cyclosporine*. *Edoxaban* is contraindicated in those with a creatinine clearance below 15 mL/min. In the U.S., but not in other countries, *edoxaban* is also not recommended in patients with a creatinine clearance over 95 mL/min because subgroup analysis of the results of the trial comparing *edoxaban* with *warfarin* for stoke prevention in atrial fibrillation revealed a higher risk of ischemic stroke with *edoxaban* than with *warfarin* in such patients.

Rivaroxaban, *apixaban*, and *edoxaban* are given in fixed doses and do not require routine coagulation monitoring. The drugs affect the PT more than the aPTT, but they prolong the PT to a variable extent, and this test does not provide a reliable measure of their anticoagulant activity. Anti–factor Xa assays using specific drug calibrators can be used to measure drug levels. Renal function should be assessed at least yearly in patients taking oral factor Xa inhibitors or more frequently in patients with renal dysfunction.

Therapeutic Uses. *Rivaroxaban*, *apixaban*, and *edoxaban* are licensed for stroke prevention in patients with atrial fibrillation (Giugliano et al., 2013; Granger et al., 2011; Patel et al., 2011) and for treatment of acute deep vein thrombosis or pulmonary embolism (Beyer-Westendorf and Ageno, 2015; Gómez-Outes et al., 2015). For the last indication, *edoxaban* is started only after a minimum 5-day course of treatment with *heparin*, LMWH, or *fondaparinux* (Buller et al., 2013). In contrast, *rivaroxaban* and *apixaban* can be started immediately (EINSTEIN Investigators, 2010, 2012; Granziera et al., 2016). Studies comparing *apixaban*, *rivaroxaban*, or *edoxaban* with *dalteparin* for treatment of cancer-associated venous thromboembolism reveal that oral direct factor Xa inhibitors are at least as effective as *dalteparin* but are associated with more bleeding in patients with intact gastrointestinal cancers, particularly those of the upper gastrointestinal tract (Mulder et al., 2020).

Rivaroxaban and *apixaban* are also licensed for postoperative thromboprophylaxis in patients undergoing hip or knee arthroplasty (Anderson et al., 2019); for this indication, the drugs are given at doses of 10 mg once daily and 2.5 mg twice daily, respectively. All three drugs are contraindicated for stroke prevention in patients with mechanical heart valves. In addition, the drugs should be avoided in patients with antiphospholipid syndrome because *warfarin* appears superior to direct oral factor Xa inhibitors for prevention of recurrent thrombosis (Ghembaza and Saadoun, 2020).

Low-dose *rivaroxaban* (2.5 mg twice daily) in combination with *aspirin* (81 or 100 mg daily) is licensed for prevention of major adverse cardiac and limb events in patients with coronary or peripheral artery disease (Capodanno et al., 2020). This combination produces dual pathway inhibition because the *rivaroxaban* attenuates thrombin generation and fibrin formation, while the *aspirin* attenuates platelet activation. Inhibition of both pathways with *rivaroxaban* plus *aspirin* proved to be more effective than single-pathway inhibition with either *rivaroxaban* or *aspirin* alone in patients with coronary or peripheral artery disease (Eikelboom et al., 2017) and was more effective than *aspirin* alone after revascularization procedures for peripheral artery disease (Bonaca et al., 2020).

Adverse Effects. As with all anticoagulants, the major adverse effect is bleeding. Rates of intracranial bleeding with *rivaroxaban*, *apixaban*, and *edoxaban* are at least 50% lower than that with *warfarin*. Rates of bleeding in other sites are similar or lower than those with *warfarin*. The sole exception is the GI tract; rates of GI bleeding with *rivaroxaban* and *edoxaban*, but not *apixaban*, are higher than that with *warfarin*. The explanation for this difference is uncertain, but it may reflect the capacity of unabsorbed anticoagulant in the gut to promote bleeding from preexisting lesions. Despite the increased risk of GI bleeding, rates of life-threatening and fatal bleeding are lower with all the oral factor Xa inhibitors than with *warfarin*. Like with other anticoagulants, the risk of bleeding with *rivaroxaban, apixaban*, or *edoxaban* is increased in patients taking concomitant antiplatelet agents or nonsteroidal anti-inflammatory agents.

Drug Interactions. All the oral factor Xa inhibitors are substrates for P-glycoprotein. Consequently, potent inhibitors or inducers of P-glycoprotein will increase or decrease drug concentrations, respectively. *Rivaroxaban* and *apixaban* are metabolized by CYP3A4, whereas *edoxaban* undergoes only minimal CYP3A4-mediated metabolism. Plasma levels of *rivaroxaban* and *apixaban* are reduced by potent inducers of both P-glycoprotein and CYP3A4, such as *carbamazepine*, *phenytoin*, *rifampin*, and St. John's wort and increased by potent inhibitors, such as *dronedarone, ketoconazole, itraconazole, ritonavir, clarithromycin, erythromycin*, and *cyclosporine*.

Reversal Agents for Direct Oral Anticoagulants

Life-threatening bleeding can occur with the direct oral anticoagulants, and patients taking these drugs may require urgent surgery or interventions. Therefore, the availability of specific reversal agents streamlines the management of such patients. *Idarucizumab* is licensed for *dabigatran* reversal in patients with serious bleeding, such as intracranial bleeding, or in those requiring urgent surgery or intervention.

Recombinant coagulation factor Xa, inactivated-zhzo, also called *andexanet alfa*, is FDA-approved for reversal of patients treated with *rivaroxaban* and *apixaban* with serious bleeding. *Andexanet alfa* has not been evaluated for reversal of *apixaban*, *rivaroxaban*, or *edoxaban* prior to urgent surgery. Because *andexanet* also reverses the anticoagulant effect of *heparin* and LMWH, it should not be used in patients undergoing procedures where *heparin* is used routinely, such as vascular or cardiac surgery.

Andexanet alfa is considerably more expensive than *idarucizumab* and is not as widely available. Because of the cost, *andexanet* is often reserved for patients with the most serious bleeds, especially intracranial hemorrhage. If *andexanet* is unavailable, the results of cohort studies suggest that four-factor prothrombin complex concentrate improves hemostasis in patients with serious bleeding. If this is ineffective, activated prothrombin complex concentrate or recombinant factor VIIa can be considered (Cuker et al., 2018).

In patients taking *dabigatran* who present with serious bleeding in the setting of acute renal failure, hemodialysis can be used to remove *dabigatran* from the circulation. Dialysis is of no value for removal of *rivaroxaban*, *apixaban*, or *edoxaban* because of their higher protein binding.

Idarucizumab

A specific reversal agent for *dabigatran*, *idarucizumab* is a humanized mouse monoclonal antibody fragment directed against *dabigatran*. The antibody binds *dabigatran* with an affinity 350-fold higher than that of *dabigatran* for thrombin, and the essentially irreversible *idarucizumab-dabigatran* complex is cleared by the kidneys. *Idarucizumab* is infused as two intravenous boluses, each of 2.5 g. It rapidly reverses the anticoagulant effects of *dabigatran*, and patients have then safely undergone major surgery (Pollack et al., 2017).

Andexanet Alfa

Andexanet alfa has been renamed by the FDA and is now referred to generically as *coagulation factor Xa (recombinant), inactivated-zhzo*. For simplicity, it is referred to as *andexanet* in this chapter. Designed as a decoy for the oral factor Xa inhibitors, *andexanet* is a recombinant analogue of factor Xa that has the active site serine residue replaced with an alanine residue to eliminate catalytic activity and the Gla domain removed to preclude its incorporation in the prothrombinase complex. *Andexanet* is administered as an intravenous bolus followed by a 2-h infusion. By sequestering circulating factor Xa inhibitors, *andexanet* rapidly reverses the anti–factor Xa activity produced by these agents and restores thrombin generation (Connolly et al., 2019). Higher doses of *andexanet* are needed to reverse *rivaroxaban* or *edoxaban* than *apixaban* (Connolly et al., 2019).

Ciraparantag

Ciraparantag is a synthetic, small cationic molecule that is reported to bind *dabigatran, rivaroxaban, apixaban*, and *edoxaban*, as well as *heparin* and LMWH. In healthy volunteers given *edoxaban*, an intravenous bolus of *ciraparantag* restored the whole-blood clotting time to normal. *Ciraparantag* is currently in phase III clinical trials.

Fibrinolytic Drugs

Fibrinolytic drugs initiate the fibrinolytic pathway, which is summarized in Figure 36–3. These agents include recombinant t-PA (*alteplase*) and its variants, *urokinase* and *streptokinase*. *Urokinase* and *streptokinase* are rarely used and no longer available in the U.S.

Tissue Plasminogen Activator

Tissue plasminogen activator is a serine protease and a poor plasminogen activator in the absence of fibrin. When bound to fibrin, t-PA activates fibrin-bound plasminogen several hundred–fold more rapidly than it activates plasminogen in the circulation. Because it has little activity except in the presence of fibrin, physiological t-PA concentrations of 5 to 10 ng/mL do not induce systemic plasmin generation. With therapeutic infusion of recombinant t-PA (*alteplase*), the concentrations rise to 300 to 3000 ng/mL, which can induce systemic fibrinogen degradation. Clearance of *alteplase* primarily occurs via hepatic metabolism, and its $t_{1/2}$ is about 5 min. *Alteplase* is effective for treatment of acute myocardial infarction (O'Gara et al., 2013), acute ischemic stroke, and life-threatening pulmonary embolism (Meyer et al., 2014).

For coronary thrombolysis, *alteplase* is given as a 15-mg intravenous bolus, followed by 0.75 mg/kg over 30 min (not to exceed 50 mg) and 0.5 mg/kg (up to 35 mg accumulated dose) over the following hour. Other recombinant variants of t-PA include *reteplase* and *tenecteplase*. They differ from *alteplase* by having longer plasma half-lives that allow convenient bolus dosing. In addition, in contrast to *alteplase*, *tenecteplase* is relatively resistant to inhibition by PAI-1. Despite these apparent advantages, these agents are like *alteplase* in terms of efficacy and toxicity.

Hemorrhagic Toxicity of Thrombolytic Therapy

The major toxicity of all thrombolytic agents is hemorrhage. It is due to (1) degradation of fibrin in hemostatic plugs at sites of vascular injury or (2) the systemic lytic state that results from the systemic generation of plasmin, which degrades fibrinogen and other coagulation factors, especially factors V and VIII. Contraindications to fibrinolytic therapy are listed in Table 36–3.

If *heparin* is used concurrently with *alteplase*, serious hemorrhage will occur in 2% to 4% of patients. Intracranial hemorrhage is the most serious problem and can occur in up to 1% of patients. For this reason, mechanical reperfusion is preferred over systemic thrombolysis in patients with acute myocardial infarction with ST-segment elevation. In patients with acute ischemic stroke, the current standard of care is mechanical thrombus extraction, which can be done with or without adjunctive *alteplase* or *tenecteplase*.

Inhibitors of Fibrinolysis

ε-Aminocaproic Acid and Tranexamic Acid

ε-Aminocaproic acid and *tranexamic acid* are lysine analogues that compete for lysine binding sites on plasminogen and plasmin, thereby blocking their interaction with fibrin. Therefore, these agents inhibit fibrinolysis and can reverse states that are associated with excessive fibrinolysis.

The main problem with their use is that thrombi that form during treatment are not degraded. For example, in patients with hematuria,

TABLE 36–3 ■ ABSOLUTE AND RELATIVE CONTRAINDICATIONS TO FIBRINOLYTIC THERAPY

Absolute Contraindications

- Prior intracranial hemorrhage
- Known structural cerebral vascular lesion
- Known malignant intracranial neoplasm
- Ischemic stroke within 3 months
- Suspected aortic dissection
- Active bleeding or bleeding diathesis (excluding menses)
- Significant closed-head trauma or facial trauma within 3 months

Relative Contraindications

- Uncontrolled hypertension (systolic blood pressure >180 mmHg or diastolic blood pressure >110 mmHg)
- Traumatic or prolonged CPR or major surgery within 3 weeks
- Recent (within 2–4 weeks) internal bleeding
- Noncompressible vascular punctures
- For streptokinase: prior exposure (more than 5 days ago) or prior allergic reaction to streptokinase
- Pregnancy
- Active peptic ulcer
- Current use of warfarin and INR >1.7

ureteral obstruction by clots may lead to renal failure after treatment with *ε-aminocaproic acid* or *tranexamic acid*. *ε-Aminocaproic acid* has been used intravenously to reduce bleeding after prostatic surgery and orally to reduce bleeding after tooth extractions in patients with hemophilia. *ε-Aminocaproic acid* is absorbed rapidly after oral administration, and 50% is excreted unchanged in the urine within 12 h. For intravenous use, a loading dose of 4 to 5 g is given over 1 h, followed by an infusion of 1 to 1.25 g/h until bleeding is controlled. No more than 30 g should be given in a 24-h period. Rarely, the drug causes myopathy and muscle necrosis.

Tranexamic acid is given intravenously in trauma resuscitation, in patients with massive hemorrhage, and in women with postpartum hemorrhage (Franchini and Mannucci, 2020). It is also used to reduce operative bleeding in patients undergoing hip or knee arthroplasty or cardiac surgery. There appears to be little or no increased risk of thrombosis. *Tranexamic acid* is excreted in the urine; therefore, dose reduction is necessary in patients with renal impairment. Oral *tranexamic acid* is approved for treatment of heavy menstrual bleeding, usually given at a dose of 1 g four times daily for 4 days.

Antiplatelet Drugs

Platelet aggregates form the initial hemostatic plug at sites of vascular injury. Platelets also contribute to the pathological thrombi that lead to myocardial infarction, stroke, and peripheral arterial thrombosis. Potent inhibitors of platelet function have been developed in recent years. These drugs act by discrete mechanisms (Figure 36–7); thus, in combination, their effects are additive or even synergistic.

Aspirin

In platelets, the major product of metabolism by *cyclooxygenase* (COX) type1 is *thromboxane A2* (TxA_2), a labile inducer of platelet aggregation and a potent vasoconstrictor. *Aspirin* blocks production of TxA_2 by acetylating a serine residue near the active site of platelet COX-1. Because platelets do not synthesize new proteins, the action of *aspirin* on platelet COX-1 is permanent, lasting for the lifetime of the platelet (7–10 days). Thus, repeated doses of *aspirin* produce a cumulative effect on platelet function.

Complete inactivation of platelet COX-1 is achieved with a daily *aspirin* dose of 75 mg. Therefore, *aspirin* is maximally effective as an antithrombotic agent at doses much lower than those required for other actions of the drug. Numerous trials indicated that *aspirin*, when used as an antithrombotic drug, is maximally effective at doses of 50 to 325 mg/day. Higher doses do not improve efficacy and potentially are less efficacious because of inhibition of prostacyclin production, which can be largely spared by using lower doses of *aspirin*. Higher doses also increase toxicity, especially bleeding. Therefore, daily *aspirin* doses of 100 mg or less are used for most indications (Arnett et al., 2019). Nonsteroidal anti-inflammatory drugs that are reversible inhibitors of COX-1 have not been shown to have antithrombotic efficacy and, in fact, may even interfere with low-dose *aspirin* regimens (see Chapters 41 and 42).

Dipyridamole

Dipyridamole interferes with platelet function by increasing the intracellular concentration of cyclic AMP. This effect is mediated by inhibition of phosphodiesterase or by blockade of uptake of adenosine, thereby increasing the dwell time of adenosine at cell surface adenosine A_2 receptors that link to the stimulation of platelet adenylyl cyclase. *Dipyridamole* is a vasodilator that, in combination with *warfarin*, inhibits embolization from prosthetic heart valves. *Dipyridamole* is approved for secondary prevention of stroke when it is combined with low-dose *aspirin*.

$P2Y_{12}$ Receptor Antagonists

Clopidogrel

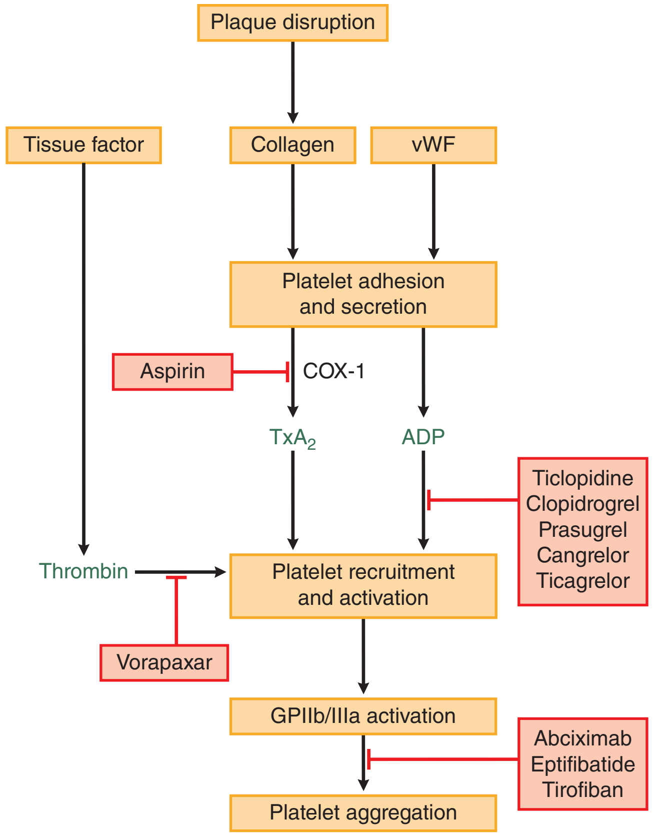


Figure 36–7 *Sites of action of antiplatelet drugs.* *Aspirin* inhibits TxA_2 synthesis by irreversibly acetylating COX-1. Reduced TxA_2 release attenuates platelet activation and recruitment to the site of vascular injury. *Ticlopidine*, *clopidogrel*, and *prasugrel* irreversibly block $P2Y_{12}$, a key ADP receptor on the platelet surface; *cangrelor* and *ticagrelor* are reversible inhibitors of $P2Y_{12}$. *Abciximab*, *eptifibatide*, and *tirofiban* inhibit the final common pathway of platelet aggregation by blocking fibrinogen and von Willebrand factor (vWF) from binding to activated GPIIb/IIIa. *Vorapaxar* inhibits thrombin-mediated platelet activation by targeting PAR-1, the major thrombin receptor on platelets.

Clopidogrel is a thienopyridine prodrug that inhibits the $P2Y_{12}$ receptor. Platelets contain two purinergic receptors, $P2Y_1$ and $P2Y_{12}$; both are G protein-coupled receptors for ADP. The ADP-activated platelet $P2Y_1$ receptor couples to the G_q-PLC-IP_3–Ca^{2+} pathway and induces platelet shape change and aggregation. The $P2Y_{12}$ receptor couples to G_i and, when activated by ADP, inhibits adenylyl cyclase, resulting in lower levels of intracellular cyclic AMP and thereby less cyclic AMP–dependent inhibition of platelet activation. Both receptors must be stimulated to result in maximal platelet activation.

Clopidogrel is an irreversible inhibitor of $P2Y_{12}$. It has largely replaced *ticlopidine* because *clopidogrel* is more potent and less toxic, with thrombocytopenia and leukopenia occurring only rarely. *Clopidogrel* is a prodrug that requires metabolic activation in the liver. Therefore, it has a slow onset of action. It also has a slow offset of action because of its irreversible effect on $P2Y_{12}$. Metabolic activation of *clopidogrel* can be affected by polymorphisms in *CYP2C19* that result in reduced or absent CYP2C19 activity. These polymorphisms contribute to the variable effect of *clopidogrel* on ADP-induced platelet aggregation. Inhibition of platelet activation is seen 2 h after ingestion of a loading dose of *clopidogrel*, and platelets are affected for the remainder of their life span.

Therapeutic Uses. *Clopidogrel* is somewhat better than *aspirin* for secondary prevention of stroke, and the combination of *clopidogrel* plus *aspirin* is superior to *aspirin* alone for prevention of recurrent ischemia in patients with unstable angina. The FDA-approved indications for *clopidogrel* are to reduce the rate of stroke, myocardial infarction, and death in patients with recent myocardial infarction, ischemic stroke, established peripheral artery disease, or acute coronary syndrome (Rahmann et al., 2019). *Clopidogrel* is routinely used in combination with *aspirin* after coronary stent implantation.

Adverse Effects. *Clopidogrel* increases the risk of bleeding, particularly when combined with *aspirin* or an anticoagulant. Thrombotic thrombocytopenic purpura can occur but is rare.

Drug Interactions. CYP2C19 inhibition by proton pump inhibitors (e.g., *omeprazole, lansoprazole, dexlansoprazole*, and *pantoprazole*) may reduce conversion to the active metabolite of *clopidogrel*, which may contribute to the lower efficacy of *clopidogrel* when coadministered with proton pump inhibitors. Other *clopidogrel* drug interactions include CYP2C19 inducers and opioids (decrease exposure to *clopidogrel*); bleeding risk is increased with anticoagulants, nonsteroidal anti-inflammatory drugs, and antidepressant drugs such as selective serotonin reuptake inhibitors. Extra caution is advised in patients coadministered *warfarin* (a CYP2C9 substrate) because high doses of *clopidogrel* inhibit CYP2C9 and the acyl-β-glucuronide metabolite of *clopidogrel* is a strong inhibitor of CYP2C8, causing drug interactions with CYP2C8 substrates (e.g., *repaglinide*).

Prasugrel

The newest member of the thienopyridine class, *prasugrel*, is a prodrug that requires metabolic activation in the liver. However, because the activation of *prasugrel* is more efficient than that of *clopidogrel*, *prasugrel* has a more rapid onset of action, and it produces greater and more predictable inhibition of ADP-induced platelet aggregation.

Prasugrel is rapidly and completely absorbed from the gut. It is hydrolyzed in the intestine to a thiolactone, which is then converted to the active metabolite in the liver. Most of the absorbed *prasugrel* undergoes activation; by comparison, only 15% of absorbed *clopidogrel* undergoes metabolic activation. Because the active metabolites of *prasugrel* bind irreversibly to the $P2Y_{12}$ receptor, its effect lasts the lifetime of the platelets. This slow offset of action can be problematic if patients require urgent surgery. *Prasugrel* is inactivated by methylation or conjugation with cysteine. Moderate renal or hepatic impairment does not appear to change the drug pharmacodynamics.

Therapeutic Uses. *Prasugrel* is indicated to reduce the rate of thrombotic cardiovascular events (including stent thrombosis) in patients with acute coronary syndrome who are managed with percutaneous coronary intervention. The incidence of cardiovascular death, myocardial infarction, and stroke is significantly lower with *prasugrel* than with *clopidogrel*, mainly reflecting a reduction in the incidence of nonfatal myocardial infarction. The incidence of stent thrombosis is also lower with *prasugrel* than with *clopidogrel*.

Adverse Effects. *Prasugrel* is associated with higher rates of fatal and life-threatening bleeding than *clopidogrel*. Because patients with a history of a prior stroke or transient ischemic attack are at particularly high risk of intracranial bleeding, the drug is contraindicated in such patients. Patients over 75 years of age should not be prescribed *prasugrel* because of the increased bleeding risk. After a loading dose of 60 mg, *prasugrel* is given once daily at a dose of 10 mg. The daily dose should be reduced to 5 mg in patients weighing less than 60 kg. No dose adjustment is required in patients with hepatic or renal impairment. If patients present with serious bleeding, platelet transfusion may be beneficial. *Prasugrel* has been reported to cause thrombotic thrombocytopenic purpura.

Drug Interactions. Concomitant administration of *prasugrel* with anticoagulants, antidepressant drugs such as selective serotonin reuptake inhibitors, or nonsteroidal anti-inflammatory drugs increases the risk of bleeding.

Ticagrelor

Ticagrelor is an orally active, reversible inhibitor of $P2Y_{12}$. The drug is given twice daily and not only has a more rapid onset and offset of action than *clopidogrel*, but also produces greater and more predictable inhibition of ADP-induced platelet aggregation. The bioavailability of *ticagrelor* is about 36%. It can be given as a whole tablet or crushed in water and administered via a nasogastric tube. *Ticagrelor* is metabolized by hepatic CYP3A4.

Therapeutic Uses. *Ticagrelor* is FDA approved for reduction in the risk of cardiovascular death, myocardial infarction, and stroke in patients with acute coronary syndrome or a history of myocardial infarction. In contrast to *prasugrel*, which is only indicated in patients with acute coronary syndrome undergoing percutaneous intervention, *ticagrelor* is indicated both in those undergoing intervention and in those managed medically.

Adverse Effects. Dyspnea is reported in 17% of patients. This is often transient and not associated with pulmonary disease. *Ticagrelor* is associated with a higher risk of intracranial bleeding than *clopidogrel* and is contraindicated in patients with a history of prior intracranial bleeding. Platelet transfusion is ineffective in patients taking *ticagrelor* who present with serious bleeding because the drug will bind to $P2Y_{12}$ on the transfused platelets. *Bentracimab* (PB2452) was developed to overcome this problem. An antibody fragment that binds *ticagrelor* and its active metabolite with high affinity, *bentracimab* is administered as an intravenous bolus followed by an infusion for up to 24 h. In volunteers given *ticagrelor*, *bentracimab* rapidly and completely reversed its antiplatelet effects (Bhatt et al., 2019). *Bentracimab* is currently under investigation for *ticagrelor* reversal in patients with life-threatening bleeding or who require urgent surgery or intervention.

Drug Interactions. Concomitant *aspirin* at a dose greater than 100 mg daily may reduce the effectiveness of *ticagrelor*. Potent inhibitors of CYP3A (e.g., *ketoconazole, itraconazole, voriconazole, clarithromycin, nefazodone, ritonavir, saquinavir, nelfinavir, indinavir, atazanavir,* and *telithromycin*) and strong inducers of CYP3A (e.g., *rifampin, phenytoin, carbamazepine*, and *phenobarbital*) should be avoided. Opioids decrease *ticagrelor* systemic exposure. *Ticagrelor* increases serum concentrations of *simvastatin* and *lovastatin* and may affect *digoxin* metabolism.

Cangrelor

Cangrelor is a parenteral reversible inhibitor of $P2Y_{12}$. When administered intravenously as a bolus followed by an infusion, *cangrelor* inhibits ADP-induced platelet aggregation within minutes, and its effect on platelet aggregation disappears within 1 h of discontinuation of the drug. *Cangrelor* has a short half-life because it is rapidly dephosphorylated in the circulation to an inactive metabolite.

Therapeutic Use. *Cangrelor* is indicated for reduction in the risk of periprocedural myocardial infarction, repeat coronary revascularization, and stent thrombosis in patients undergoing percutaneous coronary intervention who have not been treated with an oral $P2Y_{12}$ inhibitor and are not given a glycoprotein IIb/IIIa antagonist.

Adverse Effects. The risk of bleeding with *cangrelor* is greater than that with *clopidogrel* during the coronary intervention.

Drug Interactions. When transitioning to oral $P2Y_{12}$ inhibitor therapy, *ticagrelor* can be given at a loading dose of 180 mg at any time during the *cangrelor* infusion or immediately after discontinuation. In contrast, loading doses of *prasugrel* or *clopidogrel* (60 and 600 mg, respectively) should only be given after *cangrelor* is stopped because *cangrelor* blocks the interaction of their active metabolites with $P2Y_{12}$.

Thrombin Receptor Inhibitor

There are two major thrombin receptors on the platelet surface, *protease-activated receptor* (PAR) type 1 and type 4. Thrombin binds to these G protein-coupled receptors and cleaves them at their amino termini. The newly created amino termini then serve as tethered ligands to activate the receptors. PAR-1 is activated by lower concentrations of thrombin than are required to activate PAR-4.

Vorapaxar

Vorapaxar is a competitive antagonist of PAR-1 and inhibits thrombin-induced platelet aggregation. The drug is 90% bioavailable and has a rapid onset of action and a circulating half-life of 3 to 4 days. However, because *vorapaxar* remains tightly bound to PAR-1 on platelets, its effect on thrombin-induced platelet aggregation can persist for up to 4 weeks after the drug is stopped. *Vorapaxar* is metabolized in the liver by CYP3A4.

Therapeutic Uses. *Vorapaxar* is given orally in combination with either *aspirin* or *clopidogrel*. It is indicated for the reduction of thrombotic cardiovascular events in patients with a history of myocardial infarction or peripheral artery disease.

Adverse Effects. *Vorapaxar* increases the risk of bleeding and is contraindicated in patients with a history of intracranial bleeding, stroke, or transient ischemic attack.

Drug Interactions. Potent CYP3A4 inducers, such as *rifampin*, reduce drug exposure, while strong CYP3A4 inhibitors, such as *ketoconazole*, increase drug exposure. Antacids and *pantoprazole* reduce drug exposure.

Glycoprotein IIb/IIIa Inhibitors

Glycoprotein IIb/IIIa is a platelet-surface integrin, designated $\alpha_{IIb}\beta_3$ by the integrin nomenclature. This dimeric glycoprotein undergoes a conformational transformation when platelets are activated to serve as a receptor for fibrinogen and von Willebrand factor, which anchor platelets to each other, thereby mediating aggregation (Figure 36–1). Thus, inhibitors of this receptor are potent antiplatelet agents that act by a mechanism distinct from that of *aspirin* or $P2Y_{12}$ or PAR-1 inhibitors. Three agents are approved for use at present; their features are highlighted in Table 36–4. The use of these agents has decreased with the availability of potent $P2Y_{12}$ inhibitors such as *prasugrel* and *ticagrelor*.

Abciximab

Abciximab is the Fab fragment of a humanized monoclonal antibody directed against the $\alpha_{IIb}\beta_3$ receptor. It also binds to the vitronectin receptor on platelets, vascular endothelial cells, and smooth muscle cells.

The antibody is administered to patients undergoing percutaneous coronary intervention and, when used in conjunction with *aspirin* and *heparin*, has been shown to prevent recurrent myocardial infarction and death. The $t_{1/2}$ of the circulating antibody is about 30 min, but antibody remains bound to the $\alpha_{IIb}\beta_3$ receptor and inhibits platelet aggregation as measured *in vitro* for 18 to 24 h after infusion. It is given as a 0.25-mg/kg bolus followed by an infusion of 0.125 μg/kg/min (maximum 10 μg/kg/min) for 12 to 24 h.

Adverse Effects. The major side effect of *abciximab* is bleeding, and the contraindications to its use are similar to those for the fibrinolytic agents listed in Table 36–4. The frequency of major hemorrhage in clinical trials varies from 1% to 10%, depending on the intensity of concomitant anticoagulation with *heparin*. Thrombocytopenia with a platelet count below 50,000/μL occurs in about 2% of patients and may be due to the formation of antibodies directed against neoepitopes induced by bound antibody. Because the duration of action is long, if major bleeding occurs, platelet transfusion may reverse the aggregation defect because free antibody concentrations fall rapidly after cessation of infusion. *Abciximab* re-administration may be associated with human anti-chimeric antibodies, increased incidence and severity of thrombocytopenia, and enhanced risk of hypersensitivity reactions. *Abciximab* is associated with pseudothrombocytopenia (laboratory artifact requiring blood draws be collected in three separate tubes [ethylenediaminetetraacetic acid, citrate, and heparin]).

Eptifibatide

Eptifibatide is a cyclic peptide inhibitor of the fibrinogen binding site on $\alpha_{IIb}\beta_3$. It is administered intravenously and blocks platelet aggregation. In patients undergoing percutaneous coronary intervention, *eptifibatide* is typically given as a double intravenous bolus of 180 μg/kg (spaced 10 min apart), followed by an infusion of 2 μg/kg/min for 18 to 24 h. The drug is cleared by the kidneys and has a short plasma half-life of 10 to 15 min. Like *abciximab*, *eptifibatide* is mainly used in patients undergoing primary percutaneous coronary intervention for acute ST-segment elevation myocardial infarction, although it also can be used in patients with unstable angina.

Adverse Effects. The major side effect is bleeding. Thrombocytopenia occurs in 0.5% to 1% of patients and is less frequent than with *abciximab*.

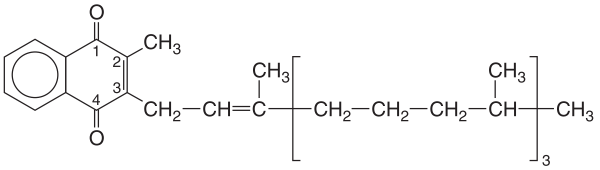
Tirofiban

Tirofiban is an intravenously administered nonpeptide, small-molecule inhibitor of $\alpha_{IIb}\beta_3$. It has a short duration of action and is used for management of patients with non–ST-segment elevation acute coronary syndrome. *Tirofiban* is administered as an intravenously bolus of 25 μg/kg followed by an infusion of 0.15 μg/kg/min for up to 18 h. The infusion dose is reduced by half in patients with a creatinine clearance below 60 mL/min. Like the other agents in this class, the major side effect of *tirofiban* is bleeding, and it may induce thrombocytopenia.

The Role of Vitamin K

Green plants are a nutritional source of vitamin K for humans, in whom vitamin K is an essential cofactor in the γ-carboxylation of multiple glutamate residues of several clotting factors and anticoagulant proteins. The vitamin K–dependent formation of Gla residues permits the appropriate interactions of clotting factors, Ca^{2+}, and membrane phospholipids and modulator proteins (see Figures 36–1, 36–2, and 36–3). Vitamin K antagonists (coumarin derivatives) block Gla formation and thereby inhibit clotting; excess vitamin K_1 can reverse the effects.

Vitamin K activity is associated with at least two distinct natural substances, designated as vitamin K_1 and vitamin K_2. Vitamin K_1, or *phytonadione* (also referred to as phylloquinone), is 2-methyl-3-phytyl-1,4-naphthoquinone; it is found in plants and is the only natural vitamin K available for therapeutic use. Vitamin K_2 is a series of compounds (the menaquinones) in which the phytyl side chain of phytonadione has been replaced by a side chain of 2 to 13 prenyl units. Synthesis of menaquinones occurs in gram-positive bacteria, and the intestinal flora synthesizes the large amounts of vitamin K contained in human and animal feces. *Menadione* (no longer available in the U.S.) is at least as active on a molar basis as *phytonadione*.



PHYTONADIONE (vitamin K_1, phylloquinone)

Physiological Functions and Pharmacological Actions

Phytonadione and menaquinones promote the biosynthesis of the factors II (prothrombin), VII, IX, and X, as well as the anticoagulant proteins C, S, and Z.

Figure 36–6 summarizes the coupling of the vitamin K cycle with glutamate carboxylation. The γ-glutamyl carboxylase and epoxide reductase are integral membrane proteins of the endoplasmic reticulum and function as a multicomponent complex. With respect to proteins affecting

TABLE 36–4 ■ FEATURES OF GPIIb/IIIa ANTAGONISTS

FEATURE	ABCIXIMAB	EPTIFIBATIDE	TIROFIBAN
Description	Fab fragment of humanized mouse mAb	Cyclical KGD-containing heptapeptide	Nonpeptidic RGD-mimetic
Specific for GPIIb/IIIa	No	Yes	Yes
Plasma $t_{1/2}$	Short (minutes)	Long (2.5 h)	Long (2.0 h)
Platelet-bound $t_{1/2}$	Long (days)	Short (seconds)	Short (seconds)
Renal clearance	No	Yes	Yes

blood coagulation, these reactions occur in the liver, but γ-carboxylation of glutamate also occurs in lung, bone, and other cell types. Mutations in γ-glutamyl carboxylase can cause bleeding disorders.

Human Requirements

In patients rendered vitamin K deficient by a starvation diet or antibiotic therapy for 3 to 4 weeks, the minimum daily requirement is estimated to be 0.03 μg/kg of body weight and possibly as high as 1 μg/kg, which is approximately the recommended daily intake for adults (70 μg).

Symptoms of Deficiency

The major clinical manifestation of vitamin K deficiency is bleeding. Ecchymoses, epistaxis, hematuria, GI bleeding, and postoperative hemorrhage are common; intracranial hemorrhage may occur. Hemoptysis is uncommon. The presence of vitamin K–dependent proteins in bone such as osteocalcin and matrix Gla protein may explain why fetal bone abnormalities can occur with maternal *warfarin* administration in the first trimester of pregnancy. Vitamin K plays a role in adult skeletal maintenance and the prevention of osteoporosis. Low concentrations of the vitamin are associated with decreased bone mineral density and subsequent fractures; vitamin K supplementation increases the carboxylation state of osteocalcin and improves bone mineral density, but the relationship between these effects is unclear. Bone mineral density in adults does not appear to be changed with long-term *warfarin* therapy, but new bone formation may be affected.

Toxicity

Phytonadione and the menaquinones are nontoxic. *Menadione* and its derivatives (synthetic forms of vitamin K) may produce hemolytic anemia and kernicterus in neonates and should not be used as therapeutic forms of vitamin K.

ADME

The mechanism of intestinal absorption of compounds with vitamin K activity varies depending on their solubility. In the presence of bile salts, *phytonadione* and the menaquinones are adequately absorbed from the intestine, *phytonadione* by an energy-dependent, saturable process in proximal portions of the small intestine and menaquinones by diffusion in the distal small intestine and the colon. After absorption, *phytonadione* is incorporated into chylomicrons in close association with triglycerides and lipoproteins. The low *phytonadione* levels in newborns may partly reflect the low plasma lipoprotein concentrations at birth and may lead to an underestimation of vitamin K tissue stores. After absorption, *phytonadione* and menaquinones are concentrated in the liver, but the concentration of *phytonadione* declines rapidly. Menaquinones, produced in the distal bowel, are less biologically active because of their long side chain. Very little vitamin K accumulates in other tissues. There is only modest storage of vitamin K in the body. Consequently, when lack of bile interferes with absorption of vitamin K, there is progressive reduction in the levels of the vitamin K–dependent clotting factors over the course of several weeks.

Therapeutic Uses

Vitamin K is used therapeutically to correct the bleeding tendency or hemorrhage associated with its deficiency. Vitamin K deficiency can result from inadequate intake, absorption, or utilization of the vitamin or as a consequence of the action of *warfarin*.

Phytonadione is available in tablet form and in a dispersion with buffered polysorbate and propylene glycol or polyoxyethylated fatty acid derivatives and dextrose. *Phytonadione* may be given by any route; however, the subcutaneous route should be avoided in patients with a coagulopathy because of the risk of bleeding. The oral route is preferred, but if more rapid reversal is required, *phytonadione* can be given by slow intravenous infusion; it should not be given rapidly because severe reactions resembling anaphylaxis can occur.

Inadequate Intake

After infancy, hypoprothrombinemia due to dietary deficiency of vitamin K is extremely rare. The vitamin is present in many foods and is synthesized by intestinal bacteria. Occasionally, the use of a broad-spectrum antibiotic may itself produce hypoprothrombinemia that responds readily to small doses of vitamin K and reestablishment of normal bowel flora. Hypoprothrombinemia can occur in patients receiving prolonged intravenous alimentation; to prevent this, it is recommended that such patients receive 1 mg of *phytonadione* per week (the equivalent of about 150 μg/day).

Hypoprothrombinemia of the Newborn

Healthy newborn infants have decreased plasma concentrations of vitamin K–dependent clotting factors for a few days after birth, the time required for adequate dietary intake of the vitamin and for establishment of normal intestinal flora. Measurements of non-**γ**-carboxylated prothrombin suggest that vitamin K deficiency occurs in about 3% of live births.

Hemorrhagic disease of the newborn has been associated with breastfeeding; human milk has low concentrations of vitamin K. In addition, the microbiome of breast-fed infants may lack microorganisms that synthesize the vitamin. Commercial infant formulas are supplemented with vitamin K. In the neonate with hemorrhagic disease of the newborn, administration of vitamin K raises the concentration of these clotting factors to levels normal for newborns and controls the bleeding tendency within about 6 h. Routine administration of 1 mg *phytonadione* intramuscularly at birth is required by law in the U.S. The dose may have to be increased or repeated if the mother has received *warfarin* or anticonvulsant drug therapy or if the infant develops a bleeding diathesis. Alternatively, some clinicians treat mothers who are receiving anticonvulsants with oral vitamin K prior to delivery (20 mg/day for 2 weeks).

Inadequate Absorption

Vitamin K is poorly absorbed in the absence of bile. Thus, hypoprothrombinemia may be associated with intrahepatic or extrahepatic biliary obstruction or with defective intestinal absorption of fat from other causes.

Biliary Obstruction or Fistula

Bleeding that accompanies obstructive jaundice or a biliary fistula responds promptly to the administration of vitamin K. Oral *phytonadione* administered with bile salts is both safe and effective and should be used in the care of the jaundiced patient, both preoperatively and postoperatively. In the absence of significant hepatocellular disease, the prothrombin level rapidly returns to normal. If oral administration is not feasible, a parenteral preparation should be used. The usual daily dose of vitamin K is 10 mg.

Malabsorption Syndromes

Among the disorders that result in inadequate absorption of vitamin K from the intestinal tract are cystic fibrosis, celiac disease, Crohn's disease, ulcerative colitis, dysentery, and extensive resection of bowel. Because drugs that reduce the bacterial population of the bowel are used frequently in many of these disorders, the availability of the vitamin may be further reduced. For immediate correction of the deficiency, parenteral vitamin K should be given.

Inadequate Utilization

Hepatocellular disease or long-standing biliary obstruction may be accompanied or followed by hypoprothrombinemia. If inadequate secretion of bile salts is contributing to the syndrome, some benefit may be obtained from the parenteral administration of 10 mg of *phytonadione* daily. Paradoxically, administration of large doses of vitamin K or its analogues in an attempt to correct the hypoprothrombinemia can be associated with severe hepatitis or cirrhosis, which may contribute to a further reduction in the level of prothrombin.

Drug- and Venom-Induced Hypoprothrombinemia

Warfarin and its congeners act as competitive antagonists of vitamin K and interfere with the hepatic biosynthesis of Gla-containing clotting factors. The treatment of bleeding caused by oral anticoagulants was described previously. Vitamin K may be of help in combating the bleeding and hypoprothrombinemia that follow the bite of the tropical American pit viper or other species whose venom degrades or inactivates prothrombin.

Drug Facts for Your Personal Formulary: *Agents That Modify Blood Coagulation*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Unfractionated Heparin		
Heparin	• Prophylaxis/treatment of venous thromboembolism • Acute coronary syndrome • Percutaneous coronary intervention • Cardiopulmonary bypass surgery • Disseminated intravascular coagulation	• Administered SC 2–3 times daily for thromboprophylaxis • Administered IV for immediate onset of action with aPTT monitoring • Can be used in renal impairment • Can be used in pregnancy
Low-Molecular-Weight Heparin		
Enoxaparin Dalteparin Tinzaparin (not in the U.S.)	• Prophylaxis against venous thromboembolism • Initial treatment of venous thromboembolism • Maintenance treatment in patients with cancer-associated venous thromboembolism • Acute coronary syndrome	• Administered SC once or twice daily • Routine anti–factor Xa monitoring not required • Dosage adjustment required when CrCL <30 mL/min • Can be used in pregnancy
Fondaparinux		
Fondaparinux	• Prophylaxis against venous thromboembolism • Initial treatment of venous thromboembolism • Heparin-induced thrombocytopenia but not for other thrombocytopenias • Acute coronary syndrome in some countries	• Once-daily SC injection • Lower dose used for thromboprophylaxis and in acute coronary syndrome • Contraindicated if CrCL <30 mL/min • Use in pregnancy less established than for low-molecular-weight heparin • Routine anti–factor Xa monitoring not required
Other Anticoagulants		
Desirudin	• Thromboprophylaxis after hip arthroplasty	• Twice-daily SC injection • Dosage adjustment required with renal impairment
Bivalirudin	• Percutaneous coronary intervention • Heparin-induced thrombocytopenia	• Administered IV • ACT or aPTT monitoring • Requires dose reduction with renal impairment
Argatroban	• Heparin-induced thrombocytopenia but not for other thrombocytopenias	• Hepatic metabolism • Can be used in renal impairment • Increases INR, which can complicate transition to warfarin
Vitamin K Antagonist		
Warfarin	• Treatment of venous thromboembolism in tandem with parenteral anticoagulation • Secondary prevention of venous thromboembolism • Prevention of stroke in atrial fibrillation • Prevention of stroke in patient with mechanical heart valves or ventricular assist devices	• Oral vitamin K antagonist • Narrow therapeutic index • Requires regular INR monitoring • Multiple drug interactions • Dietary vitamin K interactions • Can be used in renal failure • Contraindicated in pregnancy (fetal risk)
Direct Oral Thrombin Inhibitor		
Dabigatran etexilate	• Treatment of acute venous thromboembolism after at least 5 days of parenteral anticoagulation • Secondary prevention of venous thromboembolism • Prevention of stroke in atrial fibrillation • Thromboprophylaxis after hip or knee arthroplasty	• Fixed twice-daily oral dosing (once daily if used for thromboprophylaxis) • Reduce the dose with CrCL 15–30 mL/min • Contraindicated if CrCL <15 mL/min • Use with caution in patients with recent bleeding, especially GI bleeding • Can be reversed with idarucizumab
Direct Oral Factor Xa Inhibitors		
Rivaroxaban	• Treatment of acute venous thromboembolism • Secondary prevention of venous thromboembolism • Prevention of stroke in atrial fibrillation • Thromboprophylaxis after hip or knee arthroplasty • Prevention of recurrent ischemia in stabilized acute coronary syndrome patients (not in North America) • Secondary prevention of major adverse cardiovascular and limb events in patients with coronary or peripheral artery disease	• Fixed oral dosing (once daily except for initial treatment of venous thromboembolism, which starts with twice-daily dosing for 21 days and once daily thereafter, or for secondary prevention after acute coronary syndrome or in patients with coronary or peripheral artery disease where the drug is given twice daily in low doses) • Avoid in patients with renal/hepatic dysfunction • Use with caution in patients with recent bleeding, especially GI bleeding
Apixaban	• Treatment of acute venous thromboembolism • Secondary prevention of venous thromboembolism • Prevention of stroke in atrial fibrillation • Thromboprophylaxis after hip or knee arthroplasty	• Fixed oral dosing (twice daily, higher dose for the first 7 days for acute venous thromboembolism) • Reduce dose for stroke prophylaxis if any two of the following are present: age >80 years, body weight <60 kg, or serum creatinine ≥1.5 mg/dL • Use with caution in patients with recent bleeding, especially GI bleeding

Drug Facts for Your Personal Formulary: *Agents That Modify Blood Coagulation (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Edoxaban	• Treatment of acute venous thromboembolism after at least 5 days of parenteral anticoagulation • Secondary prevention of venous thromboembolism • Prevention of stroke in atrial fibrillation	• Fixed once-daily dosing • Reduce the dose if any of the following is present: CrCL 15–50 mL/min, body weight <60 kg, or concomitant potent P-glycoprotein inhibitor • Not recommended for patients with CrCL <15 mL/min • Contraindicated if CrCL >95 mL/min • Use with caution in patients with recent bleeding, especially GI bleeding
Reversal Agents for Direct Oral Anticoagulants		
Idarucizumab	• Reversal of dabigatran	• Humanized Fab fragment against dabigatran • Bolus IV administration • Rapid and complete reversal
Andexanet alfa	• Reversal of rivaroxaban or apixaban • Reversal of edoxaban (off-label use)	• Recombinant analogue of factor Xa, inactivated-zhzo • Acts as a decoy for oral factor Xa inhibitors • Given as IV bolus followed by 2-h IV infusion
Ciraparantag (not available in the U.S.)	• Reversal of dabigatran, rivaroxaban, apixaban, or edoxaban	• Synthetic small molecule • Binds target drugs • In phase III evaluation
Fibrinolytic Drugs		
Alteplase	• Thrombolysis in acute ischemic stroke, massive pulmonary embolism, or myocardial infarction	• IV bolus followed by an infusion • Risk of major bleeding, including intracranial bleeding
Reteplase	• Thrombolysis in myocardial infarction	• Two IV boluses • Risk of major bleeding, including intracranial bleeding
Tenecteplase	• Thrombolysis in pulmonary embolism and myocardial infarction	• Single IV bolus • Risk of major bleeding, including intracranial bleeding
Inhibitors of Fibrinolysis		
ε-Aminocaproic acid	• Reduce intraoperative bleeding	• Inhibits plasmin-mediated degradation of fibrin • IV infusion
Tranexamic acid	• Major head injury • Major trauma resuscitation • Reduce intraoperative bleeding • Topical application for dental bleeding and epistaxis • Menorrhagia	• Inhibits plasmin-mediated degradation of fibrin • Available in oral or IV form • Given orally in patients undergoing dental procedures or in women with menorrhagia and IV in patients with major trauma or undergoing major orthopedic surgery
Antiplatelet Drugs		
Aspirin	• Acute myocardial infarction or acute ischemic stroke • Secondary prevention in patients with stroke, coronary artery disease, or peripheral artery disease	• COX-1 inhibitor (selectivity >100× over COX-2) • Antithrombotic effect achieved with low doses (<100 mg daily) • Reduced toxicity with lower doses
Dipyridamole	• Secondary prevention of stroke when combined with aspirin	• Available as a fixed-dose combined tablet with low-dose aspirin
Clopidogrel	• Acute coronary syndrome • Secondary prevention in patients with myocardial infarction, stroke, or peripheral artery disease	• Irreversible inhibitor of $P2Y_{12}$ • Given once daily • Variable response because common genetic polymorphisms attenuate metabolic activation • Proton pump inhibitors reduce conversion to active metabolite
Prasugrel	• After coronary intervention for acute coronary syndrome	• Irreversible inhibitor of $P2Y_{12}$ • Given once daily • More predictable inhibition of ADP-induced platelet activation than clopidogrel because of more efficient metabolic activation • Contraindicated in patients with cerebrovascular disease, prior intracranial bleed, or >75 years of age • Reduce dose in patients weighing <60 kg • Higher bleeding risk than clopidogrel
Ticagrelor	• Acute coronary syndrome with or without coronary intervention	• Reversible inhibitor of $P2Y_{12}$ • Given twice daily • Does not require metabolic activation • Higher bleeding risk than clopidogrel • Contraindicated in patients with a history of intracranial bleeding

Drug Facts for Your Personal Formulary: *Agents That Modify Blood Coagulation (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Cangrelor	• Percutaneous coronary intervention	• $P2Y_{12}$ inhibitor • Rapid onset and offset IV agent • Higher bleeding risk than clopidogrel • When administered with cangrelor, clopidogrel and prasugrel will have no antiplatelet effect
Vorapaxar	• Secondary prevention in patients with a history of myocardial infarction or peripheral artery disease	• PAR-1 antagonist • Contraindicated in patients with cerebrovascular disease or prior intracranial bleed
Abciximab Eptifibatide Tirofiban	• Coronary intervention for acute coronary syndrome	• Glycoprotein IIb/IIIa antagonists • Up to 10% bleeding risk • Can cause thrombocytopenia • Eptifibatide contraindicated in renal failure • Reduce tirofiban dose if CrCL ≤60 mL/min
Vitamin Supplementation		
Vitamin K	• Reversal of warfarin • Hypoproteinemia of the newborn • Biliary obstruction • Malnutrition	• Oral administration preferred • Can be given by slow IV infusion but higher risk of adverse events

Abbreviations: SC, subcutaneous; IV intravenous; CrCL, creatinine clearance.

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Chapter 37

Drug Therapy for Dyslipidemias

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PLASMA LIPOPROTEIN METABOLISM

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- Chylomicron Remnants
- Very Low-Density Lipoproteins
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ATHEROSCLEROTIC CARDIOVASCULAR DISEASE RISK ASSESSMENT

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- Mechanism of Action
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- ATP-Citrate Lyase Inhibitor
- Inhibitor of Angiopoietin-Like Protein 3

FUTURE DEVELOPMENTS IN MANAGEMENT OF DYSLIPIDEMIAS

Dyslipidemia is a disorder of lipoprotein metabolism, including lipoprotein overproduction or deficiency. Dyslipidemia is a major cause of atherosclerotic cardiovascular disease (ASCVD), ischemic cerebrovascular disease, and peripheral vascular disease. Cardiovascular disease is the number one cause of death globally (World Health Organization, 2020). Genetic disorders, metabolic diseases such as diabetes mellitus, and lifestyle factors are common causes of dyslipidemias, including hypercholesterolemia and low levels of high-density lipoprotein (HDL) cholesterol.

In 2018, various professional organizations, including the American Heart Association (AHA) and the American College of Cardiology (ACC), published updated guidelines for the management of blood cholesterol (Grundy et al., 2019). Contrary to the 2014 ACC/AHA cholesterol guidelines (Stone et al., 2014), these updated guidelines recommend cholesterol percent reduction targets in certain high-risk groups for the primary prevention of cardiovascular disease. This change signaled a shift to previous approaches used for cholesterol management, like those highlighted in the 2004 Adult Treatment Panel III guidelines (Grundy et al., 2004; NCEP, 2002). However, fixed-dose statin recommendations, like those emphasized in the 2014 ACC/AHA guideline, remained in place in the 2018 AHA/ACC cholesterol guidelines, particularly when managing secondary prevention of cardiovascular disease and patients with diabetes mellitus. With these changes, the most recent guidelines appear to merge recommendations and varying schools of thought on cholesterol management from previously published guidelines (Table 37–1). Since its release, part of the 2018 AHA/ACC cholesterol guidelines have been updated and published separately as the 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease (Arnett et al., 2019). However, only updates on *aspirin* use and more specific recommendations on nonpharmacological aspects of ASCVD prevention were added.

Plasma Lipoprotein Metabolism

Lipoproteins are macromolecular assemblies that contain lipids and proteins. The lipid constituents include free and esterified cholesterol, triglycerides, and phospholipids. Lipoproteins are generally spherical particles, with a shell composed of free cholesterol and phospholipid, with fatty acids oriented toward the core of the particle. The protein components, known as *apolipoproteins* or *apoproteins*, provide structural stability to the lipoproteins and may function as ligands in lipoprotein-receptor interactions or as cofactors in enzymatic processes that regulate lipoprotein metabolism. The major classes of lipoproteins and their properties are summarized in Table 37–2. Apoproteins have well-defined roles in plasma lipoprotein metabolism (Table 37–3). Mutations in lipoproteins or their receptors can lead to familial dyslipidemias and premature death due to accelerated atherosclerosis.

In all spherical lipoproteins, the most water-insoluble lipids (cholesteryl esters and triglycerides) are core components, and the more polar, water-soluble components (apoproteins, phospholipids, and unesterified cholesterol) are located on the surface. Except for apo(a), the lipid-binding regions of all apoproteins contain amphipathic helices that interact with the polar, hydrophilic lipids (such as surface phospholipids) and with the aqueous plasma environment in which the lipoproteins circulate. Differences in the non–lipid-binding regions determine the functional specificities of the apolipoproteins.

Figure 37–1 summarizes the pathways involved in the uptake and transport of dietary fat and cholesterol from the intestines to adipose tissue, peripheral tissues, and the liver. Figure 37–2 shows the reverse cholesterol pathway that transports cholesterol from peripheral tissues back to the liver for excretion in the bile. These pathways involve the lipoprotein structures described in the next sections.

Chylomicrons

Intestinal cholesterol absorption is mediated by the Niemann-Pick C1–like 1 protein, which appears to be the target of *ezetimibe*, a cholesterol absorption inhibitor. After their uptake by epithelial cells in the small intestines, dietary lipids and endogenous lipids are transferred to the endoplasmic reticulum where newly synthesized apo B-48 is available to form chylomicrons. Apo B-48, synthesized only by intestinal epithelial cells, is unique to chylomicrons and functions primarily as a structural component of chylomicrons.

Chylomicrons are synthesized from the fatty acids of dietary triglycerides and cholesterol absorbed by epithelial cells in the small intestine. Chylomicrons are the largest and lowest-density plasma lipoproteins. In

Abbreviations

ABC: ATP-binding cassette
ACAT-2: type 2 isozyme of acyl coenzyme A:cholesterol acyltransferase
ACC: American College of Cardiology
ACL: ATP-citrate lyase
ACTH: adrenocorticotropic hormone
ADA: American Diabetes Association
AHA: American Heart Association
ALT: alanine aminotransferase
ANGPTL3: angiopoietin-like protein 3
apo(a): apolipoprotein (a)
ASCVD: atherosclerotic cardiovascular disease
CETP: cholesteryl ester transfer protein
CHD: coronary heart disease
CYPs: cytochrome P450s
DHA: docosahexaenoic acid
EL: endothelial lipase
EPA: eicosapentaenoic acid
ER: extended release
FH: familial hypercholesterolemia
GI: gastrointestinal
GWAS: genome-wide association studies
HDL: high-density lipoprotein
HDL-C: high-density lipoprotein cholesterol concentration
HeFH: heterozygous familial hypercholesterolemia
HIV: human immunodeficiency virus
HL: hepatic lipase
HMG-CoA: β-hydroxy β-methylglutaryl coenzyme A
HoFH: homozygous familial hypercholesterolemia
IDL: intermediate-density lipoprotein
IgG: immunoglobulin G
IR: immediate release
LCAT: lecithin:cholesterol acyltransferase
LDL: low-density lipoprotein
LDL-C: low-density lipoprotein cholesterol concentration
LDLR: LDL receptor gene
LP(a): lipoprotein (a)
LPL: lipoprotein lipase
LRP: LDL receptor–related protein
MTP: microsomal triglyceride transfer protein
NAD: nicotinamide adenine dinucleotide
NADP: nicotinamide adenine dinucleotide phosphate
NCEP: National Cholesterol Education Program
OTC: over-the-counter
PCE: pooled cohort equations
PCSK9: proprotein convertase subtilisin/kexin type 9
PPAR: peroxisome proliferator–activated receptor
SR: scavenger receptor
VLDL: very low-density lipoprotein

normolipidemic individuals, chylomicrons are present in plasma for 3 to 6 h after a fat-containing meal has been ingested. Dietary cholesterol is esterified by the acyl coenzyme A:cholesterol acyltransferase type 2 (ACAT-2). ACAT-2 is found in the intestine and in the liver, where cellular free cholesterol is esterified before triglyceride-rich lipoproteins (chylomicrons and very low-density lipoproteins [VLDLs]) are assembled.

Chylomicrons enter the systemic circulation via the thoracic duct. Chylomicron triglycerides are then metabolized to free fatty acids by an extracellular lipoprotein lipase (LPL) at the capillary luminal surface of tissues that synthesize LPL (see Figure 37–1), including adipose tissue, skeletal and cardiac muscle, and breast tissue of lactating women. The resulting free fatty acids are taken up and used by the adjacent tissues. The interaction of chylomicrons and LPL requires apo C-II as a cofactor.

Chylomicron Remnants

LPL-mediated removal of much of the dietary triglycerides generates the *chylomicron remnants*, which contain all of the dietary cholesterol. Chylomicron remnants detach from the capillary surface and are removed from the circulation by the liver within minutes (see Figure 37–1). First, the remnants are sequestered by the interaction of apo E with heparan sulfate proteoglycans on the surface of hepatocytes and are processed by the hepatic lipase (HL), further reducing the remnant triglyceride content. Next, apo E mediates remnant uptake by interacting with the hepatic low-density lipoprotein (LDL) receptor or the LDL receptor–related protein (LRP).

During the initial hydrolysis of chylomicron triglycerides by LPL, apo A-I and phospholipids are shed from the surface of chylomicrons and remain in the plasma. This is one mechanism by which nascent (precursor) HDL is generated (see Figure 37–2). Chylomicron remnants are not precursors of LDL, but the dietary cholesterol delivered to the liver by remnants increases plasma low-density lipoprotein cholesterol (LDL-C) levels. Increased liver cholesterol suppresses steroid receptor element binding protein–regulated expression of proprotein convertase subtilisin/kexin type 9 (PCSK9), thus reducing LDL receptor–mediated catabolism of LDL by the liver (see PCSK9 Inhibitors below for additional details).

Very Low-Density Lipoproteins

The VLDLs are produced in the liver when triglyceride production is stimulated by an increased flux of free fatty acids or by increased *de novo* synthesis of fatty acids by the liver. Apo B-100, apo E, and apo C-I, C-II, and C-III are synthesized constitutively by the liver and incorporated into VLDLs (see Table 37–3). Triglycerides are synthesized in the endoplasmic reticulum and, along with other lipid constituents, are transferred by the microsomal triglyceride transfer protein (MTP) to the site in the endoplasmic reticulum where newly synthesized apo B-100 is available to form nascent (precursor) VLDL. Small amounts of apo E and the C apoproteins are incorporated into nascent particles within the liver before secretion, but most of these apoproteins are acquired from plasma HDL after the VLDLs are secreted by the liver. Mutations of MTP that result in the inability of triglycerides to be transferred to either apo B-100 in the liver or apo B-48 in the intestine prevent VLDL and chylomicron production and cause the genetic disorder *abetalipoproteinemia*.

Plasma VLDL is catabolized by LPL in the capillary beds in a process similar to the lipolytic processing of chylomicrons (see Figure 37–1). When triglyceride hydrolysis is nearly complete, the VLDL remnants, usually termed *IDLs*, are released from the capillary endothelium and reenter the circulation. Apo B-100–containing small VLDLs and IDLs, which have a $t_{1/2}$ of less than 30 min, have two potential fates. About 40% to 60% are cleared from the plasma by the liver via apo B-100– and apo E–mediated interaction with LDL receptors and LRP. LPL and HL convert the remainder of the IDLs to LDLs by removal of additional triglycerides. The C apoproteins, apo E, and apo A-V redistribute to HDL.

Apolipoprotein E plays a major role in the metabolism of triglyceride-rich lipoproteins (chylomicrons, chylomicron remnants, VLDLs, and IDLs). About half of the apo E in the plasma of fasting subjects is associated with triglyceride-rich lipoproteins, and the other half is a constituent of HDL.

Low-Density Lipoproteins

Virtually all LDL particles in the circulation are derived from VLDL. The LDL particles have a $t_{1/2}$ of 1.5 to 2 days. In subjects without hypertriglyceridemia, two-thirds of plasma cholesterol is found in the LDL. Plasma clearance of LDL is mediated primarily by LDL receptors (apo B-100 binds LDL to its receptor); a small component is mediated by nonreceptor clearance mechanisms.

TABLE 37–1 ■ COMPARISON OF KEY CLINICAL GUIDELINES FOR THE MANAGEMENT OF CHOLESTEROL IN ADULTS

	ATPIII 2004	ACC/AHA 2014	AHA/ACC 2018	ACC/AHA 2019
Risk assessment strategy	10-year FRS; CHD risk factors	10-year PCE	10-year or lifetime PCE	10-year or lifetime PCE
Candidates for treatment	Patients above LDL-C goal	Patients in four statin benefit groups	Patients above LDL-C goal	Primary prevention in all patients
Recommended statin intensity	Titrated to achieve LDL-C goal	Moderate-to-high intensity	Moderate-to-high intensity (may be titrated to achieve a specific LDL-C percent reduction goal)	Moderate-to-high intensity (may be titrated to achieve a specific LDL-C percent reduction goal)

Recommendations	*Risk groups and LDL-C goals:*
	• High risk if CHD, risk equivalent, or FRS ≥20% (LDL-C goal <100 mg/dL; <70 mg/dL optional)
	• Moderate-high risk if ≥2 risk factors or FRS 10% to <20% (LDL-C goal <130 mg/dL; <100 mg/dL optional)
	• Moderate risk if ≥2 risk factors or FRS <10% (LDL-C goal <130 mg/dL; therapy started if LDL-C >160 mg/dL)
	• Lower risk if 0 or 1 risk factor (LDL-C goal <160 mg/dL; therapy started if LDL-C >190 mg/dL)
		Four statin benefit groups:
		• If ≥21 years old, clinical ASCVD, high-intensity statin (or moderate, if >75 years old)
		• If ≥21 years old and LDL-C ≥190 mg/dL, high-intensity statin
		• If 40–75 years old with DM and LDL-C 70–189 mg/dL, moderate-intensity statin (or high-intensity if ASCVD ≥7.5%)
		• If 40–75 years old with LDL-C 70–189 mg/dL, moderate- to high-intensity statin if ASCVD ≥7.5%
			Risk groups and LDL-C goals:
			• Primary Prevention (ages 40–75 years):
			  ◦ If LDL-C ≥190 mg/dL, high-intensity statin recommended regardless of ASCVD risk
			  ◦ If patient has DM, moderate-intensity statin recommended regardless of ASCVD risk
			  ◦ High risk if ASCVD ≥20% (LDL-C goal reduction of ≥50%)
			  ◦ Intermediate risk if ASCVD ≥7.5% to <20% + risk factors present (LDL-C goal reduction of 30%–49%)
			  ◦ Borderline risk if ASCVD ≥5% to <7.5% + risk factors present (moderate-intensity statin might be recommended; no specific LDL-C percent reduction stated)
			  ◦ Low risk if ASCVD <5% (lifestyle intervention only; statin therapy not recommended)
			• Secondary Prevention:
			  ◦ Very high-risk ASCVD if major events and/or high-risk conditions present: high-intensity statin recommended
			  ◦ Not very high-risk ASCVD: high-intensity statin recommended if age ≤75 years (LDL-C goal reduction of ≥50%); if age ≥75 years, moderate-intensity statin reasonable, depending on clinical assessment
				Same recommendations as those stated in AHA/ACC 2018 under "Primary Prevention"

Refer to Table 37–4 for discussion of ASCVD risk factors.

ATPIII, Adult Treatment Panel III; CHD, coronary heart disease; DM, diabetes mellitus; FRS, Framingham Risk Score.

Source: Data from ATPIII (Grundy et al., 2004; NCEP, 2002), ACC/AHA 2014 (Stone et al., 2014), AHA/ACC 2018 (Grundy et al., 2019), and ACC/AHA 2019 (Arnett et al., 2019).

The most common cause of autosomal dominant hypercholesterolemia involves loss-of-function mutations of the *LDL* receptor gene (*LDLR*). Defective or absent LDL receptors cause high levels of plasma LDL-C and the most common form of familial hypercholesterolemia (FH). Treatment of homozygous familial hypercholesterolemia (HoFH), which is associated with accelerated ASCVD and premature death at or before the age of 30, is treated by inhibiting cholesterol synthesis with statins or by inhibiting angiopoietin-like protein 3 with monoclonal antibodies. LDL becomes atherogenic when modified by oxidation, a required step for LDL uptake by the *scavenger receptors* (SRs) of macrophages. This process leads to foam cell formation in arterial lesions. At least two SRs are involved (SR-AI/II and CD36). SR-AI/II appears to be expressed more in early atherogenesis, and CD36 expression is greater as foam cells form during lesion progression. The liver expresses a large complement of LDL receptors and removes about 75% of all LDL from the plasma. Consequently, manipulation of hepatic LDL receptor gene expression is a most effective way to modulate plasma LDL-C levels. *The most effective dietary alteration (decreased consumption of saturated fat and cholesterol) and pharmacological treatment (statins) for hypercholesterolemia act by enhancing hepatic LDL receptor expression.*

High-Density Lipoproteins

The HDLs are protective lipoproteins that decrease the risk of CHD; thus, high levels of HDLs are generally desirable. This protective effect may result from the participation of HDL in reverse cholesterol transport, the process by which excess cholesterol is acquired from cells and transferred to the liver for excretion (see Figure 37–2). HDL effects also include putative anti-inflammatory, antioxidative, platelet antiaggregatory, anticoagulant, and profibrinolytic activities. Apo A-I is the major HDL apoprotein, and its plasma concentration is a more powerful inverse predictor of CHD risk than an overall high-density lipoprotein cholesterol (HDL-C) level. Apo A-I synthesis is required for normal production of HDL.

Mutations in the apo A-I gene that cause HDL deficiency often are associated with accelerated atherogenesis. In addition, two major subclasses of mature HDL particles in the plasma can be differentiated by their

TABLE 37–2 ■ CHARACTERISTICS OF PLASMA LIPOPROTEINS

LIPOPROTEIN CLASS	DENSITY (g/mL)	MAJOR LIPID CONSTITUENT	TG:CHOL	SIGNIFICANT APOPROTEINS	SITE OF SYNTHESIS	CATABOLIC PATHWAY
Chylomicrons and remnants	<1.006	Dietary triglycerides and cholesterol	10:1	B-48, E, A-I, A-IV, C-I, C-II, C-III	Intestine	TG hydrolysis by LPL; apo E–mediated remnant uptake by liver
VLDL	<1.006	"Endogenous" or hepatic triglycerides	5:1	B-100, E, C-I, C-II, C-III	Liver	TG hydrolysis by LPL
IDL	1.006–1.019	Cholesteryl esters and "endogenous" triglycerides	1:1	B-100, E, C-II, C-III	Product of VLDL catabolism	50% converted to LDL mediated by HL; 50% apo E–mediated uptake by liver
LDL	1.019–1.063	Cholesteryl esters	NS	B-100	Product of VLDL catabolism	Apo B-100-mediated uptake by LDL receptor (~75% in liver)
HDL	1.063–1.21	Phospholipids, cholesteryl esters	NS	A-I, A-II, E, C-I, C-II, C-III	Intestine, liver, plasma	Complex: transfer of cholesteryl ester to VLDL and LDL; uptake of HDL cholesterol by hepatocytes
Lp(a)	1.05–1.09	Cholesteryl esters	NS	B-100, apo(a)	Liver	Unknown

CHOL, cholesterol; NS, not significant (TG is <5% of LDL and HDL); TG, triglyceride.

content of the major HDL apoproteins, apo A-I and apo A-II. Epidemiologic evidence in humans suggests that apo A-II may be atheroprotective.

The membrane transporter ABCA1 facilitates the transfer of free cholesterol from cells to HDL. After free cholesterol is acquired by the nascent pre-β1 HDL, it is esterified by lecithin:cholesterol acyltransferase (LCAT). The newly esterified and nonpolar cholesterol moves into the core of the particle, which becomes progressively more spherical, larger, and less dense with continued cholesterol acquisition and esterification. As the cholesteryl ester content of the particle (now called HDL$_2$) increases, the cholesteryl esters of these particles begin to be exchanged for triglycerides derived from any of the triglyceride-containing lipoproteins (chylomicrons, VLDLs, remnant lipoproteins, and LDLs). This exchange, mediated by cholesterol ester transfer protein (CETP), accounts for the removal of about two-thirds of the cholesterol associated with HDL in humans. The transferred cholesterol subsequently is metabolized as part of the lipoprotein into which it was transferred. Treatments that target CETP and the ATP-binding cassette (ABC) transporters have yielded equivocal results in humans. While CETP inhibitors effectively reduce LDL-C levels, they also increase the frequency of adverse cardiovascular events (angina, revascularization, myocardial infarction, heart failure, and death).

The triglyceride that is transferred into HDL$_2$ is hydrolyzed in the liver by HL, a process that regenerates smaller, spherical HDL$_3$ particles that recirculate and acquire additional free cholesterol from tissues containing excess free cholesterol. HL is sensitive to hormone regulation, and HL activity modulates HDL-C levels. Androgens increase HL gene expression/activity, which accounts for the lower HDL-C values observed in men than in women. Estrogens reduce HL activity, but their impact on HDL-C levels in women is substantially less than that of androgens on HDL-C levels in men. HL appears to have a pivotal role in regulating HDL-C levels, as HL activity is increased in many patients with low HDL-C levels.

TABLE 37–3 ■ APOLIPOPROTEINS

APOLIPOPROTEIN (MW in kDa)	AVERAGE CONCENTRATION (mg/dL)	SITES OF SYNTHESIS	FUNCTIONS
apo A-I (~29)	130	Liver, intestine	Structural in HDL; LCAT cofactor; ligand of ABCA1 receptor; reverse cholesterol transport
apo A-II (~17)	40	Liver	Forms -*S*-*S*- complex with apo E-2 and E-3, which inhibits E-2 and E-3 binding to lipoprotein receptors
apo A-V (~40)	<1	Liver	Modulates triglyceride incorporation into hepatic VLDL; activates LPL
apo B-100 (~513)	85	Liver	Structural protein of VLDL, IDL, LDL; LDL receptor ligand
apo B-48 (~241)	Fluctuates according to dietary fat intake	Intestine	Structural protein of chylomicrons
apo C-I (~6.6)	6	Liver	LCAT activator; modulates receptor binding of remnants
apo C-II (8.9)	3	Liver	LPL cofactor
apo C-III (8.8)	12	Liver	Modulates receptor binding of remnants
apo E (34)	5	Liver, brain, skin, gonads, spleen	Ligand for LDL receptor and receptors binding remnants; reverse cholesterol transport (HDL with apo E)
apo(a) (Variable)	Variable (under genetic control)	Liver	Modulator of fibrinolysis

MW, molecular weight.

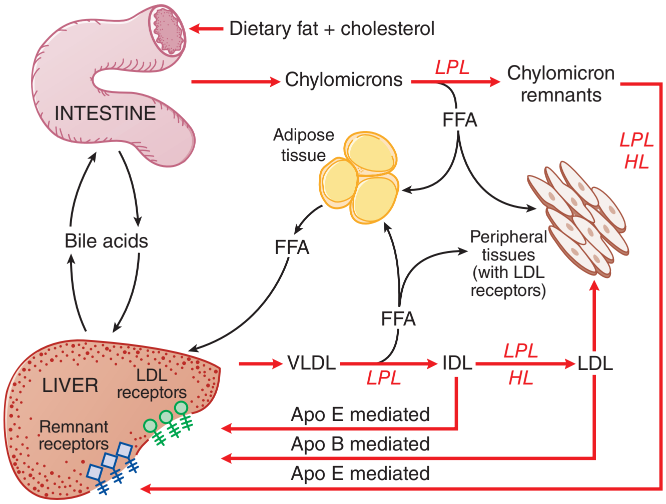


Figure 37–1 *The major pathways involved in the metabolism of chylomicrons synthesized by the intestine and VLDL synthesized by the liver.* Chylomicrons are converted to chylomicron remnants by the hydrolysis of their triglycerides by LPL. Chylomicron remnants are rapidly cleared from the plasma by the liver. “Remnant receptors” include the LRP, LDL receptors, and perhaps other receptors. Free fatty acid (FFA) released by LPL is used by muscle tissue as an energy source or taken up and stored by adipose tissue.

Lipoprotein (a)

Lipoprotein (a) [Lp(a)] is composed of an LDL particle that has a second apoprotein, apo(a), in addition to apo B-100. Apo(a) of Lp(a) is structurally related to plasminogen and appears to be atherogenic.

Atherosclerotic Cardiovascular Disease Risk Assessment

Therapy for dyslipidemias is centered around reducing the risk of fatal and nonfatal atherosclerotic cardiovascular events (ASCVD), including myocardial infarction and stroke. A flowchart that illustrates the assessment and management of ASCVD risk is shown in Figure 37–3.

The major conventional risk factors for ASCVD are elevated LDL-C, reduced HDL-C, cigarette smoking, hypertension, type 2 diabetes mellitus, advanced age, and a family history of premature CHD events (men <55 years; women <65 years) in a first-degree relative (Table 37–4). As more data have become available, ASCVD risk calculators have also begun to include race.

More recently, diagnosis and risk assessment based on genetic testing for monogenetic mutations that lead to FH (e.g., in the *LDLR*, *APOB*, *PCSK9*, and *LDLRAP1* genes) are recommended for patients suspected of having FH, as well as their at-risk relatives (Sturm et al., 2018). Early identification of individuals and their family members at risk for FH before the elevated LDL-C phenotype presents clinically could provide opportunities for interventions that reduce LDL-C before the onset of coronary artery disease. Pediatric patients identified with FH that are started on clinical interventions to reduce LDL-C have lower cumulative LDL-C burden and reduced risk of ASCVD compared to their affected parents. Screening at-risk family members (termed *cascade testing*) is considered a cost-effective means of identifying patients with FH and preventing associated morbidity and mortality but is not routinely done in the U.S. at present. In addition to monogenetic FH testing, there is significant interest in developing polygenetic risk scores based on genome-wide association studies (GWAS) to assess the aggregate contributions of many small-effect alleles on increasing LDL-C levels and consequent atherosclerotic cardiovascular risk.

Primary prevention involves management of risk factors to prevent a first-ever ASCVD event. Secondary prevention is aimed at patients who have had a prior ASCVD event (i.e., myocardial infarction, stroke, or revascularization) and whose risk factors must be reduced aggressively. In addition to cholesterol management, a comprehensive approach to ASCVD risk reduction includes smoking cessation, weight management, physical activity, healthy eating habits, appropriate use of aspirin, and management of blood glucose and blood pressure. All treatment plans to reduce ASCVD risk must include patient counseling surrounding lifestyle changes. Secondary causes of dyslipidemias (Table 37–5), including medications that affect cholesterol, should also be considered prior to initiating treatment. Patients should also be evaluated for metabolic syndrome, which affects more than one in three adults and includes insulin resistance, obesity, hypertension, low HDL-C levels, a procoagulant state, vascular inflammation, and substantially increased risk of cardiovascular disease.

The pooled cohort equation (PCE), published as part of the 2014 ACC/AHA guideline on the assessment of cardiovascular risk and still referenced in the 2018 AHA/ACC cholesterol guidelines, was developed based on data from nine cohort studies funded by the National Heart, Lung, and Blood Institute and included data from geographically and racially diverse patient populations. The PCE estimates an individual patient’s 10-year or lifetime risk of ASCVD (defined as nonfatal myocardial infarction, coronary heart disease death, or fatal or nonfatal stroke) based on age, gender, total cholesterol, HDL-C, race, systolic blood pressure, smoking status, and history of diabetes and hypertension. This risk calculator is intended for primary prevention in patients between the ages of 20 and 79 years. The ASCVD Risk Estimator is

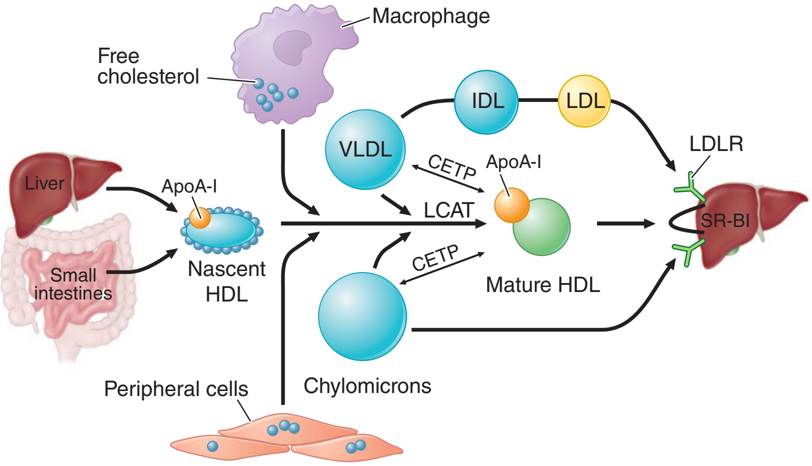


Figure 37–2 *HDL pathways and reverse cholesterol transport.* The intestines and liver generate nascent HDL, which collect free cholesterol from macrophages and peripheral tissues. The cholesterol is esterified by LCAT to cholesterol esters, leading to mature HDL particles (HDL_3 and HDL_2). Cholesterol is also acquired from cholesterol-containing lipoproteins including chylomicrons and chylomicron remnants, VLDL and VLDL remnants (IDLs), and LDL. The mature HDL particles deliver the cholesterol esters to the liver, which are then excreted into the small intestines as bile. See text for additional details. (Reproduced with permission from Jameson JL et al., eds. *Harrison’s Principles of Internal Medicine*, 20th ed. McGraw Hill, New York, 2018.)

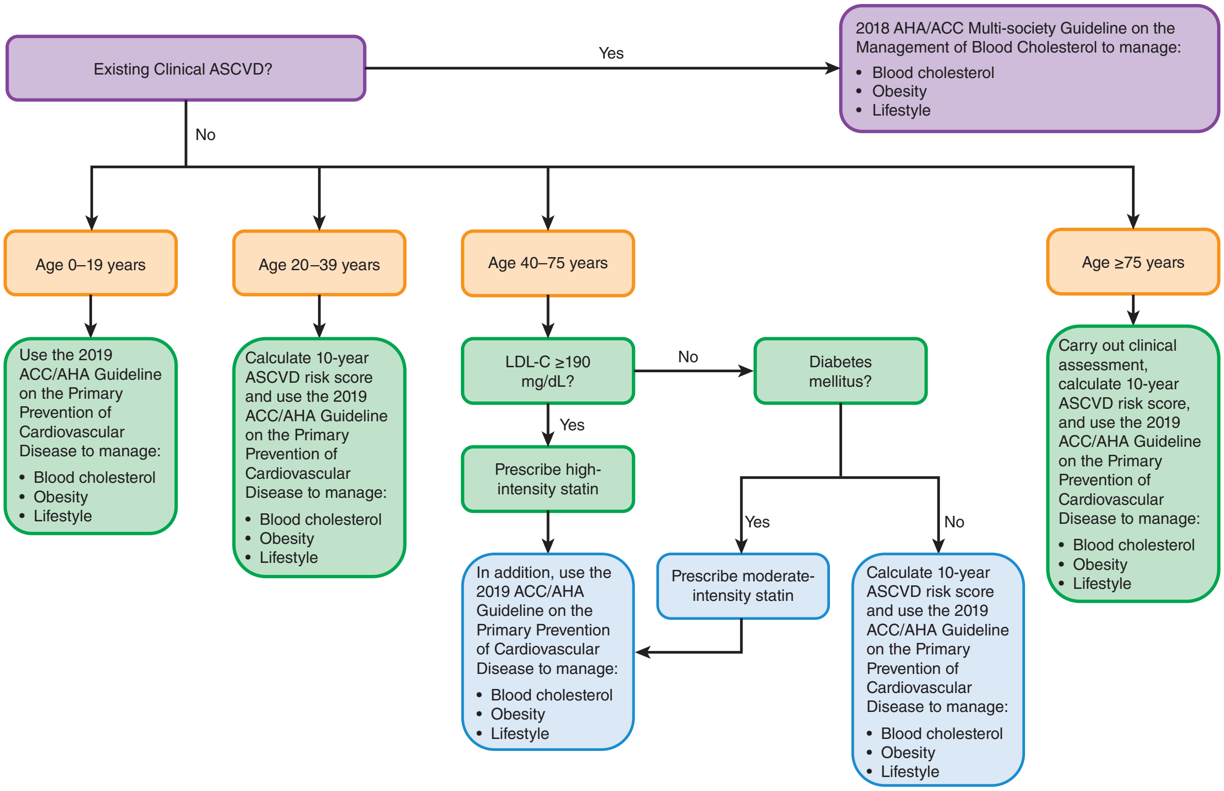

Existing Clinical ASCVD?
Yes
2018 AHA/ACC Multi-society Guideline on the Management of Blood Cholesterol to manage:
• Blood cholesterol
• Obesity
• Lifestyle
No
Age 0–19 years
Age 20–39 years
Age 40–75 years
Age ≥75 years
Use the 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease to manage:
• Blood cholesterol
• Obesity
• Lifestyle
Calculate 10-year ASCVD risk score and use the 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease to manage:
• Blood cholesterol
• Obesity
• Lifestyle
LDL-C ≥190 mg/dL?
No
Diabetes mellitus?
Yes
Prescribe high-intensity statin
Yes
No
In addition, use the 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease to manage:
• Blood cholesterol
• Obesity
• Lifestyle
Prescribe moderate-intensity statin
Calculate 10-year ASCVD risk score and use the 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease to manage:
• Blood cholesterol
• Obesity
• Lifestyle
Carry out clinical assessment, calculate 10-year ASCVD risk score, and use the 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease to manage:
• Blood cholesterol
• Obesity
• Lifestyle

Figure 37–3 *Flowchart for assessing and managing ASCVD risk.* This chart is based on the 2018 AHA/ACC Multi-society Guideline on the Management of Blood Cholesterol and the 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease. Refer to Table 37–1 and the aforementioned guidelines for additional details.

available online (https://tools.acc.org/ldl/ascvd_risk_estimator/index.html#!/calulate/estimator/) and as a mobile device application. Cardiovascular risk assessment calculators have also been developed for lifetime risk of cardiovascular disease and projected 10-year ASCVD risk when a patient is placed on specific risk-reduction therapies.

TABLE 37–4 ■ RISK FACTORS FOR ATHEROSCLEROTIC CARDIOVASCULAR DISEASE

Age	Male >45 years of age or female >55 years of age
Family history of premature CHD[a]	A first-degree relative (male <55 years of age or female <65 years of age when the first CHD clinical event occurs)
Current cigarette smoking	Defined as smoking within the preceding 30 days
Hypertension	Systolic blood pressure ≥130 mmHg, diastolic pressure ≥90 mmHg, or use of antihypertensive medication, irrespective of blood pressure
Low HDL-C	<40 mg/dL (consider <50 mg/dL as "low" for women)
Obesity	Body mass index >25 kg/m^2 and waist circumference >40 inches (men) or >35 inches (women)
Type 2 diabetes mellitus[b]	

CHD, coronary heart disease.

[a] CHD defined as myocardial infarction, coronary death, or a coronary revascularization procedure.

[b] Diabetes mellitus is considered a high- or very high-risk condition for ASCVD.

Source: Data from 2015 National Lipid Association recommendations, part 1 (Jacobson et al., 2015).

Statin Drug Therapy

Although an understanding of optimal lipoprotein levels is helpful (see ranges in Table 37–6), the 2018 AHA/ACC cholesterol guideline recommends titrating statin therapy to specific LDL-C percent decrease goals in certain patient groups, depending on their age and ASCVD risk score. Because the overwhelming body of evidence for ASCVD risk reduction with lipid-lowering therapies is from statin trials, evidence-based statin therapy of appropriate intensity is the hallmark of drug therapy of dyslipidemias. Patients aged 40 to 75 years with a known history of clinical ASCVD or diabetes mellitus and those with elevated LDL-C greater than or equal to 190 mg/dL should be offered statins regardless of their ASCVD risk score. For primary prevention in patients 40 through 79 years of age with an LDL-C between 70 and 189 mg/dL, use of the PCE is recommended to identify patients more likely to benefit from treatment. Table 37–1 summarizes 2018 AHA/ACC guideline recommendations for use of statins in adults.

Mechanism of Action

Statins exert their major effect—reduction of LDL-C levels—through a mevalonic acid-like moiety that competitively inhibits β-hydroxy β-methylglutaryl coenzyme A (HMG-CoA) reductase. By reducing the conversion of HMG-CoA to mevalonate, statins inhibit an early and rate-limiting step in cholesterol biosynthesis. Statins affect blood cholesterol levels by inhibiting hepatic cholesterol synthesis, which results in increased expression of the LDL receptor gene. Some studies suggested that statins also can reduce LDL-C levels by enhancing the removal of LDL precursors (VLDL and IDL) and by decreasing hepatic VLDL production. The reduction in hepatic VLDL production induced by statins is thought to be mediated by reduced synthesis of cholesterol, a required component of VLDLs. Higher doses of the more potent statins (e.g., *atorvastatin*, *simvastatin*, and *rosuvastatin*) also can reduce triglyceride levels caused by elevated VLDL levels. Figure 37–4 shows a representative statin structure and the reaction catalyzed by HMG-CoA reductase.

TABLE 37–5 ■ SECONDARY CAUSES OF DYSLIPIDEMIA

SECONDARY CAUSE	ELEVATED LDL-C	ELEVATED TRIGLYCERIDES
Disorders and Conditions		
Diabetes mellitus		+
Nephrotic syndrome	+	+
Excess alcohol use		+
Pregnancy	+	+
Menopause transition (declining estrogen levels)	+	+
Chronic kidney disease	+	+
Hypothyroidism	+	+
Obstructive liver disease	+	
Metabolic syndrome		+
HIV infection	+	+
Autoimmune disorders	+	+
Polycystic ovary syndrome	+	+
Drug Therapies		
Oral estrogens		+
Some progestins	+	
Glucocorticoids	+	+
Immunosuppressive drugs	+	+
Thiazide diuretics	+	+
Anabolic steroids	+	
Thiazolidinediones	+	
Rosiglitazone		+
β Blockers (especially non-β_1 selective)		+
Fibric acids (in severe hypertriglyceridemia)	+	
Bile acid sequestrants		+
Amiodarone	+	
Danazol	+	
Isotretinoin	+	
Long-chain ω-3 fatty acids (in severe hypertriglyceridemia) with docosahexanoate	+	
Tamoxifen		+
Raloxifene		+
Interferon		+
Atypical antipsychotic drugs (clozapine, olanzapine)		+
Protease inhibitors		+
L-Asparaginase		+
Cyclophosphamide		+

Source: Data from 2015 National Lipid Association recommendations, part 1 (Jacobson et al., 2015).

TABLE 37–6 ■ CLASSIFICATION OF PLASMA LIPID LEVELS (mg/dL)

Non–HDL-C	
<130	Desirable
130–159	Above desirable
160–189	Borderline high
190–219	High
≥220	Very high
HDL-C	
<40	Low (consider <50 mg/dL as low for women)
>60	High (desirable because of negative risk)
LDL-C	
<70	Optimal for very high-risk patients[a]
<100	Desirable
100–129	Above desirable
130–159	Borderline high
160–189	High
≥190	Very high
Triglycerides	
<150	Normal
150–199	Borderline high
200–499	High
≥500	Very high

[a] Some consider LDL-C <70 mg/dL the optimal goal for patients with coronary heart disease or risk equivalents.

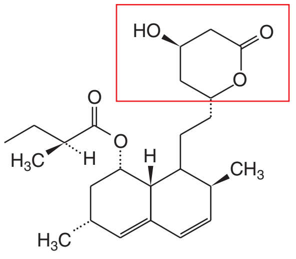
Source: Reproduced with permission from Jacobson TA, et al. National Lipid Association recommendations for patient-centered management of dyslipidemia: part 1—full report. *J Clin Lipidol*, **2015**, 9:129–169. Copyright © 2015 National Lipid Association. Published by Elsevier Inc. All rights reserved.

ADME

After oral administration, intestinal absorption of the statins is variable (30%–85%). All statins, except *simvastatin* and *lovastatin*, are administered in the β-hydroxy acid form, which is the form that inhibits HMG-CoA reductase. *Simvastatin* and *lovastatin* are administered as inactive lactones that must be transformed in the liver to their respective β-hydroxy acids, simvastatin acid and lovastatin acid. There is extensive first-pass hepatic uptake of all statins, mediated primarily by the organic anion transporter OATP1B1 (see Chapter 5). *Hepatic cholesterol synthesis is maximal between midnight and 2:00 AM. Thus, statins with* $t_{1/2}$ *of 4 h or less (all but* atorvastatin *and* rosuvastatin*) should be taken in the evening. Atorvastatin* and *rosuvastatin* both have longer half-lives and may be taken at other times of day to optimize adherence.

Due to extensive first-pass hepatic uptake, the systemic bioavailability of statins and their hepatic metabolites varies between 5% and 30% of administered doses. *Atorvastatin*, *lovastatin*, and *simvastatin* are primarily metabolized by CYPs 3A4 and 3A5. *Fluvastatin* is mostly (50%–80%) metabolized by CYP2C9 to inactive metabolites, but CYP3A4 and CYP2C8 also contribute to its metabolism. *Rosuvastatin* is excreted primarily unchanged in the stool, though around 10% is metabolized by CYP2C9. *Pravastatin*, however, is not metabolized to any appreciable extent by the CYP system and is excreted unchanged in the urine. The metabolites of all statins, except *fluvastatin* and *pravastatin*, have some HMG-CoA reductase inhibitory activity.

Under steady-state conditions, small amounts of the parent drug and its metabolites produced in the liver can be found in the systemic circulation. In the plasma, more than 95% of statins and their metabolites are protein bound, with the exception of *pravastatin* and its metabolites, which are only 50% bound. Peak plasma concentrations of statins are achieved in 1 to 4 h. The $t_{1/2}$ of the parent compounds are 1 to 4 h, except in the case of *atorvastatin* and *rosuvastatin*, which have $t_{1/2}$ of about 20 h, and *simvastatin* with a $t_{1/2}$ of about 12 h. The longer $t_{1/2}$ of *atorvastatin* and *rosuvastatin* may contribute to their greater cholesterol-lowering effect.



LOVASTATIN

Reaction Catalyzed by HMG-CoA Reductase

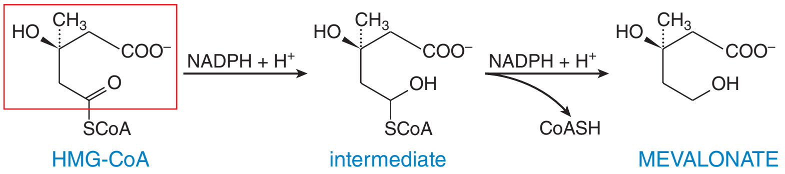


Figure 37–4 *Lovastatin and the HMG-CoA reductase reaction.*

The liver biotransforms all statins, and more than 70% of statin metabolites are excreted by the liver, with subsequent elimination in the feces.

Therapeutic Effects

Effects of Statins on LDL-C Levels

Dose-response relationships for all statins demonstrate that the efficacy of LDL-C lowering is log linear; LDL-C is reduced by about 6% (from baseline) with each doubling of the dose. Maximal effects on plasma cholesterol levels are achieved within 7 to 10 days. The statins are effective in almost all patients with high LDL-C levels. The exception is patients with HoFH, who have very attenuated responses to the usual doses of statins because both alleles of the *LDLR* gene code for dysfunctional LDL receptors.

Triglyceride Reduction by Statins

Triglyceride levels greater than 250 mg/dL are reduced substantially by statins, and the percent reduction achieved is similar to the percent reduction of LDL-C.

Effect of Statins on HDL-C Levels

Most studies of patients treated with statins have systematically excluded patients with low HDL-C levels. In studies of patients with elevated LDL-C levels and gender-appropriate HDL-C levels (40–50 mg/dL for men; 50–60 mg/dL for women), an increase in HDL-C of 5% to 10% was observed, irrespective of the dose or statin employed. However, in patients with reduced HDL-C levels (<35 mg/dL), statins may differ in their effects on HDL-C levels. More studies are needed to ascertain whether the effects of statins on HDL-C in patients with low HDL-C levels are clinically significant.

Adverse Effects and Drug Interactions

Myopathy

The major adverse effect associated with statin use is myopathy. Myopathy refers to a broad spectrum of muscle complaints, ranging from mild muscle soreness or weakness (myalgia) to life-threatening rhabdomyolysis. The risk of muscle adverse effects increases in proportion to statin dose and plasma concentrations. Consequently, factors inhibiting statin catabolism are associated with increased myopathy risk, including advanced age (especially >80 years of age), hepatic or renal dysfunction, perioperative periods, small body size, and untreated hypothyroidism. Measurements of creatinine kinase are not routinely necessary, unless the patient also is taking a drug that enhances the risk of myopathy. Concomitant use of drugs that diminish statin catabolism or interfere with hepatic uptake is associated with increased risk of myopathy and rhabdomyolysis. The most common statin interactions occur with fibrates, especially *gemfibrozil* (38%), and with *cyclosporine* (4%), *digoxin* (5%), *warfarin* (4%), macrolide antibiotics (3%), and azole antifungals (1%). Other drugs that increase the risk of statin-induced myopathy include *niacin* (rare), human immunodeficiency virus (HIV) protease inhibitors, *amiodarone*, and *nefazodone*.

Hepatotoxicity

Serious hepatotoxicity is rare and unpredictable, with a rate of about 1 case per million person-years of use. However, since 2012, the FDA has no longer recommended routine monitoring of alanine aminotransferase (ALT) or other liver enzymes following the initiation of statin therapy because routine periodic monitoring does not appear to be effective in detecting or preventing serious liver injury. Liver enzymes should be evaluated in patients with clinical symptoms suggestive of liver injury following initiation or changes in statin treatment (FDA, 2012). Statin use is contraindicated in patients with active liver disease.

Additional Precautions or Contraindications

Statins should be used with caution in patients with renal impairment. *Atorvastatin* is often the statin of choice for patients with severe renal dysfunction, as it does not require dose adjustment. *Statins are contraindicated during pregnancy and should be discontinued prior to conception, if possible.* Data regarding statin use while breastfeeding are limited, and use should be discouraged.

Drug Interactions

Gemfibrozil, the drug most commonly associated with statin-induced myopathy, both inhibits uptake of the active hydroxy acid forms of statins into hepatocytes by OATP1B1 and interferes with the transformation of most statins by glucuronidases. Coadministration of *gemfibrozil* nearly doubles the plasma concentration of statin hydroxy acids. When statins are administered with *niacin*, the myopathy is likely caused by an enhanced inhibition of skeletal muscle cholesterol synthesis (a pharmacodynamic interaction). In 2016, the FDA withdrew approval for statin drug combinations containing fibrates or *niacin* (FDA, 2016).

Drugs that interfere with statin oxidation are those metabolized primarily by CYP3A4 and include certain macrolide antibiotics (e.g., *erythromycin*); azole antifungals (e.g., *itraconazole*); *cyclosporine*; *nefazodone*, a phenylpiperazine antidepressant; HIV protease inhibitors; and *amiodarone*. These pharmacokinetic interactions are associated with increased plasma concentrations of statins and their active metabolites. Because *pravastatin*, *fluvastatin*, and *rosuvastatin* are not extensively metabolized by CYP3A4, these statins may be less likely to cause myopathy when used with one of these predisposing drugs. However, the benefits of combined therapy with any statin should be carefully weighed against the risk of myopathy.

According to a 2012 FDA warning, *simvastatin* should not be used in combination with *cyclosporine*, HIV protease inhibitors, *erythromycin*, or

TABLE 37–7 ■ INTENSITY OF STATINS BY APPROXIMATE REDUCTIONS IN LDL-C WITH DAILY DOSING

HIGH-INTENSITY STATINS	MODERATE-INTENSITY STATINS	LOW-INTENSITY STATINS
Lower LDL-C by ≥50%	Lower LDL-C by 30% to <50%	Lower LDL-C, on average, by <30%
Atorvastatin 40–80 mg **Rosuvastatin 20–40 mg**	**Atorvastatin 10 mg** (to 20 mg) **Fluvastatin 40 mg twice daily** Fluvastatin XL 80 mg **Lovastatin 40 mg** (to 80 mg) Pitavastatin 1–4 mg **Pravastatin 40 mg** (to 80 mg) **Rosuvastatin** (5 mg) to **10 mg** **Simvastatin 20–40 mg**	Fluvastatin 20–40 mg **Lovastatin 20 mg** **Pravastatin 10–20 mg** Simvastatin 10 mg

Bold type signifies statin doses used in randomized controlled trials demonstrating a reduction in major cardiovascular events.
Source: Data from *Table 3* in 2018 AHA/ACC guidelines (Grundy et al., 2019).

gemfibrozil. In patients taking *amlodipine* or *amiodarone*, the daily dose of *simvastatin* should not exceed 20 mg. No more than 10 mg of *simvastatin* should be used in combination with *diltiazem* or *verapamil*.

Other Considerations

The choice of statin should be patient-specific and based on factors such as cost, drug interactions, possible adverse effects, and desired intensity. Statin doses are characterized as low, moderate, or high intensity (Table 37–7), based on the degree of LDL-C lowering expected (range between 30% and 60%).

Rosuvastatin and *pravastatin* may be better tolerated than other statins and should be considered in patients with a history of myalgias with other statins. *Lovastatin* absorption is increased when taken with food, and patients should be encouraged to take *lovastatin* with their evening meal. Concerns have been raised about possible cognitive impairment with statins, although review of the published data do not suggest that statins harm cognition. In contrast, other studies suggest statins may have a role in the prevention of dementias. Statins, especially at higher doses, likely confer a small increased risk of developing diabetes. However, the beneficial effects of statins on ASCVD events and mortality outweigh any increased risk conferred by promoting the development of diabetes. Some statins have been approved for use in children with heterozygous FH (HeFH). *Atorvastatin*, *lovastatin*, and *simvastatin* are indicated for children aged 11 years and older. *Pravastatin* is approved for children 8 years and older.

Nonstatin Drug Therapies

The 2018 AHA/ACC cholesterol management guideline mainly focuses on the use of statins to reduce ASCVD risk. However, the guidelines do recommend the use of *ezetimibe* and/or PCSK9 inhibitors as add-on therapies for the management of cholesterol in patients with clinical ASCVD. Several important clinical trials have evaluated whether fibrates, *niacin*, *ezetimibe*, PCSK9 inhibitors, fish oil, and *icosapent ethyl* result in further reductions in ASCVD risk when used in addition to statins (ACCORD Study Group, 2010; AIM-HIGH Investigators, 2011; Bhatt et al., 2019; Cannon et al., 2015; HPS2-THRIVE Collaborative Group, 2014; ORIGIN Trial Investigators, 2012; Sabatine et al., 2017; Schwartz et al., 2018). In April 2016, the FDA withdrew approval for *niacin* extended release (ER) or *fenofibrate* used in addition to statins, citing studies that demonstrated no additional reduction in ASCVD events versus monotherapy with a statin (FDA, 2016). In October 2017, the ACC released an update to their expert consensus decision pathway that can aid clinicians in the use of nonstatins (i.e., bile acid sequestrants, PCSK9 inhibitors, or *ezetimibe*) in addition to statins for the management of ASCVD risk (Lloyd-Jones et al., 2017). Ultimately, the use of nonstatins in high-risk patient populations requires careful shared decision making between patients and their physician.

Elevated triglycerides are an important risk factor for pancreatitis. Treatment with agents most effective at reducing levels of triglycerides (fibrate or fish oil) is recommended in patients with very elevated triglycerides (>1000 mg/dL) to reduce the risk of pancreatitis. These therapies may be used in addition to statin treatment if the patient otherwise has risk factors for ASCVD that make the patient an appropriate candidate for statin therapy.

Cholesterol Absorption Inhibitor

Ezetimibe is the first compound approved for lowering total cholesterol and LDL-C levels that acts by inhibiting cholesterol absorption by enterocytes in the small intestine. It lowers LDL-C levels by about 20% and may be used as adjunctive therapy with statins.

Mechanism of Action

Ezetimibe inhibits luminal cholesterol uptake by jejunal enterocytes, by inhibiting Niemann-Pick C1–like 1 transport protein. In human subjects, *ezetimibe* reduces cholesterol absorption by 54%, precipitating a compensatory increase in cholesterol synthesis that can be inhibited with a cholesterol synthesis inhibitor (e.g., a statin or ATP-citrate lyase [ACL] inhibitor). The consequence of inhibiting intestinal cholesterol absorption is a reduction in the incorporation of cholesterol into chylomicrons; this diminishes the delivery of cholesterol to the liver by chylomicron remnants. The diminished remnant cholesterol content may decrease atherogenesis directly, as chylomicron remnants are very atherogenic lipoproteins. Reduced delivery of intestinal cholesterol to the liver by chylomicron remnants stimulates expression of the hepatic genes regulating LDL receptor expression and cholesterol biosynthesis. The greater expression of hepatic LDL receptors enhances LDL-C clearance from the plasma. *Ezetimibe* reduces LDL-C levels by 15% to 20%.

ADME

Ezetimibe is highly water insoluble, precluding studies of its bioavailability. After ingestion, it is absorbed and glucuronidated in the intestinal epithelium and then enters an enterohepatic recirculation. Pharmacokinetic studies indicated that about 70% is excreted in the feces and about 10% in the urine (as a glucuronide conjugate). Bile acid sequestrants inhibit absorption of *ezetimibe*, and the two classes of agents should not be administered together.

Therapeutic Effects

The role of *ezetimibe* as monotherapy of patients with elevated LDL-C levels is generally limited to the small group of statin-intolerant patients. The actions of *ezetimibe* are complementary to those of statins and the ACL inhibitor, *bempedoic acid*. Dual therapy with *ezetimibe* and either of these two classes of drugs prevents both the enhanced cholesterol synthesis induced by *ezetimibe* and the increase in cholesterol absorption induced by statins, providing additive reductions in LDL-C levels. LDL-C reduction at the highest *simvastatin* dose plus *ezetimibe* is similar to that of high-intensity statins. Reduction of LDL-C is greater with a statin plus *ezetimibe*/*bempedoic acid* than with a statin alone.

Preparations and Use

Ezetimibe is available as a 10-mg tablet that may be taken at any time during the day, with or without food. *Ezetimibe* may be taken in combination with other dyslipidemia medications except bile acid sequestrants, which inhibit its absorption. A combination tablet containing *ezetimibe* 10 mg and various doses of *simvastatin* (10, 20, 40, or 80 mg) has been approved. A combination of 10 mg *ezetimibe* with 180 mg *bempedoic acid* in tablet form has also been approved and can be used alone or in combination with a statin.

Adverse Effects and Drug Interactions

Other than rare allergic reactions, specific adverse effects have not been observed in patients taking *ezetimibe*. *Because all statins are contraindicated in pregnant and nursing women, combination products containing* ezetimibe *and a statin should not be used by women in childbearing years in the absence of contraception.*

Bile Acid Sequestrants

Cholestyramine, Colestipol, Colesevelam

The bile acid sequestrants *cholestyramine* and *colestipol* are among the oldest of the hypolipidemic drugs and are probably the safest because they are not absorbed from the intestine. These resins also are recommended for patients 11 to 20 years of age. Although statins are remarkably effective as monotherapy, the resins could be utilized as a second-line agent if statin therapy does not lower LDL-C levels sufficiently or in cases of statin intolerance.

Mechanism of Action

Bile acid sequestrants are highly positively charged and bind negatively charged bile acids. Because of their large size, the resins are not absorbed, and the bound bile acids are excreted in the stool. Because more than 95% of bile acids are normally reabsorbed, interruption of this process depletes the pool of bile acids, and hepatic bile acid synthesis increases. As a result, hepatic cholesterol content declines, stimulating the production of LDL receptors, an effect similar to that of statins. The increase in hepatic LDL receptors increases LDL clearance and lowers LDL-C levels, but this effect is partially offset by the enhanced cholesterol synthesis caused by upregulation of HMG-CoA reductase (see Figure 37–4). Inhibition of reductase activity by a statin substantially increases the effectiveness of the resins. The resin-induced increase in bile acid production is accompanied by an increase in hepatic triglyceride synthesis, which is of consequence in patients with significant hypertriglyceridemia (baseline triglyceride level >250 mg/dL). Use of *colesevelam* to lower LDL-C levels in patients with hypertriglyceridemia should be accompanied by frequent (every 1–2 weeks) monitoring of fasting triglyceride levels.

Therapeutic Effects

The reduction in LDL-C by resins is dose dependent. Doses of 8 to 12 g of *cholestyramine* or 10 to 15 g of *colestipol* are associated with a 12% to 18% reduction in LDL-C. *Colesevelam* lowers LDL-C by 18% at its maximum dose. Maximal doses (24 g of *cholestyramine*, 30 g of *colestipol*) may reduce LDL-C by as much as 25% but will cause GI side effects, which are often intolerable. A period of 1 to 2 weeks is sufficient to attain maximal LDL-C reduction by a given resin dose. In patients with normal triglyceride levels, triglycerides may increase transiently and then return to baseline. When used with a statin, resins are usually prescribed at submaximal doses due to poor tolerability.

Preparations and Use

The powdered forms of *cholestyramine* (4 g/dose) and *colestipol* (5 g/dose) are either mixed with a fluid (water or juice) and swallowed as a slurry or mixed with crushed ice in a blender. Ideally, patients should take the resins before breakfast and before supper, starting with 1 scoop or packet twice daily and increasing the dosage after several weeks or longer as needed and as tolerated. Patients generally will not take more than two doses (scoops or packets) twice daily. *Colesevelam hydrochloride* is available as a solid tablet containing 0.625 g of *colesevelam* and as a powder in packets of 3.75 g or 1.875 g. The starting dose is either 3 tablets taken twice daily with meals or all 6 tablets taken with a meal. The tablets should be taken with a liquid. The maximum daily dose is 7 tablets (4.375 g).

Adverse Effects and Drug Interactions

The resins are generally safe, as they are not systemically absorbed. Because they are administered as chloride salts, rare instances of hyperchloremic acidosis have been reported. Severe hypertriglyceridemia is a contraindication to the use of *cholestyramine* and *colestipol* because these resins increase triglyceride levels. At present, there are insufficient data on the effect of *colesevelam* on triglyceride levels.

Drinking a slurry of powdered *cholestyramine* or *colestipol* produces a gritty sensation that is unpleasant but generally tolerated. *Colestipol* is available in a tablet form. *Colesevelam* is available as a hard capsule that absorbs water and creates a soft, gelatinous material that allegedly minimizes the potential for GI irritation. Patients taking *cholestyramine* and *colestipol* complain of bloating and dyspepsia. These symptoms can be substantially reduced if the drug is completely suspended in liquid several hours before ingestion. Constipation may occur but sometimes can be prevented by adequate daily water intake and psyllium. *Colesevelam* may be less likely than *colestipol* to cause dyspepsia, bloating, and constipation.

The effect of *cholestyramine* and *colestipol* on the absorption of most drugs has not been studied. *Cholestyramine* and *colestipol* bind and interfere with the absorption of many drugs, including some thiazides, *furosemide*, *propranolol*, L-*thyroxine*, *digoxin*, *warfarin*, and some of the statins. *Colesevelam* does not appear to interfere with the absorption of fat-soluble vitamins or of drugs such as *digoxin*, *lovastatin*, *warfarin*, *metoprolol*, *quinidine*, and *valproic acid*. *Colesevelam* reduces the maximum concentration and the area under the curve of sustained-release *verapamil* by 31% and 11%, respectively. In the absence of information to the contrary, prudence suggests that patients take other medications 1 h before or 3 to 4 h after a dose of *colesevelam* or *colestipol*. The safety and efficacy of *colesevelam* have not been studied in pediatric patients or pregnant women.

Niacin (Nicotinic Acid)

Niacin is a water-soluble B-complex vitamin that functions as a vitamin only after conversion to nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP). Both *niacin* and its amide may be given orally as a source of niacin for its functions as a vitamin, but only *niacin* affects lipid levels. The hypolipidemic effects of *niacin* require larger doses than are required for its vitamin effects.

Mechanism of Action

In adipose tissue, *niacin* inhibits the lipolysis of triglycerides by the hormone-sensitive lipase, thereby reducing transport of free fatty acids to the liver and decreasing hepatic triglyceride synthesis. Hormone-sensitive lipase is activated by catecholamines and adrenocorticotropic hormone (ACTH). It is inhibited by insulin. *Niacin* may exert its effects on lipolysis by stimulating a G protein-coupled receptor (GPR109A) that couples to G_i and inhibits cyclic AMP production in adipocytes. In the liver, *niacin* reduces triglyceride synthesis by inhibiting both the synthesis and the esterification of fatty acids, effects that increase apo B degradation. Reduction of triglyceride synthesis reduces hepatic VLDL production, which accounts for the reduced LDL levels. *Niacin* also enhances LPL activity, an action that promotes the clearance of chylomicrons and VLDL triglycerides. *Niacin* raises HDL-C levels by decreasing the fractional clearance of apo A-I in HDL rather than by enhancing HDL synthesis.

ADME

There are various forms of *niacin* available. Crystalline *niacin* (immediate or regular, IR) refers to *niacin* tablets that dissolve quickly after ingestion, are almost completely absorbed, and lead to peak serum concentrations within 30 to 60 min. Because the $t_{1/2}$ for nicotinic acid is about 20 to 48 min, crystalline *niacin* necessitates dosing two to three times daily. Time-release (ER) *niacin* refers to preparations that continuously release *niacin* for a variable amount of time after ingestion. For example, the time to peak serum levels for ER *niacin* is 4 to 5 h. Limited data on the pharmacokinetics of nonprescription sustained-release *niacin* exist, though these dietary supplements claim to be formulated for release over time. At lower doses, most *niacin* is taken up by the liver; only the major metabolite, nicotinuric acid, is found in the urine. At higher doses, a greater proportion of the drug is excreted in the urine as unchanged nicotinic acid.

Therapeutic Effects

Niacin is indicated for the treatment of hypertriglyceridemia and elevated LDL-C. There are only two formulations of *niacin* that are FDA-approved: ER and IR. Additional formulations are sold as dietary supplements, including two "flush-free" derivatives: inositol hexanicotinate and nicotinamide riboside. However, because they are not FDA-approved, these supplements cannot claim to treat any specific disease state; instead, they can only claim to help with overall health (e.g., labels might include "to support heart health"). The potential for severe liver damage should preclude use of nonprescription formulations of *niacin* in medically unsupervised patients.

Regular or crystalline *niacin* in doses of 2 to 6 g/day reduces triglycerides by 35% to 50% (as effectively as fibrates and statins); the maximal effect occurs within 4 to 7 days. Reductions of 25% in LDL-C levels are possible with doses of 4.5 to 6 g/day; 3 to 6 weeks are required for maximal effect. *Niacin* is the most effective agent available for increasing HDL-C (30%–40%), but the effect is less in patients with HDL-C levels less than 35 mg/dL. *Niacin* also is the only lipid-lowering drug that reduces Lp(a) levels significantly. Despite salutary effect on lipids, *niacin*'s side effects limit its use (see Adverse Effects section below).

Preparations and Use

Crystalline *niacin* tablets are available without a prescription in a variety of strengths, from 50- to 500-mg tablets, and with a prescription in 500-mg tablets. *Niacin* ER is available with a prescription as 500-, 750-, and 1000-mg ER tablets. The dose of prescription *niacin* for the treatment of dyslipidemia varies depending on the formulation. *Niacin* ER should be started at 500 mg at bedtime and increased by 500 mg every 4 weeks to a maximum of 2000 mg/day. IR *niacin* should be started at 250 mg every evening and increased every 4 to 7 days to a maximum of 1500 to 2000 mg/day. IR *niacin* may then be further titrated every 2 to 4 weeks to a maximum of 6000 mg/day in patients with marked lipid abnormalities. However, the risk of experiencing adverse effects increases with doses above 2000 mg/day. Transaminases, serum albumin, fasting glucose, and uric acid levels should be measured before treatment, every 6 to 12 weeks for the first year, and every 6 months thereafter.

Adverse Effects and Drug Interactions

Two of *niacin*'s side effects, flushing and dyspepsia, limit patient compliance. The cutaneous effects include flushing and pruritus of the face and upper trunk, skin rashes, and acanthosis nigricans. Flushing and associated pruritus are prostaglandin-mediated, so taking an *aspirin* at least 30 min before each *niacin* dose can minimize flushing in many patients. Flushing can also be minimized if therapy is initiated with low doses (250–500 mg once daily) and if the drug is taken after a meal. Flushing is worse when therapy is initiated or the dosage is increased, but it ceases in most patients after 1 to 2 weeks of a stable dose. Flushing is more likely to occur when *niacin* is consumed with hot beverages or with alcohol. Dry skin, a frequent complaint, can be dealt with by using skin moisturizers, and acanthosis nigricans can be dealt with by using lotions containing *salicylic acid*. Dyspepsia and rarer episodes of nausea, vomiting, and diarrhea are less likely to occur if the drug is taken after a meal. Patients with any history of peptic ulcer disease or liver disease should not take *niacin*. Patients who consume substantial quantities of alcohol should also not take *niacin*.

The most common, medically serious side effects are hepatotoxicity, manifested as elevated serum transaminases, and hyperglycemia. Both IR (crystalline) and ER *niacin* have been reported to cause severe liver toxicity, particularly in doses above 2 g/day. Hepatotoxicity is more likely to occur with time-release formulations of *niacin*. Affected patients experience flu-like fatigue and weakness; usually, aspartate transaminase and ALT are elevated, serum albumin levels decline, and total cholesterol and LDL-C levels decline substantially.

In patients with diabetes mellitus, *niacin* should be used cautiously because *niacin*-induced insulin resistance can cause severe hyperglycemia. If *niacin* is prescribed for patients with known or suspected diabetes, blood glucose levels should be monitored at least weekly until proven to be stable. *Niacin* also elevates uric acid levels and may reactivate gout. A history of gout is a relative contraindication for *niacin* use. Rarer reversible side effects include toxic amblyopia and toxic maculopathy. Atrial tachyarrhythmias and atrial fibrillation have been reported, more commonly in elderly patients. Niacin, *at doses used in humans, has been associated with birth defects in experimental animals and should not be taken by pregnant women*.

Concurrent use of *niacin* and a statin can cause myopathy. Two randomized trials evaluating *niacin* as add-on therapy to a statin versus statin monotherapy demonstrated no further reduction in ASCVD risk, despite improved lipoprotein parameters. Given this evidence, the FDA removed the indication for *niacin* use in addition to statin therapy and withdrew approval for statin combination formulations containing *niacin* (FDA, 2016). *Niacin* could still be considered as monotherapy in a statin-intolerant patient.

Fibric Acid Derivatives

Clofibrate is a halogenated fibric acid derivative. *Gemfibrozil* is a nonhalogenated acid that is distinct from the halogenated fibrates. Several other fibric acid analogues (e.g., *fenofibrate*, *bezafibrate, ciprofibrate*) are used in Europe and elsewhere.

Mechanism of Action

The mechanisms by which fibrates lower lipoprotein levels, or raise HDL levels, remain unclear. Many of the effects of these compounds on blood lipids are mediated by their interaction with peroxisome proliferator-activated receptors (PPARs), which regulate gene transcription. Fibrates bind to PPARα and reduce triglycerides through PPARα-mediated stimulation of fatty acid oxidation, increased LPL synthesis, and reduced expression of apo C-III. Increased LPL synthesis enhances the clearance of triglyceride-rich lipoproteins. Reduced hepatic production of apo C-III, which serves as an inhibitor of lipolysis and receptor-mediated clearance, enhances the clearance of VLDLs. Fibrate-mediated increases in HDL are due to PPARα stimulation of apo A-I and apo A-II expression, which increases HDL-C levels. *Fenofibrate* is more effective than *gemfibrozil* at increasing HDL-C levels. Most fibrates have potential antithrombotic effects, including inhibition of coagulation and enhancement of fibrinolysis.

ADME

Fibrates are absorbed rapidly and efficiently (>90%) when given with a meal but less efficiently when taken on an empty stomach. Peak plasma concentrations are attained within 1 to 4 h. More than 95% of these drugs in plasma are bound to protein, nearly exclusively to albumin. The $t_{1/2}$ of fibrates range from 1.1 h (*gemfibrozil*) to 20 h (*fenofibrate*). The drugs are widely distributed throughout the body, and concentrations in liver, kidney, and intestine exceed the plasma level. *Gemfibrozil* is transferred across the placenta. The fibrate drugs are excreted predominantly as glucuronide conjugates (60%–90%) in the urine, with smaller amounts appearing in the feces. Excretion of these drugs is impaired in renal failure.

Therapeutic Effects

Effects of fibric acid agents on lipoprotein levels differ widely, depending on the starting lipoprotein profile, the presence or absence of a genetic hyperlipoproteinemia, the associated environmental influences, and the specific fibrate used. Patients with type III hyperlipoproteinemia (*dysbetalipoproteinemia*) are among the most sensitive responders to fibrates. Elevated triglyceride and cholesterol levels are dramatically lowered, and tuberoeruptive and palmar xanthomas may regress completely. Angina and intermittent claudication also improve.

In patients with mild hypertriglyceridemia (e.g., triglycerides <400 mg/dL), fibrate treatment decreases triglyceride levels by up to 50% and increases HDL-C concentrations by about 15%; LDL-C levels may be unchanged or increase. Normotriglyceridemic patients with HeFH usually experience little change in LDL-C levels with *gemfibrozil*; with the other fibric acid agents, reductions as great as 20% may occur in some patients. Fibrates usually are the drugs of choice for treating severe hypertriglyceridemia and the chylomicronemia syndrome. While the primary therapy is to remove alcohol and lower dietary fat intake as much as possible, fibrates assist by increasing triglyceride clearance and decreasing hepatic triglyceride synthesis. In patients with chylomicronemia syndrome, fibrate maintenance therapy and a low-fat diet keep triglyceride levels well below 1000 mg/dL and thus prevent episodes of pancreatitis.

Preparations and Use

Gemfibrozil usually is administered as a 600-mg dose taken twice daily, 30 min before the morning and evening meals. *Fenofibrate* is available in tablets of 48 and 145 mg or capsules containing 67, 134, or 200 mg. The choline salt of fenofibric acid is available in capsules of 45 and 135 mg.

Equivalent doses of *fenofibrate* formulations are 135 mg of choline salt, 145 mg of *fenofibrate*, and 200 mg of micronized *fenofibrate* (available in capsule form). Fibrates are the drugs of choice for treating hyperlipidemic subjects with type III hyperlipoproteinemia, as well as subjects with severe hypertriglyceridemia (triglycerides >1000 mg/dL) who are at risk for pancreatitis. A randomized clinical trial of *fenofibrate* added on to background statin therapy resulted in no further reduction of ASCVD risk (ACCORD Study Group, 2010). In 2016, the FDA withdrew approval for use of *fenofibrate* in addition to statin therapy for ASCVD risk reduction.

Adverse Effects and Drug Interactions

Fibric acid compounds usually are well tolerated. GI side effects occur in up to 5% of patients. Infrequent side effects include rash, urticaria, hair loss, myalgias, fatigue, headache, impotence, and anemia. Minor increases in liver transaminases and alkaline phosphatase have been reported. *Clofibrate*, *bezafibrate*, and *fenofibrate* reportedly potentiate the action of *warfarin*. Careful monitoring of the prothrombin time and reduction in dosage of *warfarin* may be appropriate.

A myopathy syndrome occasionally occurs in subjects taking *clofibrate*, *gemfibrozil*, or *fenofibrate* and may occur in up to 5% of patients treated with a combination of *gemfibrozil* and higher doses of statins. *Gemfibrozil* inhibits hepatic uptake of statins by OATP1B1 and competes for the same glucuronosyl transferases that metabolize most statins. Thus, levels of both drugs may be elevated when they are coadministered. Patients taking this combination should be followed at 3-month intervals with careful history and determination of creatine kinase values until a stable pattern is established. Patients taking fibrates with *rosuvastatin* should be followed especially closely even if low doses (5–10 mg) of *rosuvastatin* are employed. *Fenofibrate* is glucuronidated by enzymes that are not involved in statin glucuronidation; thus, *fenofibrate*-statin combinations are less likely to cause myopathy than combination therapy with *gemfibrozil* and statins.

All fibrates increase the lithogenicity of bile. *Clofibrate* use has been associated with increased risk of gallstone formation. Renal failure is a relative contraindication to the use of fibric acid agents, as is hepatic dysfunction. *Fibrates should not be used by children or pregnant women*.

Omega-3 Fatty Acid Ethyl Esters

Mechanism of Action

Omega-3 fatty acids, commonly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) ethyl esters, reduce VLDL triglycerides and are used as an adjunct to diet for treatment of adult patients with severe hypertriglyceridemia. The recommended daily oral dose for patients with severe hypertriglyceridemia is 3 to 4 g/day administered with food. *Icosapent ethyl*, a highly purified ethyl ester of EPA available in 1- and 0.5-g capsules, is FDA approved as adjunctive therapy for patients with hypertriglyceridemia. Daily oral dosing for adults is 4 g/day, dosed twice daily with food.

ADME

The small intestine absorbs EPA and DHA, which are mainly oxidized in the liver, similar to fatty acids derived from dietary sources. The $t_{1/2}$ of elimination is approximately 50 to 80 h.

Therapeutic Effects

Fish oil or other products containing omega-3 fatty acids are among the most common over-the-counter (OTC) herbal, vitamin, or nutritional supplements purchased by consumers each year. Doses and formulations of OTC items vary considerably. The AHA recommends that consumers eat a variety of fish at least twice a week and that fish oil supplements should only be considered for individuals with heart disease or high triglyceride levels in consultation with a medical professional. In addition to OTC fish oil products, several prescription-only products are available, generally at higher doses than those used OTC (1–1.2 g) and containing a combination of EPA and DHA. *Icosapent ethyl* does not contain DHA. Mixtures containing both EPA and DHA have increased LDL-C in patients with severe hypertriglyceridemia, whereas studies of EPA-only products suggest they may not significantly increase LDL-C while still reducing triglycerides. Controversy exists about when to treat hypertriglyceridemia. Modifiable secondary causes of high triglycerides such as uncontrolled diabetes and excessive alcohol intake should always be addressed prior to initiating therapy. While prescription omega-3 products generally have FDA indications for triglycerides of 500 mg/dL or greater, many professional organizations advocate that such products be limited to patients with levels of 1000 mg/dL or greater who are at greatest risk for pancreatitis. The ORIGIN trial found no additional reduction in ASCVD risk associated with the use of omega-3 fatty acids versus background therapy with statins alone, calling into question the common use of fish oil supplements for "heart protection" by consumers.

However, in 2019, the REDUCE-IT trial showed that patients with hypertriglyceridemia taking 4 g/day of *icosapent ethyl* experienced a decrease in cardiovascular events, including cardiovascular death, compared to patients on placebo despite the use of statins (Bhatt et al., 2019). This has brought up specific questions regarding the place of *icosapent ethyl* in therapy. Only the ADA has provided updated recommendations regarding its use. More specifically, they recommend its use in patients with diabetes and ASCVD or other cardiovascular disease risk factors who are on a statin, have well-controlled LDL-C, but still have elevated triglyceride levels between 135 and 499 mg/dL (ADA, 2021).

Adverse Effects and Drug Interactions

Adverse effects may include arthralgia, nausea, fishy burps, dyspepsia, and increased LDL-C levels. Because omega-3 fatty acids may prolong bleeding time, patients taking anticoagulants should be monitored. *Icosapent ethyl* may also increase the risk of atrial fibrillation and flutter.

PCSK9 Inhibitors

Mechanism of Action

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a protease that binds to the LDL receptor on the surface of hepatocytes and enhances lysosomal degradation of the LDL receptor, resulting in higher plasma LDL-C concentrations. Loss-of-function mutations of PCSK9 are associated with reduced LDL-C and lowered risk of ASCVD. Conversely, mutations leading to increased PCSK9 expression result in increased LDL-C levels and higher risk of ASCVD events.

Two PCSK9 inhibitors, *alirocumab* and *evolocumab*, antibodies to PCSK9, are FDA-approved (1) to lower risk of myocardial infarction, stroke, and unstable angina requiring hospitalization in patients with established cardiovascular disease, (2) to lower LDL-C as adjunctive therapy alone or in combination with other LDL-C–lowering medication in adult patients with HeFH, and (3) to lower LDL-C as adjunctive therapy in combination with other LDL-C–lowering medications in adult patients with HoFH. *Evolocumab* and *alirocumab* are fully humanized monoclonal antibodies that bind free PCSK9, thereby interfering with its binding to the LDL receptor, leading to reduced degradation of the LDL receptor and increased liver clearance of LDL from the circulation, thereby lowering serum LDL-C levels (Figure 37–5).

In 2021, the FDA approved a novel small interfering ribonucleic acid therapeutic, *inclisiran*, that targets PCSK9 mRNA and thus blocks PCSK9 protein synthesis (Wright et al., 2021). Pooled ORION trial data from three ORION phase II trials indicate *inclisiran* may be more effective in lowering LDL-C levels than maximally tolerated statin therapy in high-risk patients. Low-volume subcutaneous injections of *inclisiran* resulted in persistent reductions in LDL-C and other atherogenic lipids for more than 180 days, indicating that a biannual subcutaneous dosing regimen may be a viable dosing regimen and that once-a-year dosing might also be effective in lowering LDL-C levels. Common adverse effects of *inclisiran* include injection site reactions (8.2% vs. 1.8% in placebo) and bronchitis (4.3% vs. 2.7% in placebo).

ADME

The PCSK9 antibody inhibitors are administered as subcutaneous injections either every 2 weeks or once monthly, depending on the dose and indication. *Evolocumab* is administered as a 140-mg injection every 2 weeks or as a 420-mg injection once a month. Similarly, *alirocumab* is administered as a 75-mg injection every 2 weeks or as a 300-mg injection once a month. For the treatment of HoFH, *evolocumab* 420 mg is administered

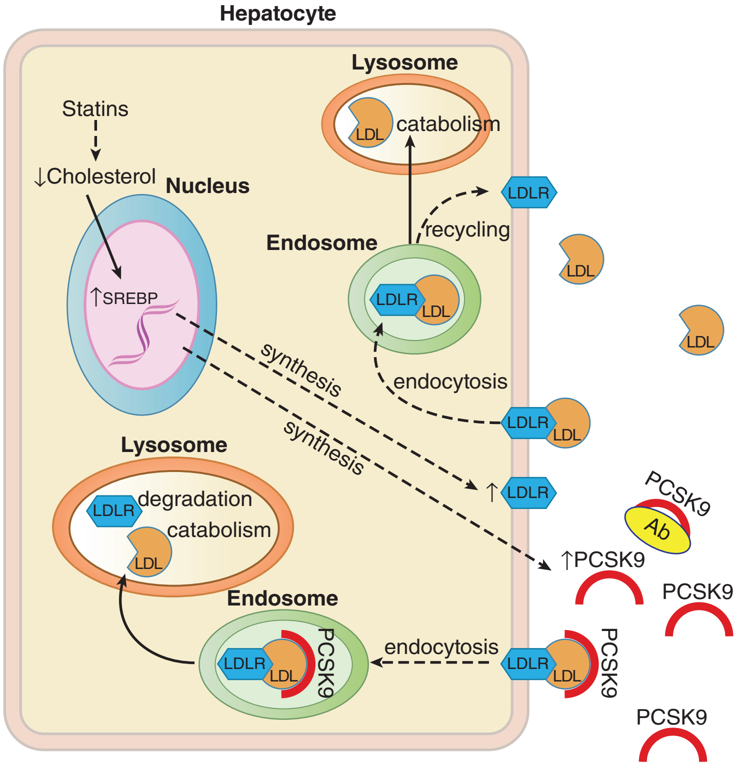


Figure 37–5 *Regulation of LDL receptor synthesis, recycling, and degradation in hepatocytes.* Statins inhibit the synthesis of cholesterol in the hepatocyte. Decreased concentrations of hepatocyte cholesterol lead to activation of sterol regulatory element–binding protein, which upregulates the transcription of hepatocyte genes including *LDLR* and *PCSK9*. This increases the synthesis of LDL receptor (LDLR) and PCSK9. Increased density of LDLR on the hepatocyte membrane increases binding of circulating LDL particles, and the LDLR-LDL complex then enters the hepatocyte by endocytosis. The LDLR-LDL complex dissociates in the endosome where LDLR is recycled to the plasma membrane and LDL enters the lysosomal pathway, leading to catabolism of the LDL particle. In the presence of circulating PCSK9, the LDLR-LDL-PCSK9 complex is removed from the plasma membrane by endocytosis and enters the lysosomal pathway for degradation. Little or no LDLR is recycled to the hepatocyte membrane, thus reducing the ability of the hepatocyte to bind LDL. There are gain-of-function mutations in *PCSK9* that enhance its activity and lead to FH (see text). Antibodies (Ab) to PCSK9 block its ability to bind the LDLR-LDL complex, thus preventing degradation of LDLR. *Inclirisan*, a small interfering RNA, acts by blocking the synthesis of PCSK9.

once monthly or every 2 weeks, whereas *alirocumab* 150 mg is administered once every 2 weeks. Administration requirements and storage of these medications are barriers when compared with the ease of oral dosage forms of other medications. *Inclisiran* is indicated as an adjunct to diet and maximally tolerated statin therapy in adults with HeFH or clinical ASCVD who require additional reduction in LDL-C. The recommended dosage is 284 mg in 1.5 mL administered as a single subcutaneous injection initially, then after 3 months, and every 6 months thereafter. *Inclisiran* can be stored at room temperature.

LDL-C plasma levels may be measured 4 to 8 weeks after initiating antibody therapy or changing doses. These antibodies inhibit PCSK9 availability for 2 to 3 weeks after administration ($t_{1/2}$ is 11–20 days), after which LDL-C levels begin to rise. Following an initial dose and 3 month dosing, inclirisan reduced serum PCSK9 levels by approximately 75% and 69% at 4 months and 6 months, respectively. After a single dose of inclirisan, reduction in LDL-C was apparent at 14 days and remained reduced by approximately 53% LDL-C at 6 months. Limited data are available in individuals with severe renal or hepatic impairment, although dose adjustments of the antibodies are not necessary in patients with mild impairment No dosage adjustments of *inclirisan* are required in patients with mild to severe renal impairment or mild to moderate liver impairment. *PCKS9 inhibitors should not be used in pregnancy because transmission across the placenta is expected. It is not known to what degree the medications will be present in breast milk, so use during lactation is also not recommended.*

Therapeutic Effects

The effects of PCSK9 inhibitors are complementary to those of statins. While statins interfere with cholesterol production and stimulate the production of LDL receptors, PCSK9 inhibitors enable more LDL receptors to be available on the surface of liver cells. PCSK9 antibody inhibitors reduce LDL-C in a dose-dependent manner by as much as 70% when used as monotherapy or by as much as 60% in patients already on statin therapy. Indications and approvals of these agents vary between countries. Currently, PCSK9 inhibitors are not FDA-approved for treatment of dyslipidemias in statin-intolerant patients without known ASCVD, although they are being used in this population elsewhere. Among patients with known ASCVD and LDL-C of 70 mg/dL or greater despite treatment with moderate- to high-intensity statins, the addition of *evolocumab* further reduced the risk of ASCVD events, but not death, in the FOURIER trial (Sabatine et al., 2017). Similar results were seen for *alirocumab* in the ODYSSEY OUTCOMES trial (Schwartz et al., 2018). Given the high cost of treatment with PCSK9 inhibitors versus relatively inexpensive statin treatment, published cost-effectiveness studies have concluded that these medications are only cost-effective in very high-risk patient populations (Fonarow et al., 2019; Grundy et al., 2019). Currently, because of these analyses, treatment with maximally tolerated doses of statins and/or *ezetimibe* is recommended prior to initiating PCSK9 inhibitors.

Adverse Effects and Drug Interactions

Several clinical trials have identified a small (<1%) risk of neurocognitive effects in patients treated with PCSK9 antibody inhibitors compared to placebo. Additional studies are under way to better understand the long-term neurocognitive effects of these medications, if any. Unlike other medications used to treat dyslipidemias, PCSK9 inhibitors do not appear to substantially increase the risk of myopathies when used as monotherapy or in combination with statins. Similar to other monoclonal antibodies, risk of infections, including nasopharyngitis, urinary tract infections, or upper respiratory infections, is slightly increased. Injection site reactions are the most frequent adverse effect of the antibodies and *inclisiran*, although these occur in less than 10% of patients. There are no known drug interactions with PCSK9 inhibitors.

Inhibitor of Microsomal Triglyceride Transfer

Lomitapide

Mechanism of Action. *Lomitapide mesylate* is the first drug that acts by inhibiting MTP, which is essential for the intracellular transfer of triglycerides into triglyceride-rich lipoproteins, thus inhibiting the formation of VLDLs in hepatocytes and chylomicrons in intestinal epithelial cells.

ADME. *Lomitapide* is administered with water and without food (or at least 2 h after the evening meal) because administration with food may increase risk of GI adverse effects. The drug is metabolized by CYP3A4 and is contraindicated with inhibitors of CYP3A4.

Therapeutic Effects. *Lomitapide* is FDA-approved as an adjunct to diet for lowering LDL-C levels, total cholesterol, apo B, and non–HDL lipoproteins in patients with HoFH. *Lomitapide* reduces LDL-C by up to 50% and should be used in combination with maximally tolerated statin therapy. The recommended starting oral dose (5 mg/day) is titrated upward at 4-week intervals to a maximum dose of 60 mg daily. The long-term cardiovascular effects of *lomitapide* are currently unknown.

Adverse Effects and Drug Interactions. Reported adverse effects include significant diarrhea, vomiting, and abdominal pain in most patients. A strict low-fat diet may improve tolerability. Serious concerns also exist regarding hepatotoxicity and liver steatosis. In clinical trials, a third of patients experienced elevations in ALT or aspartate aminotransferase greater than three times the upper limit of normal. *Lomitapide* also increases hepatic fat, with or without concomitant increases in transaminases. The agent is used under an FDA Risk Evaluation and Mitigation Strategy due to its concerning side effect profile. *Lomitapide* may be embryotoxic, and women of childbearing potential should have a negative pregnancy test before starting treatment and use effective contraception during treatment.

ATP-Citrate Lyase Inhibitor

Bempedoic Acid

Bempedoic acid, a dicarboxylic acid, is a new class of cholesterol-lowering drugs approved by the FDA in 2020 that act by inhibiting *de novo* hepatocyte cholesterol biosynthesis. Its mechanism of action complements statins and other agents that lower LDL-C. The drug is approved as an adjunct to diet and maximally tolerated statin therapy to further lower LDL-C in patients with HeFH or established ASCVD. A fixed-dose combination of *bempedoic acid* and *ezetimibe* is approved for the same indication. *Bempedoic acid* also has application in patients who are statin-intolerant.

Mechanism of Action. *Bempedoic acid* acts by blocking ACL, the cytoplasmic enzyme that converts citrate to oxaloacetate and acetyl-CoA. In the *de novo* biosynthesis of cholesterol, acetyl-CoA is converted to HMG-CoA, which is the substrate for HMG-CoA reductase, the target of statins (see Figure 37–4). *Bempedoic acid* is a prodrug that is metabolized to the active drug, bempedoic acid-CoA, by a cytosolic hepatocyte enzyme, very long-chain acyl-CoA synthase-1. Bempedoic acid-CoA is a competitive inhibitor of ACL and thereby reduces *de novo* hepatic cholesterol synthesis by reducing the flow of acetyl-CoA to HMG-CoA reductase. *Bempedoic acid*'s cholesterol-lowering effects are thus additive with those of statins.

ADME. *Bempedoic acid* is administered orally once daily (180 mg), as is the combination of *bempedoic acid* and *ezetimibe* (180 mg and 10 mg, respectively). Both the prodrug and active metabolite of *bempedoic acid* are inactivated by UGT2B7 to glucuronide conjugates, which are subsequently excreted in urine (70%) by the renal transporter OAT2. OATB1B1, OAT1B3, OAT2, and OAT3 are weakly inhibited by *bempedoic acid*. The glucuronide metabolite is an OAT3 substrate and weakly inhibits OATB1B1 and OAT1B3. Elimination $t_{1/2}$ is 21 ± 11 h, and steady-state concentrations are reached after 7 days of therapy.

Therapeutic Effects. *Bempedoic acid* is FDA-approved for the treatment of adults with either HeFH or established ASCVD who need additional LDL-C lowering. As with the statins, reduced intrahepatic cholesterol synthesis results in an upregulation of LDL receptors expressed on hepatocyte membranes, which leads to increased uptake and catabolism of LDL particles and a consequent reduction in blood LDL-C concentrations. Monotherapy with *bempedoic acid* results in a 15% to 25% reduction in LDL-C, less than with statins. In combination with *ezetimibe*, the reduction in LDL-C is around 30%, and triple therapy with a statin reduces LDL-C by 65%. Reduction in HDL-C by *bempedoic acid* is typically around 5%.

Adverse Effects and Drug Interactions. Therapy with *bempedoic acid* is associated with hyperuricemia (thought to be due to competition for OAT2 in the kidney) that can increase the risk of gout. Other adverse effects include tendon ruptures and increased serum creatine kinase, creatinine, and hepatic transaminases. Due to drug interactions that may increase the risk of myopathy, *bempedoic acid* is not used with *simvastatin* doses of greater than 20 mg/day or *rosuvastatin* doses of greater than 40 mg/day. Because ACL is an enzyme essential for lipid and cholesterol synthesis, *bempedoic acid* may cause harm to the fetus.

Inhibitor of Angiopoietin-Like Protein 3

Evinacumab-dgnb

Mechanism of Action. *Evinacumab-dgnb* is a recombinant human monoclonal antibody that binds to and inhibits angiopoietin-like protein 3 (ANGPTL3). ANGPTL3 is a member of the angiopoietin-like protein family that is expressed primarily in the liver and plays a role in the regulation of lipid metabolism by inhibiting LPL and endothelial lipase (EL). *Evinacumab-dgnb* inhibition of ANGPTL3 leads to reduction in LDL-C, HDL-C, and triglycerides. *Evinacumab-dgnb* reduces LDL-C independent of the presence of LDL receptor by promoting VLDL processing and clearance upstream of LDL formation. *Evinacumab-dgnb* blockade of ANGPTL3 lowers triglycerides and HDL-C by rescuing LPL and EL activities, respectively.

ADME. The recommended dose is 15 mg/kg administered by intravenous infusion over 60 min every 4 weeks. The exact pathway through which *evinacumab-dgnb* is metabolized has not been characterized. As a human monoclonal IgG4 antibody, *evinacumab-dgnb* is expected to be degraded into small peptides and amino acids via catabolic pathways in the same manner as endogenous immunoglobulin G (IgG). The elimination $t_{1/2}$ is a function of serum *evinacumab-dgnb* concentrations and is not a constant. Based on a population pharmacokinetic analysis, the median time for serum *evinacumab-dgnb* concentrations to decrease below the lower limit of quantitation is 19 weeks after the last steady-state dose.

Therapeutic Effects. In 2021, *evinacumab* was approved by the FDA as an addition to lipid-lowering medications and diet for patients with HoFH. Its safety and effectiveness have not been established in patients with other forms of hypercholesterolemia, including HeFH. In a randomized clinical trial of 65 HoFH patients who were on a background of other lipid-lowering therapies, including maximally tolerated statins, *ezetimibe*, PCSK9 inhibitor antibodies, *lomitapide*, and lipoprotein apheresis, *evinacumab* reduced LDL-C on average by 50% after 24 weeks.

Adverse Effects and Drug Interactions. Major adverse effects include serious hypersensitivity reactions and embryo-fetal toxicity. As such, *evinacumab* is contraindicated in pregnancy and in patients with a history of allergies to *evinacumab*. Common adverse reactions (≥5%) were nasopharyngitis, influenza-like illness, dizziness, rhinorrhea, and nausea. Drug interactions have not been documented.

Future Developments in Management of Dyslipidemias

FH is the most common inherited cardiovascular disease and is associated with significant morbidity and reduction in life expectancy. It has been appreciated for many years that inherited mutations in specific genes important for normal LDL receptor function and catabolism give rise to FH, including loss-of-function mutations in the *LDLR* gene and gain-of-function mutations in the *PCSK9* gene. An understanding of the pathophysiological sequalae of these mutations has provided valuable insights that have resulted in the invention of drugs that target key pathogenic steps and thus restore, at least in part, normal levels of LDL-C and significantly reduce the cardiovascular risk of individuals carrying these mutated genes. In addition to these monogenic causes of FH, evidence from large-scale whole-exome sequence studies has demonstrated that FH and the associated cardiovascular risks can also be caused by the aggregate effects of many small-effect alleles and that it may be possible to develop polygenic risk scores that can predict cardiovascular risk well before clinical phenotypes such as aberrant LDL-C levels are measurable. By initiating appropriate primary prevention therapy based on genetic analysis at an early age in patients with FH, there is hope that these patients could significantly reduce their risk of premature atherosclerotic disease. Moreover, the use of cascade screening to identify family members at risk of atherosclerotic disease could increase the potential benefits of genetic testing substantially. Analysis of atherosclerotic disease risk contributed by specific genes or combinations of genes in specific patient populations might identify novel drug targets that would not otherwise be discovered. Current challenges to development of polygenic risk scores based on whole-exome sequencing include wide-scale adoption by clinicians and payers, cost of whole-exome sequencing, privacy of results, development of accurate risk algorithms for genetically diverse populations, and appropriate application of risk scores by clinicians for optimal therapeutic management. Near-term benefits of polygenic risk analyses might include offset of sequencing costs by reducing inappropriate use of expensive monoclonal antibody therapies and by rapid optimization of therapy in extremely high-risk patients.

Drug Facts for Your Personal Formulary: *Therapy for Dyslipidemias*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
HMG-CoA Reductase Inhibitors (Statins)		
Atorvastatin Fluvastatin Lovastatin Pitavastatin Rosuvastatin Pravastatin Simvastatin	• The most effective and best-tolerated agents to treat dyslipidemias, especially elevated LDL-C	• Contraindicated in pregnancy • Hepatotoxicity (one case per million person-years of use); measure liver enzymes (ALT) at baseline and thereafter only when clinically indicated • Myopathy and rhabdomyolysis (one death per million prescriptions) (30-day supply); risk ↑ with dose and concomitant administration of drugs that interfere with statin catabolism or hepatic uptake
Cholesterol Absorption Inhibitor		
Ezetimibe	• Monotherapy in patients with ↑ LDL-C who are statin intolerant • Combination with statin or bempedoic acid → additive reductions in LDL-C	• Bile acid sequestrants inhibit absorption of ezetimibe; avoid concurrent use • Generally, a well-tolerated agent, but rhabdomyolysis has been reported with monotherapy and with the addition of agents known to be associated with increased risk (e.g., fibrates) • Combination therapy contraindicated in pregnancy
Bile Acid–Binding Resins (Bile Acid Sequestrants)		
Cholestyramine Colestipol Colesevelam	• Probably safest lipid-lowering drugs (not absorbed systemically) • Recommended for patients 11–20 years of age	• Common GI side effects: bloating, dyspepsia, constipation • Cholestyramine and colestipol bind and interfere with absorption of many drugs; administer all other drugs either 1 h before or 3–4 h after dose of a bile acid resin • Severe hypertriglyceridemia is a contraindication to the use of cholestyramine and colestipol; they ↑ triglyceride levels
Nicotinic Acid		
Niacin	• Favorably affects all lipid parameters; most effective agent for increasing HDL-C; also lowers triglycerides and reduces LDL-C	• Contraindicated in pregnancy, peptic ulcer disease, concurrent use of statins, gout • Flushing, pruritus, and dyspepsia limit patient compliance • Rarer episodes of nausea, vomiting, and diarrhea • Hepatotoxicity, manifested as ↑ serum transaminases • Hyperglycemia and niacin-induced insulin resistance; in patients with known or suspected diabetes, blood glucose levels should be monitored at least weekly until stable • Hepatotoxicity is more likely to occur with the use of ER forms of nicotinic acid (vs. IR form)
Fibric Acid Derivatives (Fibrates)		
Gemfibrozil Fenofibrate *Not in the U.S.:* Ciprofibrate Bezafibrate	• Usual drugs of choice for treating chylomicronemia, hyperlipidemia with type III hyperlipoproteinemia, severe hypertriglyceridemia (triglycerides >1000 mg/dL)	• GI side effects occur in up to 5% of patients • Contraindicated in children and pregnancy • A myopathy syndrome may occur in patients taking clofibrate, gemfibrozil, or fenofibrate • The FDA has withdrawn approval for coadministration of fibrates with statins • Renal failure, gallbladder disease, and hepatic dysfunction are relative contraindications

Drug Facts for Your Personal Formulary: *Therapy for Dyslipidemias (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Omega-3 Fatty Acid Ethyl Esters		

Omega-3 fatty acids (EPA, DHA, and icosapent ethyl)	• Adjunct for treating severe hypertriglyceridemia (triglycerides >1000 mg/dL)
	• *Icosapent ethyl*
	  • Adjunct to maximally tolerated statin therapy to reduce risk of cardiovascular events in adults with triglyceride levels ≥150 mg/dL
	  • Adjunct to diet in adults with severe hypertriglyceridemia (triglycerides ≥500 mg/dL)
		• Adverse effects may include arthralgia, nausea, fishy burps, dyspepsia, and increased LDL
		• Since omega-3 fatty acids may prolong bleeding time, patients taking anticoagulants should be monitored

PCSK9 Inhibitors (mAbs)		
Alirocumab Evolocumab	• To ↓risk of myocardial infarction, stroke and unstable angina requiring hospitalization in patients with established cardiovascular disease • To ↓LDL-C as adjunctive therapy alone or in combination with other LDL-C-lowering medication in adults with HeFH • To ↓LDL-C as adjunctive therapy in combination with other LDL-C-lowering medications in adult with HoFH	• Hypersensitivity or injection site reactions are possible • Most effective agents at reducing LDL-C • As with other mAbs, influenza-like symptoms, nasopharyngitis, upper respiratory infections may occur • Used in addition to maximally tolerated statin doses (complementary mechanism; see Figure 37–5) • Diabetes mellitus is associated with evolocumab (9%); large LDL-C reductions (to <25 mg/dL) are associated with alirocumab
PCSK9 Inhibitor (siRNA)		
Inclisiran	• To ↓LDL-C as adjunctive therapy with diet and maximally tolerated statin therapy for treatment of adults with HeFH who require additional lowering of LDL-C • To ↓LDL-C as adjunctive therapy with diet and maximally tolerated statin therapy for treatment of adults with clinical ASCVD who require additional lowering of LDL-C	• Injection site reactions are possible • Used in addition to maximally tolerated statin doses (complementary mechanism) • Long-lasting effects on lowering PCSK9 serum levels and LDL-C (dosing every 6 months at steady-sate) • Contraindicated in pregnancy
Liver Microsomal Triglyceride Transfer Protein Inhibitor		
Lomitapide	• Used as an adjunct to diet for lowering LDL-C, total cholesterol, apo B, and non–HDL-C in patients with HoFH	• In patients with HoFH, LDL-C reduction by 40%–50% • Adverse effects include GI symptoms, elevation of serum liver enzymes, and increased liver fat • The agent is used under an FDA Risk Evaluation and Mitigation Strategy due to hepatotoxicity (i.e., elevated transaminases and hepatic steatosis) • Patients should take daily supplements containing 400 IU vitamin E, 200 mg linoleic acid, 210 mg α-linoleic acid, 110 mg EPA, and 80 mg DHA
ATP-Citrate Lyase Inhibitor		
Bempedoic acid	• Used in patients who are statin intolerant or those who do not achieve desired LDL-C levels with statins • Monotherapy in statin-intolerant patients • Combination therapy with statins and fixed-dose combination with ezetimibe	• Low rates of adverse effects in clinical trials • May increase blood uric acid levels, leading to risk of gout
Angiopoietin-Like Protein 3 Inhibitor (Monoclonal Antibody)		
Evinacumab-dgnb	• Used as an adjunct to lipid-lowering agents and diet in patients with HoFH	• In patients with HoFH on lipid-lowering agents, LDL-C reduction by 50% • Severe hypersensitive reactions possible; contraindicated in pregnancy

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Section IV

Inflammation, Immunomodulation, and Hematopoiesis

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Chapter 38

Introduction to Immunity and Inflammation

Michael David

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- Cells of the Innate Immune System
- Cells of the Adaptive Immune System
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A NOTE ON NOMENCLATURE

ENGINEERED (RECOMBINANT) ANTIBODIES

The introduction of pathogens and foreign proteins into the human body can stimulate immune recognition, leading to inflammatory and allergic responses. Aspects of these responses are subject to pharmacological modulation. Before describing the actions of pharmacological agents affecting allergy and immunity, this chapter describes the cellular and molecular basis of immune and allergic responses and the points of pharmacological intervention. Subsequent chapters in this section cover in detail the classes of agents that can alter allergic and immune responses, as well as the biology and pharmacology of inflammation.

Cells and Organs of the Immune System

Hematopoiesis

All blood cells, including immune cells, originate from pluripotent hematopoietic stem cells (HSCs) of the bone marrow. HSCs are a population of undifferentiated progenitor cells that are capable of self-renewal. On exposure to cytokines and contact with the surrounding stromal cells, HSCs can differentiate into megakaryocytes (the source of platelets), erythrocytes (red blood cells), and leukocytes (white blood cells). This process is known as hematopoiesis (Figure 38–1).

The HSC pool can be divided in two populations: long-term (LT) and short-term (ST) HSCs. LT-HSCs are capable of lifelong self-renewal, allowing for continuous hematopoiesis throughout life. ST-HSCs have limited self-renewing capability and differentiate into multipotent progenitors—the common *myeloid* progenitor and the common *lymphoid* progenitor. The common myeloid progenitor gives rise to the myeloid lineage of cells that includes megakaryocytes, erythrocytes; granulocytes (neutrophils, eosinophils, basophils, mast cells), monocytes, macrophages, and dendritic cells (DCs). In contrast, the common lymphoid progenitor gives rise to the lymphoid lineage of cells that includes natural killer (NK) cells, B lymphocytes (B cells), and T lymphocytes (T cells) (Doulatov et al., 2012; Eaves, 2015).

Cells of the Innate Immune System

Innate immunity refers to the host defense mechanisms that are immediately available on exposure to pathogens.

Granulocytes

Granulocytes have characteristic cytoplasmic granules containing substances that, in addition to killing invading pathogens, enhance inflammation at the site of infection or injury. Neutrophils are the most abundant of the granulocytes and are generally the first cells to arrive at the site of injury. They are specialized at engulfing and killing pathogens—a process known as phagocytosis. Like neutrophils, eosinophils are also motile phagocytic cells. These cells defend against parasitic organisms such as helminths by releasing the contents of their granules, which are thought to damage the parasite membrane. Basophils and mast cells have granules that contain histamine and other pharmacologically active substances. In addition to their protective function, these cells can become dysregulated during the generation of allergic responses, in which they play an important role (see Hypersensitivity Reactions).

Mononuclear Phagocytes

Mononuclear phagocytes consist of monocytes and macrophages. Monocytes circulate in the blood and then migrate into tissues where they differentiate into macrophages, increase 5- to 10-fold in size, and acquire enhanced phagocytic and microbicidal activity. Macrophages engulf and eliminate pathogens, dead cells, and cellular debris. Macrophages can remain motile and travel throughout the tissues by amoeboid movements, and they can also take up residence in specific tissues, becoming tissue-resident macrophages. In addition to their role as phagocytes, macrophages release proinflammatory molecules, such as cytokines and eicosanoids, that recruit other immune cells to the site of infection (see Inflammation).

Natural Killer Cells

Natural killer cells (NK cells) are cytotoxic, granular lymphocytes that target tumor and virus-infected cells. NK cell receptors selectively target

Abbreviations

APC: antigen-presenting cell
BCR: B-cell receptor
C#: complement component # (e.g., C3, C5)
CAR: chimeric antigen receptor
CLL: chronic lymphocytic leukemia
CR1: complement receptor 1
CTLA-4: cytotoxic T-lymphocyte–associated protein 4
DC: dendritic cell
DN: double negative
DP: double positive
ER: endoplasmic reticulum
GI: gastrointestinal
HLA: human leukocyte antigen
HSC: hematopoietic stem cell
ICI: immune checkpoint inhibitor
IFN: interferon
Ig: immunoglobulin
IL: interleukin
iNOS: inducible nitric oxide synthase: NOS2
IRF: interferon regulatory factor
ISG: interferon-stimulated gene
ISRE: interferon-stimulated response element
LT: long term
MADCAM-1: mucosal vascular addressin cell adhesion molecule 1
MALT: mucosa-associated lymphoid tissue
MHC: major histocompatibility complex
NF-κB: nuclear factor-κB
NK cell: natural killer cell
NO: nitric oxide
PAMP: pathogen-associated molecular pattern
PD-1: programmed cell death protein 1
PRR: pattern recognition receptor
Rh: rhesus
ROS: reactive oxygen species
SARS-CoV-2: severe acute respiratory syndrome coronavirus 2
scFv: single-chain variable fragment
ST: short term
T_C: cytotoxic T cell
TCR: T-cell receptor
T_H: helper T cell
TLR: toll-like receptor
TNFα: tumor necrosis factor α
T_{Reg}: T-regulatory cells

damaged or infected host cells by recognizing abnormal expression of surface molecules seen on damaged, but not healthy, cells.

Dendritic Cells

Dendritic cells (DCs) are specialized cells that reside in tissues and stimulate adaptive immune responses. Immature DCs patrol peripheral tissues and sample their environment for infection by capturing pathogens through phagocytosis, receptor-mediated endocytosis, and pinocytosis. After maturation, DCs shift from a phenotype that promotes antigen capture to one that supports antigen presentation. Mature DCs migrate from the peripheral tissues to lymphoid organs and present antigens to activate helper and cytotoxic T cells (see Antigen Processing and Presentation).

Cells of the Adaptive Immune System

Adaptive immunity (also known as the acquired immune system) represents a branch of the immune system that is characterized by antigen specificity and immunological memory. It is mediated by B and T lymphocytes following exposure to specific antigens and is more complex than innate immunity in that it requires prior antigen processing and recognition to launch lymphocyte responses. Furthermore, in contrast to innate immune responses, which occur within hours after infection, B- and T-lymphocyte responses take days to develop.

B Cells

The B lymphocytes, also known as B cells, express cell surface pathogen receptors called immunoglobulins. When a naïve B cell (one that has not previously encountered antigen) detects a pathogen through binding of its immunoglobulin, it begins to proliferate. Its progeny can differentiate into plasma cells or memory B cells. Plasma cells are short-lived effector cells that specialize in secreting antibodies—the soluble form of immunoglobulins. Memory B cells are long-lived and persist for years following an infection. Because memory B cells express the same immunoglobulin as their parent B cell, they mount an enhanced secondary response to a pathogen on reinfection and are the basis for B cell–mediated immunity.

T Cells

The T lymphocytes, also known as T cells, express cell surface pathogen receptors called T-cell receptors (TCRs). Unlike immunoglobulins, which independently recognize antigens, TCRs only recognize antigens presented on MHC (major histocompatibility complex) molecules on the surface of DCs or other antigen-presenting cells (APCs). T cells are divided into two subpopulations—T_C (cytotoxic T) cells and T_H (helper T) cells. T_C cells or killer T cells destroy host cells that are infected with intracellular pathogens, whereas T_H cells secrete cytokines that help enhance the function of other immune cells to mediate pathogen clearance. Activated T cells can differentiate into effector cells—cells that carry out immediate functions to help clear the infection—or memory cells. Memory T cells, like memory B cells, persist for years following an infection and mount an enhanced response on re-exposure to the same pathogen (see Immunological Memory).

Organs of the Immune System

The organs of the immune system are divided into two categories based on their function: *primary lymphoid organs* and *secondary lymphoid organs*. Lymphocyte maturation and development take place in the primary lymphoid organs, whereas secondary lymphoid organs provide sites for mature lymphocytes to interact with APCs. These lymphoid organs are interconnected by blood and lymphatic vessels.

Primary Lymphoid Organs

The bone marrow and thymus make up the primary lymphoid organs. Both B-cell and T-cell precursors originate in the bone marrow from HSCs. B cells complete their maturation in the bone marrow, whereas T-cell precursors migrate to the thymus to complete their development.

The bone marrow tissue is composed of a meshwork of stromal cells (e.g., endothelial cells, adipocytes, fibroblasts, osteoclasts, osteoblasts, and macrophages). Immature B cells proliferate and differentiate within the bone marrow with direct (cell-cell contact) and indirect (cytokine release) help from stromal cells. Interleukin (IL)-1, IL-6, and IL-7 are the most important cytokines guiding the B-cell differentiation process (Hoggatt et al., 2016).

The thymus is a bilobate organ of the upper anterior mediastinum, sitting behind the sternum and in front of the heart. Each lobe is divided into smaller lobules comprising an outer compartment (cortex) and an inner compartment (medulla). Both the cortex and the medulla contain a stromal cell network of epithelial cells, DCs, and macrophages that present self-antigens to maturing T cells. This stromal cell network is responsible for the maturation process, and the cytokines IL-1, IL-2, IL-6, and IL-7 also play an important role in this process. The thymus begins to atrophy after puberty (as the thymic stroma is eventually replaced with adipose tissue), causing a decline in T-cell output. By age 35, T-cell production drops to 20% compared to that of newborn levels, and by age 65, this number further decreases to 2% (Palmer, 2013). Importantly, once the periphery is seeded with mature T cells, the host is equipped with

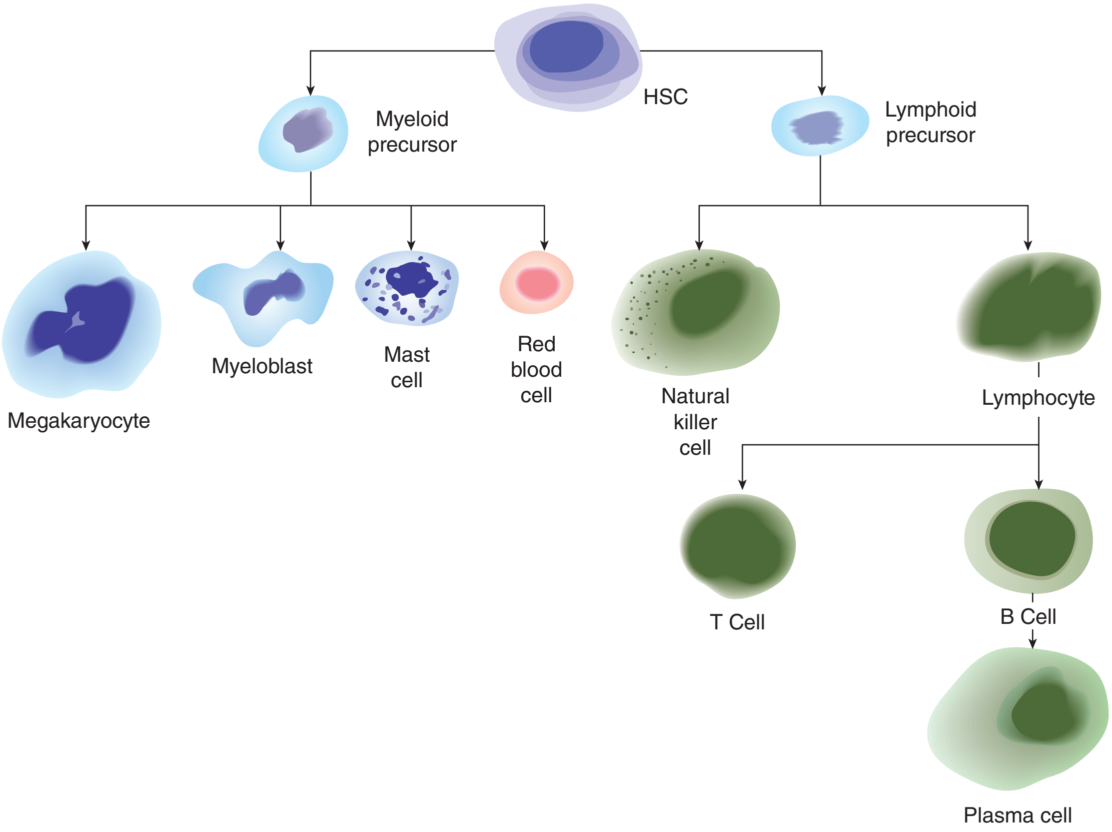

Figure 38–1 *Development of myeloid and lymphoid lineage cells from HSCs in the bone marrow.* HSCs give rise to lineage-specific precursors, which differentiate into all myeloid and lymphoid cells.

a diversity of naïve T cells that will respond to any pathogen encounter, irrespective of diminished thymic output.

Secondary Lymphoid Organs

The secondary lymphoid organs, including the spleen, lymph nodes, and mucosa-associated lymphoid tissue (MALT), are the sites where adaptive immune responses are initiated. The spleen is the largest lymphoid organ, consisting of red pulp and white pulp. The red pulp is a sponge-like tissue where old or damaged erythrocytes are recycled, whereas the white pulp region consists of lymphocytes. The spleen is the only lymphoid organ that is not connected to the lymphatic vessels. Instead, immune cells enter and exit the spleen through blood vessels.

Lymph nodes are round, specialized structures that are positioned along the lymphatic vessels like beads on a chain. They collect the lymph (containing immune cells and antigens) that drains from the skin and internal organs and provide the physical location where antigen presentation and lymphocyte activation occur. The MALTs are loosely organized lymphoid tissues located in the submucosal surfaces of the gastrointestinal (GI) tract, respiratory system, and urinary tract (Neely and Flajnik, 2016).

The Lymphatic System

The "lymphatic system" or "lymphatics" represent a network of lymphatic vessels (similar to the circulatory system's veins and capillaries) that are connected to lymph nodes. Similar to their circulatory counterparts, small lymph capillaries are made up of single endothelial cell layers, whereas in larger lymph vessels, the endothelial cells are surrounded by layers of smooth muscle cells. Additional parts of the lymphatic system are the tonsils, adenoids, spleen, and thymus. The lymphatics collect plasma continuously leaking out from blood vessels into the interstitial spaces and return this fluid, now called lymph, to the blood (after filtration in the lymph nodes) into the subclavian veins located on either side of the neck near the clavicles. Unlike blood movement, which is driven by a pump and flows throughout the body in a continuous loop, lymph flows in only one direction—upward toward the neck—and movement originates from rhythmic contractions of the smooth muscle cells, with directionality achieved via semilunar valves inside the vessels. The lymphatics therefore have an important function in regulating both immune and fluid homeostasis.

The B and T cells, unlike other blood cells, traffic through the body via both blood and lymph (hence the term *lymphocyte*). After completing their development in the primary lymphoid organs, B and T cells enter the bloodstream. When lymphocytes reach blood capillaries that empty into secondary lymphoid tissues, they enter these tissues. If a naïve lymphocyte encounters antigen, it will remain in the secondary lymphoid tissue and become activated. Otherwise, if no antigen is detected, the lymphocyte then exits through the efferent lymph and reenters the bloodstream. This pattern of movement between the blood and lymph is referred to as lymphocyte recirculation, and it allows the lymphocyte population to continuously monitor the secondary lymphoid organs for signs of infection (Masopust and Schenkel, 2013; Thomas et al., 2016).

Cytokines and Cytokine Storm

Cytokines are soluble mediators that initiate, terminate, and modulate the intensity of virtually every aspect of an organism's immune or inflammatory response. Cytokines, which are peptide based, cannot diffuse across lipid bilayers; rather, they act via cell surface receptors in an autocrine or paracrine fashion. While there is no classical definition for cytokines, the term historically encompasses interferons (IFNs), ILs, and chemokines, but does not include growth factors (which are frequently characterized by their enzymatically active receptors) and hormones (which are typically secreted into the bloodstream by cells organized into glands), in contrast to cytokines, which are released by individual, nonassembled cells of the immune system such as lymphocytes or macrophages. A feature once thought to distinguish cytokines from hormones was their differing distances of action: Whereas hormones can act over long distances (e.g., insulin or growth hormone), cytokines were thought to function primarily in microenvironments. However, discoveries over the last few decades have indicated that cytokines, too, can elicit systemic responses, adding to the difficulty of clearly defining cytokines and distinguishing them from hormones. Today, cytokines are often defined and classified based on the structural features of their cell surface receptors, which, in contrast to growth factor receptors, do not harbor intrinsic enzymatic activities but rely on cytoplasmic enzymes to accomplish postreceptor signal transduction.

Lack of specific cytokines or their receptors can result in prominent immune deficiencies; for example, defects in the γ chain of the IL-2 receptor cause severe combined immune deficiency. Conversely, overproduction of (proinflammatory) cytokines can trigger the *cytokine storm syndrome*, which is linked to systemic inflammatory responses and multiorgan failure. Cytokine storm can occur as a consequence of a severe allergic reaction (anaphylactic shock) but is also frequently associated with the excessive tissue damage resulting from responses to various pathogens; for example, many deaths caused by the 1918 influenza pandemic of H1N1 influenza A virus and by the pandemic of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2; the viral cause of the illness COVID-19) (2019–present) have been attributed to cytokine storms elicited by the respective pathogens.

Innate Immunity

Innate immunity refers to the defense mechanisms that are immediately available on exposure to pathogens. These mechanisms consist of anatomical barriers, soluble mediators, and cellular responses. To establish an infection, a pathogen must first penetrate a host's anatomical barriers, including the skin and mucous membranes. If a pathogen manages to breach these anatomical barriers, the cellular innate immune response initiates rapidly, within a matter of minutes, to activate further mechanisms of the immune response.

Anatomical Barriers

The skin and mucosal surfaces form the first line of defense against pathogens. The skin is made up of a thin outer layer (epidermis) of tightly packed epithelial cells and an inner layer (dermis) of connective tissue containing blood vessels, sebaceous glands, and sweat glands. The respiratory, GI, and urogenital tracts are lined by mucous membranes. Like skin, mucous membranes consist of an outer layer of epithelial cells and an underlying layer of connective tissue. These anatomical surfaces act as more than just passive barriers against pathogens. All epithelial surfaces secrete antimicrobial peptides called host defense peptides. Host defense peptides kill bacteria, fungi, and viruses by disrupting their membranes (Hancock et al., 2016). The sebum secreted by the sebaceous glands contains fatty acids and lactic acid that inhibit bacterial growth on the skin. Mucosal surfaces are continuously covered in mucus (a viscous fluid secreted by epithelial cells of mucous membranes) containing antimicrobial substances that trap foreign microorganisms and help limit the spread of infection. In the respiratory tract, this mucous is continually removed by the action of cilia on epithelial cells. In addition, all these anatomical surfaces harbor commensal microorganisms. These commensals help protect against disease by preventing colonization by harmful microorganisms. These physical, mechanical, chemical, and microbiological barriers prevent a majority of pathogens from gaining access to the cells and tissues of the body (Belkaid and Tamoutounour, 2016).

However, some pathogens manage to breach these barriers. Microbes can enter the skin through scratches, wounds, or insect bites, such as those from mosquitoes (e.g., *Plasmodium falciparum,* the protozoan species predominantly responsible for malaria); ticks (e.g., *Borrelia burgdorferi,* the bacterium responsible for Lyme disease); and fleas (e.g., *Yersinia pestis,* the bacterium responsible for bubonic plague). Many pathogens enter the body by penetrating mucous membranes. One example is the influenza virus, which expresses a surface molecule that allows it to attach to and invade cells in the mucous membranes of the respiratory tract.

Once a pathogen breaches these anatomical barriers, the innate immune system first responds by detecting the pathogen. This initiates an inflammatory response—mediated by soluble effectors such as complement, eicosanoids, and cytokines—that results in the recruitment of immune cells to the site of infection, direct lysis or phagocytosis of pathogens, and eventual activation of the adaptive immune response.

Pathogen Recognition

The first phase of an innate immune response involves pathogen detection, which is mediated by secreted and cell surface pathogen receptors. Innate immune cells recognize broad structural patterns that are conserved within microbial species but are absent from host tissues. These broad structural patterns are referred to as *PAMPs* (pathogen-associated molecular patterns) and the receptors that recognize them are called *PRRs* (pattern recognition receptors). PRRs can be divided into three broad classes: secreted, endocytic, and signaling PRRs.

Secreted PRRs and the Complement System

Secreted PRRs are opsonins (molecules that enhance phagocytosis) that bind to microbial cell walls and tag them for destruction by the complement system or by phagocytes. C-reactive protein and mannose-binding lectin are two examples of secreted PRRs; both are components of the acute-phase response (see Inflammation).

The plasma proteins known as the complement system are some of the first to act following pathogen entry into host tissues. Over 30 proteins and glycoproteins make up the complement system. These proteins circulate in blood and interstitial fluid in inactive forms that become activated in sequential cascades in response to interaction with molecular components of pathogens, leading to the activation of C3 (complement component 3), which plays the most important role in pathogen detection and clearance. Complement activation leads to the cleavage of C3 into C3b and C3a fragments. The large C3b fragment (an opsonin) attaches to pathogen surfaces in a process called complement fixation and can activate C5 and a lytic pathway that can damage the plasma membrane of adjacent cells and microorganisms. The C5a fragment attracts macrophages and neutrophils and can activate mast cells. The small C3a fragment (anaphylatoxin) also promotes inflammation. Thus, complement fixation has two functions: the formation of protein complexes that damage the pathogen's membrane and marking the pathogen for destruction by phagocytes (Morgan and Harris, 2015).

Endocytic PRRs

Endocytic PRRs are expressed on the surface of phagocytic cells. These receptors mediate the uptake and transport of microbes into lysosomes, where they are degraded. The degraded microbial peptides are processed and presented to T cells by members of the MHC family of cell surface proteins. (In humans, the MHC is also called *human leukocyte antigen* or *HLA*.) The mannose, glucan, and scavenger receptors are part of this class of receptors.

Signaling PRRs

On PAMP detection, signaling PRRs trigger intracellular signaling cascades that eventually result in the production of cytokines that orchestrate the early immune response. The most-studied group of signaling PRRs are the TLRs (toll-like receptors). TLRs are a family of PRRs that recognize a variety of microbial products. These transmembrane proteins are composed of an extracellular domain that detects pathogens and a cytoplasmic signaling domain that relays information to the nucleus. TLRs are expressed on the plasma membranes and endosomes of immune cells.

Signaling through TLRs leads to activation of two distinct signal transduction pathways (see Box 38–1: PAMPs, PRRs, and the Induction of Interferons). Most TLRs signal through a pathway that promotes the activation of the transcription factor nuclear factor-κB (NF-κB) and the production of proinflammatory cytokines such as IL-1, IL-6, IL-12, and tumor necrosis factor α (TNF-α). The exception is TLR3, which signals through a pathway that leads to the activation of the transcription factor IRF3 (interferon regulatory factor 3), and the production of IFN types I and III. TLR4 is unique in that it signals through both pathways (Cao, 2016).

BOX 38–1 ■ PAMPs, PRRs, and the Induction of Interferons

Cells of the innate immune system—predominantly dendritic cells and macrophages—recognize broad structural patterns that are conserved within microbial species but are absent from host tissues. These patterns are called *pathogen-associated molecular patterns* (PAMPs); *pattern recognition receptors* (PRRs) recognize PAMPs. There are three broad classes of PRRs: secreted, endocytic, and signaling PRRs.

Activation of signaling PRRs results in the production of cytokines that orchestrate the early immune response. The most well-studied group of signaling PRRs are the 11 Toll-like receptors (TLRs), each of which displays specificity for a distinct PAMP (e.g., TLR4 recognizes lipopolysaccharide; TLR3 binds double-stranded RNA; TLR9 interacts with foreign DNA). Another receptor group, C-type lectin-like receptors, recognizes unique carbohydrate structures on invading microorganisms. Other signaling PRRs are cytosolic, such as retinoic acid–inducible gene (RIG)-I-like receptors (RLRs) that are activated by cytoplasmic double-stranded and 5′-triphosphorylated RNA species, and the nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) that detect cytosolic endotoxins. Signaling through most PRRs leads to broad cytokine responses, mediated by NF-κB and resulting in the production of proinflammatory cytokines such as IL-1, IL-6, IL-12, and TNF-α.

In response to attack by viruses, bacteria, parasites, and tumor cells, membrane-bound and cytosolic (endosomal) signaling PRRs, including TLRs, work via several convergent pathways to stimulate the production of yet another class of cytokines, the IFNs. There are three types of IFNs: type I IFN (mainly IFN-α and IFN-β, plus other minor forms such as IFN-ε or IFN-ω); type II IFN (IFN-γ); and type III IFN (IFN-λ). IFNs are glycoproteins of about 145 amino acids, with molecular masses of approximately 19 to 24 kDa, depending on the extent of glycosylation. Viral infections are the major inducers of the transcription of genes encoding type I IFNs. Pathways leading to IFN production are complex. The contemporary model now encompasses the concept that PRRs trigger intracellular signaling cascades that involve receptor-associated adapters (e.g., TRIM, TIRAP, MyD88) and the assembly of a signalosome containing various protein kinases (e.g., TBK1, IKKε, TAK, ASK1). Activation of these kinases in response to pathogen recognition leads to the phosphorylation and activation of the latent cytoplasmic transcription factors termed interferon regulatory factors (IRFs). Activation of IRF3 and IRF7, sometimes in combination with other transcription factors, activates transcription of the genes encoding type I IFNs.

Actions of IFNs

The IFNs are unique among the cytokine superfamily in that they produce an array of pleiotropic effects when they bind to their specific receptor. IFNs convey antiviral, antiproliferative, and immunomodulatory functions onto their target cells.

The IFNs are the most crucial cytokines in the defense against invading microorganisms, particularly viruses. IFN-α, IFN-β, and the more recently discovered IFN-λ are vital elements in these defense mechanisms. Type I IFNs promote the production of interferon-stimulated genes (ISGs) in infected and neighboring cells, the products of which induce an intracellular antimicrobial program that limits the spread of infectious pathogens. Type I IFNs also augment antigen presentation, costimulation, and cytokine production by innate immune cells, leading to enhanced adaptive immune responses.

Produced by activated helper T (T_H) and natural killer (NK) cells, IFN-γ enhances the microbicidal activity of macrophages by inducing mammalian inducible nitric oxide synthase (iNOS, also called NOS2), thereby increasing their production of nitric oxide (NO) and their capacity to kill intracellular pathogens. Furthermore, CD8$^+$ T cells utilize IFN-γ to directly kill infected cells and tumors. Indeed, IFN-γ contributes significantly to the adaptive immune system, where it also influences developmental processes such as immunoglobulin (Ig) isotype switching in B cells and T_H1 cell differentiation.

Cellular Signaling in Responses to IFNs

Interferon signaling is a complex mechanism that elicits the appropriate antimicrobial program in target cells. IFNs bind to distinct heteromeric membrane receptors. Binding of the type I IFNs to their specific cell surface receptors leads to cross tyrosine phosphorylation, recruitment, and activation of the STAT (Janus kinase/signal transducer and activator of transcription) pathway. Several members of the STAT family of transcription factors and IRF9 cooperatively form the DNA binding protein complex ISGF3, which is required for expression of ISGs through activation of the interferon-stimulated response element (ISRE) in their promoters. Transcriptional induction of these immediate early response genes facilitates the establishment of an antiviral state, achieves antiproliferation in normal and tumor cells, and influences adaptive immune responses (e.g., via modulation of IL-2 production and expression of the α chain [CD25] of the IL-2R complex; see Figure 39–2).

Numerous genes contain an ISRE. Their gene products are components of the antiviral defense: 2′-5′ poly-A-synthase, double-stranded RNA activated protein kinase (PKR), cell surface proteins such as ICAM (intracellular adhesion molecule) and the major histocompatibility complex (MHC) I and II classes, chemokines (e.g., ISG15 and the IP10), and myriad genes of unknown function. More recently, numerous micro-RNAs have been added to the repertoire of IFN-induced response genes that contribute to control of pathogens.

Type I (IFN-α and IFN-β) and type III (IFN-λ) IFNs promote the production of ISGs (interferon-stimulated genes) in infected and neighboring cells, the products of which induce an intracellular antimicrobial program that limits the spread of infectious pathogens, particularly viruses. Type I IFNs also augment antigen presentation and cytokine production by innate immune cells, leading to enhanced adaptive immune responses (Gonzalez-Navajas et al., 2012).

Pathogen Clearance

Pathogens vary in the manner by which they live and replicate within their hosts. Extracellular pathogens replicate on epithelial surfaces, or within the interstitial spaces, blood, and lymph of their host. Intracellular pathogens establish infections within host cells, either in the cytoplasm or in cellular vesicles. Depending on the nature of the infection, different immune cells and effector mechanisms are involved in the control and elimination of the pathogen.

Extracellular Pathogens

Unlike pathogens that replicate within host cells, extracellular pathogens are accessible to soluble effector proteins. Pathogens that replicate within interstitial spaces, blood, and lymph are detected by secreted PRRs and complement proteins. Complement fixation triggers direct lysis of the pathogen and enhances pathogen uptake by phagocytic cells. The phagocytic cells involved in the clearance of extracellular pathogens are macrophages and neutrophils. Tissue-resident macrophages are long-lived cells that are present from the start of an infection. They engulf pathogens and release inflammatory mediators to alert host cells of an attack. Neutrophils, in contrast, are short-lived, circulating phagocytes. Inflammatory cues, such as those released by macrophages, recruit neutrophils to the site of infection, where they soon become the dominant phagocyte.

On entry into host tissues, the first immune cells a pathogen encounters are the tissue-resident macrophages. Macrophages phagocytize microorganisms in a nonspecific fashion through their phagocytic receptors. Proteins of the complement system enhance this process by binding to receptors expressed by macrophages. One such receptor is complement receptor 1 (CR1). CR1 molecules interact with C3b fragments that have been deposited on the pathogen's surface, facilitating the engulfment and destruction of the pathogen.

In addition to engulfing invading pathogens, macrophages alert host cells of an infection. TLR4 engagement on macrophages leads to the production of proinflammatory cytokines such as IL-1, IL-6, IL-12, TNF-α, and CXCL8 (see Inflammation). These cytokines recruit immune cells, the most prominent of which are neutrophils, to the infected tissue (Lavin et al., 2015).

Circulating neutrophils have an average life span of less than 2 days. Mature neutrophils are kept in the bone marrow for up to 5 days before being released into circulation, ensuring a large reserve that can be summoned during an infection. When neutrophils sense inflammatory signals such as cytokines, chemokines, eicosanoids, ROS (reactive oxygen species), or NO (nitric oxide), they migrate to the site of infection, where they engulf and kill the invading pathogen. In addition, neutrophils can release extracellular DNA nets that trap bacterial pathogens (von Köckritz-Blickwede and Nizet, 2009). Neutrophils die within 2 h of entry into infected tissues, forming the characteristic pus that develops at sites of infection (Kruger et al., 2015).

Intracellular Pathogens

The NK cells provide an early defense against intracellular pathogens. Like neutrophils, these circulating leukocytes migrate from the blood to the site of infection in response to inflammatory cues. Once at the site of infection, NK cells target and kill infected host cells.

The NK cells express receptors that deliver either activating or inhibitory signals. The ligands for the activating NK cell receptors are typically cell surface proteins whose expression is altered during infection or trauma. Healthy cells are protected from attack by NK cells because the signals generated from the inhibitory NK cell receptors dominate those generated from the activating receptors. In contrast, interaction between NK cells and infected or damaged cells shifts the balance of inhibitory and activating signals to favor an attack. This system allows NK cells to discriminate between healthy cells that should be protected and infected cells that should be destroyed.

The NK cells are stimulated by cytokines, including type I IFNs, IL-12, and TNF-α. IFN-α and IFN-β enhance NK cell cytotoxicity and induce NK cell proliferation, whereas IL-12 enhances cytokine production. The key cytokine produced by NK cells is IFN-γ, also called type II IFN. One function of IFN-γ is to activate macrophages. Activated macrophages exhibit enhanced microbicidal activity. One mechanism of their microbicidal activity is the induction of iNOS and the production of prodigious quantities of NO (Bjorkstrom et al., 2016).

Adaptive Immunity

Adaptive immunity refers to the arm of the immune response that changes (adapts) with each new infection. The cells responsible for adaptive immunity are B cells and T cells. The effector mechanisms used by B and T cells are similar to those used by innate immune cells; however, the important distinction between innate and adaptive immunity lies in their mode of pathogen recognition. Whereas the PRRs of the innate immune response recognize broad microbial patterns, B cells and T cells express receptors that recognize highly specific molecular structures. Following pathogen exposure, B and T cells with receptors that recognize the invading pathogen proliferate robustly and differentiate into effector lymphocytes. Soon after pathogen clearance, a large number of effector B and T cells die, but a small population of memory cells survives. Those cells have the ability to mount a rapid and specific response on re-exposure to the same pathogen. This memory response, unique to adaptive immunity, is the basis for vaccination (see Chapter 40).

Initiation of the Adaptive Immune Response

The skin and mucosal surfaces prevent the majority of pathogens from entering host tissues and causing infections. Innate immune responses generally eliminate microorganisms that breach these barriers, typically within a few days. However, some pathogens establish an infection that cannot be controlled entirely by the innate immune response. In these cases, pathogen clearance requires the adaptive immune response.

Dendritic cells provide an essential link between innate and adaptive immunity. DCs engulf pathogens at the site of infection and travel to the lymphoid organs. Once there, they activate T cells by presenting them with fragments of the engulfed pathogen loaded on MHC molecules (see section on Antigen Processing and Presentation).

Pathogen Recognition

The innate immune system detects pathogens by a fixed repertoire of soluble and cell-surface receptors that recognize broad structures shared by different pathogens. The genes encoding these pathogen receptors are inherited from one generation to the next in a stable form.

The adaptive immune system uses a more focused strategy of pathogen recognition. B and T cells recognize pathogens by using their cell surface receptors: BCRs and TCRs. In contrast to the stably inherited genes encoding innate immune pathogen receptors, the genes encoding BCRs and TCRs rearrange during the course of lymphocyte development. This gene rearrangement enables the development of millions of pathogen receptors with unique binding sites, each expressed by a small subset of lymphocytes. On pathogen exposure, only those lymphocytes with receptors that recognize specific components of the invading pathogen (referred to as the receptor's *cognate antigen*) are selected to proliferate and differentiate into effector cells.

Pathogen Receptors: BCRs and TCRs

B-cell receptors (BCRs) and TCRs are structurally related molecules. The BCR, also called immunoglobulin, is composed of two identical heavy chains and two identical light chains. Each polypeptide chain expresses an amino-terminal variable region, which contains the antigen-binding site, and a carboxy-terminal constant region. Immunoglobulins are anchored in the B-cell membrane by two transmembrane regions at the end of each heavy chain. Immunoglobulins are initially surface bound but become soluble when a B cell differentiates into a plasma cell. The soluble forms of immunoglobulins are called antibodies.

The TCR is composed of an α chain (TCRα) and a β chain (TCRβ), both anchored in the T-cell membrane by a transmembrane region. The α and β chains consist of a variable region that contains the antigen-binding site and a constant region. In contrast to immunoglobulins, TCRs remain membrane bound and are not secreted.

Both BCRs and TCRs develop through gene rearrangement. This genetic recombination process (which B cells complete in the bone marrow and T cells in the thymus) is a defining feature of the adaptive immune system. The human BCR and its soluble derivative, the antibody, are composed from genes of three loci, the *IG heavy chain*, the *IGκ light chain*, and the *IGλ light chain*, yielding a repertoire of more than 10^{11} possible combinations. In close resemblance, the TCR comprises either an α and a β chain (most common) or a γ and a δ chain. Three of the key enzymes involved are RAG1 and RAG2 (RAG, recombination-activating gene; deficiencies in these enzymes result in a complete absence of mature lymphocytes) and the terminal deoxynucleotidyl transferase, albeit the full complexity of the DNA repair machinery is required to accomplish a productive rearrangement. Failure to do so will lead to the elimination of the unsuccessful B or T cells by programmed cell death (Nemazee, 2006). These events of recombination and subsequent somatic hypermutation are vital for an optimally performing adaptive immune system. They remain unutilized as pharmacological targets.

Antigen Processing and Presentation

Immunoglobulins are capable of recognizing antigens in their native form. TCRs, in contrast, only recognize processed antigen fragments presented by specialized molecules encoded by the MHC (Figure 38–2). The MHC was first identified as a genetic complex that determines an

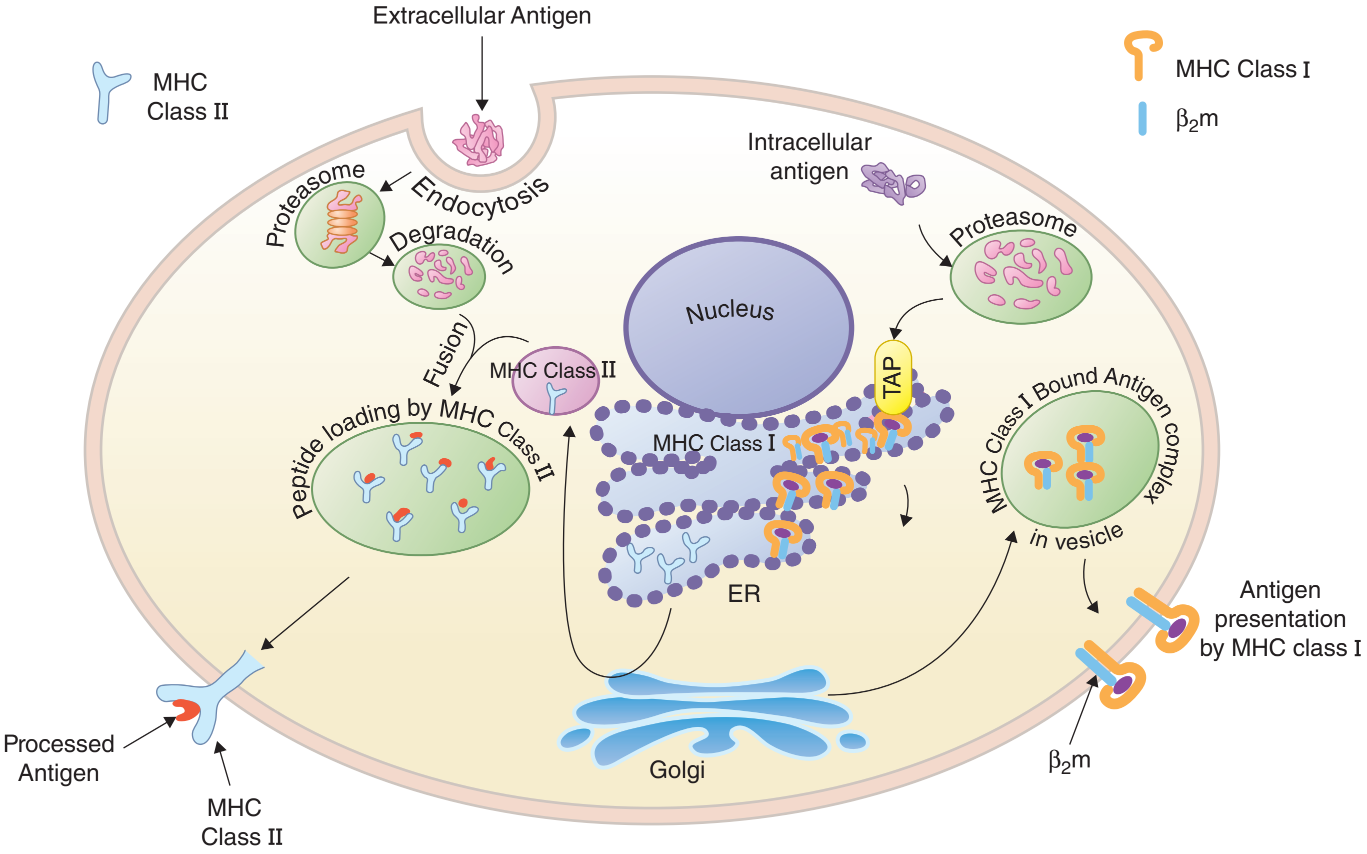

Figure 38–2 *Antigen processing and presentation via the MHC class I and II pathways.* Endogenous peptides from a variety of sources are processed by proteasomes; the resulting peptides are transported via the TAP (transporter associated with antigen processing) complex into the ER, where they encounter MHC class I–B2M (β_2-microglobulin) heterodimers. After the peptide loading of the MHC class I complex, the final peptide–MHC class I complexes migrate through the Golgi and are delivered to the cell surface to engage CD8$^+$ T cells. Exogenous antigens are endocytosed and processed by a lysosome/proteasome. The MHC class II complex is assembled in the ER, migrates through the Golgi, and subsequently fuses with the vesicle containing processed antigen fragments. These peptide cleavage products are loaded into the peptide-binding groove of the MHC class II, and the peptide–MHC class II complexes are transported to the cell surface and presented to CD4$^+$ T cells.

organism's ability to accept or reject transplanted tissue. Further studies highlighted the importance of MHC molecules in generating T_H- and T_C-cell responses.

There are two types of MHC molecules involved in antigen presentation: MHC class I and MHC class II. These structurally related molecules are expressed on different cell types but perform parallel functions in priming T-cell responses.

MHC Class I

MHC class I molecules consist of a transmembrane glycoprotein α chain noncovalently associated with a B2M (β_2-microglobulin) molecule. MHC class I molecules are expressed on the surface of nearly all nucleated cells and present peptides from endogenous antigens to CD8 T_C cells.

MHC Class II

MHC class II molecules consist of two noncovalently associated transmembrane glycoproteins, an α chain and a β chain. MHC class II molecules are primarily expressed on the surface of professional APCs (DCs, macrophages, B cells) and present peptides from exogenous antigens to CD4 T_H cells.

Antigen Processing for Presentation by MHC

Unlike immunoglobulins, which recognize a wide range of molecular structures in their native form, TCRs can only recognize antigens in the form of a peptide bound to an MHC molecule. For a pathogen to be recognized by a T cell, pathogen-derived proteins need to be degraded into peptides—an event referred to as antigen processing (see Figure 38–2). Endogenous antigens, those derived from intracellular pathogens, are processed by the cytosolic pathway for presentation by MHC class I molecules. Proteins in the cytosol are degraded into peptides by the proteosome. The resultant peptides are then transported out of the cytosol and into the endoplasmic reticulum (ER) by a protein called the TAP (transporter associated with antigen processing), which is embedded in the ER membrane. Once newly synthesized MHC class I α chains and B2M molecules are translocated into the ER membrane, the α chains and B2M molecules associate and bind peptide, forming a peptide-MHC complex. These peptide-MHC complexes make their way to the plasma membrane in membrane-enclosed vesicles of the Golgi apparatus.

Exogenous antigens, those derived from extracellular pathogens, are processed by the endocytic pathway for presentation by MHC class II molecules. In this pathway, extracellular pathogens are internalized by host cells through endocytosis or phagocytosis and are degraded by proteolytic enzymes within endocytic vesicles. Newly synthesized MHC class II α and β chains are translocated into the ER membrane, where they associate with a third chain, called the invariant chain. The invariant chain prevents MHC class II molecules from binding peptides in the ER and delivers MHC class II molecules to endocytic vesicles. Once in the endocytic vesicles, MHC class II molecules bind peptide and are carried to the cell surface by outgoing vesicles.

All T cells require peptide-MHC presentation by professional APCs for activation (see Primary Responses). If an intracellular pathogen does not infect a professional APC, CD8 T_C-cell responses can be generated through a third pathway of antigen presentation called *cross-presentation*. Cross-presentation involves the uptake of extracellular material by professional APCs and its delivery to the MHC class I presentation pathway instead of the MHC class II presentation pathway via a mechanism that remains incompletely understood (Blum et al., 2013).

Note that protein degradation occurs continuously, even in the absence of infection. In uninfected cells, MHC molecules carry self-peptides—derived from normal cellular protein turnover—to the cell surface. While these peptide-MHC complexes do not normally provoke an immune response, recognition of these self-peptides by autoreactive T cells can result in the development of *autoimmunity* (see Autoimmunity: A Breach of Tolerance).

Lymphocyte Development and Tolerance

Innate immune PRRs are fixed receptors that recognize broad microbial structures or structures associated with damaged host cells. These receptors rarely, if ever, recognize self-antigens expressed by healthy cells. In contrast, because BCRs and TCRs develop from gene rearrangement, receptors that recognize self-antigens expressed by healthy host cells can arise. The goal of lymphocyte development is to produce cells with functional pathogen receptors but eliminate cells whose receptors recognize self-antigens. Next, we describe the processes of B-cell and T-cell development and highlight the mechanisms that maintain self-tolerance.

B-Cell Development

B-cell development takes place in the bone marrow and is driven by interaction with bone marrow stromal cells and the local cytokine environment. B-cell development can be broadly divided into pro-B-, pre-B-, immature B-, and mature B-cell stages. BCR gene rearrangement starts at the early pro-B stage and continues throughout the pre-B stage. By the immature B-cell stage, B cells express fully rearranged IgM immunoglobulins on their cell surface. At this stage, immature B cells leave the bone marrow and complete their maturation in the periphery. Mature B cells express both IgM and IgD immunoglobulins on their cell surfaces (LeBien and Tedder, 2008).

Because B-cell activation depends on help from CD4 T_H cells, negative selection of T cells whose receptors recognize self-antigens also ensures that B cells whose receptors bind to the same self-antigen will not be activated. Consequently, B cells do not undergo as rigorous of a selection process as T cells. However, B cells whose receptors recognize components of the bone marrow are negatively selected and die by apoptosis.

T-Cell Development

Unlike B cells, which develop in the bone marrow, T-cell precursors complete their development in the thymus. T-cell precursors enter the thymus as $CD4^-CD8^-$ DN (double negative) cells, not yet committed to the T-cell lineage.

The DN T cells can be divided into four subsets—DN1 to DN4—based on the expression of certain cell surface molecules. Gene rearrangement of the TCRB chain begins during the DN2 stage and continues through the DN3 stage. After β-chain rearrangement is complete, the newly synthesized β-chain combines with a protein known as the pre-Tα chain, forming the pre-TCR. DN3 cells then progress to the DN4 stage and express both the CD4 and CD8 coreceptors. These cells are now referred to as $CD4^+CD8^+$ DP (double positive) cells. DP T cells proliferate rapidly, generating clones of cells expressing the same β chain. After this period of rapid proliferation, T cells begin to rearrange their α-chain genes. Because cells within each clone can rearrange a different α chain, they generate a more diverse population than if the original cell had rearranged both the β chain and α chain before proliferating. Once a DP T cell expresses a fully rearranged TCR, it undergoes the processes of positive and negative selection.

The T cells migrate into the thymic cortex to undergo positive selection. The purpose of positive selection is to select for T cells whose TCRs can interact with an individual's own MHC molecules. In the cortex, T cells interact with cortical thymic epithelial cells, which express both MHC class I and MHC class II molecules. T cells with TCRs that do not recognize self-MHC molecules die by apoptosis. T cells with TCRs that can successfully bind to self-MHC molecules are signaled to survive and proceed to the thymic medulla. As a result of positive selection, DP thymocytes mature into single-positive T cells that express just one coreceptor (CD4 or CD8). T cells that successfully interact with MHC class I molecules develop into CD8 T cells, whereas T cells that interact with MHC class II molecules become CD4 cells.

After positive selection, T cells migrate to the thymic medulla to undergo negative selection. The purpose of negative selection is to eliminate T cells whose TCRs recognize self-antigens. This is accomplished by medullary thymic epithelial cells, which promiscuously express self-peptides on their MHC molecules. If T cells interact with self-peptides with high affinity, they are deleted by apoptosis (Shah and Zuniga-Pflucker, 2014).

The positive and negative selection processes responsible for generating self-MHC restricted and self-tolerant T cells are rigorous. It is estimated that over 98% of thymocytes die by apoptosis within the thymus, with the majority failing at the positive selection stage. The T cells that manage to successfully complete both positive and negative selection leave the thymus and take up residence in the secondary lymphoid structures.

Primary Responses

The processes of lymphocyte development and gene rearrangement generate millions of unique lymphocytes that each express pathogen receptors of a single specificity. During an infection, only a small portion of these B and T cells express receptors that can recognize the invading pathogen. To increase their numbers, each lymphocyte that recognizes the invading pathogen becomes activated and proliferates, giving rise to clones expressing identical immunoglobulins or TCRs. These processes, referred to as *clonal selection* and *clonal expansion*, are essential features of lymphocyte activation and differentiation, and facilitate the effector mechanisms that B and T cells use to combat infection.

B-Cell Activation and Antibody Production

In most primary immune responses, B-cell activation and subsequent antibody production are dependent on help from CD4 T_H cells. When circulating B cells home to secondary lymphoid tissues, they first enter at the T-cell zone. If a B cell encounters its specific antigen, cross-linking of the BCR and coreceptor induces a signal transduction cascade that mediates changes in cell surface expression of adhesion molecules and chemokine receptors, preventing the B cells from leaving the T-cell zone.

After immunoglobulins bind their cognate antigen, they internalize the antigen by receptor-mediated endocytosis and process the antigen for display by MHC class II molecules. If a CD4 T_H cell recognizes its antigen, the B and T cell form a conjugate pair. This cognate interaction facilitates the delivery of T cell–derived cytokines to B cells. The most important of these cytokines is IL-4, which is essential for B-cell proliferation and differentiation into antibody-secreting plasma cells.

The initial antibodies produced by plasma cells are of generally low affinity. They help to keep the infection under control until a stronger antibody response is generated. Antibody quality improves over the course of the infection due to two processes: somatic hypermutation and isotype switching. Somatic hypermutation introduces random single-nucleotide substitutions throughout the immunoglobulin variable regions. These changes can result in immunoglobulin molecules with increased affinity for the pathogen. B cells producing these improved immunoglobulin molecules outcompete for binding to the invading pathogen and are preferentially selected to become plasma cells. As an infection proceeds, antibodies of higher affinity are produced—a process referred to as *affinity maturation* (Di Noia and Neuberger, 2007).

Isotype Switching. Immunoglobulin class switching (i.e., isotype switching or class-switch recombination) is a process that alters the type of immunoglobulin produced by proliferating B cells via rearrangement of the DNA in the immunoglobulin region. Only the constant region of the antibody's heavy chain is changed; thus, isotype switching impacts only how the antibody interacts with different effector molecules. The variable region of the antibody generated via V(D)J (*V*ariable, *D*iversity, and *J*oining segments) recombination at the immature B-cell state remains unaltered during the class switching process, and consequently, isotype switching does not affect antigen specificity (in this context, *variable* and *constant* refer to changes or lack thereof between antibodies that target different epitopes).

The immunoglobulin molecules emerging from the isotype switching process can be divided into five classes (isotypes): IgA, IgD, IgE, IgG, and IgM. These isotypes differ in their heavy chain constant regions and have specialized effector functions. As an infection proceeds, antibodies with additional effector functions are generated by isotype switching. This process is strongly influenced by cytokines secreted into the microenvironment by the B cell's cognate T cell (Xu et al., 2012). IgM and IgD are the first antibodies produced following activation of naïve mature B cells, and the antibodies tag pathogens for destruction by the complement system. As an infection proceeds, antibodies with additional effector functions are generated by isotype switching, which yields, in order, IgG, IgA, or IgE. IgA dominates immunoglobulin levels in association with mucosal membranes such as the gut-associated lymphoid tissue. IgE is implicated in the immune responses to various worms or protozoan parasites but is most prominently recognized for its role in hypersensitivity reactions where it mediates allergic reactions and the more severe anaphylactic reactions.

Role of Antibodies in Pathogen Clearance. Antibodies can aid in pathogen clearance in a number of ways. They can bind to a pathogen (or toxin) and prevent it from interacting with host cells. These antibodies are called neutralizing antibodies. Antibodies can also function as opsonins—coating of pathogens with antibodies can facilitate their engulfment by phagocytic cells, which often express receptors for the constant regions of antibodies. In addition, antibody deposition can activate the complement system, leading to the direct lysis of pathogens.

Antibody Production in Response to Vaccination. The development of these "natural antibodies" represents the core of adaptive immunity, and it not only represent the foundation of an organism's adaption to repeated encounters with potential pathogens in their natural environment but also forms the basis of the concept of immunization/vaccination. In the latter scenario, the immune system is primed by presentation with antigens that represent or resemble all or part of the actual pathogen. Historically, harmless microorganisms similar or related to the targeted pathogen were introduced into the body to elicit a protective immunity against the dangerous pathogen; for example, cowpox virus and vaccinia virus were used in inoculations to create protection against smallpox (see Chapter 40 and Kaynarcalidan et al., 2021). Over time, other strategies were developed that follow that same basic principle: instead of related, nonpathogenic microorganisms, the actual pathogen was administered in an attenuated or killed version (e.g., influenza vaccine). The advent of molecular biology facilitated the development of vaccines that are based on individual recombinant viral proteins rather than the entire pathogen. The latest technologies involve the delivery (sometimes with the help of replication-incompetent vector delivery systems) of part of a pathogen's genetic material (either RNA or DNA) to allow the host organism to *temporarily* produce these microbial antigens. The advantages of these systems are easy adaptability of the vaccine to mutations in the pathogen and the potential for quick, inexpensive, and scalable manufacturing due to complete *in vitro* manufacturing processes. For instance, mRNA-based vaccines have proved successful at inducing immunity against SARS-CoV-2.

Common to all vaccines is the inclusions of so-called adjuvants, which provide for the nonspecific stimulation of the initial steps in the innate immune response that are characterized by the production of the cytokines that promote lymphocyte development and switch recombination, as outlined above.

Another major advance harnessing the protective as well as therapeutic properties of antibodies came with the large-scale production of monoclonal antibodies that are characterized by limited but complete reproducible specificity since they originate in a laboratory environment rather than a (mammalian) organism (see Figure 39–4). This control over every property of these recombinant antibodies has recently been expanded with the aid of gene editing technologies such as CRISPR/Cas9, which allows for the manufacturing of chimeric monoclonal antibodies that are refractory to rejection by the patient's immune system and whose antigen specificity can be altered *in vitro* to improve their efficacy (see Chapter 40).

T-Cell Activation

Naïve T cells first encounter antigen presented by DCs in the secondary lymphoid tissues. For T cells to become fully activated, they need to receive two signals (Figure 38–3):

- A primary signal generated through ligation of the TCR
- A costimulatory signal generated through ligation of a T-cell surface protein called CD28

Both signals must be delivered by ligands on the same APC.

The primary signal is generated when the TCR engages a peptide-MHC complex. The TCR associates with an accessory molecule called CD3, forming the TCR-CD3 complex. CD3 does not influence the interaction of the TCR with its antigen but participates in the signal transduction that occurs after antigen engagement. The T-cell coreceptors CD4 and CD8 bind to the conserved regions of MHC molecules, strengthening and stabilizing the interaction between the TCR and the peptide-MHC complex. CD4 and CD8 also participate in signal transduction.

The costimulatory signal is generated when CD28 binds to its ligands, called B7-1 (CD80) and B7-2 (CD86). These costimulatory B7 molecules are expressed only on activated professional APCs, highlighting their importance in T-cell activation. Additional stimulatory and inhibitory coreceptors can modulate T cell ativaion by binding to their ligands on APCs or tumor cells. Activating and blocking monoclonal antibodies can interfere with this fine-tuning of TCR signaling, thus permitting the pharmacological modulation of the immune response (Figure 38–4).

Engagement of the TCR complex activates signal transduction cascades that induce the expression of multiple genes, including NFAT, AP-1, and NF-κB. One of the most important downstream targets of these genes is IL-2, a cytokine that is essential for T-cell proliferation and survival. The IL-2 receptor, CD25, is expressed on activated T cells. When T cells become activated, they begin to express a cell surface protein called CTLA-4 (cytotoxic T-lymphocyte–associated protein 4). This protein resembles CD28 and binds to the costimulatory B7 molecules with higher affinity than does CD28. Whereas CD28 ligation promotes T-cell activation, CTLA-4 ligation dampens T-cell activation. This inhibitory molecule serves to keep T-cell responses in check (Brownlie and Zamoyska, 2013). In addition to CTLA-4, T cells upregulate expression of other inhibitory coreceptors such as PD-1 (programmed cell death protein 1) and PSGL-1 (P-selectin glycoprotein ligand-1) that help to fine-tune the ensuing T-cell response (Attanasio and Wherry, 2016; Tinoco et al., 2016).

T-Cell Anergy. For a naïve T cell to become fully activated, it must receive a signal through the TCR and CD28. If a T cell engages a peptide-MHC

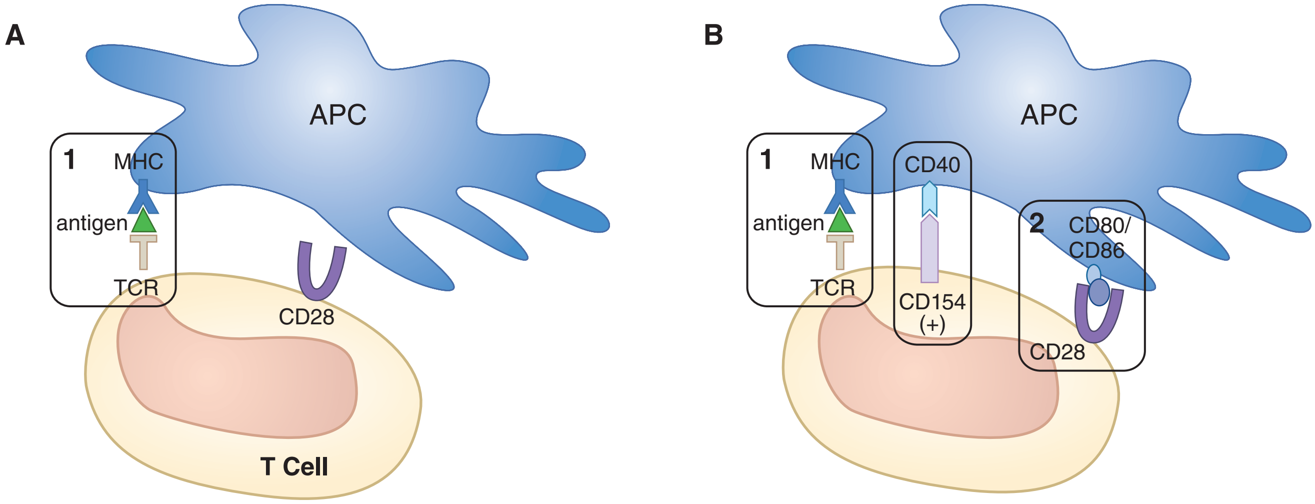

Figure 38–3 *T-cell activation: costimulation.* Two signals are required for T-cell activation: presentation of an antigen ligand to the TCR and signaling by an additional "costimulatory" pair. **A.** The primary signal, *signal 1*, is the interaction of the TCR with the MHC-antigen complex on the APC. Activation requires a second, costimulatory interaction. **B.** *Signal 2*, the costimulatory interaction between CD28 on the T cell (the costimulatory receptor) and the costimulatory ligand on the APC, CD80/CD86, leads to T-cell activation. Additional costimulatory signals, such as the interaction of CD154 with CD40 on the APC, can further enhance T-cell activation (+). In the absence of costimulation, a T cell can become anergic or unresponsive. Additional APC–T-cell interactions can occur after T-cell activation, and some can be inhibitory, providing *immune checkpoints* that are important for reducing autoimmunity and for regulating the size and extent of immune responses, See Figures 39–2 and 39–5 for details.

complex in the absence of a sufficient costimulatory signal, it enters a state of nonresponsiveness referred to as clonal anergy. Anergy is defined by the inability of T cells to proliferate after engaging a peptide-MHC complex due to a lack of IL-2 production and signaling (Figure 38–3; see also Figure 39–2).

CD4 T_H-Cell Differentiation and Effector Functions. Following activation, naïve CD4 T_H cells can differentiate into specialized T_H-cell subsets. These T_H-cell subsets display unique patterns of cytokine production and perform distinct effector functions. The initial studies on T_H-cell differentiation generated a biphasic model in which activated T_H cells differentiate into either T_H1 cells, which defend mainly against intracellular pathogens, or T_H2 cells, which aid in the clearance of extracellular pathogens. More recent models of T_H-cell differentiation have been expanded to include T_H9, T_H17, T_H22, T_{FH} (follicular helper T), and T_{Reg} (T-regulatory) cells (DuPage and Bluestone, 2016).

As their name implies, CD4 T_H cells help activate other immune cells. T_H1 cells secrete IFN-γ and TNF-α, which activate macrophages to kill pathogens located within their phagosomes. These cytokines also activate CD8 T_C cells to kill infected host cells. T_H2 cells, which produce IL-4 and IL-5, defend against extracellular pathogens by enhancing humoral immunity. IL-4 activates B cells to differentiate into antibody-secreting plasma cells. T_H2-derived cytokines also induce class switching to IgA and IgE. Another subset of CD4 T_H cells, the T_{Reg} cell, is responsible for maintaining peripheral tolerance. Through various mechanisms, these cells suppress the proliferation of effector T cells, keeping the T-cell response under control.

CD8 T_C-Cell Effector Functions. The main role of CD8 T_C cells is to induce cytolysis of infected host cells expressing peptide-MHC class I complexes. Activated CD8 T_C cells kill their target cells by two distinct pathways: the granule exocytosis pathway and the Fas-FasL pathway. The granule exocytosis pathway involves the release of perforin and granule enzymes (granzymes) A and B. Perforin molecules form pores in the target cell membrane, allowing the granzyme molecules to enter the cell. Upregulation of FasL (CD95L) on activated T_C cells induces the aggregation of Fas (CD95) on target cells. Both pathways activate the caspase cascade in the target cell, resulting in programmed cell death.

In addition to their cytolytic activity, activated CD8 T_C cells release proinflammatory cytokines, including IFN-γ and TNF-α. These cytokines further aid in pathogen clearance by enhancing the activity of macrophages and neutrophils (Harty et al., 2000).

Leukocyte Extravasation: Diapedesis

Leukocytes fulfill most of their immunological functions outside the bloodstream in the surrounding tissues. Consequently, traversing the capillary endothelial cell barrier is a crucial step in this process. Extravasation (diapedesis) refers to the movement of leukocytes out of the blood into the site of infection or physical tissue damage (Figure 38–5). In the case of blood monocytes, extravasation also occurs in the absence of pathophysiological events and facilitates their conversion into tissue macrophages. On a molecular level, diapedesis can be dissected into four mechanistic steps: *chemoattraction*, *rolling adhesion*, *tight adhesion,* and *transmigration* (Vestweber, 2015).

While initially believed to play its most important role in innate immunity, diapedesis has garnered more attention in recent years as a pharmacological target in the treatment of chronic (inflammatory) autoimmune diseases such as *multiple sclerosis* or *Crohn's disease* (see Autoimmunity). The leukocyte cell surface adhesion molecule $\alpha_4\beta_1$ integrin (VLA-4) that facilitates extravasation of $CD4^+$ T cells interacts with VCAM-1 on vascular endothelial cells. *Natalizumab* is a humanized monoclonal antibody directed against α_4 integrin; *natalizumab's* interference with the $\alpha_4\beta_1$ integrin–VCAM-1 interaction leads to a blockade of autoreactive T-cell diapedesis into the brain and thus prevents attack on the myelin composing the nerve shielding. Similarly, *natalizumab*-mediated prevention of $\alpha_4\beta_7$ integrin binding to the adhesion molecule MADCAM-1 found on endothelial cells of venules is responsible for the efficacy of the drug against Crohn's disease. Another monoclonal antibody recently approved for the treatment of Crohn's disease and ulcerative colitis is *vedolizumab*, which produces fewer side effects due to its $\alpha_4\beta_7$-restricted binding specificity. Preventing entry of effector cells to inflammatory sites through the use of neutralizing antibodies has shown high therapeutic potential in multiple disease settings.

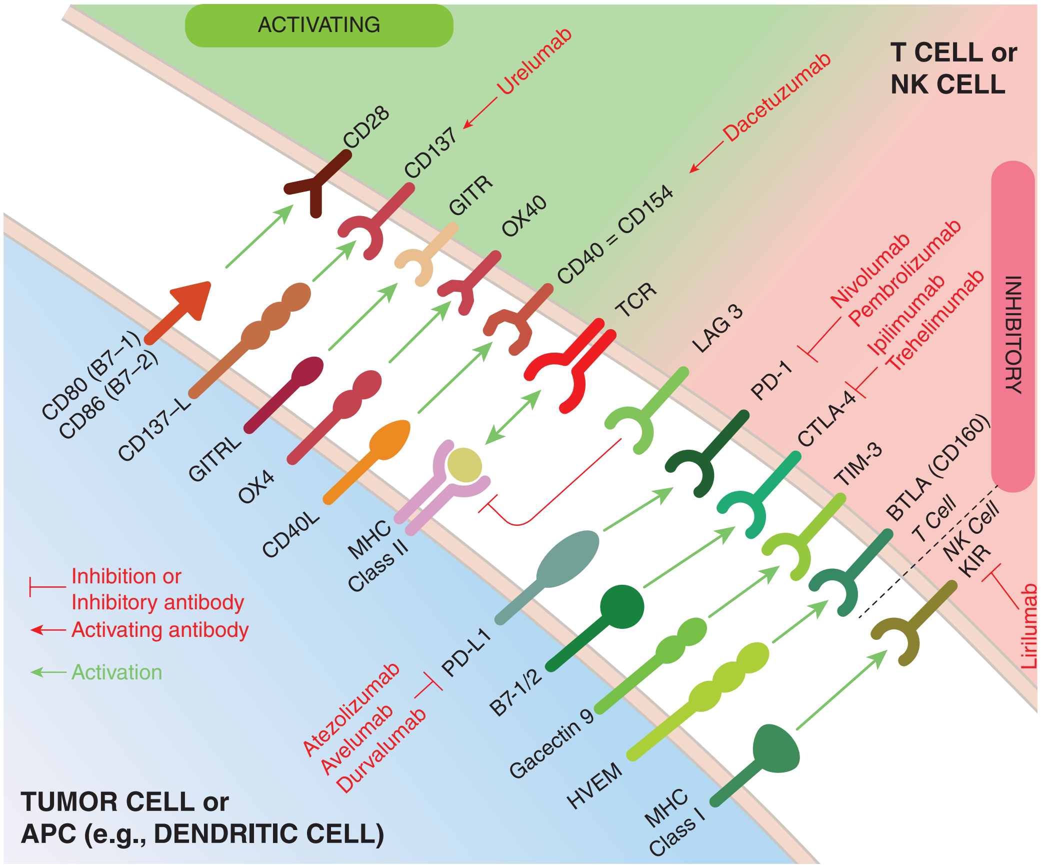
ACTIVATING
T CELL or NK CELL
Urelumab
Dacetuzumab
CD28
CD137
GITR
OX40
CD40 = CD154
TCR
LAG 3
Nivolumab
Pembrolizumab
Ipilimumab
Tremelimumab
INHIBITORY
PD-1
CTLA-4
TIM-3
BTLA (CD160)
T Cell
NK Cell
KIR
Lirilumab
CD80 (B7–1)
CD86 (B7–2)
CD137–L
GITRL
OX4
CD40L
MHC Class II
Inhibition or Inhibitory antibody
Activating antibody
Activation
Atezolizumab
Avelumab
Durvalumab
PD-L1
B7-1/2
Gacectin 9
HVEM
MHC Class I
TUMOR CELL or APC (e.g., DENDRITIC CELL)

Figure 38–4 *T-cell activation and its modulation.* TCR signaling on $CD4^+$ cells after engagement with an MHC class II–peptide complex is enhanced by activating coreceptors (green-shaded area) or attenuated by inhibitory coreceptors (red-shaded area) after these bind their respective ligands on APCs or tumor cells. Numerous activating (→) or blocking (—|) monoclonal antibodies interfere with this fine-tuning of TCR signaling, thus allowing for the pharmacological modulation of the resulting immune response.

Immunological Memory

The numbers of B and T cells decline after pathogen clearance, leaving behind a small population of memory cells. These memory cells have the ability to mount an enhanced secondary immune response upon re-exposure to the same pathogen.

Due to their expression of certain cell surface molecules, memory T cells are more sensitive to TCR-mediated activation by peptide-MHC complexes than naïve T cells. In addition, memory T cells have less-stringent requirements for costimulatory signals, allowing them to respond to peptide-MHC complexes displayed on cells that lack the costimulatory B7 molecules (Farber et al., 2014). Memory B cells produce better antibodies than naïve B cells because they express immunoglobulins that underwent somatic hypermutation and isotype switching during the first antigen encounter (Kurosaki et al., 2015). Combined, these properties allow for a faster and stronger secondary immune response, features that form the foundation of vaccination and subsequent "booster" or "refresher" inoculations (see Chapter 40).

Summary: Innate and Adaptive Immunity in Infectious Diseases

The innate and adaptive immune systems work together to keep the host healthy. The innate immune response is the body's first line of defense and eliminates the majority of pathogens on its own. In the case that the innate immune system is insufficient to eliminate the pathogen, it keeps the infection in check until the adaptive immune system is able to mount a response. Pathogens will be cleared (acute infections), or they may evade the immune response and persist (chronic infections). Chronic infections such as HIV/AIDS and hepatitis B and C lead to immune system suppression that results in susceptibility to secondary infections or cancers associated with infection.

Inflammation

What Is Inflammation, and What Purpose Does It Serve?

The inflammatory response, or inflammation, is a physiological response to tissue injury and infection, although it should be clear that *inflammation* is not a synonym for *infection*. The Romans described the characteristics of this response almost 2000 years ago: pain (*dolor*), heat (*calor*), redness (*rubor*), and swelling (*tumor*). Within minutes of tissue injury and infection, plasma proteins mediate an increase in vascular diameter (vasodilation) and vascular permeability. Vasodilation increases blood flow to the area of injury, resulting in the heating and reddening of the tissue. Increased vascular permeability allows leakage of fluid from the blood vessels into the damaged tissue, resulting in swelling (edema). Within a few hours of these vascular changes, leukocytes arrive at the site of injury. They adhere to activated endothelial cells in the inflamed region and pass through the capillary walls into the tissue (extravasation). These leukocytes phagocytize the invading pathogens and release soluble mediators—cytokines, prostaglandins, leukotrienes—that further contribute to the inflammatory response and the recruitment and activation of effector cells.

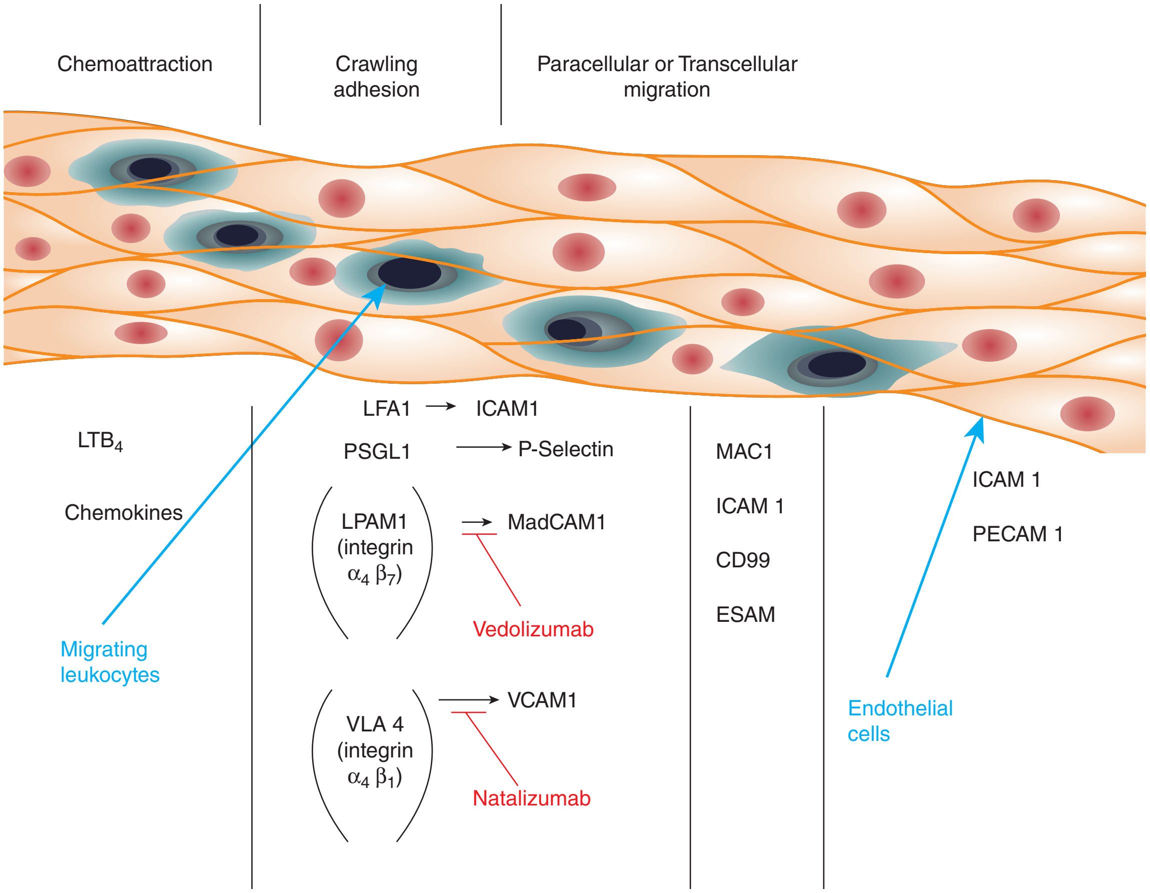

Figure 38–5 *Leukocyte diapedesis.* Leukocytes are recruited to the site of injury or infection by various chemoattractants. The expression of specific, complementary adhesion molecules on the surfaces of both the endothelial cells and the leukocytes facilitates the initial capture and subsequently the "rolling" binding of the leukocyte. After engagement of additional adhesion molecules, the leukocyte enters the subendothelial space, either by squeezing between endothelial cells (paracellular migration) or via movement through individual endothelial cells (transcellular migration). CAM, cellular adhesion molecule; CD99, cluster of differentiation 99 antigen; ESAM, endothelial CAM; ICAM, inter-CAM; MAC-1, macrophage-1 antigen; MADCAM-1, mucosal vascular addressin cell adhesion molecule 1; PSGL-1, P-selectin glycoprotein ligand 1; VCAM-1, vascular CAM 1.

Inflammation can be *acute*, as in response to tissue injury, or it may be *chronic*, leading to progressive tissue destruction, as seen in chronic infections, autoimmunity, and certain cancers. Below are details of both forms of inflammation, including their triggers, the soluble mediators and cell types involved, and the resulting tissue pathology.

The leukocyte inflammatory response is a multistep process whose individual stages can be defined based on the speed of their occurrence, which in turn depends on the availability of the factors that dominate their phases (Figure 38–6). The earliest response (phase 1) is the mobilization of Ca^{2+} that occurs within milliseconds of activation (ligand-receptor interaction). This elevation of cellular Ca^{2+} is the trigger for the release of preexisting factors and mediators stored in intracellular compartments (phase 2), factors such as histamine and proteases. Slightly delayed are phases 3 and 4, the release of mediators whose generation requires *de novo* synthesis but which is generally limited to simple, enzymatic steps that can take place within minutes of the initiating events. The synthesis and release of eicosanoids and the generation of ROS illustrate phases 3 and 4 of the inflammatory response. Phase 5, the slowest (>10 min) but most diverse and complex phase of immune and inflammatory responses, is characterized by processes that involve transcriptional and/or translational events, both stimulatory (e.g., production of cytokines) and inhibitory (e.g., transcriptional suppression by glucocorticoids). Phase 5 not only takes longer to initiate but also persists for a significantly longer period, with the consequences often still apparent days after the initiating events.

Acute Inflammatory Response

The acute inflammatory response provides protection following tissue injury and infection by restricting damage to the localized site, recruiting immune cells to eliminate the invading pathogen, and initiating the process of wound repair.

Following tissue damage, a number of plasma proteins are activated, including those of the clotting and kinin systems. The enzymatic cascade of the clotting system produces fibrin strands that accumulate to form clots, limiting the spread of infection into the blood (see Chapter 36). The enzymatic cascade of the kinin system results in the production of bradykinin—a peptide that induces vasodilation and enhanced vascular permeability (see Chapter 43). In addition, the complement products C3a and C5a bind to receptors on local mast cells, facilitating their degranulation. The resulting release of histamine, prostaglandins, and leukotrienes contributes to vascular changes by inducing vasodilation and enhancing vascular permeability. Prostaglandins and leukotrienes also serve as chemoattractants for neutrophils (see Chapter 41).

Within a few hours of these vascular changes, neutrophils bind to the endothelial cells of the inflamed region and extravasate into the tissue (see previous section, Diapedesis) where they phagocytize the invading pathogens and release soluble inflammatory mediators, including macrophage inflammatory proteins (MIPs) 1α and 1β, which are chemokines that attract macrophages to the site of inflammation. Macrophages arrive at the damaged tissue 5 to 6 h after the onset of the inflammatory response. Activated macrophages secrete three major

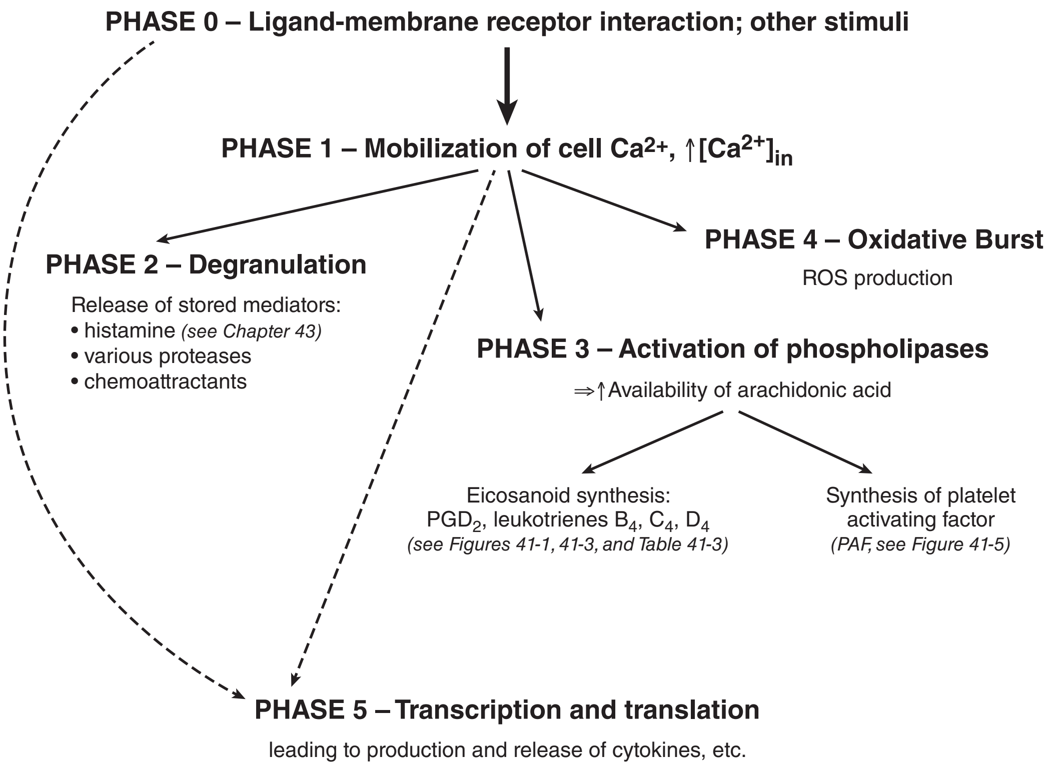
PHASE 0 – Ligand-membrane receptor interaction; other stimuli
PHASE 1 – Mobilization of cell Ca2+, ↑[Ca2+]in
PHASE 2 – Degranulation
Release of stored mediators:
• histamine (see Chapter 43)
• various proteases
• chemoattractants
PHASE 4 – Oxidative Burst
ROS production
PHASE 3 – Activation of phospholipases
⇒↑Availability of arachidonic acid
Eicosanoid synthesis:
PGD2, leukotrienes B4, C4, D4
(see Figures 41-1, 41-3, and Table 41-3)
Synthesis of platelet activating factor
(PAF, see Figure 41-5)
PHASE 5 – Transcription and translation
leading to production and release of cytokines, etc.

Figure 38–6 *The leukocyte inflammatory response.* Steps 1 through 4 occur immediately following ligand-receptor activation and in rapid succession. Step 5, which involves transcription and translation, results in a slower (>10 min) but more persistent response with effects that are apparent for days. See text for details.

proinflammatory cytokines: IL-1, IL-6, and TNFα. These cytokines induce coagulation, increase vascular permeability, and promote the acute-phase response. IL-1 and TNFα also induce increased expression of adhesion molecules on endothelial cells, allowing for circulating leukocytes (neutrophils, macrophages, granulocytes, and lymphocytes) to interact with the endothelium and extravasate into the inflamed tissues. Acute inflammation displays a rapid onset following tissue injury and resolves relatively quickly. The resulting tissue pathology is typically mild and localized.

Chronic Inflammation

Chronic inflammation results from continuous exposure to the offending element. This can be due to pathogen persistence, autoimmune diseases in which self-antigens continuously activate T cells, and cancers. The hallmark of chronic inflammation is the accumulation and activation of macrophages and lymphocytes, as well as fibroblasts that replace the original, damaged, or necrotic tissue. Soluble factors released by macrophages and lymphocytes play an important role in the development of chronic inflammation. Whereas non-protein–based soluble factors (e.g., eicosanoids, bioamines) dominate the landscape during acute inflammation, in chronic inflammation, cytokines, chemokines, growth factors, and secreted/released enzymes and ROS are the primary actors. For instance, cytotoxic T cells and T_H1 cells release IFN-γ, which activates macrophages and DCs. These, in turn, release a variety of soluble factors, such as IL-6 and TNFα, that ultimately result in tissue injury and cell death. Replacement of tissue lost in this manner by fibroblasts leads to fibrosis due to excessive amounts of growth factors (platelet-derived growth factor, transforming growth factor β), fibrogenic cytokines (IL-1 and TNFα), and angiogenic factors (fibroblast growth factor [FGF], vascular endothelial growth factor [VEGF]). Chronic inflammation can also lead to the formation of granulomas, cellular masses consisting of activated macrophages surrounded by activated lymphocytes.

There are myriad anti-inflammatory drugs available (see Chapter 42). The newest group of anti-inflammatory agents, whose use is limited to chronic inflammatory conditions, aims to eliminate proinflammatory cytokines through the use of monoclonal antibodies or soluble receptors (typically a truncated receptor encompassing only the ligand-binding, extracellular domain). *Infliximab*, *adalimumab*, *certolizumab*, and *golimumab* are monoclonal antibodies that bind and neutralize TNFα; *etanercept* is a TNFα receptor fusion protein with the same goal.

Immune System–Related Conditions and Immune Checkpoint Modulators

There are pathological conditions to which the immune system contributes, such as overreactions (allergy, autoimmunity, transplant rejection) or insufficient responses (immune deficiencies, cancer). The basic elements that lead to the activation of B and T cells have been identified for several decades; understanding the fine-tuning of these triggering steps has taken longer. In recent years, the area of immune checkpoints and immune system modulation has advanced rapidly to provide clinically important treatments. These basic elements can exist as soluble factors or as cell-surface receptors (co-receptors).

The components of the immune modulation fall into two categories, *those that enhance* the responses that are triggered by engagement of the antigen receptor, and *those that attenuate* immune responses; the latter play a role in terminating immune responses that are no longer necessary and in preventing excessive immune responses that have potentially fatal consequences (e.g., anaphylactic shock, septic shock). Both types of modulation primarily alter T-cell responses, affecting B-cell function only indirectly for the most part. Engagement of these modulatory co-receptors *per se* produces little if any response; co-engagement with the antigen receptor is required to produce their immunomodulatory function. The ultimate T-cell response depends on the effects of simultaneous occupation of the TCR, of inhibitory or activating co-receptors, and of various cytokine receptors. The following sections present examples of immune system–related conditions and the utility of immunomodulatory biological agents.

Hypersensitivity Reactions

The immune system mobilizes a number of effector mechanisms to eliminate pathogens from the body. These effector mechanisms typically generate a localized inflammatory response that effectively eliminates the pathogen, with minimal collateral damage to the surrounding tissue. Besides pathogens, humans come into contact with numerous foreign antigens, such as plant pollen and food. Contact with these environmental antigens does not elicit an immune response in the majority of individuals. However, in certain predisposed individuals, the immune system can mount a response to these generally innocuous antigens, resulting in tissue damage that ranges from mild irritation to life-threatening anaphylactic shock. These immune responses are referred to as allergic reactions or hypersensitivity reactions. Hypersensitivity reactions can be divided into four categories, type I to type IV, distinguished by the cell types and effector molecules involved (Burmester et al., 2003).

Type I Hypersensitivity: Immediate Hypersensitivity Reactions

Type I hypersensitivity reactions require that an individual first produces IgE antibodies on initial encounter with an antigen, also referred to as an allergen. After the antigen is cleared, the remaining antigen-specific IgE molecules will be bound by mast cells, basophils, and eosinophils that express receptors for the IgE constant region (FcεR1). This process is referred to as sensitization. On subsequent exposure to antigen, cross-linking of the IgE molecules on sensitized cells induces their immediate degranulation. The release of inflammatory mediators such as histamine, leukotrienes, and prostaglandins causes vasodilation, bronchial smooth muscle contraction, and mucus production similar to that seen during inflammatory responses to tissue injury and infection. Type I hypersensitivity reactions can be local or systemic. Systemic reactions against peanut or bee venom antigens can result in anaphylaxis, a potentially life-threatening condition.

Allergic asthma is an example of type I hypersensitivity. On exposure to certain allergens (typically inhaled), individuals with allergic asthma experience inflammation of the airways, characterized by tissue swelling and excessive mucus production. This narrowing of the airways makes it difficult to breathe (see Chapter 44).

Type II Hypersensitivity: Antibody-Mediated Cytotoxic Reactions

Type II hypersensitivities are antibody-mediated cytotoxic reactions. One example is the immunization to erythrocyte antigens during pregnancy. In an Rh-negative mother with an Rh-positive fetus (Rh inherited from the father), the mother forms antibodies against the Rh antigen when fetal blood cells come into contact with the maternal immune system, typically during delivery. If a subsequent pregnancy with an Rh-positive fetus occurs, maternal IgG antibodies can cross the placenta and cause hemolysis of fetal Rh-positive erythrocytes. Close monitoring and adequate symptomatic treatments (e.g., plasma exchange, intrauterine infusion, Rh immunoglobulin) are prescribed, as fetal symptoms can range from mild to potential fetal death from heart failure.

Type III Hypersensitivity: Immune Complex–Mediated Reactions

Type III hypersensitivity reactions are mediated by antibody-antigen complexes that form during an immune response. When not properly cleared, these immune complexes can settle into various tissues, where they induce complement activation. These immune complexes are of particular concern in the kidney, where they can lead to glomerulonephritis and kidney failure. In the past, type III hypersensitivity reactions fell largely in the realm of autoimmune diseases (e.g., systemic lupus erythematosus); their incidence rate has significantly risen with the introduction of nonhuman or nonhumanized monoclonal antibodies as pharmacological agents (human antimouse antibodies). Murine or murine-human (mu-hu) chimeric (chim) therapeutic monoclonal antibodies are "mistaken" by the patient's immune system as potentially dangerous, foreign antigens. The resulting immune response not only "defuses" the therapeutic antibody but also promotes the formation of antibody(mu)-antibody(hu) or antibody(chim)-antibody(hu) complexes that trigger type III hypersensitivity reactions.

Type IV Hypersensitivity: Delayed Hypersensitivity Reactions

Unlike types I to III hypersensitivity reactions, which are antibody mediated, type IV reactions are mediated by T cells. However, all these hypersensitivity reactions are memory responses. Haptens are molecules that are too small to function as antigens on their own. These molecules penetrate the epidermis and bind to carrier proteins in the skin. Hapten-carrier complexes are detected by APCs in the skin (Langerhans cells), which then migrate to the lymph nodes and prime T-cell responses. When an individual is re-exposed to the hapten, antigen-specific T cells migrate to the skin, causing local inflammation and edema. Nickel in clothing and jewelry is a common trigger of type IV hypersensitivity reactions.

Autoimmunity, Immune Deficiency, and Transplant Rejection

Just as for a regular and appropriate immune response, autoimmunity is founded in either humoral (autoantibody) or cellular (T-cell) responses. As described in the section on lymphocyte development, the process of central tolerance limits the development of autoreactive B and T cells. This process is imperfect, and mechanisms of peripheral tolerance are in place to limit the activity of self-reactive lymphocytes that manage to escape thymic deletion. Peripheral tolerance is primarily mediated by two mechanisms: the action of T_{Reg} cells (see section on CD4 T_H-cell effector functions) and the induction of T-cell anergy. Naïve T cells require costimulatory signals to become activated. Consequently, autoreactive T cells typically will not become activated if they interact with an MHC molecule expressing self-antigen because most tissues do not express costimulatory molecules. Induction of anergy leaves T cells unresponsive, even on subsequent exposure to antigen with sufficient costimulation.

Autoimmunity: A Breach of Tolerance

There are several theories about the origins of individual autoimmune disorders:

- *Molecular Mimicry.* The hypothesis of molecular mimicry reasons that unique pathogen-derived antigens resemble endogenous host antigens. If an infection occurs, the immune system's defensive arsenal (antibodies, cytotoxic T lymphocytes, and NK cells) not only attack the pathogen-derived antigen but also assault the host's structurally similar antigen, thus causing autoimmunity in the form of collateral damage.
- *Relationship Between Autoimmunity and the HLA System.* Individuals with specific HLA types are more likely to develop certain autoimmune diseases (e.g., type 1 diabetes, ankylosing spondylitis, celiac disease, systemic lupus erythematosus). A reasonable explanation for this observation might be found in the fact that particular HLA proteins are more "efficient" than others in presenting antigens and consequently might erroneously activate T cells.
- *Altered Thymic Function.* Thymic T-cell selection is crucial to central tolerance; type I IFNs, which are highly induced during infectious events, also govern several steps in T-cell selection. Therefore, pathogen-induced disturbances to thymic events might negatively affect elimination of autoreactive T cells. Regardless of the mechanism, central tolerance has thus far not been exploited for pharmacological intervention.

Immune Deficiencies

Primary immunodeficiency encompasses genetic or developmental defects in the immune system that leave the individual susceptible to infections to various degrees. Severe forms (severe combined immunodeficiency) are typically diagnosed in early childhood and are associated with significantly reduced life expectancy. Presently, nine classes of primary immunodeficiency are recognized, totaling over 120 unique conditions. Unfortunately, current treatment options are limited to supportive therapy in the form of antiviral, antifungal, and antibacterial drugs.

Acquired immunodeficiency refers to the loss of immune function due to environmental exposure. These conditions encompass patients receiving immune-suppressive therapy for autoimmune disorders or to prevent transplant rejections. Acquired immunodeficiency is also commonly observed in patients suffering from hematopoietic malignancies, as tumor cells outcompete functional leukocytes for space in the bone marrow or blood. Probably the most common use for the term, however, is in connection with HIV infection, the underlying cause for AIDS (see Chapter 64).

Transplant Rejection

"Host-versus-graft disease" or "graft-versus-host disease" results from the immunological rejection of a transplanted tissue by the recipient's immune system, or in cases where bone marrow is transplanted, the "new" immune system might attack the host's tissues. The intensity of rejection is minimized with increased compatibility between donor and recipient; however, a lifelong regimen of immunosuppressive drugs is unavoidable (see Chapter 39).

Classical immunosuppressive therapy employs glucocorticoids (e.g., *prednisone*), inhibitors of T-cell activation (e.g., *cyclosporine*), T-cell proliferation inhibitors (e.g., mycophenolic acid), or mTOR inhibitors (e.g., *sirolimus*) that inhibit production of IL-2, a cytokine essential for T-cell activation and proliferation. Treatment of transplant rejection also has benefitted from advances in monoclonal antibody therapy. Antibodies directed against the IL-2 receptor (e.g., *daclizumab*) or CD20 (e.g., *rituximab*) are now available to prevent transplant rejection (see Figure 39–2).

Cancer Immunotherapy: Immune Checkpoints and Their Inhibitors

As described previously, T-cell responses are modulated by a balance between costimulatory signals, exemplified by CD28 ligation, and coinhibitory signals, such as those provided by CTLA-4 or PD-1 ligation (Figure 38–7). *Immune checkpoints* refer to inhibitory (often negative feedback) regulators that limit the amplitude and duration of an immune response. Under normal physiological conditions, immune checkpoints protect tissues from damage during an immune response and contribute to the maintenance of self-tolerance. Thus, immune checkpoints act as physiological brakes on the immune system and are essential for the maintenance of immune homeostasis and the prevention of autoimmunity.

Cancer cells express a variety of genetic and epigenetic alterations that distinguish them from healthy cells. These tumor-associated antigens can be recognized by the host antitumor T cells, which can kill the transformed tumor cells. Unfortunately, a tumor can often progress despite a high infiltration of T cells as a result of T-cell dysfunction and the development of immuno-evasive mechanisms by the tumor cells. In particular, *immune checkpoint molecules* become highly expressed on the surface of T cells in the tumor microenvironment, an expression upregulated by T-cell exhaustion. The two most widely studied immune checkpoint molecules are CTLA-4 and PD-1.

CTLA-4 and PD-1 act through different mechanisms to inhibit TCR signaling and reduce T-cell activation and function. B7 (CD80/CD86) on the tumor cell has a higher affinity for the CTLA-4 expressed on T cells than for CD28 on the T cell; thus, a coinhibitory interaction forms in preference to a costimulatory interaction (see Figure 38–7). By effectively displacing stimulatory CD28/B7 interactions, CTLA-4 can prevent costimulation, thereby dampening TCR signaling. PD-1 on T cells can bind PD-L1 expressed by APCs and tumors, and this interaction dampens TCR and CD28 signaling, also reducing T-cell proliferation and immune function. Thus, these inhibitory signals induce a dysfunctional "exhausted" state in T cells that limits their ability to kill and destroy tumor cells, thereby permitting tumors to grow and metastasize.

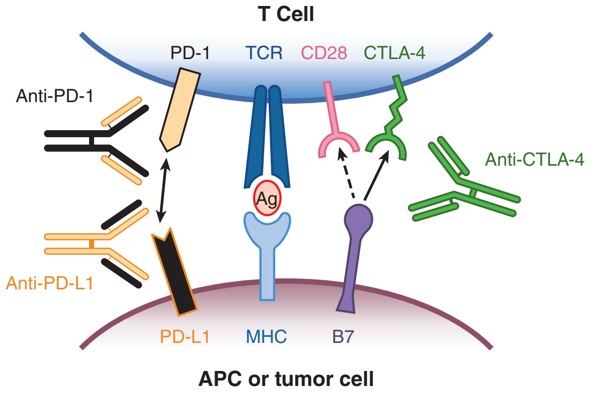

Figure 38–7 *Immune checkpoint evasion.* Via the TCR, a T cell can detect and bind an antigen (Ag) presented by the MHC of a tumor cell. If there is a satisfactory costimulatory receptor, the T cell will be activated to proliferate and mount an immune response against the tumor cell. However, in the tumor microenvironment, immune checkpoint molecules such as CTLA-4 and PD-1 may be highly expressed on the surface of the T cell, an expression upregulated by T-cell exhaustion. If the tumor cell expresses immune checkpoint ligands (B7 and PD-L1 are shown), then binding of the cognate coinhibitory ligands on the T cell can occur. The affinity of B7 for CTLA-4 exceeds its affinity for CD28, thereby replacing a costimulatory pairing with a coinhibitory one. Thus, in this example, two coinhibitory pairings can occur. The result is an inhibition of T-cell function. Monoclonal antibodies to PD-1 or PD-L1 can remove that checkpoint, and anti–CTLA-4 can remove that inhibitory checkpoint, restoring T-cell function. James Allison and Tasuku Honjo shared the 2018 Nobel Prize in Medicine/Physiology "for their discovery of cancer therapy by inhibition of negative immune regulation."

Immune checkpoint inhibitors (ICIs) are antibodies that block CTLA-4, PD-1, or PD-L1, and their mechanism of action is to ligate these proteins to prevent their TCR inhibitory function (see Figure 38–7) (Cruz et al., 2020; Pardoll, 2012; Tang et al., 2016). ICIs have had impressive efficacy in patients with a number of cancers (e.g., melanoma) and are now the standard therapy. While subsets of cancer patients respond to ICIs, combination therapies targeting multiple immune checkpoints can increase clinical responses. ICIs have induced clinical responses and long-term progression-free survival in patients with metastatic melanoma and partial responses in patients with lung, kidney, and colon cancer. ICIs work through various mechanisms that reinvigorate antitumor T cells within the tumor and the lymph nodes draining the tumor.

In addition to these immune checkpoints, additional ICIs have been identified and are being tested in clinical trials. Figure 38–4 provides an overview of activating and inhibitory coreceptors on T cells and the drugs (monoclonal antibodies) that target these inhibitory pathways. In general, these antibodies work by releasing the "brake" on antitumor T cells, which reinvigorates these to kill tumors. It is important to be aware that some monoclonal antibodies block the immune checkpoint itself (PD-1), while others block their respective ligand (PD-L1). The clinical utility of modulating these pathways in cancer therapy is discussed in Chapter 72.

Immune checkpoint inhibitors hold great promise for treating cancer patients with advanced disease, as evidenced by the recent success of clinical trials and FDA approvals. Biologics to stimulate antitumor T cells have been approved by the FDA and are the first line of treatment of cancers such as metastatic melanoma, non–small cell lung cancer, and renal cell carcinoma. In addition, anti–PD-1, anti–PD-L1, and anti–CTLA-4 therapies are currently in clinical trials to assess their efficacy in head and neck cancers, breast cancer, small cell lung cancer, Hodgkin lymphoma, gastric cancer, hepatocellular carcinoma, bladder cancer, ovarian cancer, colon cancer, and Merkel cell carcinoma. It is important to note that only a small fraction of patients respond to ICIs; this frequency can increase when patients are given combination therapy, such as administering both anti–PD-1 and anti–CTLA-4 antibodies or combining ICIs with radiation or chemotherapy. One consequence of ICI therapy is the development of immune-related adverse events in which patients can develop toxicities including hepatitis, pneumonitis, colitis, rash, vitiligo, and endocrine pathology. Increased efficacy of ICIs will likely be achieved when additional drugs are developed to target other inhibitory pathways in combination, but caution must be used to ensure patient safety (Callahan et al., 2016).

In addition to solid tumors, liquid tumors like CLL (chronic lymphocytic leukemia) are also being targeted by immunotherapeutic approaches. Patient T cells can be engineered to express chimeric antigen receptors (CARs) comprising antibody-binding domains connected to domains that activate T cells. In the case of CLL, CAR-T cells recognize CD19 on B cells, and their chimeric receptor sustains T activation. CAR-T cells are engineered from patient blood, expanded *in vitro*; then, millions are infused into the same patient. These cells circulate in the patient and recognize and destroy all B cells expressing CD19. This cellular therapy has shown promise in patients with CLL, resulting in highly durable objective responses (Kalos et al., 2011). Complementing ICIs and CAR-T cells, many additional immunotherapies are currently being investigated in clinical trials. Examples include cancer vaccines, adaptive cell therapies using DCs and tumor-infiltrating lymphocytes, oncolytic viruses, antibody-antigen conjugates, antibody-drug conjugates, and cytokines.

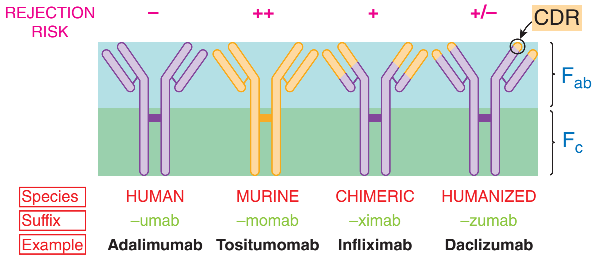

Figure 38–8 *Former nomenclature of therapeutic monoclonal antibodies.* This older nomenclature, still in use by some workers, focused primarily on the source of the antibody (murine, human, chimeric, or humanized). Current nomenclature (Table 38-1) incorporates information on the target tissue as well. Abbreviations: Fab, antigen-binding fragment; Fc, crystallizable fragment; CDR, complementarity-determining regions of the variable domains, also called hypervariable regions.

A Note on Nomenclature

The naming of monoclonal antibodies has changed over the years. The current system, shown in Table 38–1, is a four-component system, with each antibody having a unique signifying syllable followed by syllables for target tissue, source organism, and the conserved suffix, *mab*; for example, ri-tu-xi-mab. A further revision of the nomenclature is in progress.

A former system, pictured in Figure 38–8, is also still in use.

TABLE 38–1 ■ CURRENT NOMENCLATURE FOR THERAPEUTIC MONOCLONAL ANTIBODIES

UNIQUE PREFIX	TARGET TISSUE		SOURCE ORGANISM		CONSERVED SUFFIX
variable	*-o(s)-*	bone	*-u-*	human	-mab
	-vi(r)-	viral	*-o-*	mouse	
	-ba(c)-	bacterial	*-a-*	rat	
	-li(m)-	immune	*-e-*	hamster	
	-le(s)-	infectious lesions	*-i-*	primate	
	-ci(r)-	cardiovascular	*-xi-*	chimeric	
	-mu(l)-	musculoskeletal	*-zu-*	humanized	
	-ki(n)-	interleukin	*-axo-*	rat/murine hybrid	
	-co(l)-	colonic tumor			
	-me(l)-	melanoma			
	-ma(r)-	mammary tumor			
	-go(t)-	testicular tumor			
	-go(v)-	ovarian tumor			
	-pr(o)-	prostate tumor			
	-tu(m)-	miscellaneous tumor			
	-neu(r)-	nervous system			
	-tox(a)-	toxin as target			
Examples: Beva	ci		zu		mab
Ri	tu		xi		mab
Ala	ci		zu		mab
Glemba	tum		u		mab

Current nomenclature incorporates information on the source of the antibody as well as the intended target tissue. An older nomenclature, still used by some workers, focuses on the source of the antibody (see Figure 38–8).

Engineered (Recombinant) Antibodies

Most engineered antibodies are based on the use of the heavy and light chain, or fragments thereof, of the variable region of immunoglobulins. They are derived from hybridomas created through the fusion of mortal, antibody-producing primary B cells with immortal myeloma cells. The resulting immortal antibody-producing cell can be expanded into a large culture to produce unlimited quantities of antibodies of identical specificity (hence, "monoclonal"), whereby each fusion cell will give rise to a unique hybridoma with a distinct antibody product. The antigen specificity of each Ig molecule depends on the makeup of the paratope (antigen-binding site), which comprises six complementarity-determining regions. A large number of mAbs are currently used therapeutically (see Table 40–4).

F(ab) fragments represent enzymatic cleavage products derived from monoclonal antibodies (papain can cleave an immunoglobulin monomer into two monovalent F(ab) fragments and an Fc fragment). Similarly, pepsin cleaves below the hinge region, producing a bivalent $F(ab')_2$ fragment (Figure 38–9). These antibody derivatives can be produced by cell-based methods rather than molecular biological techniques. The valency of the Ig or F(ab) molecule will determine the functional properties of the product: bivalent Igs and their derivatives can either bind their target and neutralize it (e.g., blocking or competing Igs) or act as activators of cell surface receptors by forcing two of them via their two antigen-binding sites into proximity sufficient to trigger transactivation. In these cases, the antigen specificities of both F(ab) domains are identical; intact Igs or $F(ab')_2$ fragments can promote the formation only of homodimers of their antigens. F(ab) fragments of single valency can interact with only a single antigen molecule and thus can act only as neutralizing or blocking agents.

Engineered bivalent *bispecific* Ig or $F(ab')_2$ fragments create the opportunity for a single Ig or $F(ab')_2$ molecule to bind *two* cognate yet distinct antigens. This feature allows for the forced formation of various heterodimers (e.g., activation of cell surface receptors that are made up of two distinct chains).

The bivalent antibody *amivantamab* employs this engineered duality to expand treatment of EGFR-mutated non-small cell lung cancer (NSCLC). *Amivantamab* targets both EGFR and c-MET (mesenchymal-epithelial transition receptor) on the surface of the NSCLC cells. This bivalent complex provides three modes of attack:

1. Interaction of the bivalent mAB with EGFR and c-MET downregulates oncogenic signaling via these receptors and causes their heterodimerization and internalization.

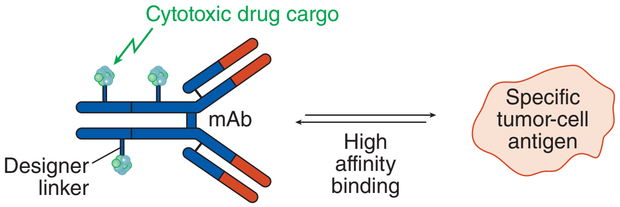

Figure 38-10 *Antibody-drug conjugates (ADCs).* The antibody of an ADC should have low immunogenicity, a long half-life, and a high affinity for the tumor antigen. The target antigen should as specific to the tumor cell as as possible, with high expression on the tumor cell and little or no expression elsewhere. The cytotoxic drug cargo, usually a small molecule, should be effective in the sub-nM range. The linker should keep the drug and mAb attached in the plasma and until the mAb has bound to the tumor antigen, when the drug-linker bonds may be severed to release active drug at the tumor site in response to local conditions (pH, proteolytic enzymes: a "cleavable" linker) or via lysosomal degradation of the ADC within the tumor cell following endocytosis (a "non-cleavable" linker). The average number of drug molecules bound per mAb molecule ranges from 2 to 8 amongst currently approved ADCs. Only a fraction of administered drug reaches the targeted cells; thus the cytotoxic drug should be potent and one must anticipate off-target effects of the drug.

2. The mAb's Fc-dependent interaction with NK cells and with the EGFR promotes NK cell-mediated cellular toxicity of the cancer cell.
3. The mAb's Fc-dependent interaction with macrophages promotes macrophage-mediated trogocytosis (Guo, et al, 2021).

Antibodies can also be designed to carry, target, and deliver small molecule drugs to discrete sites, such as to the surface of a tumor cell. Antibody-drug conjugates (ADCs) are increasingly being approved for use in cancer chemotherapy to deliver a cytotoxic cargo to specific cells expressing the target antigen. Figure 38–10 shows the basic design of an ADC. Molecules of the drug (the cargo or payload) are connected to the mAb by a bespoke linker that attaches to amino acid side chains on the antibody (usually cys or lys residues) and to the drug molecule. The linker is designed to prevent the drug's release in the plasma following injection but to permit the drug's release in conditions at the tumor site (e.g., low pH, proteolytic enzymes) or after endocytosis of the ADC into the tumor cell. Table 38–2 shows the ADCs currently FDA-approved as

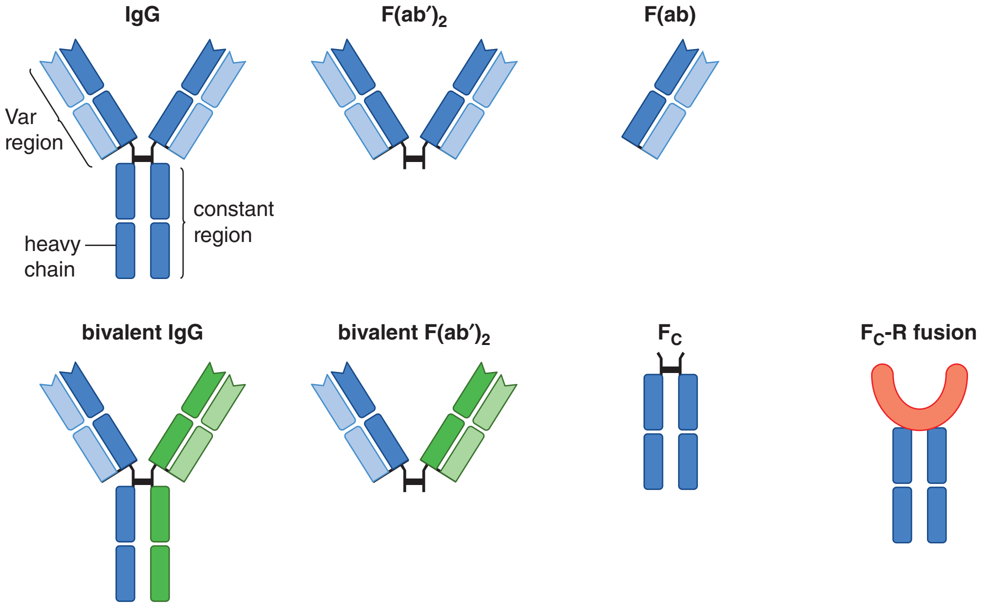

Figure 38–9 *Engineered antibodies and fragments.* See text for details.

TABLE 38–2 ■ FDA-APPROVED ANTIBODY-DRUG CONJUGATES FOR CANCER THERAPY*

mAb	mAb TARGET	CYTOTOXIC DRUG CARGO	DRUG ACTION
Belantamab[h]	BCMA	methyl auristatin F	microtubule inhibition
Brentuximab[h]	CD30	methyl auristatin E	microtubule inhibition
Enfortumab[s]	Nectin 4	methyl auristatin E	microtubule inhibition
Famtrastumab[s]	HER2	Dxd-camptothecin	topoisomerase 1 inhibition
Gemtusumab[h]	CD33	Ozogamicin-calicheamicin	DNA cleavage
Inotuzumab[h]	CD22	Ozogamicin-calicheamicin	DNA cleavage
Loncastuximab[h]	CD19	pyrrolobenzodiazepine dimer	DNA cleavage
Polatuzumab[h]	CD79b	methyl auristatin E	microtubule inhibition
Sacituzumab[s]	Trop2	SN-38/campothecin	topoisomerase 1 inhibition
Tisotumab[s]	Tissue factor	methyl auristatin E	microtubule inhibition
Trastuzumab[s]	HER2	mertansine (DM1)	microtubule inhibition

*These ADCs are designed for use in treating hemotological malignancies[h] and certain solid tumors[s]. See current FDA-approved label for approved indications and prescribing information. See also Chapter 72. Similar conjugates, known as immunotoxins, use a targeting protein coupled to a toxin. For example, *moxetumomab pasudotox-tdfk* is the Fv portion of an antibody to CD22, linked to a 38kDa fragment of Pseudomonas endotoxin A and used in treating refractory hairy cell leukemia in adults. Other examples of FDA-approved immunotoxins are *denileukin diftitox* and *tagraxofusp-erzs*.

Abbreviations: BCMA, B cell maturation antigen; HER2, human epidermal growth factor receptor 2; Trop2, tumor-associated Ca^{2+} signal transducer 2. For more detailed information, see Tong, et al. (2021), Ceci et al. (2022), and Fu, et al (2022).

cancer therapeutics, noting the molecular target of the mAb, the cargo, and that drug's action.

Molecular biology facilitates the advanced rearrangement, fusion, and editing of the antibody coding genes. These genes typically consist of heavy and light chain domains of the variable region of Ig. The simplest form of an antigen binding protein is the so-called single-chain variable fragment (scFv) in which the heavy and light chain fragments that comprise the antigen binding site are assembled as a linear fusion protein with a connecting linker region rather than being held together by disulfide bonds as in the natural Ig. Functionally, scFvs mimic F(ab) fragments. A rather unique approach is the fusion of the Ig Fc domain to the ligand binding domain of a cytokine receptor (as in *etanercept*). The resulting product binds and neutralizes the respective cytokine via its ligand binding domain, and the Fc portion extends the half-life of the recombinant fusion protein in the bloodstream.

Gene editing techniques such as CRISPR/Cas9 bring previously unimaginable opportunities in the creation of antibodies with desired specificities. Previous cloning techniques allowed for the reassembling of Ig domains into novel orders and facilitated the generation of chimeric or humanized monoclonal antibodies, but these techniques did not alter the antigen specificity of the resulting new molecule. Site-specific mutations within the complementarity-determining region created using CRISPR/Cas9 open up the possibility to introduce mutations into the antigen binding site of all of the aforementioned antibodies and their derivatives, theoretically eliminating the need for new immunizations.

As the clinically oriented chapters of this book demonstrate, combinations of these various techniques are yielding an unprecedented expansion in the availability of new antibodies against pathological conditions ranging from cancer to autoimmunity, and from immune hyperreactions and chronic inflammation to immune deficiencies.

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Chapter 39

Immunosuppressants, Immunomodulation, and Tolerance

Carla V. Rothlin and J. Silvio Gutkind

THE IMMUNE RESPONSE

IMMUNOSUPPRESSION

- General Approach to Organ Transplantation Therapy
- Glucocorticoids
- Calcineurin Inhibitors
- Antiproliferative and Antimetabolic Drugs
- Sphingosine-1-Phosphate Receptor Modulators
- Other Antiproliferative and Cytotoxic Agents
- Immunosuppression Antibodies and Fusion Receptor Proteins

IMMUNOMODULATION

- Immunotherapy and the Nature of Costimulation and Inhibition
- Interleukin Antagonists
- Inhibition of Cytokine Signaling: JAK Inhibitors
- Inhibition of Lymphocyte Function–Associated Antigen
- Biologicals Targeting Integrins
- Cytokine Therapy
- Targeting B Cells

TOLERANCE

- Costimulatory Blockade
- Donor Cell Chimerism
- Antigens
- Soluble HLA

IMMUNOTHERAPY FOR MULTIPLE SCLEROSIS

- Clinical Features and Pathology
- Pharmacotherapy

This chapter reviews the components of the immune response and drugs that modulate immunity via immunosuppression, tolerance, or neutralization of cytokine signaling. Four major classes of immunosuppressive drugs are discussed: glucocorticoids, calcineurin inhibitors, antiproliferative and antimetabolic agents, and antibodies or small molecules that target cytokine signaling. While there are similarities, the approach to the use of immunosuppressant drugs in transplant rejection has evolved separately from the approaches used to treat autoimmune disease and thus is presented separately.

The Immune Response

The immune system evolved to discriminate self from non-self. *Innate immunity* (natural immunity) is primitive, based on the recognition of conserved molecular patterns of microorganisms and, as such, broadly reactive. *Adaptive immunity* (learned immunity) is antigen specific, depends on antigen exposure or priming, and can be of very high affinity. The two arms of immunity work closely together, with the innate immune system most active early in an immune response and adaptive immunity becoming progressively dominant over time.

The major effectors of *innate immunity* are complement, granulocytes, monocytes/macrophages, natural killer (NK) cells, innate lymphoid cells, mast cells, and basophils. The major effectors of *adaptive immunity* are B and T lymphocytes. B lymphocytes make antibodies; T lymphocytes function as helper, cytolytic, and regulatory (suppressor) cells. These cells not only are important in the normal immune response to infection and tumors but also mediate transplant rejection and autoimmunity.

Immunoglobulins (antibodies) produced by B lymphocytes can recognize a large variety of specific structural conformations. In contrast, T lymphocytes recognize antigens as peptide fragments in the context of major histocompatibility complex II (MHC II) proteins (called human leukocyte antigens [HLAs] in humans) on the surface of antigen-presenting cells (APCs), such as dendritic cells and macrophages, or on MHC I, which is expressed by all nucleated cells. Once activated by specific antigen recognition, both B and T lymphocytes are triggered to divide and differentiate, leading to release of soluble mediators (cytokines, chemokines) and antibodies in the case of B cells that perform as effectors and regulators of the immune response. Chapter 38 presents a more detailed view of the immune system at the levels of the molecules, cells, and organs involved in immunity.

Immunosuppression

Immunosuppressive drugs are used to dampen the immune response in organ transplantation and autoimmune disease. In transplantation, the major classes of immunosuppressive drugs used today are:

- Glucocorticoids
- Calcineurin inhibitors
- Antiproliferative/antimetabolic agents
- Biologicals (antibodies)

Table 39–1 summarizes the sites of action of representative immunosuppressants on T-cell activation. These drugs are successful in treating conditions such as acute immune rejection of organ transplants and autoimmune diseases. However, such therapies often require lifelong use and nonspecifically suppress the entire immune system, exposing patients in some instances to higher risks of infection and cancer. The calcineurin inhibitors and daily glucocorticoids, in particular, are nephrotoxic and diabetogenic, respectively, thus restricting their usefulness in a variety of clinical settings.

Monoclonal and polyclonal antibody preparations directed at both T cells and B cells or against cytokines such as tumor necrosis factor (TNF) α are important therapies providing an opportunity to more specifically target immune pathways. Biologicals designed to deplete B cells (anti-CD20) are approved for the treatment of relapsing-remitting and primary progressive multiple sclerosis (MS). Newer small molecules and antibodies have expanded the arsenal of immunosuppressives. In particular, inhibitors of the mammalian target of rapamycin (mTOR; *sirolimus, everolimus, temsirolimus*) (Budde et al., 2011; Euvrard et al., 2012) and anti-CD25 (interleukin 2 receptor [IL-2R]) and various antibodies (e.g., *basiliximab, daclizumab*) (Nashan, 2005) target growth factor pathways. *Belatacept* and others inhibit T-cell costimulation (see Figures 39–2 and 39–4). Thus, there

Abbreviations

ALG: antilymphocyte globulin
APC: antigen-presenting cell
ATG: antithymocyte globulin
AUC: area under the plasma concentration–time curve
CAPS: cryopyrin-associated periodic syndromes
CLL: chronic lymphocytic leukemia
CTLA-4: cytotoxic T-lymphocyte–associated antigen 4
CYP: cytochrome P450
GA: glatiramer acetate
GI: gastrointestinal
GM-CSF: granulocyte-macrophage colony-stimulating factor
GVHD: graft-versus-host disease
HLA: human leukocyte antigen
HSC: hematopoietic stem cell
IFN: interferon
Ig: immunoglobulin
IL: interleukin
IL-1RA: IL-1 receptor antagonist
IL-2R: interleukin 2 receptor
JAK: Janus kinase
JAKinib: Janus kinase inhibitor
JCV: John Cunningham virus
LDL: low-density lipoprotein
LFA: lymphocyte function–associated antigen
mAb: monoclonal antibody
MAO: monoamine oxidase
MHC: histocompatibility complex
MMF: mycophenolate mofetil
6-MP: 6-mercaptopurine
MPA: mycophenolic acid
MPAG: MPA glucuronide
MS: multiple sclerosis
mTOR: mammalian target of rapamycin
NFAT: nuclear factor of activated T lymphocytes
NK: natural killer
PD-1: programmed cell death protein 1
PD-L1: programmed death ligand 1
PML: progressive multifocal leukoencephalopathy
S1P: sphingosine-1-phosphate
S1PR: sphingosine-1-phosphate receptor
STAT: signal transducer and activator of transcription
TCR: T-cell receptor
TNF: tumor necrosis factor
TYK2: tyrosine kinase 2
VZV: varicella-zoster virus

are useful pharmacological tools that can substantially limit clonal expansion and potentially promote tolerance (Goldfarb-Rumyantzev et al., 2006; Halloran, 2004; Krensky et al., 1990).

General Approach to Organ Transplantation Therapy

Organ transplantation therapy is organized around five general principles.

1. Carefully prepare the patient and select the best available ABO blood type–compatible HLA match for organ donation.
2. Employ multitier immunosuppressive therapy and simultaneously use several agents, each of which is directed at a different molecular target within the allograft response. Synergistic effects permit use of the various agents at relatively low doses, thereby limiting specific toxicities while maximizing the immunosuppressive effect.
3. Employ intensive induction and lower-dose maintenance drug protocols. Greater immunosuppression is required to gain early engraftment or to treat established rejection than to maintain long-term immunosuppression. The early high risk of acute rejection is replaced over time by the increased risk of the medications' side effects, necessitating a slow reduction of immunosuppressive drugs used for maintenance.
4. Investigate each episode of transplant dysfunction, evaluating for recurrence of the disease, rejection, drug toxicity, and infection (keeping in mind that these various problems can, and often do, coexist).
5. Reduce dosage or withdraw a drug if its toxicity exceeds its benefit (Danovitch et al., 2007).

TABLE 39–1 ■ SITES OF ACTION OF SELECTED IMMUNOSUPPRESSIVE AGENTS ON T-CELL ACTIVATION

DRUG	SITE (AND MECHANISM) OF ACTION
Glucocorticoids	Glucocorticoid response elements in DNA (regulate gene transcription)
Cyclosporine	Calcineurin (inhibits phosphatase activity)
Tacrolimus	Calcineurin (inhibits phosphatase activity)
Azathioprine	DNA (false nucleotide incorporation)
Mycophenolate mofetil	Inosine monophosphate dehydrogenase (inhibits activity)
Sirolimus	mTOR, protein kinase involved in cell-cycle progression (inhibits activity)
Everolimus	mTOR, protein kinase involved in cell-cycle progression (inhibits activity)
Belatacept	Costimulatory ligands (CD80 and CD86) present on antigen presenting cells (inhibits activity)
Alemtuzumab	CD52 protein, widely expressed on B cells, T cells, macrophages, NK cells (induces lysis)
Muromonab-CD3	T-cell receptor complex (blocks antigen recognition)
Daclizumab, basiliximab	IL-2R (block IL-2–mediated T-cell activation)

Biological Induction Therapy

In many transplant centers, induction therapy with biological agents is used to delay the use of the nephrotoxic calcineurin inhibitors or to intensify the initial immunosuppressive therapy in patients at high risk of rejection (i.e., repeat transplants, broadly presensitized patients, African American patients, or pediatric patients). This strategy has been an important component of immunosuppression since the 1960s, when Starzl and colleagues demonstrated the beneficial effect of antilymphocyte globulin (ALG) in the prophylaxis of rejection. Two preparations are FDA-approved for use in transplantation: lymphocyte immune globulin (Atgam) and antithymocyte globulin (ATG; Thymoglobulin) (Brennan et al., 2006; Nashan, 2005). ATG is the most frequently used depleting agent. *Alemtuzumab*, a humanized anti-CD52 monoclonal antibody (mAb) that produces prolonged lymphocyte depletion, is approved for use in chronic lymphocytic leukemia (CLL) and MS but is increasingly used off-label as induction therapy in transplantation (Jones and Coles, 2014).

Most limitations of murine-based mAbs generally were overcome by the introduction of chimeric or humanized mAbs that lack antigenicity and have a prolonged serum $t_{1/2}$. Antibodies derived from transgenic mice carrying human antibody genes are labeled "humanized" (90%–95% human) or "fully human" (100% human); antibodies derived from human cells are labeled "human." However, all three types of antibodies are of equal efficacy and safety. Chimeric antibodies generally contain about 33% mouse protein and 67% human protein and produce an antibody response that results in reduced efficacy and shorter $t_{1/2}$ compared to humanized antibodies.

Biological agents for induction therapy in the prophylaxis of rejection currently are used in about 70% of *de novo* transplant patients. Biological agents for induction can be divided into two groups: the *depleting agents* and the *immune modulators*. The depleting agents consist of lymphocyte immune globulin, ATG, *muromonab*-CD3 mAb (discontinued in the U.S.), and *teplizumab*-CD3 mAb (FDA-approved as breakthrough therapy in 2019). Their efficacy derives from their ability to deplete the recipient's CD3-positive cells at the time of transplantation and antigen presentation. The second group of biological agents, the anti–IL-2R mAbs, do not deplete T lymphocytes; rather, they block interleukin (IL)-2–mediated T-cell activation by binding to the α chain of IL-2R (CD25). For patients with high levels of anti-HLA antibodies and humoral rejection, more aggressive therapies include plasmapheresis, intravenous immunoglobulin, and *rituximab*, a chimeric anti-CD20 mAb (Brennan et al., 2006; Chan et al., 2011; Guerra et al., 2011; Nashan, 2005; Sureshkumar et al., 2012).

Maintenance Immunotherapy

Basic immunosuppressive therapy uses multiple drugs simultaneously, typically a calcineurin inhibitor, glucocorticoids, and *mycophenolate* (an inhibitor of purine metabolism), each directed at a discrete step in T-cell activation (Vincenti et al., 2008). Glucocorticoids, *azathioprine, cyclosporine, tacrolimus, mycophenolate, sirolimus, belatacept*, and various mAbs and polyclonal antibodies all are approved for use in transplantation.

Therapy for Established Rejection

Low doses of *prednisone*, calcineurin inhibitors, purine metabolism inhibitors, *sirolimus*, or *belatacept* are effective in preventing acute cellular rejection; they are less effective in blocking activated T lymphocytes and thus are not very effective against established, acute rejection or for the total prevention of chronic rejection. Therefore, treatment of established rejection requires the use of agents directed against activated T cells. These include glucocorticoids in high doses (pulse therapy) or polyclonal antilymphocyte antibodies.

Glucocorticoids

The introduction of glucocorticoids as immunosuppressive drugs in the 1960s played a key role in making organ transplantation possible. *Prednisone*, *prednisolone*, and other glucocorticoids are used alone and in combination with other immunosuppressive agents for treatment of transplant rejection and autoimmune disorders. The pharmacological properties of glucocorticoids are described in Chapter 50.

Mechanism of Action

Glucocorticoids have broad anti-inflammatory effects on multiple components of cellular immunity but relatively little effect on humoral immunity. Glucocorticoids bind to receptors inside cells and regulate the transcription of numerous other genes. Glucocorticoids also curtail activation of nuclear factor-κB, suppress formation of proinflammatory cytokines such as IL-1 and IL-6, inhibit T cells from making IL-2 and proliferating, and inhibit the activation of cytotoxic T lymphocytes. In addition, glucocorticoid-treated neutrophils and monocytes display poor chemotaxis and decreased release of lysosomal enzymes.

Therapeutic Uses

There are numerous therapeutic indications for glucocorticoids. They commonly are combined with other immunosuppressive agents to prevent and treat transplant rejection. Glucocorticoids also are efficacious for treatment of graft-versus-host disease (GVHD) in bone marrow transplantation. Glucocorticoids are routinely used to treat autoimmune disorders such as rheumatoid and other arthritides, systemic lupus erythematosus, systemic dermatomyositis, psoriasis and other skin conditions, asthma and other allergic disorders, inflammatory bowel disease, inflammatory ophthalmic diseases, autoimmune hematological disorders, and acute exacerbations of MS (see section on MS). In addition, glucocorticoids limit allergic reactions that occur with other immunosuppressive agents and are used in transplant recipients to block the first-dose cytokine storm caused by treatment with ATG (see Antithymocyte Globulin). Due to the long-term side effects of continuous steroid treatment, immunosuppressive protocols in transplantation include the option of early steroid withdrawal, particularly in pediatric patients (Gajardo et al., 2021). The risks of this practice continue to be evaluated, particularly in recipients of organs from deceased donors (Bae et al., 2020).

Toxicity

Extensive glucocorticoid use often results in disabling and life-threatening adverse effects. These effects include growth retardation in children, avascular necrosis of bone, osteopenia, increased risk of infection, poor wound healing, cataracts, hyperglycemia, and hypertension (see Chapter 50). The advent of combined glucocorticoid/calcineurin inhibitor regimens has allowed reduced doses or rapid withdrawal of steroids, resulting in lower steroid-induced morbidities (Vincenti et al., 2008).

Calcineurin Inhibitors

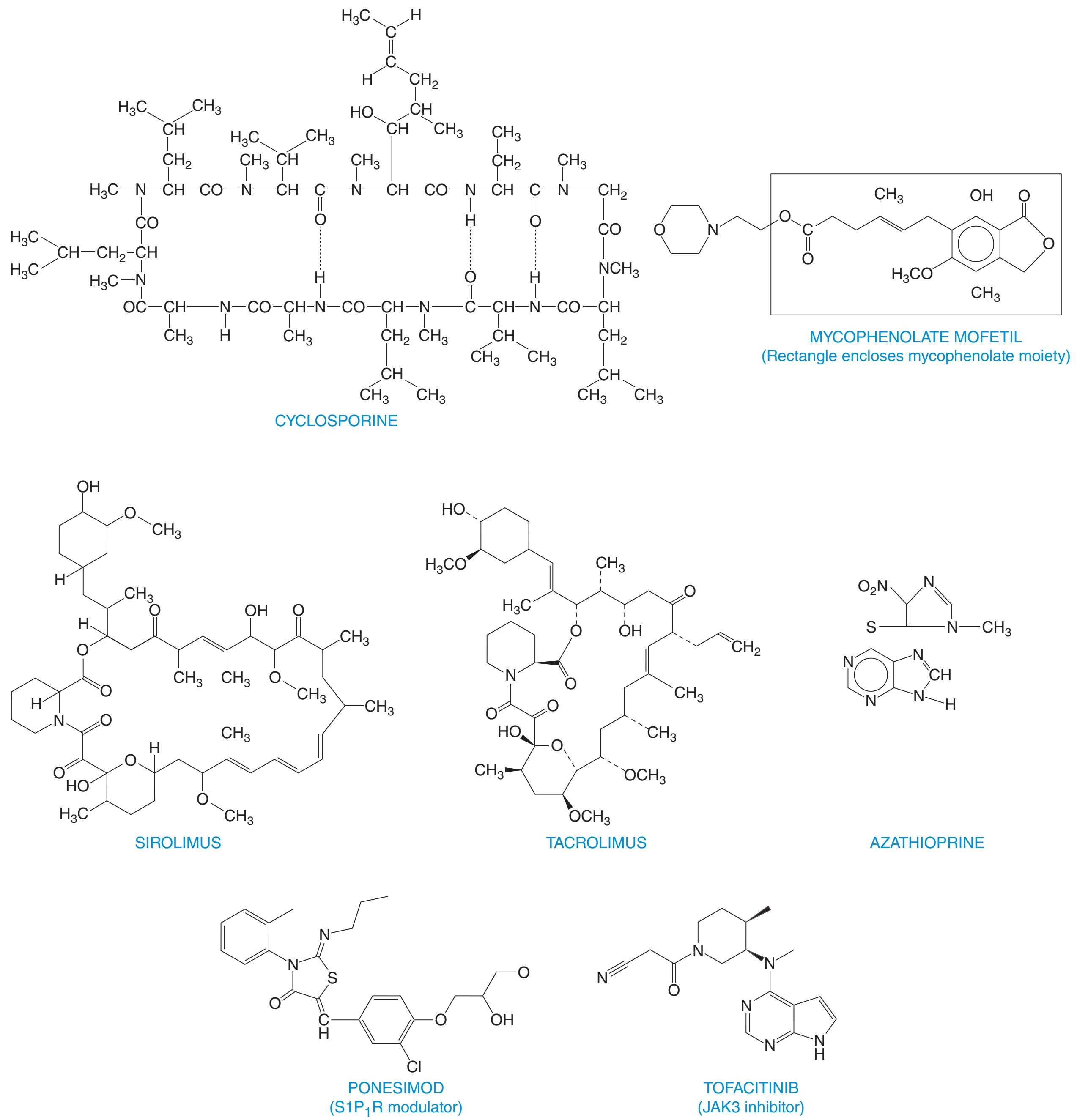
Among the most effective immunosuppressive drugs in routine use are the calcineurin inhibitors *cyclosporine* and *tacrolimus* (Figure 39–1), which target intracellular signaling pathways induced as a consequence of T-cell receptor (TCR) activation (Figure 39–2). *Cyclosporine* and *tacrolimus* bind to an immunophilin (cyclophilin for *cyclosporine* or FKBP-12 for *tacrolimus*), resulting in subsequent interaction with calcineurin to block its phosphatase activity. A *cyclosporine* analogue, *voclosporin*, with distinct binding for cyclophilin and immunosuppressive activity superior to that of *cyclosporine* was FDA-approved in 2021 for the treatment of lupus nephritis (Kuglstatter et al., 2011). Calcineurin-catalyzed dephosphorylation is required for movement of a component of the nuclear factor of activated T lymphocytes (NFAT) into the nucleus. NFAT, in turn, is required to induce a number of cytokine genes, including the gene for IL-2, a prototypic T-cell growth and differentiation factor (Verghese et al., 2014).

Tacrolimus

Tacrolimus is a macrolide antibiotic produced by *Streptomyces tsukubaensis*. Because of perceived slightly greater efficacy and ease of blood level monitoring, *tacrolimus* has become the preferred calcineurin inhibitor in most transplant centers (Ekberg et al., 2007).

Mechanism of Action. Like *cyclosporine*, *tacrolimus* inhibits T-cell activation by inhibiting calcineurin. *Tacrolimus* binds to an intracellular protein, FKBP-12, an immunophilin structurally related to cyclophilin. A complex of *tacrolimus*–FKBP-12, Ca^{2+}, calmodulin, and calcineurin then forms, and calcineurin phosphatase activity is inhibited (see Figure 39–2). Inhibition of phosphatase activity prevents dephosphorylation and nuclear translocation of NFAT and inhibits T-cell activation. Thus, although the intracellular receptors differ, *cyclosporine* and *tacrolimus* target the same pathway for immunosuppression.

ADME. *Tacrolimus* is available for oral administration as capsules and extended-release capsules (0.5, 1, and 5 mg); extended-release tablets (0.75, 1, and 4 mg); and a solution for injection (5 mg/mL). Sublingual *tacrolimus* has been used off-label for the short term in patients who are unable to receive medications orally. Because of intersubject variability in pharmacokinetics, individualized dosing is required for optimal therapy. For *tacrolimus*, whole blood is the preferred sampling compartment; the trough drug level in whole blood seems to correlate better with clinical events for *tacrolimus* than for *cyclosporine*. Target concentrations are 10 to 15 ng/mL in the early preoperative period and 6 to 8 ng/mL at 3 months posttransplantation. Target concentrations are dependent on sampling technique, product-release characteristics, and immediate- versus extended-release forms. Gastrointestinal (GI) absorption is incomplete and variable. Food decreases the rate and extent of absorption. Plasma protein binding of *tacrolimus* is 75% to 99%, involving



CYCLOSPORINE

MYCOPHENOLATE MOFETIL
(Rectangle encloses mycophenolate moiety)

SIROLIMUS

TACROLIMUS

AZATHIOPRINE

PONESIMOD
($S1P_1R$ modulator)

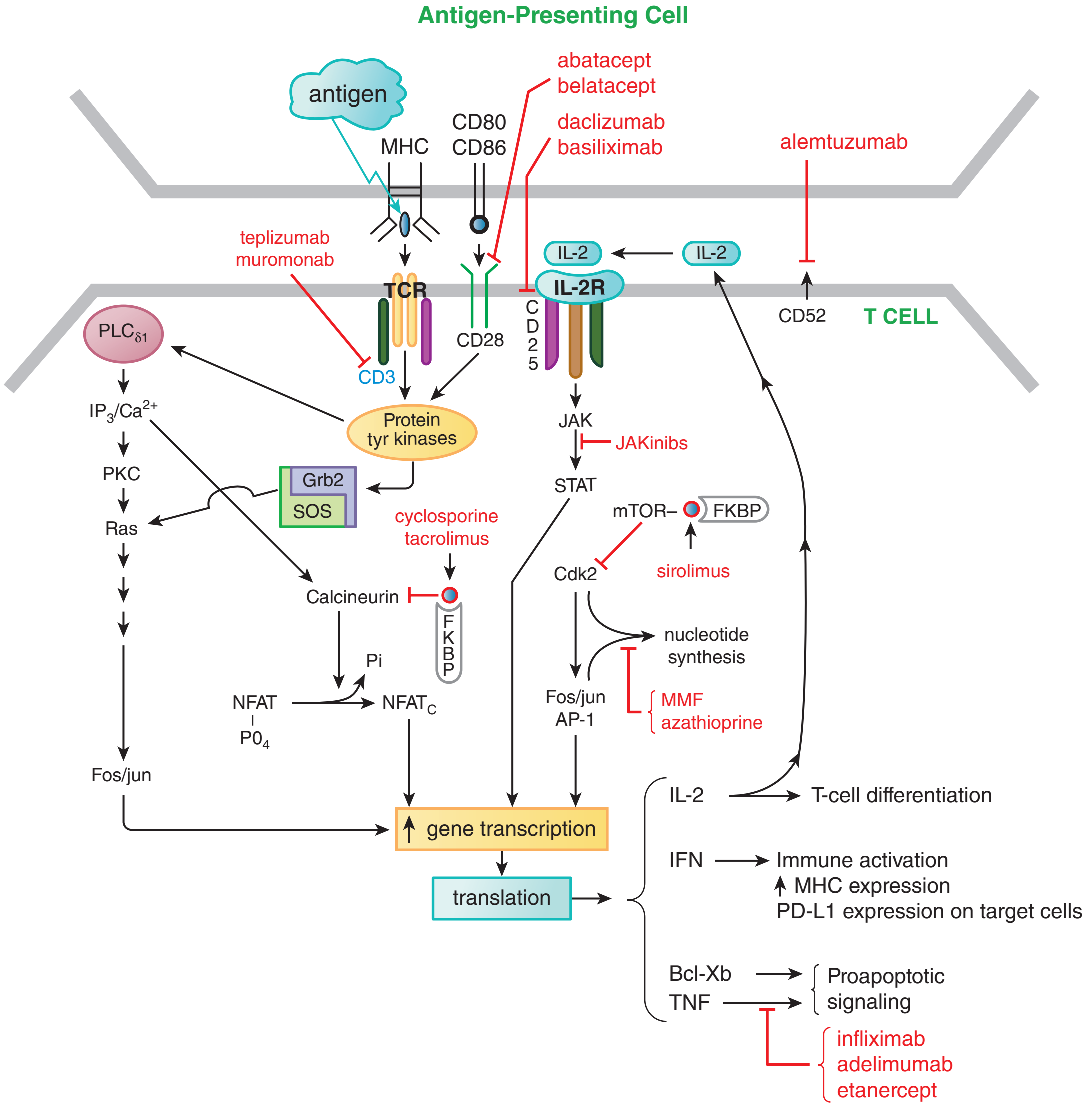
TOFACITINIB
(JAK3 inhibitor)

Figure 39–1 *Structures of selected immunosuppressive drugs.*

primarily albumin and α_1-acid glycoprotein. The $t_{1/2}$ of *tacrolimus* is about 12 h. *Tacrolimus* is extensively metabolized in the liver by hepatic CYPs 3A4 and 3A5. Some of the metabolites are active. The bulk of excretion of the parent drug and metabolites is in the feces.

Therapeutic Uses. *Tacrolimus* is indicated for the prophylaxis of solid-organ allograft rejection in a manner similar to *cyclosporine* (see Cyclosporine) and is used off-label as rescue therapy in patients with rejection episodes despite "therapeutic" levels of *cyclosporine*. Recommended initial oral doses are 0.2 mg/kg per day for adult kidney transplant patients, 0.1 to 0.15 mg/kg per day for adult liver transplant patients, 0.075 mg/kg per day for adult heart transplant patients, and 0.15 to 0.2 mg/kg per day for pediatric liver transplant patients in two divided doses 12 h apart. These dosages are intended to achieve typical blood trough levels in the 5- to 20-ng/mL range (Goring et al., 2014). Note that the oral dose of *tacrolimus* depends on product release characteristics (immediate- vs. extended-release formulation) and the specific cocktail of medications selected for prophylaxis.

Toxicity. Nephrotoxicity, neurotoxicity (e.g., tremor, headache, motor disturbances, seizures), GI complaints, hypertension, hyperkalemia, hyperglycemia, and diabetes all are associated with *tacrolimus* use. *Tacrolimus* has a negative effect on pancreatic islet β cells, and glucose intolerance and diabetes mellitus are well-recognized complications of *tacrolimus*-based immunosuppression. While combined use of calcineurin inhibitors and glucocorticoids is particularly diabetogenic, new-onset diabetes after transplantation incidence was significantly higher with *tacrolimus* than with *cyclosporine*, the other calcineurin inhibitor. Obese patients, African American or Hispanic transplant recipients, or those with a family history of type 2 diabetes or obesity are especially at risk. As with other immunosuppressive agents, there is an increased risk of secondary tumors and opportunistic infections. Notably,


Antigen-Presenting Cell
antigen
MHC
CD80
CD86
abatacept
belatacept
daclizumab
basiliximab
alemtuzumab
teplizumab
muromonab
TCR
CD3
CD28
IL-2
IL-2
IL-2R
CD25
CD52
T CELL
PLCδ1
IP3/Ca2+
PKC
Ras
Protein tyr kinases
Grb2
SOS
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STAT
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FKBP
sirolimus
cyclosporine
tacrolimus
Calcineurin
FKBP
Cdk2
nucleotide synthesis
MMF
azathioprine
Pi
NFAT
P04
NFATC
Fos/jun
AP-1
Fos/jun
gene transcription
translation
IL-2
T-cell differentiation
IFN
Immune activation
MHC expression
PD-L1 expression on target cells
Bcl-Xb
TNF
Proapoptotic signaling
infliximab
adelimumab
etanercept

Figure 39–2 *T-cell activation and sites of action of immunosuppressive agents.* The TCR recognizes antigens bound to the MHC. A costimulatory signal is required for T-cell activation: The CD80/CD86-CD28 interaction from the APC to the T cell. Activation leads to IL-2 production (in a positive-feedback loop) and a host of other events, some of which are bracketed in the lower right-hand corner. Numerous agents are available to suppress T-cell activation. *Cyclosporine* and *tacrolimus* bind to immunophilins (cyclophilin and FKBP, respectively), forming a complex that inhibits the phosphatase calcineurin and the calcineurin-catalyzed dephosphorylation that permits translocation of NFAT into the nucleus. NFAT is required for transcription of IL-2 and other growth- and differentiation-associated cytokines (lymphokines). *Sirolimus* (rapamycin) works downstream of the IL-2R, binding to FKBP; the FKBP-*sirolimus* complex binds to and inhibits the mTOR, a kinase involved in cell cycle progression (proliferation). MMF and *azathioprine* inhibit nucleic acid synthesis, thereby inhibiting T-cell proliferation. The antibody *muromunab* inhibits TCR function via interaction with its CD3 component. *Daclizumab* and *basiliximab* block IL-2 signaling by interacting with the alpha subunit of the IL-2R complex (CD25). Several antibodies can block the systemic effects of released TNF. *Alemtuzumab*, by binding to CD52, marks the cell for destruction, thereby depleting $CD52^+$ cells. Small-molecule inhibitors ("JAKinibs") targeting members of the Janus kinase (JAK) family block the tyrosine phosphorylation of STAT transcription factors in response to IL-2 and multiple cytokines, thereby inhibiting many of their biological functions. APC, antigen-presenting cell.

tacrolimus does not adversely affect uric acid or low-density lipoprotein (LDL) cholesterol. Diarrhea and alopecia are common in patients on concomitant *mycophenolate* therapy.

Drug Interactions. Because of its potential for nephrotoxicity, *tacrolimus* blood levels and renal function should be monitored closely. Coadministration with *cyclosporine* results in additive or synergistic nephrotoxicity; therefore, a delay of at least 24 h is required when switching a patient from *cyclosporine* to *tacrolimus*. Because *tacrolimus* is metabolized mainly by CYP3A4/5, the potential interactions described in the following section for *cyclosporine* also apply for *tacrolimus*. Per the label, concomitant use of *tacrolimus* with *cyclosporine* or *sirolimus* is not recommended for prophylaxis against renal transplant rejection.

Cyclosporine

Cyclosporine (cyclosporin A), a cyclic polypeptide of 11 amino acids produced by the fungus *Beauveria nivea*, inhibits calcineurin activity (Azzi et al., 2013).

Mechanism of Action. *Cyclosporine* forms a complex with cyclophilin, a cytoplasmic-receptor protein present in target cells (see Figure 39–2). This complex binds to calcineurin, inhibiting Ca^{2+}-stimulated dephosphorylation of the cytosolic component of NFAT. When cytoplasmic NFAT is dephosphorylated, it translocates to the nucleus and complexes with nuclear components required for complete T-cell activation, including transactivation of IL-2 and other lymphokine genes. Calcineurin phosphatase activity is inhibited after physical interaction with the *cyclosporine*/cyclophilin complex.

At the level of immune system function, *cyclosporine* suppresses some humoral immunity but is more effective against T-cell–dependent immune mechanisms such as those underlying transplant rejection and some forms of autoimmunity. It preferentially inhibits antigen-triggered signal transduction in T lymphocytes, blunting expression of many lymphokines, including IL-2, and the expression of antiapoptotic proteins. *Cyclosporine* also increases expression of transforming growth factor-β, a potent inhibitor of IL-2–stimulated T-cell proliferation and generation of cytotoxic T lymphocytes (Colombo and Ammirati, 2011; Molnar et al., 2015).

ADME. Because *cyclosporine* is lipophilic and highly hydrophobic, it is formulated for clinical administration using castor oil or other strategies to ensure solubilization. *Cyclosporine* can be administered intravenously or orally. The intravenous preparation is provided as a solution in an ethanol–polyoxyethylated castor oil vehicle that must be further diluted in 0.9% sodium chloride solution or 5% dextrose solution before injection. The oral dosage forms include soft gelatin capsules and oral solutions. *Cyclosporine* supplied in the original soft gelatin capsule is absorbed slowly, with 20% to 50% bioavailability. A modified microemulsion formulation, NEORAL, has become the most widely used preparation. It has more uniform and slightly increased bioavailability compared to the original formulation. It is provided as 25- and 100-mg soft gelatin capsules and a 100-mg/mL oral solution. The original and microemulsion formulations are *not bioequivalent* and cannot be used interchangeably without heightened monitoring of drug concentrations and assessment of graft function. A second modified formulation, GENGRAF, is also marketed, and like NEORAL, is *not interchangeable* with nonmodified *cyclosporine* formulations. Transplant units need to educate patients that the *cyclosporine* preparation known as SANDIMMUNE and its generics are not the same as NEORAL and its generics, such that one preparation cannot be substituted for another without risk of inadequate immunosuppression or increased toxicity. The danger of unauthorized, inadvertent, unmonitored, or inappropriate substitution of nonequivalent formulations can result in graft loss and other adverse patient outcomes.

Blood levels taken 2 h after a dose administration (so-called C_2 levels) may correlate better with the AUC (area under the plasma concentration–time curve) than other single points, but no single time point can simulate the exposure better than more frequent drug sampling. In practice, if a patient has clinical signs or symptoms of toxicity or if there is unexplained rejection or renal dysfunction, a pharmacokinetic profile can be used to estimate that person's systemic exposure to the drug.

Cyclosporine absorption is incomplete following oral administration and varies with the individual patient and the formulation used. *Cyclosporine* is distributed extensively outside the vascular compartment. After intravenous dosing, the steady-state volume of distribution reportedly is as high as 3 to 5 L/kg in solid-organ transplant recipients. The elimination of *cyclosporine* from the blood generally is biphasic, with a terminal $t_{1/2}$ of 5 to 18 h. After intravenous infusion, clearance is about 5 to 7 mL/min/kg in adult recipients of renal transplants, but results differ by age and between different patient populations. For example, clearance is slower in cardiac transplant patients and more rapid in children. Thus, the intersubject variability is so large that individual monitoring is required.

After oral administration of *cyclosporine* (as NEORAL), the time to peak blood concentrations is 1.5 to 2 h. Administration with food delays and decreases absorption. High- and low-fat meals consumed within 30 min of administration decrease the AUC by about 13% and the maximum concentration by 33%. This makes it imperative to individualize dosage regimens for outpatients. *Cyclosporine* is extensively metabolized by hepatic CYP3A4 and to a lesser degree in the GI tract and kidneys. At least 25 metabolites have been identified in human bile, feces, blood, and urine. All of the metabolites have reduced biological activity and toxicity compared to the parent drug. *Cyclosporine* and its metabolites are excreted principally through the bile into the feces, with only about 6% excreted in the urine. *Cyclosporine* is also excreted in human milk. In the presence of hepatic dysfunction, dosage adjustments are required. No adjustments generally are necessary for patients on dialysis or with renal failure.

Therapeutic Uses. Clinical indications for *cyclosporine* are kidney, liver, heart, and other organ transplantation; rheumatoid arthritis; psoriasis; and xerophthalmia. Its use in dermatology is discussed in Chapter 75. *Cyclosporine* usually is combined with other agents, especially glucocorticoids and either *azathioprine* or *mycophenolate*, and, most recently, *sirolimus*. The dose of *cyclosporine* varies, depending on the organ transplanted and the other drugs used in the specific treatment protocol(s). The initial dose generally is not given before the transplant because of the concern about nephrotoxicity. For renal transplant patients, therapeutic algorithms have been developed to delay *cyclosporine* or *tacrolimus* introduction until a threshold renal function has been attained. Dosing is guided by signs of rejection (too low a dose), renal or other toxicity (too high a dose), and close monitoring of blood levels. Great care must be taken to differentiate renal toxicity from rejection in kidney transplant patients. Ultrasound-guided allograft biopsy is the best way to assess the basis for renal dysfunction. Because adverse reactions have been ascribed more frequently to the intravenous formulation, this route of administration is discontinued as soon as the patient can take the drug orally.

In rheumatoid arthritis, *cyclosporine* is used in severe cases that have not responded to *methotrexate*. *Cyclosporine* can be combined with *methotrexate*, but the levels of both drugs must be monitored closely. In psoriasis, *cyclosporine* is indicated for treatment of adult immunocompetent patients with severe and disabling disease for whom other systemic therapies are contraindicated or have failed. Because of its mechanism of action, there is a theoretical basis for the use of *cyclosporine* in a variety of other T-cell–mediated diseases. *Cyclosporine* reportedly is effective in Behçet's disease, acute ocular syndrome, endogenous uveitis, atopic dermatitis, inflammatory bowel disease, and nephrotic syndrome, even when standard therapies have failed.

Toxicity. The principal adverse reactions to *cyclosporine* therapy are renal dysfunction and hypertension. Tremor, hirsutism, hyperlipidemia, and gum hyperplasia also are frequently encountered. Hypertension occurs in about 50% of renal transplant and almost all cardiac transplant patients. Hyperuricemia may lead to worsening of gout, increased P-glycoprotein activity, and hypercholesterolemia (see Chapters 4, 37, and 42). Nephrotoxicity occurs in the majority of patients and is the major reason for cessation or modification of therapy. Combined use of calcineurin inhibitors and glucocorticoids is particularly diabetogenic, although this seems more problematic in patients treated with *tacrolimus* (see previous Tacrolimus section). *Cyclosporine*, as opposed to *tacrolimus*, is more likely to produce elevations in LDL cholesterol.

Drug Interactions. *Cyclosporine* interacts with a wide variety of commonly used drugs, and close attention must be paid to drug interactions. Any drug that affects CYPs, especially CYP3A4, may affect *cyclosporine* blood concentrations. Substances that inhibit this enzyme can decrease *cyclosporine* metabolism and increase blood concentrations. These include Ca^{2+} channel blockers (e.g., *verapamil, nicardipine*); antifungal agents (e.g., *fluconazole, ketoconazole*); antibiotics (e.g., *erythromycin*); glucocorticoids (e.g., *methylprednisolone*); HIV-protease inhibitors (e.g., *indinavir*); and other drugs (e.g., *allopurinol, metoclopramide*). Grapefruit juice inhibits CYP3A4 and the P-glycoprotein multidrug efflux pump and thereby can increase *cyclosporine* blood concentrations. In contrast, drugs that induce CYP3A4 activity can increase *cyclosporine* metabolism and decrease blood concentrations. Such drugs include antibiotics (e.g., *nafcillin, rifampin*); anticonvulsants (e.g., *phenobarbital, phenytoin*); and others (e.g., *octreotide, ticlopidine*).

Interactions between *cyclosporine* and *sirolimus* require that administration of the two drugs be separated by time. *Sirolimus* aggravates *cyclosporine*-induced renal dysfunction, while *cyclosporine* increases *sirolimus*-induced hyperlipidemia and myelosuppression. Additive nephrotoxicity may occur when *cyclosporine* is coadministered with nonsteroidal anti-inflammatory drugs (NSAIDs) and other drugs that cause renal dysfunction; elevation of *methotrexate* levels may occur when the two drugs are coadministered, as can reduced clearance of other drugs, including *prednisolone*, *digoxin*, and statins (Azzi et al., 2013; Ekberg et al., 2007).

Antiproliferative and Antimetabolic Drugs

Sirolimus

Sirolimus is a macrocyclic lactone produced by *Streptomyces hygroscopicus*.

Mechanism of Action. *Sirolimus* inhibits T-lymphocyte activation and proliferation downstream of the IL-2 and other T-cell growth factor receptors (see Figure 39–2). Like *cyclosporine* and *tacrolimus*, therapeutic action of *sirolimus* requires formation of a complex with an immunophilin, in this case *FKBP-12*. The *sirolimus–FKBP-12 complex* does not affect calcineurin activity; rather, it binds to and inhibits the protein kinase mTOR, which is a key enzyme in cell cycle progression. Inhibition of mTOR blocks cell cycle progression at the $G_1 \rightarrow S$ phase transition.

In animal models, *sirolimus* not only inhibits transplant rejection, GVHD, and a variety of autoimmune diseases but also has effects for several months after discontinuation, suggesting a tolerizing effect (see Tolerance). A newer indication for *sirolimus* is to avoid the use of calcineurin inhibitors, even when patients are stable, to protect kidney function (Schena et al., 2009).

ADME. After oral administration, *sirolimus* is absorbed rapidly and reaches a peak blood concentration within about 1 h after a single dose in healthy subjects and within about 2 h after multiple oral doses in renal transplant patients. Systemic availability is about 15%, and blood concentrations are proportional to dose between 3 and 12 mg/m^2. A high-fat meal decreases peak blood concentration by 34%; *sirolimus* therefore should be taken consistently either with or without food, and blood levels should be monitored closely. About 40% of *sirolimus* in plasma is protein bound, especially to albumin. The drug partitions into formed elements of blood (blood-to-plasma ratio = 38 in renal transplant patients). *Sirolimus* is extensively metabolized by CYP3A4 and is transported by P-glycoprotein. The bulk of total excretion is via the feces. Although some of its metabolites are active, *sirolimus* itself is the major active component in whole blood and contributes more than 90% of the immunosuppressive effect. The blood $t_{1/2}$ after multiple doses in stable renal transplant patients is 62 h. A loading dose of three times the maintenance dose will provide nearly steady-state concentrations within 1 day in most patients.

Therapeutic Uses. *Sirolimus* is indicated for prophylaxis of organ transplant rejection, usually in combination with a reduced dose of calcineurin inhibitor and glucocorticoids. *Sirolimus* has been used with glucocorticoids and *mycophenolate* to avoid permanent renal damage. *Sirolimus* dosing regimens are relatively complex, with blood levels generally targeted between 5 and 15 ng/mL. It is recommended that the daily maintenance dose be reduced by approximately one-third in patients with hepatic impairment. *Sirolimus* also has been incorporated into stents to inhibit local cell proliferation and blood vessel occlusion (Moes et al., 2015).

Toxicity. The use of *sirolimus* in renal transplant patients is associated with a dose-dependent increase in serum cholesterol and triglycerides that may require treatment. Although immunotherapy with *sirolimus per se* is not considered nephrotoxic, patients treated with *cyclosporine* plus *sirolimus* have impaired renal function compared to patients treated with *cyclosporine* alone. *Sirolimus* can worsen proteinuria and should be used with caution in patients with a glomerular filtration rate below 30% or proteinuria; these conditions can worsen renal failure. Renal function and proteinuria therefore must be monitored closely in such patients. Lymphocele, a known surgical complication associated with renal transplantation, is increased in a dose-dependent fashion by *sirolimus*, requiring close postoperative follow-up.

Other adverse effects include anemia, leukopenia, thrombocytopenia, mouth ulcer, hypokalemia, and GI effects. Delayed wound healing may occur with *sirolimus* use. This mTOR inhibitor has been shown to have anticancer effects, especially on skin cancer; it is considered the immunosuppressant of choice in patients with a history of malignancy. *Temsirolimus* is specifically approved for kidney (but not skin) cancer, while *everolimus* is approved for a variety of cancers (but not skin cancer). As with other immunosuppressive agents, there is an increased risk of infections.

Drug Interactions. Because *sirolimus* is a substrate for CYP3A4 and is transported by P-glycoprotein, close attention to interactions with other drugs that are metabolized or transported by these proteins is required (see Chapters 4 and 5). Dose adjustment may be required when *sirolimus* is coadministered with CYP3A4 and P-glycoprotein inhibitors (e.g., *diltiazem*) or strong inducers (e.g., *rifampin*) (Alberú et al., 2011; Euvrard et al., 2012).

Everolimus

Everolimus [40-*O*-(2-hydroxyethyl)-rapamycin] is FDA-approved for treatment of astrocytoma, breast cancer, kidney and liver transplant rejection prophylaxis, pancreatic neuroendocrine tumor, renal angiomyolipoma, and renal cell cancer. It is chemically closely related to sirolimus but has distinct pharmacokinetics. The main difference is a shorter $t_{1/2}$ and thus a shorter time to achieve steady-state concentrations of the drug. Dosage on a milligram per kilogram basis is similar to (but not the same as) that of *sirolimus*. In kidney transplant rejection prophylaxis, the initial dose of *everolimus* is 0.75 mg twice daily, with later adjustment based on serum concentrations. As with *sirolimus*, the combination of a calcineurin inhibitor and an mTOR inhibitor produces worse renal function at 1 year than does calcineurin inhibitor therapy alone, suggesting a drug interaction between the mTOR inhibitors and the calcineurin inhibitors that reduces rejection but enhances toxicity. The toxicity of *everolimus* and the potential for drug interactions seem to be the same as with *sirolimus* (Budde et al., 2011; Moes et al., 2015). Like *sirolimus*, individualization of drug dose through therapeutic drug monitoring is required.

Azathioprine

Azathioprine is a purine antimetabolite. It is an imidazolyl derivative of 6-mercaptopurine, metabolites of which can inhibit purine synthesis.

Mechanism of Action. Following exposure to nucleophiles such as glutathione, *azathioprine* is cleaved to 6-mercaptopurine (6-MP), which in turn is converted to additional metabolites that inhibit *de novo* purine synthesis (see Chapter 70). A fraudulent nucleotide, 6-thio-IMP, is converted to 6-thio-GMP and finally to 6-thio-GTP, which is incorporated into DNA. Cell proliferation thereby is inhibited, impairing a variety of lymphocyte functions. *Azathioprine* appears to be a more potent immunosuppressive agent than 6-MP (Hardinger et al., 2013).

ADME. *Azathioprine* is well absorbed orally and reaches maximum blood levels within 1 to 2 h after administration. The $t_{1/2}$ of *azathioprine* is about 10 min, and the $t_{1/2}$ of 6-MP is about 1 h. Other metabolites have a $t_{1/2}$ of up to 5 h. Blood levels have limited predictive value because of extensive metabolism, significant activity of many different metabolites, and high tissue levels attained. *Azathioprine* and *mercaptopurine* are moderately bound to plasma proteins and are partially dialyzable. Both are rapidly removed from the blood by oxidation or methylation in the liver or erythrocytes. Renal clearance has little impact on the biological effectiveness or toxicity.

Therapeutic Uses. *Azathioprine* is indicated as an adjunct for prevention of organ transplant rejection and in severe rheumatoid arthritis. The usual starting dose of *azathioprine* is 3 to 5 mg/kg per day. Lower initial doses (1 mg/kg per day) are used in treating rheumatoid arthritis. Complete blood count and liver function tests should be monitored.

Toxicity. The major side effect of *azathioprine* is bone marrow suppression, including leukopenia (common), thrombocytopenia (less common), or anemia (uncommon). Other important adverse effects include increased susceptibility to infections (especially varicella and herpes simplex viruses), hepatotoxicity, alopecia, GI toxicity, pancreatitis, and increased risk of neoplasia.

Drug Interactions. Xanthine oxidase, an enzyme of major importance in the catabolism of *azathioprine* metabolites, is blocked by *allopurinol*. Hence, the combination of *azathioprine* with *allopurinol* should be avoided.

Adverse effects resulting from coadministration of *azathioprine* with other myelosuppressive agents or angiotensin-converting enzyme inhibitors include leukopenia, thrombocytopenia, and anemia as a result of myelosuppression.

Mycophenolate Mofetil

Mycophenolate mofetil (MMF) is the 2-morpholinoethyl ester of mycophenolic acid (MPA).

Mechanism of Action. *Mycophenolate mofetil* is a prodrug that is rapidly hydrolyzed to the active drug MPA, a selective, noncompetitive, reversible inhibitor of inosine monophosphate dehydrogenase, an enzyme in the *de novo* pathway of guanine nucleotide synthesis. B and T lymphocytes are highly dependent on this pathway for cell proliferation; MPA thus selectively inhibits lymphocyte proliferation and functions, including antibody formation, cellular adhesion, and migration.

ADME. *Mycophenolate mofetil* undergoes rapid and complete metabolism to MPA after oral or intravenous administration. MPA is then metabolized to the inactive MPA glucuronide (MPAG). The parent drug is cleared from the blood within a few minutes. The $t_{1/2}$ of MPA is approximately 16 h. Most (87%) is excreted in the urine as MPAG. Plasma concentrations of MPA and MPAG are increased in patients with renal insufficiency.

Therapeutic Uses. *Mycophenolate mofetil* is indicated for prophylaxis of transplant rejection, and it typically is used in combination with glucocorticoids and a calcineurin inhibitor but not with *azathioprine*. Combined treatment with *sirolimus* is possible, although potential drug interactions necessitate careful monitoring of drug levels. The approved dose for liver transplantation rejection prophylaxis is 1 g twice daily. For renal transplants, 1 g is administered orally or intravenously (over 2 h) twice daily (2 g/day). A higher dose, 1.5 g twice daily (3 g/day), may be recommended for African American renal transplant patients and all liver and cardiac transplant patients. MMF is increasingly used off-label in systemic lupus. MMF has been used to treat a number of different inflammatory disorders, including MS and sarcoidosis. A delayed-release formulation of MPA is available; it does not release MPA under acidic conditions (pH <5), as in the stomach, but is soluble in neutral pH, as in the intestine. The enteric coating results in a delay in the time to reach maximum MPA concentrations (Darji et al., 2008).

Toxicity. The principal toxicities of MMF are GI and hematological: leukopenia, pure red cell aplasia, diarrhea, and vomiting. The MPA formulation has been introduced to reduce the frequent GI upset and has had variable results. There also is an increased incidence of some infections, especially sepsis associated with cytomegalovirus. *Tacrolimus* in combination with MMF has been associated with activation of polyoma viruses such as BK virus, which can cause interstitial nephritis. The use of MMF in pregnancy is associated with congenital anomalies and increased risk of pregnancy loss.

Drug Interactions. *Tacrolimus* delays elimination of MMF by impairing the conversion of MPA to MPAG. This may enhance GI toxicity. Coadministration with antacids containing aluminum or magnesium hydroxide leads to decreased absorption of MMF; thus, these drugs should not be administered simultaneously. MMF should not be administered with *cholestyramine* or other drugs that affect enterohepatic circulation. Such agents decrease plasma MPA concentrations, probably by binding free MPA in the intestines. *Acyclovir* and *ganciclovir* may compete with MPAG for tubular secretion, possibly resulting in increased concentrations of both MPAG and the antiviral agents in the blood, an effect that may be compounded in patients with renal insufficiency. MMF serum level monitoring is not performed routinely (Darji et al., 2008; Goldfarb-Rumyantzev et al., 2006).

Sphingosine-1-Phosphate Receptor Modulators

Sphingosine-1-phosphate (S1P) is a sphingolipid metabolite that affects inflammation and immunity, among numerous other biological functions (McGinley and Cohen, 2021; Spiegel and Milstien, 2011). The relevance of S1P to immunity derives from its role in promoting the egress of lymphocytes from lymphoid tissues.

Sphingosine-1-phosphate is produced enzymatically by phosphorylation of sphingosine derived from ceramide (Figure 39–3A). S1P acts intracellularly and, once exported from the cell, also extracellularly, where it interacts with transmembrane receptors on the cell surface. The five cell surface receptors for S1P (S1PR) are G protein-coupled receptors, S1P$_1$R through S1P$_5$ R, which couple to effectors via the G$_i$/G$_o$ family (see Chapter 3). These receptors are differentially expressed in different tissues: S1P$_5$Rs are found in the CNS; S1P$_4$Rs seem largely confined to the immune system; S1P$_2$R and S1P$_3$R are widely expressed; endothelial cells and B and T lymphocytes express predominantly S1P$_1$R.

Sphingosine-1-phosphate receptor modulators are being explored as pharmacotherapy for a wide variety of conditions, including CNS degenerative diseases, chemotherapy-induced neuropathy, stroke, lupus erythematosus, psoriasis, and inflammatory bowel disease (McGinley and Cohen, 2021). The pharmacological profiles for these drugs are presented below.

Available S1P Modulators

There are currently four approved modulators of S1PRs: *fingolimod*, *siponimod*, *ozanimod*, and *ponesimod*.

Mechanism of Action and Specificities

Although the approved S1PR inhibitors differ slightly in their specificity for S1PRs, these compounds cause irreversible downregulation, internalization, and degradation of S1P$_1$R; thus, these compounds all function as irreversible agonists at S1P$_1$R. B cells and CCR7-positive T cells normally respond to a gradient of S1P and exit the lymphoid tissue. However, inhibition or blockade of the S1P$_1$R reduces lymphocyte egress, causing sequestration of host lymphocytes in lymph nodes and Peyer patches and thus away from the circulation, thereby protecting lesions and grafts from T-cell–mediated attack and reducing allergic inflammation.

All but *fingolimod* act directly. *Fingolimod* is a prodrug. It is phosphorylated by sphingosine kinase 2 (SK2); *fingolimod phosphate* is the active form (Figure 39-3B). *Fingolimod* has broad receptor affinity, interacting with all subtypes of S1PRs except S1P$_2$R. *Siponimod* and *ozanimod* have high affinity for S1P$_1$R and S1P$_5$R. *Ponesimod* shows the greatest specificity, interacting with high affinity only at S1P$_1$R.

ADME

Administration. These agents are effective orally with good bioavailability; see individual package inserts for required predose testing and specific regimens for initiating dosing to reduce risk of bradycardia and atrioventricular conduction delays. When switching from immunosuppressant agents with prolonged half-lives of effect, such as *natalizumab*, *teriflunomide*, or *mitoxantrone*, account for the duration and mode of action of these drugs to avoid additive immunosuppressive effects when initiating an inhibitor of S1P action. Due to the adverse effect profile of these agents, a number of precautions must be taken in advance of initiating therapy. Do not administer S1PR modulators to patients with active infections. Before initiating treatment, follow recommendations on the FDA-approved package insert with respect to complete blood count, infections, immunizations (especially against varicella-zoster virus [VZV]) and the timing of vaccinations, monitoring electrocardiogram and blood pressure, determining whether patient has conduction abnormalities or is taking any drugs that slow heart rate, and assessing serum transaminase and bilirubin levels and condition of the optic fundus.

Metabolism and Excretion, Drug Interactions, and Contraindications. *Fingolimod* is cleared by phosphorylation to its active form ($t_{1/2}$ 6–9 h) and by the action of CYP4F2, which *ketoconazole* can inhibit. Severe hepatic disease increases exposure.

Ozanimod metabolism produces a series of active metabolites with similar specificity for S1P$_1$R and S1P$_5$R; thus, although the plasma $t_{1/2}$ of the parent drug is approximately 21 h, the $t_{1/2}$ of the effect is approximately 11 days. An active metabolite of *ozanimod* inhibits monoamine oxidase (MAO)-B, providing the potential of serious hypertensive interactions

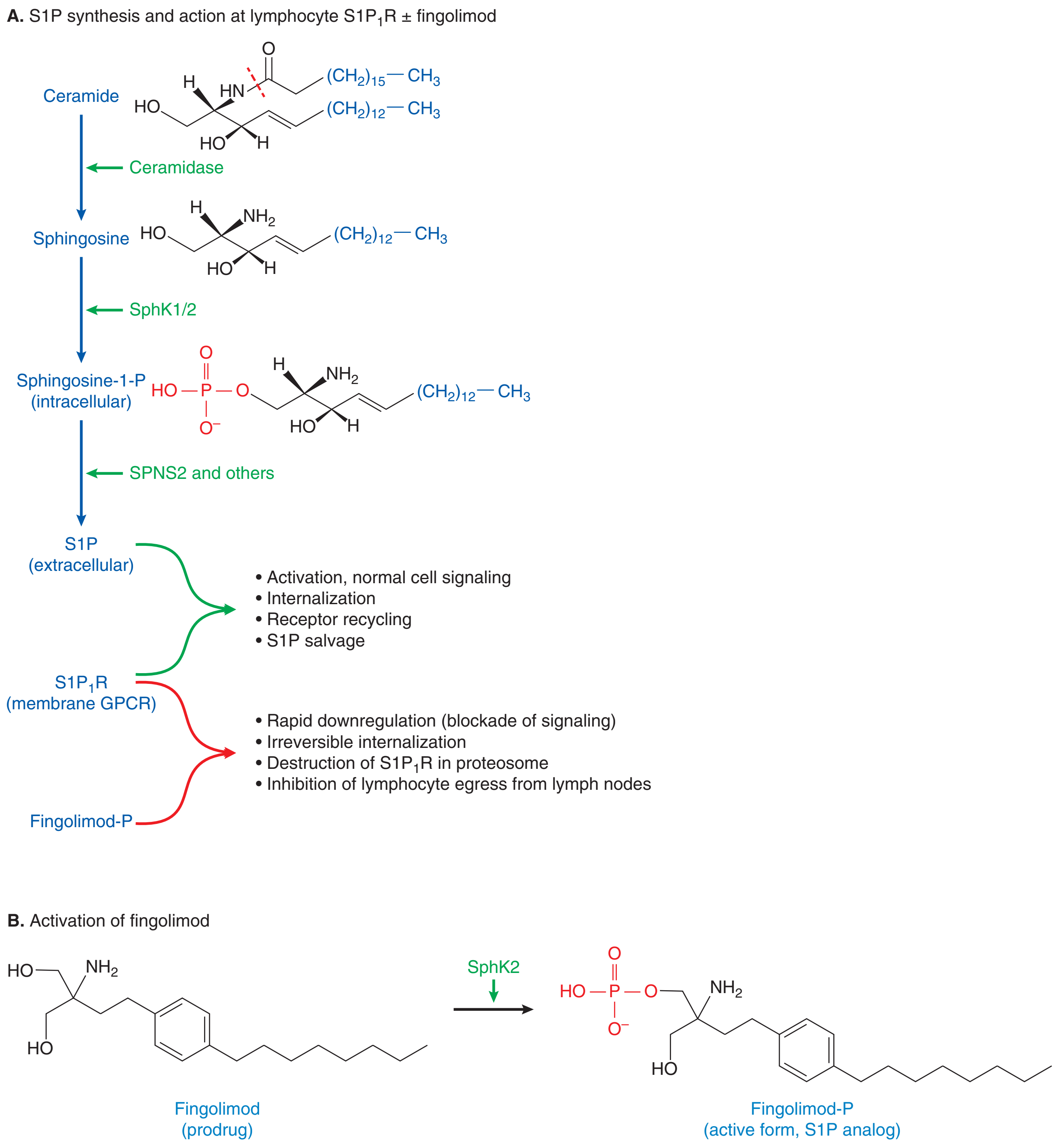
A. S1P synthesis and action at lymphocyte S1P1R ± fingolimod
Ceramide
Ceramidase
Sphingosine
SphK1/2
Sphingosine-1-P (intracellular)
SPNS2 and others
S1P (extracellular)
S1P1R (membrane GPCR)
Fingolimod-P
• Activation, normal cell signaling
• Internalization
• Receptor recycling
• S1P salvage
• Rapid downregulation (blockade of signaling)
• Irreversible internalization
• Destruction of S1P1R in proteosome
• Inhibition of lymphocyte egress from lymph nodes
B. Activation of fingolimod
SphK2
Fingolimod (prodrug)
Fingolimod-P (active form, S1P analog)
HO
HN
NH2
O
O–
HO—P—O
(CH2)15—CH3
(CH2)12—CH3
H

Figure 39–3 **A.** *Sphingosine-1-phosphate formation and action at $S1P_1R$.* **B.** *The prodrug fingolimod and its active metabolite.* GPCR, G protein-coupled receptor; SphK, sphingosine kinase; SPNS2, S1P transporter.

between *ozanimod* and agents that increase sympathetic tone, norepinephrine, or serotonin (opioids, selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, tricyclic antidepressants, MAO inhibitors, tyramine, and foods containing tyramine). *Siponimod* is metabolized by CYP2C9, which is highly polymorphic, and subsequently by CYP3A4. Concomitant use of moderate to strong inducers (e.g., *rifampin*, *carbamazepine*) or inhibitors (e.g., *fluconazole*) of these CYPs is not recommended. CYP2C9*3/*3 and CYP2C9*2/*2 polymorphs show decreased clearance and increased exposure; use of *siponimod* is contraindicated in patients with the homozygous CYP2C9*3/*3 genotype. Excretion is mainly via the biliary/fecal route. *Ponesimod* is metabolized by CYP3A4 and UGT1A1, with a $t_{1/2}$ of approximately 26 h. Coadministration with strong inducers of these enzymes (e.g., *rifampin*, *phenytoin*, *carbamazepine*) is not recommended. Conversely, *ponesimod* is not recommended for patients with moderate or severe hepatic impairment (Child-Pugh class B or C). Excretion is predominantly via the feces.

Adverse Effects

The shared common adverse effects include increased risk of infections (upper respiratory, urinary tract infection), bradyarrhythmias and impaired atrioventricular conduction, hypertension (monitor blood pressure), hepatic transaminase elevation (monitor), altered vision (macular edema), and dyspnea. An increased risk of cutaneous malignancies and breast cancer has been reported, as has posterior reversible encephalopathy syndrome. Lymphopenia and heart rate reduction reverse upon discontinuation of the drug. To avoid fetal harm, women of childbearing age should use effective contraception during treatment and from

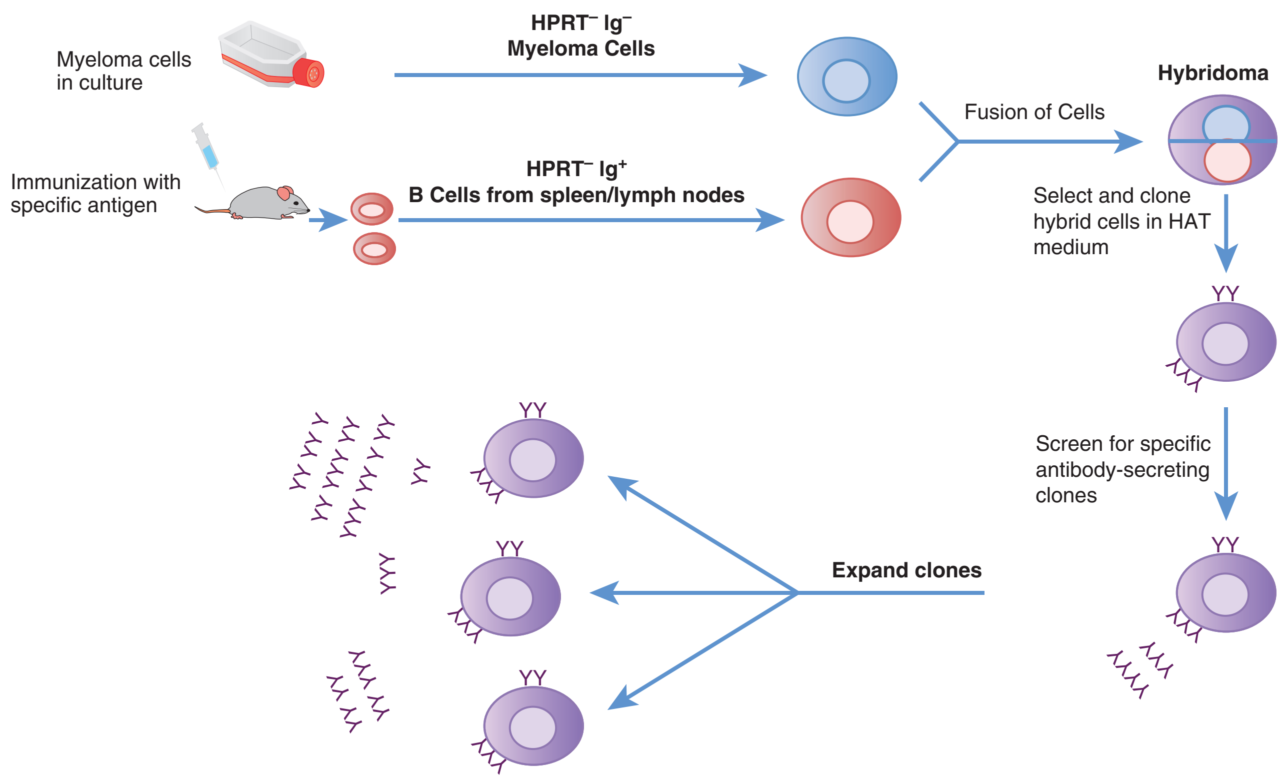
Myeloma cells in culture
HPRT$^-$ Ig$^-$ Myeloma Cells
Immunization with specific antigen
HPRT$^-$ Ig$^+$ B Cells from spleen/lymph nodes
Fusion of Cells
Hybridoma
Select and clone hybrid cells in HAT medium
Screen for specific antibody-secreting clones
Expand clones

Figure 39–4 *Generation of mAbs.* Mice are immunized with the selected antigen, and the spleen or lymph node is harvested and B cells separated. These B cells are fused to a suitable B-cell myeloma selected for its ability to grow in medium supplemented with HAT (hypoxanthine, aminopterin, and thymidine). Only myeloma cells that fuse with B cells can survive in HAT-supplemented medium. The hybridomas expand in culture. Hybridomas of interest are selected based on a specific screening technique and then cloned by limiting dilution. The mAbs can be used directly as supernatants or ascites fluid for experimental use but are purified for clinical use.

2 weeks to 3 months thereafter (depending on half-life of active drug; see requirements of the individual agents).

Therapeutic Uses

Ozanimod is approved for ulcerative colitis (see Chapter 55). All four agents are approved for treatment of relapsing-remitting MS. That use is discussed further in the final section of this chapter, Immunotherapy for Multiple Sclerosis.

Other Antiproliferative and Cytotoxic Agents

Many of the cytotoxic and antimetabolic agents used in cancer chemotherapy (see Chapter 70) are immunosuppressive due to their action on lymphocytes and other cells of the immune system. Other cytotoxic drugs that have been used both on- and off-label as immunosuppressive agents include *methotrexate*, *cyclophosphamide*, *thalidomide*, and *chlorambucil*. *Methotrexate* is used for prophylaxis against GVHD and treatment of rheumatoid arthritis, psoriasis, bullous pemphigoid, and some cancers. *Cyclophosphamide* and *chlorambucil* are used in leukemia and lymphomas and a variety of other malignancies. *Cyclophosphamide* also is FDA-approved for childhood nephrotic syndrome and is used widely off-label for treatment of severe systemic lupus erythematosus, MS, and vasculitides such as granulomatosis with polyangiitis (formerly known as Wegener's granulomatosis). *Leflunomide* is a pyrimidine synthesis inhibitor indicated for the treatment of adults with rheumatoid arthritis. This drug has found increasing empirical use in the treatment of polyomavirus nephropathy seen in immunosuppressed renal transplant recipients. There are no controlled studies showing efficacy compared with control patients treated with only withdrawal or reduction of immunosuppression alone in BK virus nephropathy. The drug inhibits dihydroorotate dehydrogenase in the *de novo* pathway of pyrimidine synthesis. It is hepatotoxic and can cause fetal injury when administered to pregnant women.

Immunosuppression Antibodies and Fusion Receptor Protein

Polyclonal and monoclonal antibodies against lymphocyte cell surface antigens are widely used for prevention and treatment of organ transplant rejection. Polyclonal antisera are generated by repeated injections of human thymocytes (for ATG) or lymphocytes (for ALG) into animals and then purifying the serum immunoglobulin fraction. These preparations vary in efficacy and toxicity from batch to batch.

The capacity to produce mAbs (Figure 39–4) has overcome the problems of variability in efficacy and toxicity seen with the polyclonal products, but mAbs are more limited in their target specificity. The first-generation murine mAbs have been replaced by newer humanized or fully human mAbs that lack antigenicity, have a prolonged $t_{1/2}$, and can be mutagenized to alter their affinity to Fc receptors.

Another class of biological agents developed for both autoimmunity and transplantation are fusion receptor proteins. These agents consist of the ligand-binding domains of receptors bound to the Fc region of an immunoglobulin (usually immunoglobulin [Ig] G1) to provide a longer $t_{1/2}$ (Baldo, 2015). Examples include *abatacept* (a first-generation cytotoxic T-lymphocyte–associated antigen 4 [CTLA-4]-Ig) and *belatacept* (a second-generation CTLA-4-Ig); these agents comprise the Fc region of IgG1 fused to the extracellular domain of CTLA-4. For more information, see the section on Costimulatory Blockade.

Antithymocyte Globulin

Antithymocyte globulin is a purified gamma globulin from the serum of rabbits immunized with human thymocytes (Thiyagarajan et al., 2013). It is provided as a sterile, freeze-dried product for intravenous administration after reconstitution with sterile water. ATG is one of many immune globulin preparations used therapeutically, generally for passive immunization (see Table 39–2 and Chapter 40).

TABLE 39–2 ■ SELECTED IMMUNE GLOBULIN PREPARATIONS

GENERIC NAME	COMMON SYNONYMS	ORIGIN
Antithymocyte globulin	ATG	Rabbit
Botulism immune globulin intravenous	BIG-IV	Human
Cytomegalovirus immune globulin intravenous	CMV-IGIV	Human
Hepatitis B immune globulin	HBIG	Human
Immune globulin intramuscular	Gamma globulin, IgG, IGIM	Human
Immune globulin intravenous	IVIG	Human
Immune globulin subcutaneous	IGSC	Human
Lymphocyte immune globulin	ALG, antithymocyte globulin (equine), ATG (equine)	Equine
Rabies immune globulin	RIG	Human
Rho(D) immune globulin intramuscular	Rho[D] IGIM	Human
Rho(D) immune globulin intravenous	Rho[D] IGIV	Human
Rho(D) immune globulin microdose	Rho[D] IG microdose	Human
Tetanus immune globulin	TIG	Human
Vaccinia immune globulin intravenous	VIGIV	Human

Mechanism of Action. Antithymocyte globulin contains cytotoxic antibodies that bind to CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, and HLA class I and II molecules on the surface of human T lymphocytes. The antibodies deplete circulating lymphocytes by direct cytotoxicity (both complement and cell mediated) and block lymphocyte function by binding to cell surface molecules involved in the regulation of cell function.

Therapeutic Uses. Antithymocyte globulin is used for induction immunosuppression, although the approved indications are for the treatment and prophylaxis of acute renal transplant rejection in combination with other immunosuppressive agents and for the treatment of aplastic anemia. Antilymphocyte-depleting agents (Thymoglobulin, Atgam) are not registered for use as induction immunosuppression. A course of ATG often is given to renal transplant patients with delayed graft function to avoid early treatment with the nephrotoxic calcineurin inhibitors, thereby aiding in recovery from ischemic reperfusion injury. The recommended dose of Thymoglobulin for acute rejection of renal grafts is 1.5 mg/kg per day (over 4–6 h) for 7 to 14 days. Mean T-cell counts fall by day 2 of therapy. The recommended dose of Atgam for acute rejection of renal grafts is 10 to 15 mg/kg per day for 14 days. ATG also is used for acute rejection of other types of organ transplants and for prophylaxis of rejection.

Toxicity. Polyclonal antibodies are xenogeneic proteins that can elicit major side effects, including fever and chills with the potential for hypotension. Premedication with corticosteroids, *acetaminophen*, or an antihistamine and administration of the antiserum by slow infusion (over 4–6 h) into a large-diameter vessel minimize such reactions. Serum sickness and glomerulonephritis can occur; anaphylaxis is rare. Hematological complications include leukopenia and thrombocytopenia. As with other immunosuppressive agents, there is an increased risk of infection and malignancy, especially when multiple immunosuppressive agents are combined. No drug interactions have been described; anti-ATG antibodies develop but do not limit repeated use.

Immunomodulation

Immunosuppression refers to the overall reduction in the magnitude of the immune response. Here we define as immunomodulators those therapeutic approaches that are targeted to a specific molecular driver of the immune response (e.g., cytokines).

Immunotherapy and the Nature of Costimulation and Inhibition

Multiple costimulatory and inhibitory molecules interact to regulate T-cell responses. Immune activation requires two signals that emanate from the interaction of membrane proteins on APCs and T cells (Figures 39–5A and 39–5B). A growing number of antibodies directed at these interacting proteins permits interruption of immune activation to produce a state of immune suppression. Figures 39–2 and 39–5 point out some of these antibodies, which are especially useful in preventing rejection after organ transplantation, as summarized in the material that follows.

In what might be considered an antiparallel system to activation, inhibitory regulation of T-cell activity can also result from the interaction of paired membrane ligands of APCs and T cells (Figure 39–5C). These points of negative regulation are called *immune checkpoints*. By targeting and blocking these immune checkpoints, antibodies can permit T-cell activation to proceed, unfettered by downregulation (Figures 39–5C and 39–5D). Activating immune attacks of tumor cells by blockade of immune checkpoints is producing new therapeutic options for cancer therapy (Callahan et al., 2016; Topalian et al., 2015). Chapter 72 presents the use of immunotherapy in cancer treatment.

Anti-CD3 Monoclonal Antibodies

CD3 is a component of the TCR complex on the surface of human T lymphocytes (see Figure 39–2). Antibodies directed at the ε chain of CD3 have been used with considerable efficacy in human transplantation. The anti-CD3 antibody is monoclonal and targets the CD3 chain of the TCR, inducing its endocytosis and T-cell inactivation and removal through phagocytosis. The original mouse IgG2a antihuman CD3 mAb, *muromonab-CD3* (OKT3), is no longer marketed due to its side effects: It frequently caused cytokine release syndrome and severe pulmonary edema.

Genetically altered anti-CD3 mAbs have been developed that are “humanized” to minimize the occurrence of antiantibody responses and mutated to prevent binding to Fc receptors. In initial clinical trials, a humanized anti-CD3 mAb that does not bind to Fc receptors reversed acute renal allograft rejection without causing the first-dose cytokine release syndrome. Humanized anti-CD3 mAbs are also in clinical trials in patients with type 1 autoimmune diabetes (Mignogna et al., 2021). Interestingly, a prevention trial in nondiabetic patients at high risk for type 1 diabetes resulted in a significant extension on the median diagnosis in patients treated with *teplizumab* (an Fc receptor nonbinding anti-CD3 mAb) in comparison to placebo (Sims et al., 2021).

Anti-CD52 Monoclonal Antibody (Alemtuzumab)

Alemtuzumab is a depleting humanized anti-CD52 mAb.

Mechanism of Action. *Alemtuzumab* binds the CD52 protein that is wildly expressed on B cells and T cells, as well as macrophages, NK cells, and some granulocytes. *Alemtuzumab* binding to CD52 induces an antibody-dependent lysis of cells and a profound leukopenia that may last for more than a year (Jones and Coles, 2014).

Therapeutic Uses. *Alemtuzumab* is used mainly for induction of immunosuppressive therapy and allows the avoidance of the early high dose of steroids. For transplants, the most common regimen is a single intraoperative dose of 30 mg. *Alemtuzumab* is also used for the treatment of refractory acute cellular- and antibody-mediated rejections with the same

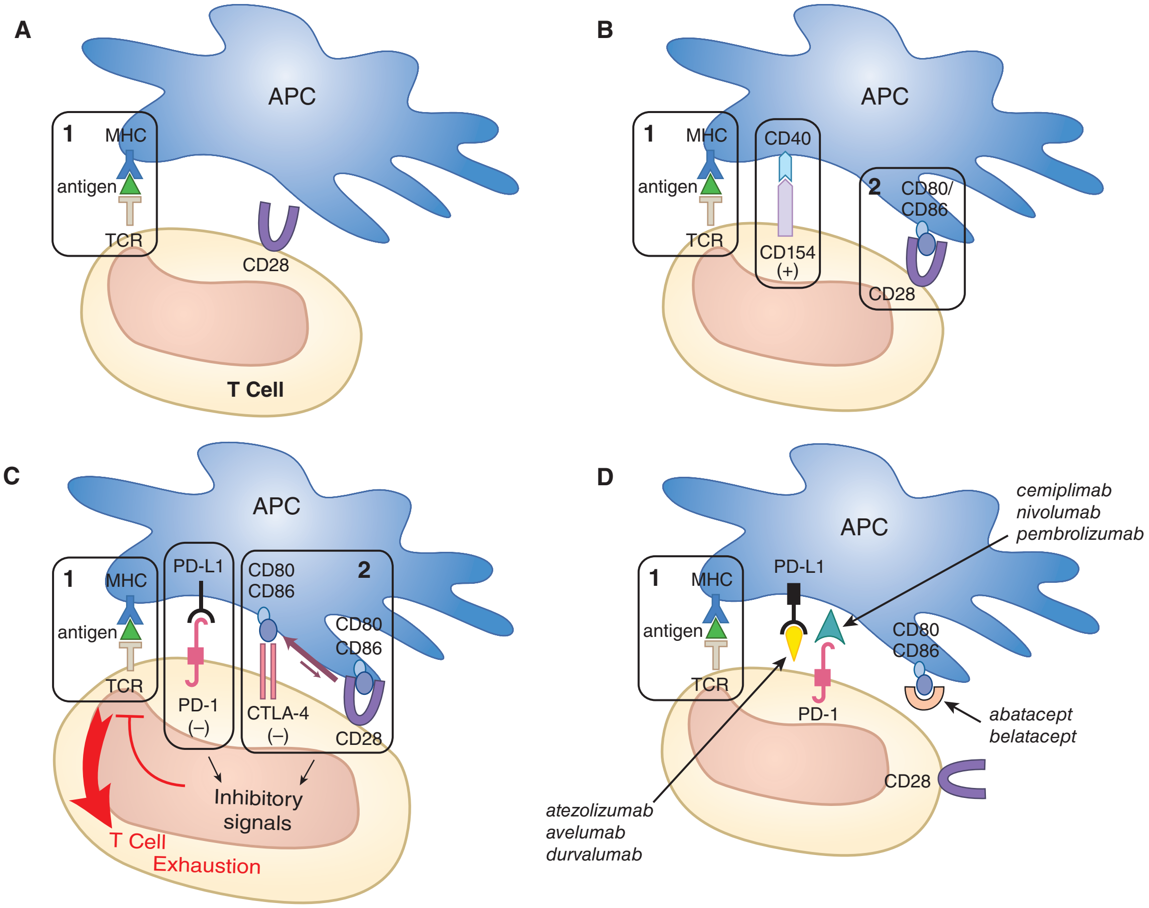

Figure 39–5 *T-cell activation: costimulation and coinhibitory checkpoints.* Numerous membrane CD proteins may be expressed on the APC and the T cell that lead to signaling interactions between ligands and receptors. These interactions can enhance or reduce the activation state of the T cell. Two signals are required for T-cell activation: presentation of an antigen ligand to the TCR and signaling by an additional "costimulatory" pair. **A.** The primary signal, *signal 1*, is the interaction of the TCR with the MHC-antigen complex on the APC. Activation requires a second, costimulatory interaction. **B**. *Signal 2*, the costimulatory interaction between CD28 on the T cell (the costimulatory receptor) and the costimulatory ligand on the APC, CD80/CD86, leads to T-cell activation. Additional costimulatory signals, such as the interaction of CD154 with CD40 on the APC, can further enhance T-cell activation (+). In the absence of costimulation, a T cell can become anergic or unresponsive. **C.** Additional APC–T-cell interactions can occur after T-cell activation, and some can be inhibitory, providing *immune checkpoints* that are important for reducing autoimmunity and for regulating the size and extent of immune responses. For example, the interaction of CTLA-4 (CD152) with CD80/86 produces inhibitory signals that attenuate T-cell activation and proliferation (–). CD28 and CTLA-4 compete for binding to CD80/CD86. As the figure suggests, the affinity of CTLA-4 for CD80/CD86 exceeds that of CD28, and the equilibrium lies toward the formation of the inhibitory signaling complex, CTLA-4-CD80/CD86. T cells may express varying amounts of another important modifier, PD-1 (programmed cell death protein 1, also called CD279). When liganded by PD-L1 (programmed death ligand 1), PD-1 produces inhibitory signals (↑ protein phosphatase activity, ↓ signaling by TCR, ↓ MAPK activity; see Figure 39–2) and reduces T-cell proliferation, leading to T-cell exhaustion, a state of hyporesponsiveness. When PD-1 is highly expressed, as during conditions of chronic viral infection and cancer, suppression of T-cell activity via this pathway can be very effective; this pathway can facilitate continued viral replication and tumor progression. **D.** These immune checkpoints are useful sites for pharmacological regulation of T-cell activation. For instance, the agents *abatacept* and *belatacept* are fusion proteins that contain the extracellular CTLA-4 domain and act as decoys. These agents block costimulation of T cells by binding CD80/CD86 (see additional examples in Figure 39–2). *Nivolumab, pembrolizumab*, and *cemiplimab* (antibodies to PD-1) and *atezolizumab, avelumab,* and *durvalumab* (antibodies to PD-L1) block interaction of PD-1 with PD-L1, thereby blocking the immune suppression that would normally ensue and producing a state of immune hyperactivity. Checkpoint inhibitors that enhance immune responses are being used in cancer therapy (see Chapter 72). Antibodies can also be designed to aid in generating a state of immune suppression that would be useful in treating autoimmune diseases. APC, antigen-presenting cell; CD, cluster of differentiation.

dose used during induction. The drug is licensed for the management of CLL and MS (CAMMS223 Investigators et al., 2008).

Toxicity. Neutropenia remains the most common adverse effect seen with *alemtuzumab.* Almost half of the patients will also experience thrombocytopenia and anemia. Another major side effect is autoimmune hemolytic anemia and other autoimmune diseases thought to be due to immune reconstitution after the profound lymphocyte depletion.

Anti–IL-2 Receptor (Anti-CD25) Antibodies

Daclizumab is a humanized murine complementarity-determining region/human IgG1 chimeric mAb. *Basiliximab* is a murine-human chimeric mAb. Both are licensed for use in conjunction with *cyclosporine* and corticosteroids for the prophylaxis of acute organ rejection in patients receiving renal transplants.

Mechanism of Action. The anti-CD25 mAbs bind with high affinity to the α subunit of the IL-2 receptor (see Figure 39–2) and act as receptor antagonists, inhibiting T-cell activation and proliferation without inducing cell lysis (see Table 39–1). *Daclizumab* has a somewhat lower affinity but a longer $t_{1/2}$ (20 days) than *basiliximab* (Brennan et al., 2006). In addition, the induction of $CD56^+$ $CD4^+$ T cells is associated with response to therapy in patients with MS (D'Amico et al., 2015).

Therapeutic Uses. Anti-CD25 mAbs are used for induction therapy in solid-organ transplantation. They are also in phase III clinical trials in patients with MS. The long $t_{1/2}$ of *daclizumab* (20 days) results in saturation of the IL-2Rα on circulating lymphocytes for up to 120 days after transplantation. *Daclizumab* is administered in five doses (1 mg/kg given intravenously over 15 min in 50–100 mL of normal saline) starting immediately preoperatively and subsequently at biweekly intervals.

The $t_{1/2}$ of *basiliximab* is 7 days. In trials, *basiliximab* was administered in a fixed dose of 20 mg preoperatively and on days 0 and 4 after transplantation. This regimen of *basiliximab*-saturated IL-2R acts on circulating lymphocytes for 25 to 35 days after transplantation. *Basiliximab* was used with a maintenance regimen consisting of *cyclosporine* and *prednisone* and was found to be safe and effective when used in a maintenance regimen consisting of *cyclosporine*, MMF, and *prednisone*.

While *daclizumab* and *basiliximab* are comparable in effectiveness, *daclizumab* has a more costly dosing regimen. The higher cost has reduced demand, and *daclizumab* is now produced only for use in treating MS.

Toxicity. *Basiliximab* and *daclizumab* seem to be relatively safe as induction agents, with most of the clinical trials reporting adverse reactions rates comparable to placebo. No cytokine release syndrome has been noted, but anaphylactic reactions, rare lymphoproliferative disorders, and opportunistic infections may occur. No drug interactions have been described.

Belatacept, a Fusion Protein

Belatacept is a fusion protein composed of a modified Fc fragment of a human immunoglobulin linked to the extracellular domain of the CTLA-4 (CD152) that is present on T cells (Figure 39–6). This second-generation CTLA-4-Ig has two amino acid substitutions, increasing its affinity for CD80 (2-fold) and CD86 (4-fold), yielding a 10-fold increase in potency *in vitro* compared to CTLA-4-Ig (Chinen et al., 2015).

Mechanism of Action. Induction of specific immune responses by T lymphocytes requires two signals: an antigen-specific signal via the TCR and a costimulatory signal provided by the interaction of molecules such as CD28 on CD4 lymphocyte with CD80 and CD86 on APCs and CD2 engagement by lymphocyte function–associated antigen-3 (LFA-3, also known as CD58) on CD8 cells (see Figure 39–5) (Riella and Sayegh, 2013). *Belatacept* is a selective T-cell costimulation blocker that potently binds the cell surface costimulatory ligands (CD80 and CD86) present on APCs, interrupting their interaction with CD28 on T cells (signal 2). The inhibition of signal 2 inhibits T-cell activation, promoting anergy and apoptosis.

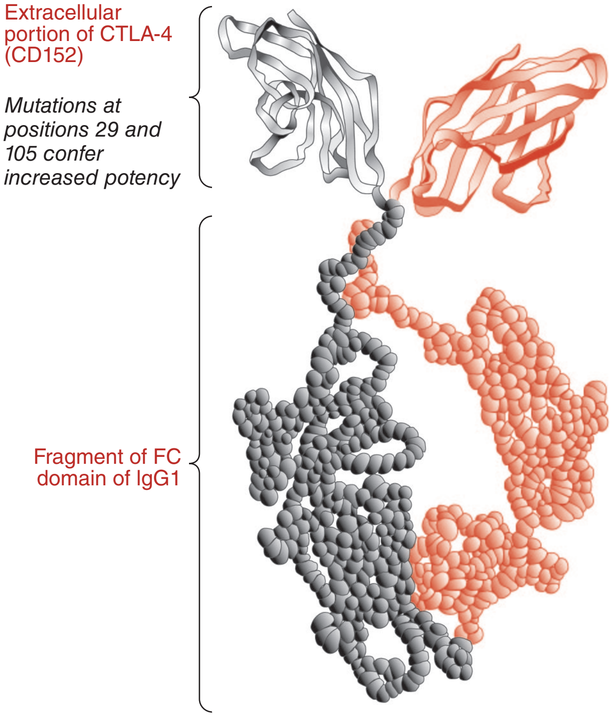
Extracellular portion of CTLA-4 (CD152)
Mutations at positions 29 and 105 confer increased potency
Fragment of FC domain of IgG1

Figure 39–6 *Structure of belatacept, a CLTA-4-Ig congener.* For details, see the text and Figure 39–4.

Disposition and Pharmacokinetics. *Belatacept* is the first intravenous maintenance therapy in solid-organ transplantation. *Belatacept*'s pharmacokinetics were determined to be linear, with zero-order intravenous infusion and first-order elimination within the standard dose range of 5 to 10 mg/kg. The $t_{1/2}$ of *belatacept* is about 11 days.

Therapeutic Uses. Preclinical renal transplant studies showed that *belatacept* did not induce tolerance but did prolong graft survival. *Belatacept* is FDA-approved as an alternative to calcineurin inhibitors as a strategy to prevent long-term calcineurin inhibitor toxicity (Satyananda and Shapiro, 2014; Talawila and Pengel, 2015). *Belatacept* has been approved specifically for prophylaxis of organ rejection in adult patients receiving a kidney transplant in combination with *basiliximab* induction, MMF, and corticosteroids.

The BENEFIT trial compared two *belatacept*-based regimens to *cyclosporine* and showed better kidney function and metabolic profile with *belatacept*-treated patients compared to *cyclosporine*. Patients were induced with *basiliximab* and maintained on MMF and a *prednisone* taper. While infusions of *belatacept* are required relatively frequently early after transplantation, it becomes once per month by the end of the first or third month, depending on the dosage regimen chosen (Masson et al., 2014).

Toxicity. An increased risk of posttransplant lymphoproliferative disorder in Epstein-Barr virus–seronegative patients has been observed with *belatacept* treatment. Hence, its use is restricted to Epstein-Barr virus–seropositive patients. Infusion-related reactions occur infrequently, and the drug is generally well tolerated (Masson et al., 2014).

Drug Interactions. No specific pharmacokinetic drug-drug interactions have been reported with *belatacept* (Pestana et al., 2012).

Interleukin Antagonists

Antibodies to the IL-2 Receptor (CD25)

Anti–IL-2R mAbs discussed previously have been FDA-approved as a second-line drugs for patients with MS.

Anti-CD52

Mature lymphocytes express CD52 (CAMPATH-1 antigen), a negatively charged membrane dodecapeptide. *Alemtuzumab,* discussed previously, is a humanized mAb that binds to CD52 and targets the lymphocyte for destruction. In addition to the uses mentioned, *alemtuzumab* is approved for use in CLL and MS.

Anti-TNF Reagents

Tumor necrosis factor α is a proinflammatory cytokine that has been implicated in the pathogenesis of several immune-mediated intestinal, skin, and joint diseases. Several diseases (rheumatoid arthritis, Crohn's disease) are associated with elevated levels of TNFα. As a result, a number of anti-TNF agents have been developed as treatments.

Infliximab is a chimeric IgG1 mAb containing a human constant (Fc) region and a murine variable region. It binds with high affinity to TNFα and prevents the cytokine from binding to its receptors. *Infliximab* is approved in the U.S. for treating the symptoms of rheumatoid arthritis and is typically used in combination with *methotrexate* in patients who do not respond to *methotrexate* alone. *Infliximab* also is approved for treatment of moderate-to-severe Crohn's disease in patients who have failed to respond to conventional therapy (see Chapter 55). Other FDA-approved indications include ankylosing spondylitis, plaque psoriasis, psoriatic arthritis, and ulcerative colitis. About one in six patients receiving *infliximab* experiences an infusion reaction characterized by fever, urticaria, hypotension, and dyspnea within 1 to 2 h after antibody administration.

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The development of antinuclear antibodies and, rarely, a lupus-like syndrome have been reported after treatment with *infliximab* (Meroni et al., 2015).

Etanercept is a fusion protein that targets TNFα. *Etanercept*, with the ligand-binding portion of a human TNFα receptor fused to the Fc portion of human IgG1, binds to TNFα and prevents it from interacting with its receptors. It is approved for treatment of the symptoms of rheumatoid arthritis, ankylosing spondylitis, plaque psoriasis, polyarticular juvenile idiopathic arthritis, and psoriatic arthritis. *Etanercept* can be used in combination with *methotrexate* in patients who have not responded adequately to *methotrexate* alone. Injection-site reactions (i.e., erythema, itching, pain, or swelling) have occurred in more than one-third of *etanercept*-treated patients.

Adalimumab is another anti-TNF product for subcutaneous use. This recombinant human IgG1 mAb is approved for use in rheumatoid arthritis, ankylosing spondylitis, Crohn's disease, juvenile idiopathic arthritis, plaque psoriasis, psoriatic arthritis, and ulcerative colitis.

Golimumab is a human IgG1 (anti-TNFα) monoclonal antibody. *Golimumab* alone or in combination with *methotrexate* is approved for treatment of moderately to severely active rheumatoid arthritis and active psoriatic arthritis. It is also approved for treatment of patients with ankylosing spondylitis and moderately to severely active ulcerative colitis. *Golimumab* is administered by subcutaneous injections and is available in 50- and 100-mg doses.

Certolizumab pegol is a humanized pegylated antibody specific to TNFα. Pegylation of the Fab' fragment provides sustained activity. This agent is approved for the treatment of adults with Crohn's disease and rheumatoid arthritis, active psoriatic arthritis, and active ankylosing spondylitis. It is available as 200-mg lyophilized powder or 200-mg/mL prefilled sterile injections for subcutaneous administration.

Toxicity. All anti-TNF agents (i.e., *infliximab, etanercept, adalimumab, golimumab, certolizumab*) carry an FDA black-box warning concerning increase in the risk of serious infections, lymphomas, and other malignancies. For example, fatal hepatosplenic T-cell lymphomas have been reported in adolescent and young adult patients with Crohn's disease treated with *infliximab* in conjunction with *azathioprine* or *6-MP*.

IL-1 Inhibition

Plasma IL-1 levels are increased in patients with active inflammation (see Chapter 38). In addition to the naturally occurring IL-1 receptor antagonist (IL-1RA), several IL-1RAs are in development, and a few have been approved for clinical use.

Anakinra is an FDA-approved recombinant, nonglycosylated form of human IL-1RA for the management of joint disease in rheumatoid arthritis. *Anakinra* is also approved for cryopyrin-associated periodic syndromes (CAPS), a group of rare inherited inflammatory diseases associated with overproduction of IL-1 that includes familial cold autoinflammatory and Muckle-Wells syndromes, and for treatment of neonatal-onset multisystem inflammatory disease. It can be used alone or in combination with anti-TNF agents such as *etanercept*, *infliximab*, or *adalimumab*.

Canakinumab is an IL-1β mAb that is FDA-approved for CAPS and active systemic juvenile idiopathic arthritis. *Canakinumab* is being evaluated for use in chronic obstructive pulmonary disease.

Rilonacept, a fusion protein that binds IL-1, is being evaluated for gout. IL-1 is an inflammatory mediator of joint pain associated with elevated uric acid crystals.

Other Interleukin Antagonists

Tocilizumab, an IL-6 receptor antagonist, is FDA-approved for treatment of rheumatoid arthritis and systemic juvenile idiopathic arthritis, giant cell arteritis, polyarticular juvenile idiopathic arthritis, and cytokine release syndrome. Two other biologicals targeting the IL-6 receptor have been developed: *sarilumab* and *satralizumab*. *Satralizumab* is approved for the treatment of neuromyelitis optica spectrum disorder in adult patients who are positive for anti–aquaporin-4 antibody. *Siltuximab* is an IL-6 antagonist approved for treatment of multicentric Castleman disease if the patient is HIV and human herpesvirus 8 negative. A series of biologicals target the IL-12 and IL-23 pathways. *Ustekinumab* is directed against the IL-12 β subunit, which is a common subunit of IL-12 and IL-23, and is indicated for the treatment of plaque psoriasis and psoriatic arthritis. Selective targeting of the IL-23 pathway was achieved with the development of mAbs that target the IL-23 p19 subunit (*guselkumab*, *risankizumab*, *tildrakizumab*). *Tildrakizumab* and *risankizumab* have significantly longer half-lives than *guselkumab* and are administered in a maintenance regimen every 12 weeks versus 8 weeks, respectively. *Secukinumab* is a human anti–IL-17A antagonist and *ixekizumab* is a humanized anti–IL-17A antagonist that are indicated for treatment of plaque psoriasis, psoriatic arthritis and ankylosing spondylitis. *Brodalumab* also inhibits the IL-17 pathway, although by targeting IL-17 receptor A. The IL-17 receptor A is a coreceptor for multiple IL-17 cytokines, including IL-17A, IL-17A/F, IL-17F, IL-17E, and IL-17C. *Brodalumab* is also indicated for the treatment of plaque psoriasis but has been reported to lead in some patients to suicidal ideation and behavior.

A decoy-resistant variant of IL-18 (ST-067) is in a phase I clinical trial in a broad array of solid tumors. This variant was engineered to be resistant to IL-18 binding protein, which is frequently upregulated in tumors.

Therapeutic approaches targeting cytokine pathways associated with type II immune responses such as those triggered by IL-4 and IL-5 have been approved for asthma and moderate-to-severe atopic dermatitis. They are discussed in more detail in Chapters 44 and 75 along with biologicals directed to IL-13, IL-33, and thymic stromal lymphopoietin, which are in clinical development.

Inhibition of Cytokine Signaling: JAK Inhibitors

The use of "biologicals" such as antibodies and proteins targeting a diversity of immune cell functions and immune modulating cytokines and their receptors has revolutionized the treatment of most autoimmune diseases. However, not all patients respond fully to these biological agents, and there is a potential cross talk among the multiple cytokines that accumulate in the inflammatory microenvironment. The latter may render ineffective some of the single targeting agents, including some of the widely used mAbs. A recently developed strategy instead targets a shared repertoire of intracellular molecules that transduce the signals generated by multiple cytokine receptors, thereby inhibiting their function concomitantly. Among them, a growing family of cytokine signaling inhibitors represents an active area of drug development, with multiple small-molecule inhibitors recently approved (see Table 39–2), primarily inhibitors of the Janus kinase (JAK) family.

JAK Inhibitors

Mechanism of Action. Janus kinases are protein tyrosine kinases that are stimulated by agonist-occupied cytokine receptors; in turn, JAKs initiate intracellular signaling cascades mediating most biological functions regulated by these cytokines (Aaronson and Horvath, 2002; Stark and Darnel, 2012). These include the direct tyrosine phosphorylation of signal transducer and activator of transcription (STAT) transcription factors, such as STAT2, STAT3, and STAT5, which initiate the expression of growth-promoting and inflammatory gene programs (Aaronson and Horvath, 2002; Stark and Darnel, 2012) (see Figure 39–2). The available JAK inhibitors, known as JAKis or JAKinibs, have varying specificities for the JAK isoforms, which include JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2). Most JAKinibs target the ATP binding site of the JAK tyrosine kinase domain, while some of the most recently developed JAKinibs act as allosteric inhibitors targeting specific JAKs (Alexander et al., 2021; Gadina et al., 2020).

Therapeutic Uses. In the past few years, 10 JAKinibs have been approved for use in humans (two JAK1 and JAK2 inhibitors: *ruxolitinib* and *baricitinib*; three JAK1 inhibitors: *upadacitinib*, *filgotinib*, and *abrocitinib*; two JAK2 inhibitors: *fedratinib* and *pacritinib*; a JAK1, JAK2, and JAK3 inhibitor: *tofacitinib*; and two pan-JAK [JAK1/JAK2/JAK3/TYK2] inhibitors, *peficitinib* and *delgocitinib*) for multiple indications, including rheumatoid arthritis, psoriatic arthritis, ulcerative colitis, polycythemia vera, GVHD, myelofibrosis, ankylosing spondyloarthritis, and allergic dermatitis and atopic dermatitis. Among these, *abrocitinib*, *baricitinib*, *fedratinib*, *ruxolitinib*, *tofacitinib*, and *upadacitinib* are FDA-approved; *filgotinib* is approved by the European Medicines Agency; *peficitinib* is approved in South Korea and Japan only; and *delgocitinib* is approved by the Japanese Pharmaceutical and Medical Devices Agency.

Many clinical trials are ongoing using these JAKinibs for multiple autoimmune and inflammatory conditions, including alopecia areata, lupus, Sjögren's syndrome, and inflammatory eye disease (Alexander et al., 2021). Numerous new JAKinibs are under current development, including TYK2-specific inhibitors, JAK3-specific inhibitors, and multitarget agents inhibiting JAKs and other kinases. Development includes nonabsorbable JAKinibs aimed at achieving local responses while limiting systemic exposures. JAKinibs represent an attractive area for future drug development, building on the well-established therapeutic activity of the approved agents and the opportunities to identify new indications and develop next-generation JAKinibs with distinct kinase selectivity. See Chapter 75 for additional information on JAKinibs and their clinical use.

ADME.

Administration. JAKinibs are delivered by oral administration and are readily absorbed, reaching peak plasma concentrations shortly after administration.

Ruxolitinib is metabolized primarily by hepatic CYP3A4. Hence, CYP3A4 inhibitors such as *ketoconazole* can reduce the clearance rate and increase *ruxolitinib* half-life. Conversely, *rifampin*, which induces CYP3A4, decreases the exposure and half-life of *ruxolitinib*.

Tofacitinib is also primarily metabolized by hepatic CYPs and is affected as expected by CYP inhibitors and inducers. Unmetabolized *tofacitinib* is eliminated by the kidneys, and dose adjustment is necessary in patients with liver and renal impairment.

Baricitinib is a substrate for the organic anion transporter OAT3, the multidrug and toxin extrusion protein (MATE) 2-K, P-glycoprotein, and breast cancer resistance protein (BCRP); *baricitinib* is actively secreted in the kidney, and probenecid, an OAT3 inhibitor, decreases renal clearance and increases the AUC of *baricitinib* (Posada et al., 2017).

Drug Interactions. Most JAKinibs exhibit drug interactions with potent inhibitors of CYP3A4, such as *ketoconazole*, or moderate inhibitors of CYP3A4 and potent inhibitors of CYP2C19 such as *fluconazole*. The combination of JAKinibs with other strong systemic immunosuppressants is generally not recommended. All age-appropriate vaccinations (see Chapter 40) should be completed prior to therapy, including prophylactic herpes zoster vaccinations. Immunizations with live vaccines should be avoided.

Toxicities. As with most immunosuppressive agents, JAKinibs are associated with a risk for infection. Most frequent side effects are anemia, thrombocytopenia, leukopenia, lymphopenia, and neutropenia, as well as hyperlipidemia diarrhea, and in some cases, headache and high blood pressure. *Baricitinib*, *tofacitinib*, and *upadacitinib* increase the risk of venous thromboembolism, deep venous thromboembolism, and pulmonary embolism. The FDA has recently issued a boxed warning for oral JAKinibs used chronically. The black-box warning for these agents includes the following:

- Increased risk of serious bacterial, fungal, viral, and opportunistic infections leading to hospitalization or death
- Higher rate of all-cause mortality, including sudden cardiovascular death
- Higher rate of certain cancers
- Higher rate of cardiovascular death, myocardial infarction, and stroke
- Thrombosis

JAK inhibitors should be used with awareness of the latest information from the FDA on initiating therapy and patient monitoring and with knowledge of the recommended usages and potential adverse reactions.

Inhibition of Lymphocyte Function–Associated Antigen

Alefacept is a human LFA-3–IgG1 fusion protein. The LFA-3 portion of *alefacept* binds to CD2 on T lymphocytes, blocking the interaction between LFA-3 and CD2 and interfering with T-cell activation. *Alefacept* is approved for use in psoriasis. Treatment with *alefacept* has been shown to produce a dose-dependent reduction in T-effector memory cells (CD45, RO$^+$) but not in naïve cells (CD45, RA$^+$) (Vincenti and Kirk, 2008). This effect has been related to its efficacy in psoriatic disease and is of significant interest in transplantation because T-effector memory cells are associated with costimulation blockade-resistant and depletional induction-resistant rejection. *Alefacept* delays rejection in cardiac transplantation in nonhuman primates and has synergistic potential when used with costimulation blockade or *sirolimus*-based regimens in nonhuman primates (Vincenti and Kirk, 2008). A phase II multicenter study to assess the safety and efficacy of maintenance therapy with *alefacept* in kidney transplant recipients showed no difference from placebo controls (Rostaing et al., 2013).

Biologicals Targeting Integrins

Mechanism of Action

Leukocyte migration through the endothelium is a critical step in the inflammatory response that depends, in part, on the interaction between integrins and their ligands. Binding of integrins expressed on leukocytes and their corresponding ligands—cell adhesion molecules expressed on endothelial cells—allows for leukocyte arrest and subsequent transmigration (Dustin, 2019; Luo et al., 2007). Integrins are heterodimeric receptors, composed of α and β subunits, and biologicals targeting integrins, such as *natalizumab* and *vedolizumab*, have been developed to inhibit leukocyte migration to inflamed tissues. While *natalizumab* targets α4, a shared subunit between the α4β1 and α4β7 integrins, *vedolizumab* selectively blocks the α4β7 integrin. Interestingly, the expression of cell adhesion molecules can be tissue specific. Such is the case of MAdCAM-1 (mucosal addressin cell adhesion molecule 1), the ligand for the α4β7 integrin, which is expressed on endothelial cells at mucosal sites. Thus, neutralization of α4β7-MAdCAM-1 interaction reduces leukocytes homing to the inflamed intestine. By contrast, interaction of α4β1 integrin with VCAM-1 (vascular cell adhesion molecule 1) is critical for T-cell trafficking across the blood-brain barrier; blocking this interaction has been highly effective in inhibiting T-cell infiltration in the CNS.

Therapeutic Uses

Natalizumab is indicated for the treatment of adult patients with relapsing-remitting MS as well as active, secondary progressive disease. *Natalizumab* is also indicated for the treatment of moderate to severe Crohn's disease that does not adequately respond to conventional therapies or TNFα inhibitors. *Vedolizumab*, a selective α4β7 integrin antagonist, is indicated for moderately to severely active Crohn's disease and ulcerative colitis.

Toxicities

A significant increase the risk of progressive multifocal leukoencephalopathy (PML) has been observed after *natalizumab* administration. PML is a rare, opportunistic viral infection of the brain caused by the human polyomavirus 2, also known as John Cunningham virus (JCV). PML can lead to death or severe disability. A series of factors are known to increase the risk of PML in patients treated with *natalizumab*. These include the presence of anti-JCV antibodies, prolonged treatment with *natalizumab*, or prior treatment with immunosuppressants such as *mitoxantrone*, *azathioprine*, *methotrexate*, *cyclophosphamide*, or MMF. While only one case of PML has been reported with the use of *vedolizumab*, monitoring of patients for new or worsening neurological signs or symptoms is recommended in patients receiving this biological. Both *natalizumab* and *vedolizumab* increase the risk of infections. Given the selective ability of *natalizumab* to inhibit T-cell trafficking to the CNS, this drug is associated with an increased risk of herpes encephalitis and meningitis.

Cytokine Therapy

Interferon

For a description of interferon (IFN) induction and signaling and the major actions of IFN, see Chapter 38. IFN-β was among the first cytokines used for the treatment of autoimmune diseases, particularly MS. IFNs are endogenous regulatory cytokines that increase or decrease transcriptional initiation of hundreds of genes in a cell-dependent fashion with multiple mechanisms of action, including induction of IL-10. The different IFN-β formulations have modest therapeutic efficacy, decreasing the exacerbation rate in MS by approximately 30%. They are relatively safe; fatigue is the major side effect. There are multiple preparations of IFN-β in the market that are administered either by the intramuscular or subcutaneous routes. IFN-β preparations are usually used for MS, and IFN-α/γ preparations are used for infections. There are no significant differences between these IFN preparations, and as more efficacious drugs are now available, they should no longer be considered first-line drugs for the treatment of MS (see below).

Granulocyte-Macrophage Colony-Stimulating Factor

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that functions as a growth factor that stimulates the differentiation of hematopoietic stem cells (HSCs) into granulocytes such as neutrophils, eosinophils, and basophils. It also promotes the differentiation of HSCs into monocytes. A recombinant form of GM-CSF, *sargramostim*, is approved for the acceleration of myeloid reconstitution following autologous bone marrow or peripheral blood progenitor cell transplantation. *Sargramostim* is also indicated for the induction of neutrophil production following chemotherapy and in patients who have been acutely exposed to myelosuppressive doses of radiation.

Targeting B Cells

Most of the advances in transplantation can be attributed to drugs designed to inhibit T-cell responses. As a result, T-cell–mediated acute rejection has been become much less of a problem, while B-cell–mediated responses such as antibody-mediated rejection and other effects of donor-specific antibodies have become more evident. Both biologicals and small molecules with B-cell–specific effects now are in development for transplantation, including humanized mAbs to CD20 and inhibitors of the two B-cell–activation factors, BLYS and APRIL, and their respective receptors. *Belimumab*, an mAb that targets BLYS, is approved for use in patients with systemic lupus erythematosus.

The CD20 antibodies *rituximab* and *ocrelizumab* deplete circulating mature B lymphocytes (though they may remain to some degree in lymph nodes), and positive results from clinical trials in patients with rheumatoid arthritis and MS strongly suggest that B cells play a critical part in disease pathogenesis. Genetic fine mapping studies demonstrated a potentially pathogenic role of B cells in MS and rheumatoid arthritis that were not limited to antibody production. In particular, a definitive genetic modeling study pointed to the crucial role of B cells as APCs (Farh et al., 2015).

Tolerance

Immunosuppression has concomitant risks of opportunistic infections and secondary tumors. Therefore, the ultimate goal of research on organ transplantation and autoimmune diseases is to induce and maintain immunological tolerance, the active state of antigen-specific nonresponsiveness (Krensky and Clayberger, 1994). Tolerance, if attainable, would represent a true cure for conditions discussed previously in this section without the side effects of the various immunosuppressive therapies. The calcineurin inhibitors prevent tolerance induction in some, but not all, preclinical models. In these same model systems, *sirolimus* does not prevent tolerance and may even promote tolerance (Kawai et al., 2014; Krensky and Clayberger, 1994). In experimental animals, *sirolimus* promotes regulatory T cells, a subtype of T cells shown to suppress all immunity and promotes tolerance. Studies in kidney transplant recipients showed that *sirolimus* spared regulatory T cells in the periphery, unlike calcineurin inhibitors, which reduced their percentage (Segundo et al., 2006).

Costimulatory Blockade

Inhibition of the costimulatory signal has been shown to induce tolerance (Figure 39–5).

Abatacept is a fusion protein (see previous discussion) that contains the binding region of CTLA-4 (CD152), which is a CD28 homolog, and the Fc region of the human IgG1. CTLA-4-Ig competitively inhibits CD28 binding to CD80 and CD86 and thus inhibits the activation of T cells. CTLA-4-Ig is effective in the treatment of rheumatoid arthritis in patients resistant to other drugs.

A second costimulatory pathway involves the interaction of CD40 on activated T cells with CD40 ligand (CD154) on B cells, endothelium, or APCs (see Figure 39–5). Among the purported activities of anti-CD154 antibody treatment is the blockade of B7 expression induced by immune activation. Two humanized anti-CD154 mAbs have been used in clinical trials in renal transplantation and autoimmune diseases. The development of these antibodies, however, was discontinued due to associated thromboembolic events. Alternative approaches to block CD154 with improved safety profile, including modified antibodies, recombinant proteins, and biologicals that target CD40 are under clinical trial.

Donor Cell Chimerism

A promising approach is induction of chimerism (coexistence of cells from two genetic lineages in a single individual) by first dampening or eliminating immune function in the recipient with ionizing radiation, drugs such as *cyclophosphamide*, or antibody treatment, and then providing a new source of immune function by adoptive transfer (transfusion) of bone marrow or hematopoietic stem cells. On reconstitution of immune function, the recipient no longer recognizes new antigens provided during a critical period as "nonself." Such tolerance is long lived and less likely to be complicated by the use of calcineurin inhibitors.

Antigens

Specific antigens induce immunological tolerance in preclinical models of diabetes mellitus, arthritis, and MS. *In vitro* and preclinical *in vivo* studies demonstrated that one can selectively inhibit immune responses to specific antigens without the associated toxicity of immunosuppressive therapies. With these insights comes the promise of specific immune therapies to treat an array of immune disorders from autoimmunity to transplant rejection (Riedhammer and Weissert, 2015). To date, this approach has worked only in animal models of autoimmune disease.

Soluble HLA

In the precyclosporine era, blood transfusions were shown to be associated with improved outcomes in renal transplant patients. These findings gave rise to donor-specific transfusion protocols that improved outcomes. After the introduction of *cyclosporine*, however, these effects of blood transfusions disappeared, presumably due to the efficacy of this drug in blocking T-cell activation. Nevertheless, the existence of tolerance-promoting effects of transfusions is irrefutable. It is possible that this effect is due to HLA molecules on the surface of cells or in soluble forms. Soluble HLA and peptides corresponding to linear sequences of HLA molecules can induce immunological tolerance in animal models via a variety of mechanisms (Murphy and Krensky, 1999).

Immunotherapy for Multiple Sclerosis

Clinical Features and Pathology

Multiple sclerosis is a genetically mediated demyelinating inflammatory disease of the CNS white matter that includes a loss of immune tolerance and the development of apparent autoimmunity toward components of the CNS. The detailed etiology of MS remains unknown. The disease occurs with prevalence increasing from late adolescence to 35 years of age and then declining. MS is 2- to 3-fold more common in females than in males and occurs mainly in higher latitudes of the temperate climates. Epidemiological studies suggest a role for environmental factors in the pathogenesis of MS, including low vitamin D, smoking, increases in body mass index, and high salt intake (Ransohoff et al., 2015).

Genome-wide association studies have identified 200 genetic variants associated with MS susceptibility (International Multiple Sclerosis Genetics Consortium et al., 2007). Many of these variants fall within specific signaling cascades, which suggests that alterations in pathways—rather than individual genes—may be useful in predicting response to therapy. Over half of genetic variants associated with MS risk are also found in other putative autoimmune diseases, and risk alleles are primarily associated with genes that regulate immune function. Single-nucleotide variants were strongly enriched within binding sites for immune-related transcription factors (Farh et al., 2015). In patients with MS, there are activated T cells that are reactive to different myelin antigens, including myelin basic protein, and these T cells secrete proinflammatory cytokines, whereas in healthy controls, T cells secrete the anti-inflammatory IL-10 cytokine (Cao et al., 2015). IL-2–IL-2R signaling features prominently in current considerations of understanding the progression of MS (Peerlings et al., 2021).

Attacks are classified by type and severity and likely correspond to specific degrees of CNS damage and pathological processes. Thus, physicians refer to relapsing-remitting MS (the form in 85% of younger patients), secondary progressive MS (progressive neurological deterioration following a long period of relapsing-remitting disease), and primary progressive MS (~15% of patients, wherein deterioration with relatively little inflammation is apparent at onset).

Pharmacotherapy

There are no pharmacological cures for MS. Specific therapies are aimed at resolving acute attacks, reducing recurrences and exacerbations, and slowing the progression of disability. There are now both parenteral and oral immunomodulatory agents for the treatment of MS that are classified as "disease-modifying" agents. Disease-modifying agents for the various stages of MS are summarized below. See Table 39–3 for the efficacy ranking of approved therapies; pharmacological profiles for most of them are presented earlier in this chapter. The high annual cost of these newer disease-modifying agents is an issue (Medical Letter, 2021a, 2021b).

Acute exacerbations of MS can be treated with a short course of *methylprednisolone* (3–5 days of 500–1000 mg/day, administered orally or intravenously) (Comi and Radaelli, 2015). To varying degrees, the agents listed reduce relapse rates in relapsing-remitting MS and secondary progressive MS. Relapsing-remitting MS is often treated initially with an antibody to CD20 (i.e., *ocrelizumab* or *ofatumumab*) or with *natalizumab*. The American Academy of Neurology's guidelines for using disease-modifying therapies for adults with MS (Rae-Grant et al., 2018) suggest *alemtuzumab*, *natalizumab*, or an S1PR modulator for MS patients with highly active disease. In general, the disease-modifying immunomodulatory agents for MS should not be coadministered with other immunosuppressive drugs. In administering any of these immunomodulatory agents, it is essential to follow the FDA-approved instructions for initiation and maintenance of treatment and monitoring during the process, with awareness of potential adverse effects, drug interactions, and risks of fetal harm. Earlier rather than later use of these drugs in the course of MS is more effective in preventing disease relapses. The reductions in annual relapse rates achieved by these disease-modifying agents in clinical trials has varied from 34% to 68% (Goldschmidt and McGinley, 2021; Medical Letter, 2021a).

Parenteral Agents

First-Generation Agents. The so-called first-generation (but not necessarily "first-line") drugs include IFN-β (discussed above in Cytokine Therapy) and random polymers that contain amino acids commonly used as MHC anchors. *Glatiramer acetate* (GA), a random-sequence polypeptide consisting of four amino acids (alanine [A], lysine [K], glutamate [E], and tyrosine [Y]) with an average length of 40 to 100 amino acids, binds efficiently to MHC class II DR molecules *in vitro*. In

TABLE 39–3 ■ EFFICACY RANKING OF APPROVED THERAPIES FOR MULTIPLE SCLEROSIS[a]

DRUG	ERA OF DEVELOPMENT	MECHANISM OF ACTION	KEY CONSIDERATIONS
Most effective			
Natalizumab	Second	Monoclonal antibody against integrin α4	Risk of PML must be assessed via presence of JCV antibodies.
Ocrelizumab	Third	mAb against CD20 (B cells)	Low risk of PML, slight increase in infections
Alemtuzumab	Third	mAb against CD52	High risk of second-degree thyroiditis and other autoimmune disease
Highly effective			
Fingolimod	Second	Sphingosine S1P-R modulator	Cardiac complications preclude use in individuals over the age of 50 and those with history of cardiac disease. VZV antibody testing must be conducted to mitigate risk of disseminated herpes zoster.
Dimethyl fumarate	Third	Immunomodulator	Necessary to monitor lymphocyte count as risk mitigation against PML. Gastrointestinal complications may limit use.
Moderately effective			
IFN-β	First	Immunomodulator	Well-characterized long-term safety and efficacy profiles. Patients should not be required to "fail" before receiving alternative treatments.
Glatiramer acetate	First	Immunomodulator	Best safety profile for pregnant women with mild disease. Patients should not be required to "fail" before receiving alternative treatments.
Teriflunomide	Third	Pyrimidine-synthesis inhibitor	Risk of teratogenicity precludes use in women who are, or intend to become, pregnant.

[a] Rankings are estimated on the basis of clinical trials, postapproval studies, and a few head-to-head comparisons. The factors that determine drug efficacy in any individual patient are largely undefined, and good clinical judgment is essential for treatment selection. For details, see Ransohoff et al., 2015. Reproduced with permission from Ransohoff RM, et al. Multiple sclerosis—a quiet revolution. *Nat Rev Neurol*, **2015**, 11:134–142. Copyright © 2015 Nature Publishing Group.

clinical trials, GA, administered subcutaneously to patients with relapsing-remitting MS, decreased the rate of exacerbations by about 30%, similar to the efficacy of IFN-β. *In vivo* administration of GA induces highly cross-reactive CD4$^+$ T cells that are immune-deviated to secrete anti-inflammatory T_H2 cytokines such as IL-4 and IL-13. Administration of GA also prevents the appearance of new lesions detectable by magnetic resonance imaging (Duda et al., 2000). GA provided an early illustration of the potential of an agent that ameliorates autoimmune disease by altering signals through the TCR complex. However, the long-term treatment of patients with MS has moved from the use of IFN-β and GA to more effective and directed treatments, and there is no rationale for the use of step therapy with IFN-β and GA before using the more effective drugs such as *ocrelizumab* and *natalizumab*.

Antibodies to α4 Integrin. *Natalizumab* is a recombinant humanized antibody to the adhesion molecule α4 integrin. An interaction of α4β1 integrin with vascular cell adhesion molecule 1 (VCAM1) is critical for T-cell trafficking from the periphery into the CNS; blocking this interaction has been highly effective in inhibiting exacerbations of MS. *Natalizumab* is administered via a 60-min IV infusion every 4 weeks. Its use is associated with the development of PML; consequently, the availability of *natalizumab* is restricted by a Risk Evaluation and Mitigation Strategy program that dictates measurement of JCV antibodies. Patients negative for JCV are often recommended to begin *natalizumab*, while JCV-positive persons are tested for VZV to evaluate for treatment with an S1PR modulator.

Antibodies to CD20. B-cell depletion therapy with *ocrelizumab*, a humanized antibody to CD20, and with *ofatumumab*, a recombinant human antibody to CD20, is an efficacious treatment (Hauser et al., 2017). *Ocrelizumab* must be administered by IV infusion at 6-month intervals; *ofatumumab* is administered subcutaneously on a monthly basis. Both antibodies are approved for relapsing-remitting MS and secondary progressive MS. *Ocrelizumab* is also approved for primary progressive MS. *Rituximab,* a chimeric antibody to CD20 (see Chapter 72), has been used off-label for the treatment of MS.

Antibodies to CD52. *Alemtuzumab* is a humanized mAb directed against CD52 that depletes CD52$^+$ B and T cells. It is approved for relapsing-remitting MS. *Alemtuzumab* has a long-lasting effect, but due to adverse infusion responses, serious autoimmune reactions, and association with malignancies, it is recommended for patients who have had suboptimal response to two other disease-modifying agents. The antibody is administered by a daily intravenous infusion for 5 consecutive days, then again 12 months later for 3 consecutive days. *Alemtuzumab* is more effective than IFN-β1a in preventing relapses. Due to the risk of fetal harm, women of childbearing potential should use effective contraception while taking *alemtuzumab* and for 4 months thereafter.

Anthracenediones. *Mitoxantrone*, an inhibitor of DNA replication, has shown effect in reducing relapse rates and slowing progression of MS. The drug is given by a short intravenous infusion every 3 months. Long-term cumulative risks (cardiotoxicity, chronic myeloid leukemia) are significant, reducing its safety profile.

Oral Agents

S1PR Modulators. The pharmacology of S1PR modulators has been described earlier in the chapter. The relevance of S1P to immunity derives from the role of S1P in promoting the egress of lymphocytes from lymphoid tissues and their infiltration into the CNS. The efficacy of S1PR modulators in MS resides in their capacity to act as irreversible activators of S1PRs and cause irreversible downregulation of S1P signaling (see Figure 39–3), thereby reducing the egress of lymphocytes from lymphoid tissue and disrupting the targeting of circulating lymphocytes to the CNS. There are four FDA-approved S1PR modulators: *ponesimod, siponimod, ozanimod,* and *fingolimod.* All but *fingolimod* act directly; *fingolimod* is phosphorylated to its active form (see Figure 39–3). The individual agents differ in their specificity for the five S1PRs and thus in the breadth of their effects and side effects. *Fingolimod* interacts with all subtypes of S1PRs except $S1P_2R$. *Siponimod* and *ozanimod* have high affinity for $S1P_1R$ and $S1P_5R$. *Ponesimod* shows the greatest specificity, interacting with high affinity only at $S1P_1R$, the S1PR found on lymphocytes and endothelial cells. All four S1PR modulators are approved for use in treating relapsing-remitting MS. *Ponesimod* is approved for secondary progressive MS as well. S1PR modulators are FDA-approved as a first-line therapy in MS, decreasing the exacerbation rate by about 50%. MS may worsen when S1PR modulators are withdrawn.

Fumarates. *Monomethyl fumarate, dimethyl fumarate*, and *diroximel fumarate* appear to have multiple immunomodulatory effects and are used for relapsing-remitting and secondary progressive MS in adults. A detailed mechanism of action is unknown. *Dimethyl fumarate* and *diroximel fumarate* are metabolized to *monomethyl fumarate*, the active form, which is an activator of *nrf2* that mediates an antioxidative stress response. *Monomethyl fumarate* also is a nicotinic acid receptor agonist, an action that may contribute to the cutaneous flushing associated with the initiation of fumarates. The fumarates reduce annualized relapse rates by about 50% as compared with placebo. These agents appear to be safe, although GI side effects can occasionally cause difficulties. Fumarates have been associated with lymphopenia, PML, and opportunistic infections (viral [e.g., herpes zoster virus], fungal, bacterial). The different preparations have different dosing, but all begin with a 7-day twice-daily initiation dosage followed by a double-dose twice-daily maintenance regimen. Prior to initiation, patient blood count, serum aminotransferase, alkaline phosphatase, and bilirubin should be assessed and then monitored throughout the drug's use for signs of lymphopenia and liver injury.

Teriflunomide. *Teriflunomide* reduces pyrimidine synthesis by reversibly inhibiting dihydro-orotate dehydrogenase, thereby causing a reduction in proliferation of activated B and T cells (Bar-Or et al., 2014). The drug carries an FDA boxed warning about risks of hepatotoxicity and teratogenicity, and a complete blood count before treatment and assessments and monitoring of serum enzymes and bilirubin before and during the treatment period are required. Physicians should be aware of drug interactions due to effects of *teriflunomide* on CYPs 2C8 and 1A2; administration of *teriflunomide* with *warfarin* may reduce *warfarin*'s effect. *Teriflunomide* is approved for treating relapsing-remitting MS; in clinical trials, it has reduced annual relapse rates by approximately 34%.

Cladribine. The purine antimetabolite *cladribine* (chloro-2′-deoxy-β -D-adenosine) is FDA-approved for treatment of relapsing-remitting MS and secondary progressive MS in adults. The drug selectively depletes B cells. *Cladribine* carries an FDA boxed warning about risks of malignancies and teratogenicity and requires cancer screening and other assessments prior to initiation. Patients taking *cladribine* are at increased risk for opportunistic infections and hepatic damage. The drug is administered in four doses at prescribed times over the course of a year, followed by a 2-year hiatus. In clinical trials, *cladribine* has reduced annual relapse rates by approximately 50%.

Drug Facts for Your Personal Formulary: *Immunosuppressants, Immunomodulators, and tolerogens*

	Therapeutic Uses	Clinical Pharmacology and Tips
Glucocorticoids		
Prednisone (prednisone → prednisolone in liver)	• Prevent and treat transplant rejection; treat GVHD in bone marrow transplant, autoimmune disease, rheumatoid arthritis, ulcerative colitis, MS, systemic lupus erythematosus	• Broad effects on cellular immunity • Affects transcription of many genes; ↓ nuclear factor-κB activation, ↓ proinflammatory cytokines IL-1 and IL-6 • ↓ T-cell proliferation, cytotoxic T-lymphocyte activation and neutrophil and monocyte function • Can cause ↑ blood glucose, hypertension, cushingoid habitus, ↑ weight, ↑ risk of infection, osteoporosis, glaucoma, cataracts, depression, anxiety, psychosis • Long-term treatment → adrenal suppression; withdraw slowly on alternate days
Prednisolone	• Rheumatoid arthritis, uveitis, ulcerative colitis, vasculitis, sarcoidosis, systemic lupus erythematosus	• As above
Methylprednisolone	• Systemic lupus erythematosus, MS	• As above
Dexamethasone	• Rheumatoid arthritis, idiopathic thrombocytopenic purpura	• As above
Calcineurin Inhibitors		
Cyclosporine	• Transplant rejection prophylaxis, transplant rejection rescue therapy, rheumatoid arthritis, psoriasis and other skin diseases, xerophthalmia	• Use algorithms to delay dosing until renal function okay in kidney transplant patients • Monitor C_p to avoid side effects • Side effects: tremor, hallucinations, drowsiness, coma, nephrotoxicity, hypertension, hirsutism, hyperlipidemia, gum hyperplasia • Metabolized by CYP3A → drug interactions • Severe interactions with antiarrhythmics
Tacrolimus	• Transplant rejection prophylaxis, transplant rejection rescue therapy	• GI absorption is incomplete and variable • Side effects include nephrotoxicity, neurotoxicity, GI complaints, and hypertension • Glucose intolerance and diabetes mellitus • Metabolized by CYP3A → drug interactions • Monitor blood levels to avoid nephrotoxicity
Voclosporin	• Lupus nephritis	• Side effects include nephrotoxicity, high blood pressure, neurotoxicity • Metabolized by CYP3A4 → drug interactions • ↑ risk of skin cancers and lymphomas • ↑ risk of infections
Antiproliferative and Antimetabolic Agents		
Azathioprine (inhibits purine synthesis and DNA replication)	• Adjunct for prevention of organ transplant rejection, rheumatoid arthritis	• Renal clearance has little effect on efficacy or toxicity • Side effects include bone marrow suppression (leukopenia > thrombocytopenia > anemia) • Susceptibility to infections, hepatotoxicity, alopecia, GI toxicity • Avoid allopurinol
Mycophenolate mofetil (inhibits GMP synthesis → selective inhibition of DNA synthesis in B and T cells)	• Prophylaxis of transplant rejection, used off-label for systemic lupus erythematosus, MS, sarcoidosis	• Side effects include GI (diarrhea and vomiting) and hematological (leukopenia, pure red cell aplasia) problems • Contraindicated in pregnancy
Sirolimus (mTOR inhibitor)	• Prophylaxis of organ transplant rejection, incorporated into stents to inhibit occlusion	• Monitor blood levels • Hyperlipidemia • Anemia, leukopenia, thrombocytopenia • GI effects, mouth ulcers, hyperkalemia • Anticancer effects • Metabolized by CYP3A → possible drug interactions
Everolimus (mTOR inhibitor)	• Astrocytoma, breast cancer, kidney and liver transplant reception prophylaxis, pancreatic neuroendocrine tumor, renal angiomyolipoma, renal cell cancer	• Pharmacokinetics distinct from sirolimus • Toxicity similar to that of sirolimus
Temsirolimus (mTOR inhibitor)	• Advanced renal cell carcinoma	• Weekly intravenous administration • Pharmacokinetics distinct from sirolimus and everolimus • Toxicity similar to that of sirolimus

Drug Facts for Your Personal Formulary: *Immunosuppressants, Immunomodulators, and tolerogens (continued)*

	Therapeutic Uses	Clinical Pharmacology and Tips
T-Cell Costimulatory Blocker		
Belatacept (binds to CD80/ CD86, blocking CD28 costimulation)	• Prevention of renal transplant rejection	• ↑ Risk of posttransplant lymphoproliferative disorder involving the CNS, PML, and serious CNS infections → administration of higher or more frequent dosing than the recommended doses is *not* recommended
Antibodies Against Cell Surface Molecules		
Antilymphocyte globulin/ antithymocyte globulin Atgam Thymoglobulin	• Prevention and treatment of organ transplant rejection, aplastic anemia	• Contains antibodies against numerous T-cell surface molecules • Can elicit fever, chills, hypotension; use premedication (steroid/ acetaminophen/antihistamine) • Watch for leukopenia, thrombocytopenia
Abetacept Belatacept	• Prophylaxis of organ transplant rejection, autoimmunity trials	• CTLA-4-Ig fusion protein • Risk for posttransplant lymphoproliferative disorder
Anti-CD52 Alemtuzumab	• Chronic lymphocytic leukemia, MS, prevention and treatment of transplant rejection	• Prolonged lymphocyte depletion (neutropenia, thrombocytopenia as side effects) • Secondary autoimmunity
Anti-CD20 Rituximab Ocrelizumab	• Rheumatoid arthritis, MS	• Deplete circulating mature B lymphocytes
Anti-CD2 Alefacept	• Psoriasis	
Anti-BLyS Belimumab	• Systemic lupus erythematosus, lupus nephritis	• ↑ risk of infections • Cases of PML have been reported • Depression
Biologicals Targeting Cytokines and Their Receptors		
Anti–IL-1 Anakinra (recombinant IL-1 receptor antagonist) Canakinumab (IL-1β antibody) Rilonacept (fusion protein: IL-binding domains of IL-1R$_1$ and IL-RAcP linked to Fc region of IgG1)	• Rheumatoid arthritis, cryopyrin-associated syndromes	• IL-1 can be neutralized by endogenous IL-1R antagonist (IL-1Ra); levels of IL-1Ra are insufficient to neutralize the increased amounts of IL-1 produced in disease • IL-1 blockade can ↑ risk of serious opportunistic infections; discontinue anti–IL-1 therapy in patients with infections • ↑ in serious infections in patients with rheumatoid arthritis treated with anakinra and etanercept → this combination is not recommended • Live vaccines should not be administered in patients treated with anti–IL-1 therapies
Anti–IL-2 Basiliximab (antibody to IL-2R α chain/CD25) Daclizumab (antibody to IL-2R α chain)	• Prophylaxis of acute organ transplant rejection	• β Adrenergic blocking effects (worsening of heart failure and bronchospasm) • Block T-cell activation • Do not deplete • Good safety profile
Anti–IL-4 Dupilumab (mAb to IL-4Rα)	• Moderate-to-severe atopic dermatitis, maintenance treatment of moderate-to-severe asthma with eosinophilic phenotype or glucocorticoid-dependent asthma, maintenance treatment of chronic rhinosinusitis with nasal polyposis	• Associated with a reduction of oral glucocorticoid use for severe asthma; however, reduction of glucocorticoid dose, if deemed appropriate, should be gradual
Anti–IL-5 Benralizumab (blocking antibody to IL-5Rα) Mepolizumab (antibody to IL-5) Reslizumab (antibody to IL-5)	• Maintenance treatment of severe asthma (eosinophilic phenotype) • Mepolizumab is also indicated for eosinophilic granulomatosis with polyangiitis and hypereosinophilic syndrome	• Reslizumab is an intravenous infusion only • Benralizumab is a cytolytic mAb that induces efficient depletion of eosinophils in circulation

Drug Facts for Your Personal Formulary: *Immunosuppressants, Immunomodulators, and tolerogens* (*continued*)

	Therapeutic Uses	Clinical Pharmacology and Tips
Biologicals Targeting Cytokines and Their Receptors (cont.)		
Anti–IL-6 Sarilumab (antibody to IL-6R) Satralizumab (antibody to IL-6R) Tocilizumab (antibody to IL-6R) Siltuximab (chimeric mAb to IL-6)	• Tocilizumab and sarilumab: approved for the treatment of rheumatoid arthritis in patients who do not adequately respond to one or more disease-modifying agents • Tocilizumab: also approved for the treatment of giant cell arteritis, polyarticular juvenile idiopathic arthritis, systemic juvenile idiopathic arthritis, and cytokine release syndrome • Satralizumab: approved for the treatment of neuromyelitis optica spectrum disorder in adult patients who are positive for anti–aquaporin-4 antibody • Siltuximab: approved for treatment of multicentric Castleman disease if patient is negative for HIV and human herpesvirua-8	• IL-6 blockade can ↑ the risk of serious infections • Serious cases of hepatoxicity have been reported • Avoid use of live vaccines in patients treated with tocilizumab
Anti–IL-12/IL-23 Ustekinumab (antibody to IL-12β subunit, aka p40; IL-12β is a common subunit of IL-12 and IL-23)	• Moderate-to-severe plaque psoriasis, active psoriatic arthritis, moderately to severely active Crohn's disease, and moderately to severely active ulcerative colitis	• IL-12 and IL-23 blockade can ↑ the risk of serious infections • Consider discontinuation if patients develop serious infections
Anti–IL-17 Brodalumab (antibody to IL-17RA) Ixekizumab (antibody to IL-17A antibody) Secukinumab (antibody to IL-17A antibody)	• Patients aged 6 years or older with moderate-to-severe plaque psoriasis who are candidates for systemic therapy or phototherapy • Adults with active psoriatic arthritis, active ankylosing spondylitis, or active nonradiographic axial spondyloarthritis with objective signs of inflammation	• IL-17 blockade can ↑ the risk of serious infections; discontinue therapy in patients who develop serious infections • ↑ risk of inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis; monitor for onset or exacerbation of IBD; if IBD is diagnosed, discontinue anti–IL-17 treatment • Suicidal ideation and behavior have occurred in patients treated with brodalumab
Anti–IL-23 Guselkumab Risankizumab Tildrakizumab (antibodies to IL-23 p19 subunit)	• Moderate-to-severe plaque psoriasis • Guselkumab is also approved for active psoriatic arthritis	• May ↑ risk of infections • Guselkumab is administered by subcutaneous injection on weeks 0 and 4 and every 8 weeks thereafter • Tildrakizumab and risankizumab: administered subcutaneously on weeks 0 and 4 and every 12 weeks thereafter
Anti-TNFα Adalimumab (human mAb to TNFα) Certolizumab (anti-TNFα, humanized Fab fragment) Golimumab (human mAb to TNFα) Infliximab (chimeric antibody to TNFα) Etanercept (decoy receptor)	• Rheumatoid arthritis, Crohn's disease, ankylosing spondylitis, plaque psoriasis, psoriatic arthritis, ulcerative colitis	• TNFα blockade can ↑ risk of serious infections; administration of anti-TNFα therapies should be discontinued in patients who develop serious infections • ↑ risk of lymphoma and other malignancies
GM-CSF Sargramostim (recombinant GM-CSF)	• Acceleration of myeloid reconstitution following autologous bone marrow or peripheral blood progenitor cell transplantation • Promotion of neutrophil recovery following chemotherapy • Mobilization of hematopoietic progenitor cells into peripheral blood for collection by leukapheresis and autologous transplantation in adult patients • Acute exposure to myelosuppressive doses of radiation	• Can cause infusion-related reactions, characterized by respiratory distress, hypoxia, flushing, hypotension, syncope, and/or tachycardia

Drug Facts for Your Personal Formulary: *Immunosuppressants, Immunomodulators, and tolerogens* (*continued*)

	Therapeutic Uses	Clinical Pharmacology and Tips
Small-Molecule Inhibitors of Cytokine Receptor Signaling		
JAK inhibitors		• *Clinical tips for all JAK inhibitors:* Note FDA boxed warning and instructions for baseline testing, initiating therapy, and subsequent monitoring. Side effects: thrombocytopenia, anemia, and neutropenia; ↑ risk of infections (upper respiratory, sinus, common cold; also hepatitis C virus, etc.). Risk of fetal harm; nonuse during breastfeeding. Avoid concurrent use of live vaccines. Be aware of drug-drug interactions for each agent. JAKinibs have an FDA boxed warning for: • Increased risk of serious bacterial, fungal, viral, and opportunistic infections leading to hospitalization or death • Higher rate of all-cause mortality, including sudden cardiovascular death • Higher rate of certain cancers • Higher rate of thrombosis, cardiovascular death, myocardial infarction, and stroke
JAK1/JAK2 inhibitors Ruxolitinib Baricitinib	• Rheumatoid arthritis, graft-versus-host disease, myelofibrosis, polycythemia vera	
JAK1 inhibitors Upadacitinib Abrocitinib	• Rheumatoid arthritis, psoriatic arthritis, atopic dermatitis	
JAK2 inhibitors Fedratinib Pacritinib	• Myelofibrosis	• Avoid in patients at high risk of pulmonary embolism • Drug interactions via CYPs 3A4 and 2C19
JAK1/JAK3 inhibitor Tofacitinib	• Rheumatoid arthritis, psoriasis, ulcerative colitis, polyarticular course juvenile idiopathic arthritis	
Pan-JAK inhibitor Peficitinib (inhibits JAK1, JAK2, JAK3, and TYK2; modest selectivity for JAK3)	• Peficitinib (approved in Japan and Korea): rheumatoid arthritis	• In trials, well tolerated over 6-year period • ↑ risk of infections (herpes zoster virus, others) • Disease-modifying antirheumatic drug therapy: concurrent methotrexate does not affect pharmacokinetics of either drug • Drug interactions via P-glycoprotein, OATP1B1, and CYP3A4
Biologicals Targeting LFA		
Alefacept (anti-CD2)	Psoriasis	
Biologicals Targeting Integrins		
Natalizumab (targets α4, blocking both α4β1 and α4β7 integrin)	• MS (relapsing forms of and active secondary progressive disease), Crohn's disease (moderate-to-severe active disease that does not adequately respond to conventional therapies or anti-TNFα)	• Targets α4 integrin, blocking T-cell trafficking • ↑ risk of infection • Contraindicated in patients who have or have had PML • ↑ risk of developing herpes encephalitis and meningitis • ↑ risk of infections • Hepatotoxicity
Vedolizumab (blocks α4β7 but not α4β1 integrin)	• Ulcerative colitis and Crohn's disease (moderate-to-severe active disease)	• ↑ risk of PML; monitor patients for new or worsening of neurological signs and symptoms • ↑ risk of infections • Hepatotoxicity
S1PR Modulators		
Ponesimod (S1P$_1$R) Siponimod (S1P$_{1,5}$R) Ozanimod (S1P$_{1,5}$R) Fingolimod (S1P$_{1,3,4,5}$R)	• Relapsing-remitting MS (all), secondary progressive MS (siponimod, ponesimod), ulcerative colitis (ozanimod)	• These agents ↓ lymphocyte egress and homing • ↑ risk of infection • Follow FDA guidelines for administration and monitoring • First dose effects: bradycardia, ↓ atrioventricular conduction, ↑ QTc interval; monitor before and during initiation of therapy • Risk of macular edema; evaluate and monitor • Fetal risk • Monitor for cutaneous malignancies • Siponimod: contraindicated in patients with the homozygous CYP2C9*3/*3 genotype • Ozanimod: active metabolite inhibits MAO-B ⇒ potential for hypertensive crisis with agents that ↑ sympathetic tone
Additional Drugs for MS (see Table 39–3 and text for further information)		
Oral Agents: Fumarates, teriflunomide, cladribine *Parental Agents:* Glatiramer acetate, interferon β		

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Chapter 40

Immune Globulins and Vaccines

Roberto Tinoco and James E. Crowe, Jr.

HISTORICAL PERSPECTIVE

VACCINATION INDUCES DEVELOPMENT OF IMMUNOLOGICAL MEMORY

IMMUNIZATION STRATEGIES
- Passive
- Active

VACCINE TYPES
- Live Attenuated
- Inactivated
- Subunit Vaccines
- DNA Vaccines
- mRNA Vaccines
- Recombinant Vectors

IMMUNOGLOBULINS
- Structure
- Antibody Classes and Functions
- Monoclonal Antibodies for RSV
- Monoclonal Antibodies for EBOV
- Monoclonal Antibodies for SARS-CoV-2

SPECIFIC CONVENTIONAL VACCINES RECOMMENDED IN THE U.S.
- Vaccines for Bacteria
- Vaccines for Viruses

MATERNAL IMMUNIZATION

VACCINES FOR TRAVEL
- Japanese Encephalitis Virus Vaccine
- Yellow Fever Virus Vaccine
- Typhoid Vaccine
- Rabies Virus Vaccine

SPECIALTY VACCINES
- Anthrax Vaccine
- Cholera Vaccines
- Vaccinia Virus (Smallpox Vaccine)
- Other Vaccines for Biodefense and Special Pathogens

INTERNATIONAL VACCINES
- Dengue Virus Vaccine
- Malaria Vaccine
- BCG Vaccine

SARS-COV-2 AND COVID-19
- SARS-CoV-2 Vaccines

THE FUTURE OF VACCINE TECHNOLOGY

VACCINE SAFETY: MYTHS, TRUTHS, AND CONSEQUENCES
- Vaccine Adjuvants and Safety
- Vaccines Do Not Cause Autism
- Preservatives, Including Thimerosal
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LICENSURE AND MONITORING OF VACCINES
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Historical Perspective

The historical impact of infectious diseases is evident in the high mortality rates in young children and adults and the disruption that these diseases have caused in emerging societies. The rise of civilization in conjunction with the domestication of plants and animals permitted people to live in denser communities with each other and with their animals. Such proximity provided ideal breeding grounds for infectious pathogens, and their spread resulted in epidemics throughout the world. As people began to question the underlying causes of disease and the apparent protection to reinfection afforded to some survivors of a disease, ideas of immunity and disease prevention were born, apparently as early as the 5th century.

The concept of immunity goes back at least to the 17th century when emperor K'ang of China documented his practice of variolation, or inoculation, of his troops and his own children with smallpox to confer protection from the disease (Hopkins, 2002). Variolation involved taking liquid from a smallpox pustule of an infected patient, cutting the skin of an uninfected person, and then introducing the inoculum. Records from the 18th century note that enslaved Africans brought to the U.S. bore scars from smallpox variolation and were thought to be immune to the disease. Variolation against smallpox was also reported by Lady Mary Montagu during her time in Constantinople (1716–1718). Lady Montagu, herself a survivor of smallpox, reported that certain Turkish women would open a wound in healthy individuals and introduce the contents of a smallpox vesicle with a large needle, thereby providing a level of protection against smallpox. About 2% to 3% died after variolation, whereas 20% to 30% died from natural infection. Lady Montagu had herself and a son variolated and later had a daughter successfully variolated in London under the auspices of physicians of the Royal Society. Positive outcomes notwithstanding, fear of the procedure persisted.

Around the same time, in Boston, Cotton Mather and Dr. Zabdiel Boylston began a program of variolation against smallpox. The program met with general success but was opposed by many physicians, fearful that inoculation spread the disease and worried by deaths after inoculation (~2% of those inoculated). One Puritan religious leader, Edmund Massey, preached against inoculation, quoting from the book of Job (Job 2:7: "So Satan went forth from the presence of the Lord and smote Job with sore boils.") and arguing that Satan was the prime practitioner of inoculation and that such diseases as smallpox were a necessary trial of

Abbreviations

ACIP: Advisory Committee on Immunization Practices
AID: activation-induced cytidine deaminase
APC: antigen-presenting cell
ASD: autism spectrum disorder
BCG: bacille Calmette-Guérin
BCR: B-cell receptor
CDC: Centers for Disease Control and Prevention
CoP: correlate of protection
COVID-19: coronavirus disease 2019
CRM: cross-reactive material
DTaP: diphtheria and tetanus toxoids and acellular pertussis
EBOV: *Zaire ebolavirus*, Ebola virus
EMA: European Medicines Agency
EUA: Emergency Use Authorization
Fab: fragment, antigen-binding
Fc: fragment crystallizable
GBS: Guillain-Barré syndrome
GPCR: G protein-coupled receptor
H1N1: hemagglutinin subtype 1 and neuraminidase subtype 1
H3N2: hemagglutinin subtype 3 and neuraminidase subtype 2
HA: hemagglutinin
Hib: *Haemophilus influenzae* type b
HIV: human immunodeficiency virus
HPV: human papillomavirus
Ig: immunoglobulin
IIV: inactivated influenza vaccine
IOM: Institute of Medicine (now National Academy of Medicine)
IPV: inactivated poliovirus (vaccine)
JE: Japanese encephalitis
mCoP: mechanistic correlate of protection
MeV: measles virus
MMR: measles-mumps-rubella
MMRV: measles-mumps-rubella-varicella
mAbs: monoclonal antibodies
mRNA: messenger RNA
nCoP: nonmechanistic correlate of protection
PCV: pneumococcal conjugate vaccine
PRP: polyribosylribitol phosphate
RBD: receptor binding domain
RSV: respiratory syncytial virus
SAE: serious adverse event
SARS-CoV-2: severe acute respiratory syndrome coronavirus 2
SIDS: sudden infant death syndrome
TB: *Mycobacterium tuberculosis*
Td: tetanus toxoid and reduced diphtheria toxoid
Tdap: tetanus toxoid, reduced diphtheria toxoid, acellular pertussis
VZV: varicella-zoster virus
WHO: World Health Organization

faith or punishment for sins, the fear of which "is a happy restraint upon many people" (Gross and Sepkowitz, 1998). Medical practice in Boston has come a long way since that time.

In 1796, Edward Jenner, who coined the term *vaccination*, from *vacca*, Latin for "cow," helped to advance vaccine safety. He tested the hypothesis that smallpox protection could be achieved by using cowpox, a nonfatal, self-limited disease in humans caused by a virus of the Poxviridae family that includes monkeypox and smallpox and that can spread from cows to humans. Jenner infected a boy with cowpox pus from an infected milkmaid; the boy got mildly ill from cowpox, recovered, and when challenged with smallpox collected from scabs of a smallpox patient, was unaffected, showed no symptoms, and was fully protected against the disease. Thus, it was possible to inoculate against a disease using material from a related but less-harmful disease.

By the early to mid-19th century, vaccination was accepted widely, and governments in the U.S. and Europe began to require vaccination of children. As in our own era, there was organized resistance from antivaccination groups. There was also a sense that immunity waned with time, and revaccinations were introduced, producing a sustained diminution of smallpox.

The work of Pasteur and Koch established a link between microorganisms and disease and provided the scientific understanding to develop more specific vaccines. Preservatives (glycerol was an early additive) and refrigeration increased shelf life of vaccines and permitted their wider distribution. The cells of the immune system began to be identified around 1890, followed by the discovery of antibodies and hyperimmune serum and the demonstration of the efficacy of adjuvants (aluminum was the first) to increase immunogenicity (Marrack et al., 2009). In the 1950s, freeze-drying became standard, permitting worldwide distribution of purified vaccines. Through the coordinating efforts of the World Health Organization (WHO), smallpox was declared "eliminated" in 1979.

Other scourges were attacked by vaccination in the mid-20th century. One was polio, an incurable neurological disease causing muscle wasting, paralysis, and death if the diaphragm is affected. In 1955, Jonas Salk released a vaccine against poliovirus. The Salk vaccine, an inactivated virus preparation administered by injection, was followed in 1961 by the Sabin oral vaccine, which employs an attenuated poliovirus that provides immunity to all three types of poliovirus. As a result of the polio vaccines, the annual number of cases in the U.S. fell to 161 in 1961 from 35,000 in 1955 (Hinman, 1984). Eradication of polio depends on interruption of person-to-person transmission, which requires that a high percentage of the susceptible population be inoculated. Most adults in developed countries are immune, but when a significant fraction of children are unvaccinated, there is the potential for an outbreak because wild polioviruses circulate.

These fundamental observations and experiments paved the way for the modern vaccines that have reduced mortality and morbidity rates from infectious pathogens across the globe. Modern laboratory technologies have rendered vaccines safe and highly effective against infectious pathogens and virus-transforming cancers and against neoantigens on cancerous cells. Vaccination strategies are a public health success, as shown by the complete worldwide eradication of smallpox and the elimination of polio in the Americas in 1994, Europe in 2002, and Southeast Asia in 2014, with remaining endemic cases only in Pakistan, Afghanistan, and Nigeria in 2016, according to WHO. In 2016, WHO and the Pan American Health Organization declared the Americas free of endemic measles, credited to immunization campaigns.

Table 40–1 summarizes the current recommendations for childhood vaccinations. A later section of the chapter discusses the issue of nonvaccinators.

Vaccination Induces Development of Immunological Memory

The hallmarks of an immune response to pathogens are the recognition and activation of the innate immune response that limits pathogen spread when microbes breach the host's natural protective barriers, such as the skin, the respiratory epithelium, or the gastrointestinal epithelium. If the pathogen is not controlled, the innate immune system then recruits the humoral (antibody-secreting B cell) and cellular (T cell) arms of the adaptive immune response to specifically target and destroy the invading pathogen. Once the microbe is eliminated during this primary response, small numbers of pathogen-specific B and T cells survive long term, sometimes for the entire life of the host, as *memory B and T cells*. These memory cells confer host protection against reinfection with the same pathogen. During a second response, memory cells use their specific

TABLE 40–1 ■ RECOMMENDED IMMUNIZATION SCHEDULE FOR CHILDREN AND ADOLESCENTS AGED 18 YEARS OR YOUNGER, U.S., 2022*

These recommendations must be read with the notes that follow. For those who fall behind or start late, provide catch-up vaccination at the earliest opportunity as indicated by the green bars. To determine minimum intervals between doses, see the catch-up schedule.*

Vaccine	Birth	1 mo	2 mos	4 mos	6 mos	9 mos	12 mos	15 mos	18 mos	19–23 mos	2–3 yrs	4–6 yrs	7–10 yrs	11–12 yrs	13–15 yrs	16 yrs	17–18 yrs
Hepatitis B (HepB)	🟨 1st dose	🟨 ◄--- 2nd dose ---►	🟨	🟩	🟨 ◄--- 3rd dose ---►	🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
Rotavirus (RV): RV1 (2-dose series), RV5 (3-dose series)	⬜	⬜	🟨 1st dose	🟨 2nd dose	🟨 See Notes	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Diphtheria, tetanus, acellular pertussis (DTaP <7 yrs)	⬜	⬜	🟨 1st dose	🟨 2nd dose	🟨 3rd dose	🟩	🟩	🟨 ◄--- 4th dose ---►	🟨	🟩	🟩	🟨 5th dose	⬜	⬜	⬜	⬜	⬜
***Haemophilus influenzae* type b (Hib)**	⬜	⬜	🟨 1st dose	🟨 2nd dose	🟨 See Notes	🟩	🟨 ◄ 3rd or 4th dose, See Notes ►	🟨	🟩	🟩	🟩	🟩🟪	🟪	🟪	🟪	🟪	🟪
Pneumococcal conjugate (PCV13)	⬜	⬜	🟨 1st dose	🟨 2nd dose	🟨 3rd dose	🟩	🟨 ◄--- 4th dose ---►	🟨	🟩	🟩	🟩	🟩🟪	🟪	🟪	🟪	🟪	🟪
Inactivated poliovirus (IPV <18 yrs)	⬜	⬜	🟨 1st dose	🟨 2nd dose	🟨 ◄--- 3rd dose ---►	🟨	🟨	🟨	🟨	🟩	🟩	🟨 4th dose	🟩	🟩	🟩	🟩	🟩⬜
Influenza (IIV4)	⬜	⬜	⬜	⬜	🟨 Annual vaccination 1 or 2 doses	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨🟨 Annual vaccination 1 dose only	🟨	🟨	🟨	🟨
or													**or**				
Influenza (LAIV4)	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟨 Annual vaccination 1 or 2 doses	🟨	🟨🟨 Annual vaccination 1 dose only	🟨	🟨	🟨	🟨
Measles, mumps, rubella (MMR)	⬜	⬜	⬜	⬜	🟪 See Notes	🟪	🟨 ◄--- 1st dose ---►	🟨	🟩	🟩	🟩	🟨 2nd dose	🟩	🟩	🟩	🟩	🟩
Varicella (VAR)	⬜	⬜	⬜	⬜	⬜	⬜	🟨 ◄--- 1st dose ---►	🟨	🟩	🟩	🟩	🟨 2nd dose	🟩	🟩	🟩	🟩	🟩
Hepatitis A (HepA)	⬜	⬜	⬜	⬜	🟪 See Notes	🟪	🟨 2-dose series, See Notes	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	🟩	🟩
Tetanus, diphtheria, acellular pertussis (Tdap ≥7 yrs)	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟩	🟨 1 dose	🟪 🟩	🟪 🟩	🟪 🟩
Human papillomavirus (HPV)	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜🟪 ⬜▨	🟨 See Notes	🟩	🟩	🟩
Meningococcal (MenACWY-D ≥9 mos, MenACWY-CRM ≥2 mos, MenACWY-TT ≥2years)	⬜	⬜	🟪 See Notes	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟨 1st dose	🟩	🟨 2nd dose	🟩
Meningococcal B (MenB-4C, MenB-FHbp)	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜🟪 See Notes ⬜	🟪 ⬜	🟪 ⬜	🟪 🟦	🟪 🟦
Pneumococcal polysaccharide (PPSV23)	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟪 See Notes	🟪	🟪	🟪	🟪	🟪	🟪
Dengue (DEN4CYD; 9-16 yrs)	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜🟨 Seropositive in endemic areas only (See Notes)	🟨	🟨	🟨	⬜

🟨 Range of recommended ages for all children
🟩 Range of recommended ages for catch-up vaccination
🟪 Range of recommended ages for certain high-risk groups
▨ Recommended vaccination can begin in this age group
🟦 Recommended vaccination based on shared clinical decision-making
⬜ No recommendation/ not applicable

*These recommendations are reprinted from the website of the Centers for Disease Control and Prevention (CDC) and should be read with Tables 2 and 3 and the notes provided on the CDC website:** https://www.cdc.gov/vaccines/schedules/downloads/child/0-18yrs-child-combined-schedule.pdf. **For those who fall behind or start late, provide catch-up vaccination at the earliest opportunity during the time periods indicated by the green bars. To determine minimum intervals between doses, see the catch-up schedule (Table 2) on the CDC website.**

antigen receptors to recognize the invading pathogen. This results in their activation and expansion to directly kill infected cells (via T cells) or generate antibodies (via B cells) that will neutralize the pathogen.

Vaccination technology takes advantage of this paradigm. As a means of generating immunological memory, uninfected individuals are given a controlled infection or exposed to antigen that elicits an immune response. When these vaccinated individuals are subsequently infected with these pathogens in their environment, the responses of their memory T and B cells outpace the invading microbes to neutralize and prevent their spread in a much more rapid secondary response of greater magnitude.

B-cell clonal expansion results in the differentiation of long-lived memory B cells and emergence of shorter-lived plasma cells that produce antibodies. During the primary response, following the vaccination, B cells will undergo this differentiation process and will initially secrete immunoglobulin (Ig) M antibodies. IgM antibodies are large and provide some protection. Days after the response is initiated, B cells will undergo clonal selection and will produce IgG, which is a higher-affinity antibody with enhanced pathogen neutralization capacity.

Differentiated plasma cells can also produce other antibody classes, such as IgA, IgD, and IgE, that have unique functions. IgD can be expressed on the surface of B cells; its function continues to be investigated. IgA antibodies are concentrated in mucous secretions, breast milk, and tears. IgE antibodies are important in the elimination of parasitic infections. *Because IgG antibodies have undergone a selection process that increases their affinity, these antibody types are the goals of vaccine design*. Secondary responses after vaccination therefore elicit a faster and larger B-cell response, and these B cells primarily make IgG antibodies (Clem, 2011).

Cellular immunity involving both CD4$^+$ and CD8$^+$ T cells is also a goal of vaccine design. Unlike B cells, T cells target intracellular pathogens that have infected host cells. CD4$^+$ T cells (helper T cells) stimulate B cells to produce antibody. CD8$^+$ T cells kill infected cells. Like B cells, antigen-memory T cells survive long term and provide protection for future encounters with their specific antigen.

Immunization Strategies

Immunity can be achieved from either passive or active methods involving exposure to natural infection or through artificial human-made antigens. Individuals can develop antibodies from natural infection or after vaccination.

Passive

Passive immunity involves the transfer of preformed antibodies from an immune individual to a nonimmune individual to confer temporary immunity. An example of passive natural immunity is the transfer of antibodies from mother to fetus during pregnancy and through breast milk and colostrum consumed by an infant. These antibodies enter the body

TABLE 40–2 ■ AVAILABLE IMMUNE GLOBULINS

Human intravenous immune globulin
Human subcutaneous immune globulin
Human intramuscular immune globulin
Human hyperimmune globulins
Anthrax immune globulin, intravenous
Botulism immune globulin, intravenous
Cytomegalovirus immune globulin, intravenous
Hepatitis B immune globulin, intravenous
Rabies immune globulin
Rho(D) immune globulin, intravenous
Vaccinia immune globulin, intravenous
Varicella-zoster immune globulin
Animal-derived immune globulins
Lymphocyte immune globulin, anti-thymocyte globulin (equine)
Centruroides (scorpion) immune F(ab′)2 (equine) injection
Crotalidae immune F(ab′)2 (equine)
Antivenin (*Latrodectus mactans*) (equine) [i.e., black widow spider antivenin]
Botulism antitoxin bivalent (equine) types A and B
Botulism antitoxin heptavalent (A, B, C, D, E, F, G) (equine)
Antivenin (*Micrurus fulvius*) (equine origin) [i.e., North American coral snake antiven]
Crotalidae polyvalent immune Fab (ovine)
Digoxin immune Fab (ovine)
Antithymocyte globulin (rabbit)

and provide a first line of defense to the fetus or infant, who otherwise has no immunity to any pathogen.

An example of *artificial passive immunization* is the injection of antivenom antibodies. Animals are immunized with venom antigen, and their hyperimmunized serum is transfused into the patient. Antivenom can be monovalent, effective against one type of venom, or polyvalent and effective against venom from multiple species. An antivenom binds and neutralizes a toxin. Early administration after injury is critical because antivenom can halt but not reverse venom damage. Even though antivenom is purified, trace proteins remain, and these can trigger anaphylaxis or serum sickness in patients. Most antivenoms are administered intravenously but can also be injected intramuscularly against the venoms of stonefish and redback spiders. Antivenoms have been developed against venomous spiders, acarids, insects, scorpions, marine animals, and snakes. Passive immunization is used for a variety of toxins and infections; a list of available immunoglobulins is shown in Table 40–2.

Active

A natural infection that stimulates the immune response in uninfected individuals may lead to development of immunological memory and protection from reinfection, as in the case of infection with the measles virus (MeV). This induction of immunity occurs only if the individual survives the primary infection, which is not always the case for viruses like MeV, influenza virus, or Ebola virus (EBOV). Active immunization through injection of artificial antigens elicits a controlled immune response leading to the generation of immunological memory. This type of immunization, compared to natural infection, does not cause infectious disease or compromise the life of the individual. Thus, vaccine technologies through active stimulation of the immune system ensure that the individual survives and has protection against the pathogen in the natural environment.

Vaccine Types

Advanced technologies are currently used to generate vaccines to prevent many infectious diseases and to deter infectious pathogens that cause cancer, such as hepatitis viruses that can lead to hepatocellular carcinoma, and human papillomaviruses (HPVs) that can cause cervical, anal, vaginal, and penile cancers. Effective vaccines activate both the innate and the adaptive immune systems. There are many different types of vaccines, each with advantages and disadvantages. Vaccine design involves an understanding of the nature of the microbe, the tropism of the pathogen, and the practical need in certain regions of the world. The following section summarizes current methods used in vaccine design. For a list of vaccines approved by the FDA, see Table 40–3.

Live Attenuated

Live attenuated vaccines use a weakened form of a virus that contains antigens that appropriately stimulate an immune response. Such viruses have been passaged to reduce their virulence but retain immunogenic antigens that elicit strong humoral and cellular responses and the development of memory cells after one or two doses. A virus, for example, can be isolated from humans and then used to infect monkey cells. After several passages, the virus can no longer infect human cells but retains immunogenic capacity. These attenuated viruses can elicit a robust immune response because they are similar in many respects to the natural pathogen.

Several drawbacks exist with these vaccines. Because these are live viruses, they generally must be refrigerated to retain their activity. In remote areas of the world where refrigeration is not available, obtaining and storing this type of vaccine can be limiting. Because viruses can mutate and change in the host, it may be possible that viruses can become virulent again and cause disease, although the frequency of adverse reactions using these vaccines is very low. Furthermore, attenuated vaccines cannot be used in immune-compromised individuals (e.g., patients with HIV [human immunodeficiency virus] or cancer). In addition, these vaccines are usually not given during pregnancy. MeV, polio, rotavirus, yellow fever, and varicella viruses are examples of pathogens from which live attenuated vaccines have been generated. Attenuated vaccines for bacteria are more challenging to generate than for viruses because bacteria have more complex genomes; however, recombinant DNA technology can be utilized to remove virulence but retain immunogenicity. A vaccine against *Vibrio cholerae* has been generated this way. A live attenuated vaccine for tuberculosis also has been developed.

Inactivated

Polio, influenza, and rabies viruses and typhoid and plague bacteria have been utilized to generate inactivated vaccines. Killing pathogens by heat, radiation, or chemicals to inactivate them generates the antigenic starting materials. The dead pathogens can no longer replicate or mutate to their disease-causing state and thus are safe. These types of vaccines are useful because they can be freeze-dried and transported without refrigeration, an important consideration in reaching developing countries. A drawback with inactivated vaccines is that they induce an immune response that may be weaker than that induced by the natural infection; thus, patients often require multiple doses of inactivated vaccines or periodic booster vaccinations to sustain immunity to the pathogen. In areas where people have limited access to healthcare, ensuring that these multiple doses are delivered on time can be problematic and may result in reduced immunity to the pathogen, as in the case of poliovirus endemic disease.

Subunit Vaccines

As with inactivated vaccines, subunit vaccines do not contain live pathogens; rather, subunit vaccines use a component of the microorganism as a vaccine antigen to mimic exposure to the organism itself. Subunit vaccines typically contain *polysaccharides* or proteins (*surface proteins* or *toxoids*). Compared to live attenuated vaccines, subunit vaccines induce a less-robust immune response. The selection of antigenic subunit and the design and development of the vaccine can be lengthy and costly because the pathogen’s subunit antigens and their combination must be thoroughly tested to ensure they elicit an effective immune response. Scientists can identify the more immunogenic antigens in the laboratory and manufacture these antigen molecules via recombinant DNA technology,

TABLE 40–3 ■ APPROVED VACCINES IN THE U.S.
Toxoids
Tetanus and diphtheria toxoids adsorbed
Tetanus and diphtheria toxoids adsorbed for adult use
Tetanus toxoid adsorbed
Tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine, adsorbed
Bacterial polysaccharide
Meningococcal polysaccharide vaccine, groups A, C, Y, and W-135 combined
Pneumococcal vaccine, polyvalent
Typhoid Vi polysaccharide vaccine
Bacterial conjugate vaccines
Haemophilus b conjugate vaccine (meningococcal protein conjugate)
Haemophilus b conjugate vaccine (tetanus toxoid conjugate)
Pneumococcal 7-valent conjugate vaccine (diphtheria CRM$_{197}$ protein)
Pneumococcal 13-valent conjugate vaccine (diphtheria CRM$_{197}$ protein)
Pneumococcal 20-valent conjugate vaccine
Meningococcal (groups A, C, Y, and W-135) oligosaccharide diphtheria CRM$_{197}$ conjugate vaccine
Meningococcal groups C and Y and *Haemophilus* b tetanus toxoid conjugate vaccine
Meningococcal (groups A, C, Y, and W-135) polysaccharide diphtheria toxoid conjugate vaccine
Meningococcal group B vaccine
Live bacterial
BCG live
Typhoid vaccine live oral Ty21a
Cholera vaccine live oral
Inactivated or subunit bacterial
Plague vaccine
Anthrax vaccine adsorbed
Live viral
Measles and mumps virus vaccine, live
Measles, mumps, and rubella virus vaccine, live
Measles, mumps, rubella, and varicella virus vaccine, live
Varicella virus vaccine, live
Zoster vaccine, live (Oka/Merck)
Rotavirus vaccine, live, oral
Rotavirus vaccine, live, oral, pentavalent
Influenza vaccine, live, intranasal (quadrivalent, types A and types B)
Adenovirus type 4 and type 7 vaccine, live, oral
Yellow fever vaccine
Smallpox (vaccinia) vaccine, live
Smallpox and monkeypox vaccine, live, nonreplicating
Dengue tetravalent vaccine, live
Inactivated or subunit viral
Poliovirus vaccine inactivated (human diploid cell)
Poliovirus vaccine inactivated (monkey kidney cell)
Hepatitis A vaccine, inactivated
Hepatitis B (recombinant) vaccine
Hepatitis A vaccine, inactivated, and hepatitis B (recombinant) vaccine
Influenza A (H1N1) 2009 monovalent vaccine
Influenza virus vaccine, H5N1 (for national stockpile)
Influenza A (H5N1) virus monovalent vaccine, adjuvanted
Influenza virus vaccine (trivalent, types A and B)
Influenza virus vaccine (quadrivalent, types A and B)
Human papillomavirus bivalent (types 16, 18) vaccine, recombinant
Human papillomavirus quadrivalent (types 6, 11, 16, 18) vaccine, recombinant
Human papillomavirus 9-valent vaccine, recombinant
Japanese encephalitis virus vaccine, inactivated
Japanese encephalitis virus vaccine, inactivated, adsorbed
Rabies vaccine
Rabies vaccine adsorbed
Zoster vaccine recombinant, adjuvanted
Vectored viral
Ebola Zaire vaccine, live

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producing *recombinant subunit vaccines*. For example, the hepatitis B vaccine is generated by the insertion into yeast of hepatitis B genes coding for selected antigens. The yeast cells express these antigens, which are then purified and used in making a vaccine. A drawback to these vaccines is that even though they elicit an immune response, immunity is not guaranteed. Subunit vaccines usually are considered safe because they have no live replicating pathogen present.

Polysaccharides

Polysaccharide subunit vaccines utilize polysaccharide (sugar) antigens to induce an immune response. Bacterial cell walls are composed of peptidoglycan polysaccharides that help pathogens evade the immune system. This evasion mechanism is highly effective in infants and young children, making them more susceptible to infection. Unfortunately, these polysaccharides are not very immunogenic. Furthermore, the vaccines produced using polysaccharide antigens often induce suboptimal immune responses that result in only short-term immunity. Meningococcal infection caused by *Neisseria meningitidis* (groups A, C, W-135, and Y) and *pneumococcal* disease are polysaccharide subunit vaccines against bacterial pathogens. Conjugate subunit vaccines use a technology to bind polysaccharide from the bacterial capsule to a carrier protein, often diphtheria or tetanus toxoid. This sort of antigen combination can induce long-term protection in infants and adults. These vaccines provide protection against pathogens where plain polysaccharide vaccines fail to work in infants and provide more long-term protection in young children and adults. The *Haemophilus influenzae* type b (Hib) and *pneumococcal* (pneumococcal conjugate vaccine [PCV] 7 valent, PCV10 valent, PCV13 valent) vaccines are conjugate subunit vaccines recommended for children (see Table 40–1). The *meningococcal A* vaccine used in Africa is also an example of a conjugate subunit vaccine.

Surface Protein Subunit Vaccines

Protein-based subunit vaccines use purified proteins from the pathogen or recombinant proteins based on pathogen sequences to induce an immune response. Because these proteins may not be presented in fully native conformations (i.e., as in the live pathogen), some of the antibodies generated against these antigens may not bind efficiently to the live pathogen. Acellular pertussis and hepatitis B vaccines are examples of protein-based subunit vaccines. The hepatitis B vaccine contains the hepatitis B virus envelope protein made as an antigen produced in yeast cell culture.

Toxoids

Pathogenic bacteria such as *Clostridium tetani* and *Corynebacterium diphtheriae* induce disease (tetanus or diphtheria, respectively) through production of their toxins. Vaccines against these toxins, known as toxoid vaccines, are effective because they elicit an immune response that results in the production of toxin-specific neutralizing antibodies, preventing cell damage in the patient. Inactivated toxins (*toxoids*) are used as the immunogen; however, because these proteins often are not highly immunogenic, they must be adsorbed to adjuvants (aluminum or calcium salts) to increase their capacity to stimulate the immune response. Toxoid vaccines generally are safe because they do not contain live pathogens. In addition, these proteins usually are stable over a wide range of temperatures and humidities (Baxter, 2007).

DNA Vaccines

Sequencing the genome of a pathogen provides information that enables the production of a DNA vaccine against selected genetic material. A microbe's antigenic genes are selected and incorporated in synthetic DNA. Intramuscular or intradermal injection delivers this engineered DNA to antigen-presenting cells (APCs), which uptake the DNA and transcribe and translate it to produce antigenic proteins. These APCs present these antigens to both humoral and cellular immune system components to generate immunity. This type of vaccine poses no risk of infection, can easily be developed and produced, is cost-effective, is stable, and may provide long-term protection (Robinson et al., 2000). Disadvantages include its limit to protein antigens and the possibility of generating tolerance to that antigen because of low immunogenicity, thereby rendering ineffective immunity

Many DNA vaccines are currently in experimental phases, but none has been licensed in the U.S. DNA vaccines for influenza virus, herpesvirus, flaviviruses like Zika virus, and others are in the early stages of development. A DNA vaccine against West Nile virus has been approved for veterinary use. Delivery platforms for enhancing efficacy of DNA vaccines (e.g., electroporation) are being developed.

mRNA Vaccines

One of the barriers to effectiveness of DNA vaccines is that the nucleic acids must not only be delivered into the cytoplasm of cells but also achieve nuclear import to localize with host cell DNA polymerases to produce messenger RNA (mRNA). Delivering mRNA instead leaps over this problem, but historically, RNA was difficult to use for several reasons. RNA is inherently unstable as it is susceptible to degradation by enzymes that are ubiquitous in biological settings, is difficult to deliver into cells, and can trigger cell programs inhibiting translation following recognition by Toll-like receptors and other host cell sensing mechanisms. Decades of research have overcome these obstacles, with recent successes. The cytoplasmic delivery and stability issues were surmounted by development of novel lipid nanoparticle formulations that protect the RNA and facilitate delivery to cells. The use of engineered RNAs, such as incorporation of modified nucleosides, allowed evasion of rapid host cell detection. As a result, several major successes occurred with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines in 2020 (discussed below), and these technologies are being investigated for novel vaccines for influenza, HIV-1, and other major pathogens.

Recombinant Vectors

A vector is a virus or bacterium that is used to deliver heterologous microbial genes to cells for expression in the vaccinee to elicit an immune response. Once the vector infects or transduces host cells, the selected antigens will be presented during the immune response to generate immunity. Both viruses and bacteria are being investigated as recombinant vectors for candidate vaccines. Virus vectors that have been used in candidate vaccines include many poxviruses (vaccinia virus, modified vaccinia Ankara, avian poxviruses, and others), many adenoviruses (of both human and primate origin), and other families of viruses.

Immunoglobulins

Structure

Vaccination results in the expansion and differentiation of B cells into long-lived memory cells, which provide long-term protection to secondary challenge, and plasma cells, which are immunoglobulin (antibody)-generating cells that produce large quantities of these proteins. Antibodies in the body are found in two forms, either membrane bound on B cells as B-cell receptors (BCRs) that can deliver signals to activate and induce B-cell differentiation after antigen ligation or as soluble effector molecules that neutralize antigens throughout the body. Antibodies are heterodimeric proteins composed of two chains, the light and heavy chains. Both light and heavy chains contain variable regions in the N-terminal region of the protein that engage antigens. Naïve B cells express BCRs with low affinity to antigen. These BCRs can be selected through VDJ (variable, diversity, joining) recombination via the activity of RAG (recombination-activating gene) enzymes. Antibody diversity is achieved through antigen-binding site region variation, combinatorial diversity of gene segments, and combination of light and heavy regions, an overall diversity program that can result in an antibody repertoire of potentially 10^{16} to 10^{18} different molecules, ensuring that a unique B cell in the body will exist to recognize any foreign antigen. In addition to this diversity, antibodies also can undergo class-switch recombination in which the constant region of the heavy chain can be switched, based on cytokine signals by T cells, to tailor antibody specificity and function. It is this portion of the antibody that determines the five main isotypes: IgM, IgD, IgG, IgA, and IgE. These isotypes differ in size, Fc (fragment crystallizable) receptor binding, ability to fix complement, and appropriate isotypes for specific pathogens (Schroeder and Cavacini, 2010).

Antibody diversity can be further enhanced following antigen recognition by memory B cells and help from $CD4^+$ T cells. B cells can further strengthen their antibody affinity by mutating their variable regions, and with repetitive antigen stimulation, the affinity of the B-cell clones selected for expansion by binding to antigen can increase further. This mechanism explains why some vaccines, like the one for hepatitis B, are most immunogenic when delivered in three doses. This repeated antigen stimulation induces somatic hypermutation of antibody variable genes to increase antibody potency. Activation-induced cytidine deaminase (AID) is a key enzyme that mediates class-switch recombination and somatic hypermutation. Human patients with defective AID suffer from hyper-IgM syndrome and are unable to class-switch their antibodies, which makes them more susceptible to certain infections.

Manufactured antibodies can be used for passive immunization; for a list of available antibodies, see Table 40–4. Such monoclonal antibodies (mAbs) are biologicals that have become some of the most important drug classes of our era. To date, mAbs have been implemented most effectively for use in cancer immunotherapy and management of autoimmune diseases. Several mAbs have been advanced for infectious diseases, including for respiratory syncytial virus (RSV), EBOV, SARS-CoV-2, and *Clostridium difficile* toxin. As the cost of production of mAbs continues to fall, more of these biologicals will likely be used for prophylaxis or treatment of infectious diseases.

Antibody Classes and Functions

Immunoglobulin M

The first antibody class expressed by B cells is IgM. IgM molecules are membrane-bound monomers found on circulating mature B cells. When mature B cells are antigen stimulated, they generate IgM pentamers that are secreted. IgM antibodies, also called natural antibodies, have low affinity as monomers, but their avidity can increase in their pentameric structure, which improves epitope binding to repeating antigens on pathogens. These antibodies are associated with a primary immune response; they are found at mucosal surfaces and constitute 10% of the antibody content of serum. IgM molecules function by coating their specific antigen to target the pathogen for destruction via phagocytosis or to induce complement fixation to kill the pathogen (Schroeder and Cavacini, 2010).

Immunoglobulin D

Like IgM molecules, IgD molecules are also expressed on naïve B cells that have not been activated by their specific antigen and thus have not undergone somatic hypermutation. They are expressed as monomers on the surface of B cells and can also be secreted; they represent less than 0.5% of the antibody in the serum (Schroeder and Cavacini, 2010). The exact function of IgD antibody is not fully known, but it can bind bacterial proteins through its constant region (Riesbeck and Nordstrom, 2006).

Immunoglobulin G

The IgG antibodies exist as monomers, represent about 70% of the antibody in circulation, and have been the most studied. Among immunoglobulins, IgGs have the longest $t_{1/2}$ in serum and are generated with high affinity after affinity maturation. The constant region of the heavy chain can further lead to diversity in the structure of these antibodies to generate four subclasses: IgG1, IgG2, IgG3, and IgG4. These subclasses are named based on their concentrations in serum, with IgG1 the most abundant and IgG4 the least. IgG1, IgG2, and IgG3 subclasses can activate complement to opsonize pathogens, but IgG4 cannot. These antibodies can also differ in their ability and affinity to engage Fc receptors, which further enhances their effector functions. All IgG subclasses cross the placenta to provide passive immunity to the fetus. Allotypes for IgG exist, conferring differences between individuals, especially for IgG3 in which the exact length of the antibody hinge region varies between allotypes.

TABLE 40–4 ■ THERAPEUTIC MONOCLONAL ANTIBODIES APPROVED IN THE EUROPEAN UNION AND THE U.S., JULY 2021

ANTIBODY	TARGET; *ANTIBODY TYPE*	THERAPEUTIC USE
Abciximab	GPIIb/IIIa; *chimeric IgG1 Fab*	Prevention of blood clots in angioplasty
Adalimumab	TNF; *human IgG1*	Rheumatoid arthritis
Ado-trastuzumab emtansine	HER2; *humanized IgG1; immunoconjugate*	Breast cancer
Aducanumab	Amyloid beta; *human IgG1*	Alzheimer's disease
Alemtuzumab	CD52; *humanized IgG1*	Multiple sclerosis
Alirocumab	PCSK9; *human IgG1*	Lowering cholesterol
Amivantamab, amivantamab-vmjw	EGFR, cMET; *human bispecific IgG1*	Non–small cell lung cancer with EGFR exon 20 insertion mutations
Ansuvimab-zykl	Ebola virus glycoprotein; *human IgG1*	Ebola virus infection
Atezolizumab[a]	PD-L1; *humanized IgG1*	Bladder cancer
Atoltivimab, maftivimab, and odesivimab-ebgn	Ebola virus; *cocktail of 3 human IgG1*	Ebola virus infection
Avelumab	PD-L1; *human IgG1*	Merkel cell carcinoma
Basiliximab	IL-2R; *chimeric IgG1*	Prevention of kidney transplant rejection
Belantamab mafodotin, belantamab mafodotin-blmf	B-cell maturation antigen; *humanized IgG1 ADC*	Multiple myeloma
Belimumab	BLyS; *human IgG1*	Systemic lupus erythematosus
Benralizumab	IL-5Rα; *humanized IgG1*	Asthma
Bevacizumab	VEGF; *humanized IgG1*	Colorectal cancer
Bezlotoxumab	*Clostridium difficile* toxin B; *human IgG1*	*C. difficile* infections
Blinatumomab	CD19, CD3; *murine bispecific tandem scFv*	Acute lymphoblastic leukemia
Brentuximab vedotin	CD30; *chimeric IgG1; immunoconjugate*	Hodgkin lymphoma, systemic anaplastic large cell lymphoma
Brodalumab	IL-17RA; *human IgG2*	Plaque psoriasis
Brolucizumab, brolucizumab-dbll	VEGF-A; *humanized scFv*	Neovascular age-related macular degeneration
Burosumab, burosumab-twza	FGF23; *human IgG1*	X-linked hypophosphatemia
Canakinumab	IL-1β; *human IgG1*	Muckle-Wells syndrome
Caplacizumab, caplacizumab-yhdp	von Willebrand factor; *humanized nanobody*	Acquired thrombotic thrombocytopenic purpura
Catumaxomab[b]	EPCAM/CD3; *rat/mouse bispecific mAb*	Malignant ascites
Cemiplimab, cemiplimab-rwlc	PD-1; *human mAb IgG4*	Cutaneous squamous cell carcinoma
Certolizumab pegol	TNF; *humanized Fab, pegylated*	Crohn disease
Cetuximab	EGFR; *chimeric IgG1*	Colorectal cancer
Crizanlizumab; crizanlizumab-tmca	CD62 (aka P-selectin); *humanized IgG2*	Sickle cell disease
Daclizumab	IL-2R; *humanized IgG1*	Multiple sclerosis
Daratumumab	CD38; *human IgG1*	Multiple myeloma
Denosumab	RANK-L; *human IgG2*	Bone loss
Dinutuximab	GD2; *chimeric IgG1*	Neuroblastoma
Dostarlimab, dostarlimab-gxly	PD-1; *humanized IgG4*	Endometrial cancer
Dupilumab	IL-4Rα/*human IgG4*	Eczema
Durvalumab	PD-L1/*human IgG1*	Urothelial carcinoma
Eculizumab	C5; *humanized IgG2/4*	Paroxysmal nocturnal hemoglobinuria
Efalizumab	CD11a; *humanized IgG1*	Psoriasis
Elotuzumab	SLAMF7; *humanized IgG1*	Multiple myeloma
Emapalumab, emapalumab-lzsg	IFNγ; *human IgG1*	Primary hemophagocytic lymphohistiocytosis

(*Continued*)

TABLE 40–4 ■ THERAPEUTIC MONOCLONAL ANTIBODIES APPROVED IN THE EUROPEAN UNION AND THE U.S., JULY 2021 (*CONTINUED*)

ANTIBODY	TARGET; *ANTIBODY TYPE*	THERAPEUTIC USE
Emicizumab	Factor Ixa, X; *humanized IgG4, bispecific*	Hemophilia A
Enfortumab vedotin, enfortumab vedotin-ejfv	Nectin-4; *human IgG1 ADC*	Urothelial cancer
Eptinezumab, eptinezumab-jjmr	CGRP; *humanized IgG1*	Migraine prevention
Erenumab, erenumab-aooe	CGRP receptor; *human IgG2*	Migraine prevention
Evinacumab	Angiopoietin-like 3; *human IgG4*	Homozygous familial hypercholesterolemia
Evolocumab	PCSK9; *human IgG2*	Lowering cholesterol
Fremanezumab, fremanezumab-vfrm	CGRP; *humanized IgG2*	Migraine prevention
Galcanezumab, galcanezumab-gnlm	CGRP; *humanized IgG4*	Migraine prevention
Gemtuzumab ozogamicin[a]	CD33; *humanized IgG4*	Acute myeloid leukemia
Golimumab	TNF; *human IgG1*	Rheumatoid and psoriatic arthritis, ankylosing spondylitis
Ibalizumab, ibalizumab-uiyk	CD4; *humanized IgG4*	HIV infection
Ibritumomab tiuxetan	CD20; *murine IgG1*	Non-Hodgkin lymphoma
Idarucizumab	Dabigatran; *humanized Fab*	Dabigatran excess (reversing anticoagulation)
Inebilizumab, inebilizumab-cdon	CD19; *humanized IgG1*	Neuromyelitis optica spectrum disorders
Infliximab	TNF; *chimeric IgG1*	Crohn disease
Inotuzumab ozogamicin	CD22; *humanized IgG4; ADC*	Acute lymphoblastic leukemia
Isatuximab, isatuximab-irfc	CD38; *chimeric IgG1*	Multiple myeloma
Guselkumab	IL-23 p19; *human IgG1*	Plaque psoriasis
Ipilimumab	CTLA-4; *human IgG1*	Metastatic melanoma
Ixekizumab	IL-17A; *humanized IgG4*	Psoriasis
Lanadelumab, lanadelumab-flyo	Plasma kallikrein; *human IgG1*	Hereditary angioedema attacks
Loncastuximab tesirine, loncastuximab tesirine-lpyl	CD19; *humanized IgG1 ADC*	Diffuse large B-cell lymphoma
Margetuximab-cmkb	HER2; *chimeric IgG1*	HER2-positive metastatic breast cancer
Mepolizumab	IL-5; *hIgG1*	Severe eosinophilic asthma
Mogamulizumab, mogamulizumab-kpkc	CCR4; *humanized IgG1*	Mycosis fungoides or Sézary syndrome
Moxetumomab pasudotox, moxetumomab pasudotox-tdfk	CD22; *murine IgG1 dsFv immunotoxin*	Hairy cell leukemia
Muromonab-CD3	CD3; *murine IgG2a*	Reversal of kidney transplant rejection
Natalizumab	α4 integrin; *humanized IgG4*	Multiple sclerosis
Naxitamab-gqgk	GD2; *humanized IgG1*	High-risk neuroblastoma and refractory osteomedullary disease
Necitumumab	EGFR; *human IgG1*	Non–small cell lung cancer
Nivolumab	PD1; *human IgG4*	Melanoma, non–small cell lung cancer, renal cell carcinoma
Obiltoxaximab[a]	Protective antigen *of Bacillus anthracis* exotoxin[c]; *chimeric IgG1*	Prevention of inhalational anthrax
Obinutuzumab	CD20; *humanized IgG1; glycoengineered*	Chronic lymphocytic leukemia
Ocrelizumab	CD20; *human IgG1*	Multiple sclerosis
Ofatumumab	CD20; *human IgG1*	Chronic lymphocytic leukemia
Olaratumab	PDGFR; *human IgG1*	Soft-tissue sarcoma
Omalizumab	IgE; *humanized IgG1*	Asthma
Palivizumab	RSV; *humanized IgG1*	Prevention of respiratory syncytial virus infection
Panitumumab	EGFR; *human IgG2*	Colorectal cancer

(*Continued*)

TABLE 40–4 ■ THERAPEUTIC MONOCLONAL ANTIBODIES APPROVED IN THE EUROPEAN UNION AND THE U.S., JULY 2021 (*CONTINUED*)

ANTIBODY	TARGET; *ANTIBODY TYPE*	THERAPEUTIC USE
Pembrolizumab	PD1; *humanized IgG4*	Melanoma, non–small cell carcinoma
Pertuzumab	HER2; *humanized IgG1*	Breast cancer
Polatuzumab vedotin, polatuzumab vedotin-piiq	CD79b; *humanized IgG1 ADC*	Diffuse large B-cell lymphoma
Ramucirumab	VEGFR2; *human IgG1*	Gastric cancer
Ranibizumab	VEGF; *humanized IgG1 Fab*	Macular degeneration
Ravulizumab, ravulizumab-cwvz	C5; *humanized IgG2/4*	Paroxysmal nocturnal hemoglobinuria
Raxibacumab[a]	*B. anthracis* protective antigen[c]; *human IgG1*	Prevention of inhalational anthrax
Reslizumab	IL-5; *humanized IgG4*	Asthma
Risankizumab, risankizumab-rzaa	IL-23 p19; *humanized IgG1*	Plaque psoriasis
Rituximab	CD20; *chimeric IgG1*	Non-Hodgkin lymphoma
Romosozumab, romosozumab-aqqg	Sclerostin; *humanized IgG2*	Osteoporosis in postmenopausal women at increased risk of fracture
Sacituzumab govitecan; sacituzumab govitecan-hziy	TROP-2; *humanized IgG1 ADC*	Triple-negative breast cancer
Sarilumab	IL-6R; *human IgG1*	Rheumatoid arthritis
Satralizumab, satralizumab-mwge	IL-6R; *humanized IgG2*	Neuromyelitis optica spectrum disorder
Secukinumab	IL-17A; *human IgG1*	Psoriasis
Siltuximab	IL-6; *chimeric IgG1*	Castleman disease
Tafasitamab, tafasitamab-cxix	CD19; *humanized IgG1*	Diffuse large B-cell lymphoma
Teprotumumab, teprotumumab-trbw	IGF-1R; *human IgG1*	Thyroid eye disease
Tildrakizumab; tildrakizumab-asmn	IL-23 p19; *humanized IgG1*	Plaque psoriasis
Tocilizumab	IL-6R; *humanized IgG1*	Rheumatoid arthritis
Tositumomab-I^{131}[a]	CD20; *murine IgG2a*	Non-Hodgkin lymphoma
Trastuzumab	HER2; *hIgG1*	Breast cancer
[fam-]trastuzumab deruxtecan, fam-trastuzumab deruxtecan-nxki	HER2; *humanized IgG1 ADC*	HER2-positive metastatic breast cancer
Ustekinumab	IL-12/23; *human IgG1*	Psoriasis
Vedolizumab	α4β7 integrin; *humanized IgG1*	Ulcerative colitis, Crohn disease

[a] Not approved in the European Union.
[b] Not approved in the U.S.
[c] Inhibits the binding of the protective antigen to its membrane receptors, thereby preventing the intracellular entry of the anthrax lethal factor and edema factor, the enzymatic toxin components responsible for the pathogenic effects of anthrax toxin.

Vaccines predominantly induce these antibody types, which become important during the secondary immune response to inactivate pathogens. Different subclasses are selected during the secondary antibody response. In designing vaccines, scientists must determine which antibody subclass will provide the optimal response. In addition to complement and opsonization, IgG antibodies can directly neutralize toxins and viruses (Schroeder and Cavacini, 2010).

Immunoglobulin A

The IgA antibody class is expressed as monomers or dimers and represents about 15% of the antibodies in serum, slightly higher than IgM antibodies. IgA antibodies are found at the highest concentrations at mucosal surfaces and in saliva and breast milk (Woof and Mestecky, 2005). In late pregnancy and the early postnatal period, female mammary glands produce colostrum; more than half of the protein content of colostrum that breastfeeding neonates consume is IgA antibodies. IgA is primarily a monomer in the serum but a dimer at mucosal sites.

IgA antibodies have two subclasses, IgA1 and IgA2, that differ only slightly in their structures. IgA1 antibodies are longer than IgA2 antibodies and are therefore more sensitive to degradation. IgA2 is more stable and is found primarily in mucosal secretions, in contrast to IgA1, which predominates in serum. IgA antibodies work via direct neutralization of viruses, bacteria, and toxins to protect mucosal tissues and to prevent antigen binding to host cells that damage or infect mucosal tissues. IgA antibodies within cells may also prevent pathogen tropism. Even though IgA antibodies do not lead to complement fixation, neutrophils can uptake them to mediate antibody-dependent cell-mediated cytotoxicity (Schroeder and Cavacini, 2010).

Immunoglobulin E

The IgE antibody class is present at the lowest serum concentration, less than 0.01% of circulating antibodies, and has the shortest $t_{1/2}$. IgE binds with very high affinity to Fcγ receptors, receptors that Langerhans and mast cells, basophils, and eosinophils express. Fc receptor engagement also results in FcγR upregulation on bound cells. These antibodies recognize antigens on parasitic worms when they are cross-linked on granulocytes; the cells degranulate to release inflammatory mediators to destroy the parasite. IgE antibodies are also relevant in mediating allergic reactions by recognizing innocuous antigens, such as bee venom and peanut antigen.

Patients who develop allergic reactions generate memory B cells that produce IgE antibodies to specific antigens. The granulocytes become coated with IgE antibodies and on antigen reexposure (e.g., a bee's sting or peanuts), the antigen cross-links IgEs, leading to granulocyte degranulation, which can result in anaphylactic shock. Therapies are in development to generate and use antibodies against soluble IgE molecules to prevent their uptake by granulocytes. For a list of approved mAbs, see Table 40–4.

Monoclonal Antibodies for RSV

Palivizumab is a humanized murine mAb that is licensed for use in high-risk infants to prevent hospitalization due to RSV. The drug was first approved in 1998, but the recommendations for use have evolved over time. The following recommendations are provided by the American Academy of Pediatrics as a policy statement in the organization's publication *Red Book*, with the policy last reaffirmed in February 2019.

In the first year of life, *palivizumab* prophylaxis is recommended for infants born before 29 weeks, 0 days' gestation. Prophylaxis is not recommended for otherwise healthy infants born at or after 29 weeks, 0 days' gestation. Prophylaxis in the first year of life is recommended for preterm infants with chronic lung disease of prematurity, defined as birth at less than 32 weeks, 0 days' gestation and a requirement for greater than 21% oxygen for at least 28 days after birth. Clinicians may administer prophylaxis in the first year of life to certain infants with hemodynamically significant heart disease. Clinicians may administer up to a maximum of 5 monthly doses of *palivizumab* (15 mg/kg per dose) during the RSV season to infants who qualify for prophylaxis in the first year of life. Qualifying infants born during the RSV season may require fewer doses. For example, infants born in January would receive their last dose in March. Prophylaxis is *not* recommended in the second year of life except for children who required at least 28 days of supplemental oxygen after birth and who continue to require medical intervention (supplemental oxygen, chronic corticosteroid, or diuretic therapy). Monthly prophylaxis should be discontinued in any child who experiences a breakthrough RSV hospitalization. Children with pulmonary abnormality or neuromuscular disease that impairs the ability to clear secretions from the upper airways may be considered for prophylaxis in the first year of life. Children younger than 24 months who will be profoundly immunocompromised during the RSV season may be considered for prophylaxis. Insufficient data are available to recommend *palivizumab* prophylaxis for children with cystic fibrosis or Down syndrome. The burden of RSV disease and costs associated with transport from remote locations may result in a broader use of *palivizumab* for RSV prevention in Alaska Native populations and possibly in selected other American Indian populations. *Palivizumab* prophylaxis is not recommended for prevention of healthcare-associated RSV disease.

Monoclonal Antibodies for EBOV

Atoltivimab/maftivimab/odesivimab (developed as REGN-EB3; brand name Inmazeb) is a cocktail of three mAbs for the treatment of EBOV. In the PALM clinical trial and as part of an expanded access program conducted in the Democratic Republic of the Congo during an EBOV outbreak in 2018 to 2019, of participants who received the cocktail, 33.8% died after 28 days, compared to 51% of the participants who received a control. This combination was the first FDA-approved treatment for EBOV, approved for use in the U.S. in October 2020 with orphan drug and breakthrough therapy designations. The cocktail also was given an orphan drug designation by the European Medicines Agency (EMA).

Ebanga (*ansuvimab*; originally mAb114) is a single human mAb for treating EBOV infection in adults and children. Ebanga halved the mortality rate of EBOV infection (from ~70% to ~34%). The FDA issued authorization for Ebanga in late 2020.

Monoclonal Antibodies for SARS-CoV-2

Human mAbs against SARS-CoV-2 are a rapidly evolving group of agents. Many were developed rapidly during the SARS-CoV-2 outbreak and tested in the clinic. Several anti–SARS-CoV-2 mAbs have received Emergency Use Authorization (EUA) from the FDA.

Bamlanivimab (also known as LY-CoV555 and LY3819253) is a neutralizing mAb that targets the receptor binding domain (RBD) of the S protein of SARS-CoV-2. *Etesevimab* (also known as LY-CoV016 and LY3832479) is a second mAb that binds to a different but overlapping epitope in the RBD of the SARS-CoV-2 spike protein. *Casirivimab* (also designated REGN10933) and *imdevimab* (REGN10987) are recombinant human mAbs that bind to nonoverlapping epitopes of the spike protein RBD. Two combinations (*bamlanivimab* plus *etesevimab* and *casirivimab* plus *imdevimab*) obtained EUA for the treatment of mild to moderate coronavirus disease 2019 (COVID-19) in nonhospitalized patients with laboratory-confirmed SARS-CoV-2 infection who are at high risk for progressing to severe disease and/or hospitalization. Because SARS-CoV-2 antigenic variants of concern emerged in circulation that evaded the action of *bamlanivimab*, the *bamlanivimab* plus *etesevimab* combination, and the *casirivimab* plus *imdevimab* combination, the FDA subsequently revoked the EUA for those drugs. In May 2021, the FDA granted an EUA for *sotrovimab* (VIR-7831), an investigational single-dose mAb, for the treatment of mild-to-moderate COVID-19 in adults and pediatric patients (≥12 years of age weighing ≥40 kg) with positive results of direct SARS-CoV-2 viral testing and who are at high risk for progression to severe COVID-19, including hospitalization or death. A combination of two long-acting human mAbs (AZD7442) that were modified in an Fc format for long-half life (long-acting antibody) obtained FDA EUA in December 2021 for prevention of disease in high-risk individuals and obtained a marketing authorization from the European Marketing Agency in March 2022 for any individual 12 years or older. Many other mAbs for SARS-CoV-2 are in earlier stages of clinical development.

Specific Conventional Vaccines Recommended in the U.S.

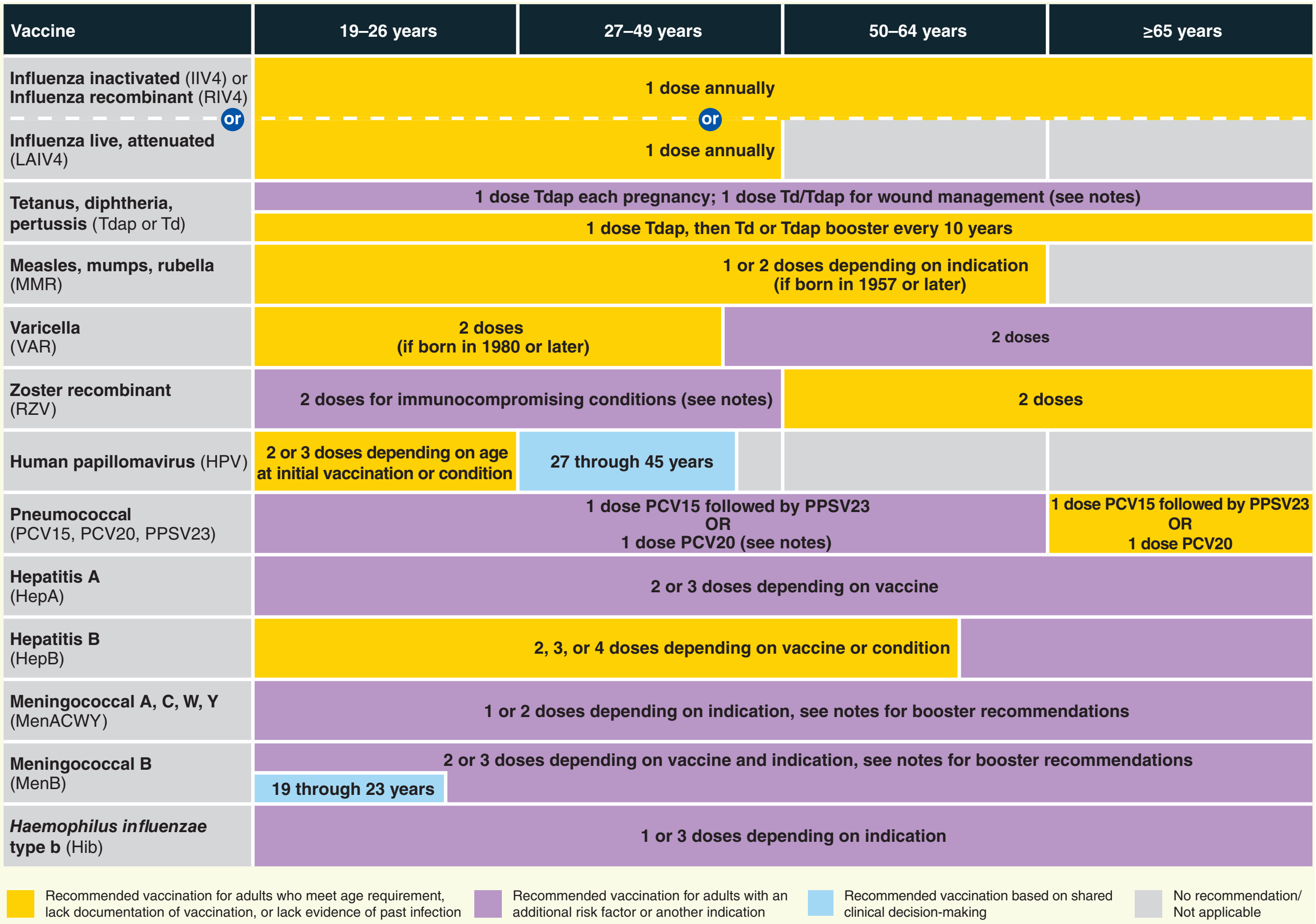
The Centers for Disease Control and Prevention (CDC) maintains tables listing currently recommended vaccinations for various susceptibilities throughout life. Next is a discussion of the properties and schedule of administration for the vaccinations recommended from birth to elder adulthood. The vaccines are grouped by the target type (bacterium, virus, etc.) and then by vaccine type, as discussed in the previous section. See Table 40–1 for infant and childhood vaccination schedules. For a complete list of the adult recommended immunizations and immunization schedule, see Tables 40–5 and 40–6.

Vaccines for Bacteria

Bacterial Toxoid Vaccines: Diphtheria and Tetanus

Tetanus Toxoid Vaccine. Tetanus is a disease characterized by prolonged spasms and tetany caused by the toxin secreted by the bacterium *C. tetani*, which enters from environmental sources through wounds. Tetanus toxin enters the nervous system and by retrograde transport reaches the inhibitory interneurons of the spinal cord, where the active fragment cleaves synaptobrevin (see Figures 10–3 to 10–6), thereby inhibiting exocytosis of neurotransmitter from these nerve cells and resulting in

TABLE 40–5 ■ RECOMMENDED IMMUNIZATION SCHEDULE FOR ADULTS AGED 19 YEARS OR OLDER BY AGE GROUP, U.S., 2022



Vaccine	19–26 years	27–49 years	50–64 years	≥65 years
Influenza inactivated (IIV4) or **Influenza recombinant** (RIV4)	🟨 1 dose annually	🟨	🟨	🟨
or	or			
Influenza live, attenuated (LAIV4)	🟨 1 dose annually	🟨	⬜	⬜
Tetanus, diphtheria, pertussis (Tdap or Td)	🟪 1 dose Tdap each pregnancy; 1 dose Td/Tdap for wound management (see notes) 🟨 1 dose Tdap, then Td or Tdap booster every 10 years	🟪 🟨	🟪 🟨	🟪 🟨
Measles, mumps, rubella (MMR)	🟨 1 or 2 doses depending on indication (if born in 1957 or later)	🟨	🟨	⬜
Varicella (VAR)	🟨 2 doses (if born in 1980 or later)	🟨 🟪	🟪 2 doses	🟪
Zoster recombinant (RZV)	🟪 2 doses for immunocompromising conditions (see notes)	🟪	🟨 2 doses	🟨
Human papillomavirus (HPV)	🟨 2 or 3 doses depending on age at initial vaccination or condition	🟦 27 through 45 years ⬜	⬜	⬜
Pneumococcal (PCV15, PCV20, PPSV23)	🟪 1 dose PCV15 followed by PPSV23 OR 1 dose PCV20 (see notes)	🟪	🟪	🟨 1 dose PCV15 followed by PPSV23 OR 1 dose PCV20
Hepatitis A (HepA)	🟪 2 or 3 doses depending on vaccine	🟪	🟪	🟪
Hepatitis B (HepB)	🟨 2, 3, or 4 doses depending on vaccine or condition	🟨	🟨 🟪	🟪
Meningococcal A, C, W, Y (MenACWY)	🟪 1 or 2 doses depending on indication, see notes for booster recommendations	🟪	🟪	🟪
Meningococcal B (MenB)	🟪 2 or 3 doses depending on vaccine and indication, see notes for booster recommendations 🟦 19 through 23 years	🟪	🟪	🟪
***Haemophilus influenzae* type b** (Hib)	🟪 1 or 3 doses depending on indication	🟪	🟪	🟪

🟨 Recommended vaccination for adults who meet age requirement, lack documentation of vaccination, or lack evidence of past infection

🟪 Recommended vaccination for adults with an additional risk factor or another indication

🟦 Recommended vaccination based on shared clinical decision-making

⬜ No recommendation/ Not applicable

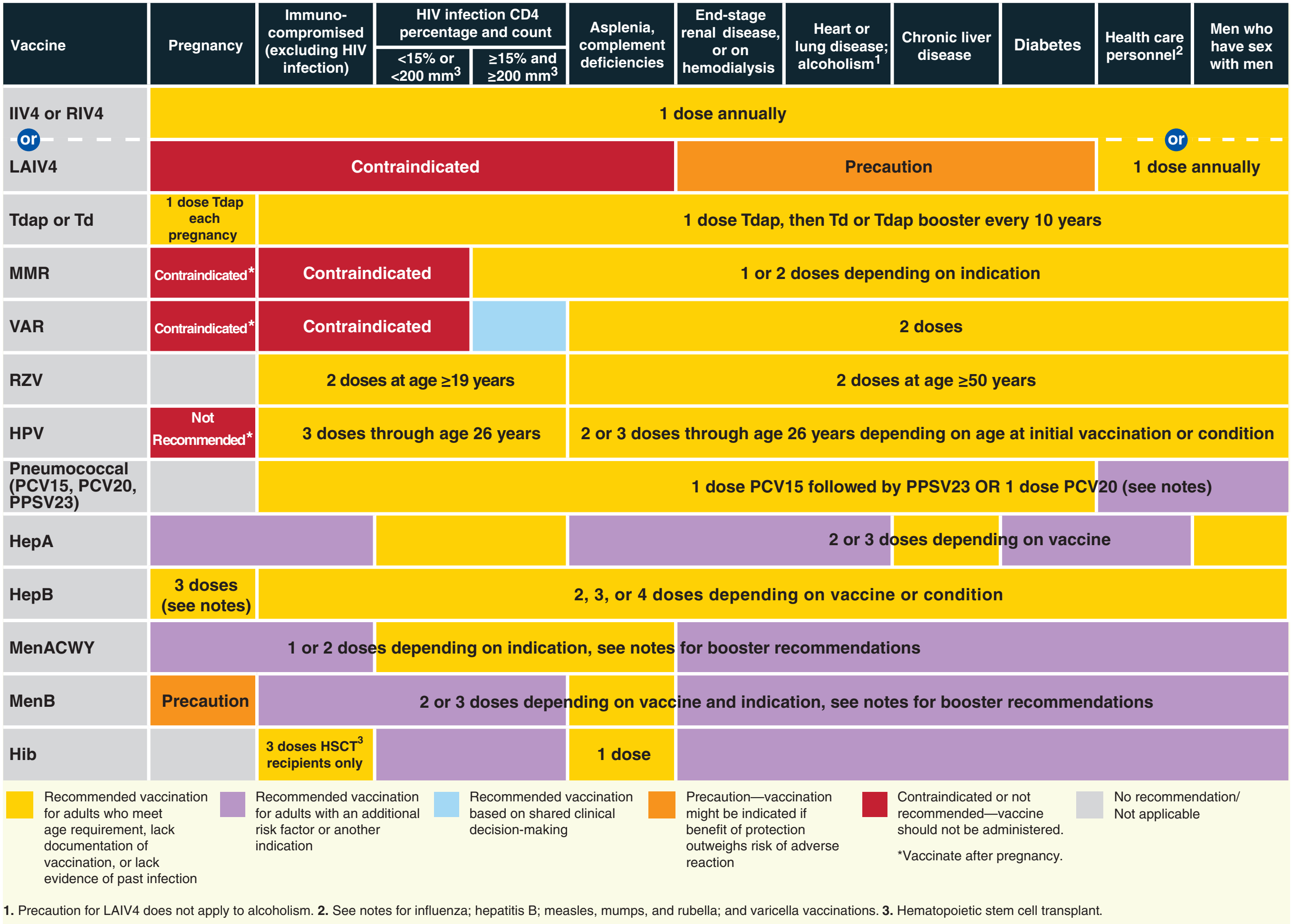
NOTE: The above recommendations are reprinted from the website of the Centers for Disease Control and Prevention (CDC) and should be read along with the footnotes of this schedule, available on the CDC website: https://cdc.gov/vaccines/schedules/.

uninhibited skeletal muscle contraction. The toxoid is produced by deactivating toxin isolated from the bacterium using formaldehyde. Immunization usually begins at about age 2 months, as a component of the combination vaccine DTaP (diphtheria and tetanus toxoids and acellular pertussis) that is given to infants. Tetanus toxoid is included in several combination vaccine formulations. DTaP is the vaccine used in children younger than age 7; Tdap (tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis) and Td (tetanus toxoid and reduced diphtheria toxoid), given at later ages, are booster immunizations that offer continued protection from those diseases for adolescents and adults. In these designations, upper- and lowercase letters represent the comparative quantity of antigen present. Thus, the shared uppercase *T* indicates there is about the same amount of tetanus toxoid in DTaP, Tdap, and Td. The uppercase *D* and *P* in the childhood formulation indicate that there is more diphtheria and pertussis antigen in DTaP than in Tdap or Td.

Diphtheria Toxoid Vaccines. Diphtheria is a disease caused by a secreted toxin of the aerobic gram-positive bacterium *C. diphtheriae*; toxin production is under control of the bacterial systems, but the structural gene for toxin production is contributed by a β phage that infects all pathogenic strains of *C. diphtheriae*. The A subunit of the toxin is an ADP-ribosylase; following its entry into a cell, this subunit ADP-ribosylates eukaryotic elongation factor 2 (eEF-2) and thereby inhibits protein translation in human cells (Gill et al., 1973). The throat of the victim becomes swollen and sore during infection, and the toxin causes damage to myelin sheaths in the nervous system, leading to loss of sensation or motor control. The vaccine, which has been used for nearly 80 years, is a toxoid that is produced by treating toxin with formalin. The toxoid is used to immunize infants beginning at about 2 months, typically as part of the combination DTaP vaccine. The diphtheria toxin also has been detoxified genetically by introduction of point mutations that abrogate enzymatic activity but allow retention of binding activity; for instance, the mutant diphtheria toxin protein CRM$_{197}$ (cross-reactive material) is the protein carrier for a licensed Hib vaccine.

Pertussis Vaccines. Pertussis, or whooping cough, is a respiratory tract disease characterized by prolonged paroxysmal coughing and sometimes respiratory failure; it is caused by the gram-negative coccobacillus *Bordetella pertussis*. The secreted pertussis toxin has an A subunit that, once in the cell cytosol, ADP-ribosylates the α subunit of the G$_i$ protein that couples inhibitory G protein-coupled receptor (GPCR) signaling to adenylyl cyclase to reduce cyclic AMP production. After ADP-ribosylation, G$_{i\alpha}$ becomes inactive, and GPCR-mediated reduction of cyclic AMP production is abolished. The physiological sequelae of this action of pertussis toxin are thought to contribute to the constellation of symptoms of whooping cough. Routine vaccination typically begins as part of the childhood combination DTaP vaccine series. It is also appropriate to immunize healthy adults, adolescents, and pregnant mothers as pertussis does occur throughout life due to waning immunity. There are two licensed pertussis vaccines, the historical inactivated organism "whole-cell" vaccine used in the past in the U.S. and still in many other countries and a second "acellular" formulation that incorporates antigen fragments derived from the organism. Both vaccines are immunogenic and protective. The whole-cell vaccine appears to induce more durable

TABLE 40–6 ■ RECOMMENDED IMMUNIZATION SCHEDULE FOR ADULTS AGED 19 YEARS OR OLDER BY MEDICAL CONDITION AND OTHER INDICATIONS, U.S., 2022



Vaccine	Pregnancy	Immuno-compromised (excluding HIV infection)	HIV infection CD4 percentage and count		Asplenia, complement deficiencies	End-stage renal disease, or on hemodialysis	Heart or lung disease; alcoholism[1]	Chronic liver disease	Diabetes	Health care personnel[2]	Men who have sex with men
			<15% or <200 mm^3	≥15% and ≥200 mm^3							
IIV4 or RIV4	🟨 1 dose annually	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
or										or	
LAIV4	🟥 Contraindicated	🟥	🟥	🟥	🟥	🟧 Precaution	🟧	🟧	🟧	🟨 1 dose annually	🟨
Tdap or Td	🟨 1 dose Tdap each pregnancy	🟨 1 dose Tdap, then Td or Tdap booster every 10 years	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
MMR	🟥 Contraindicated*	🟥 Contraindicated	🟥	🟨 1 or 2 doses depending on indication	🟨	🟨	🟨	🟨	🟨	🟨	🟨
VAR	🟥 Contraindicated*	🟥 Contraindicated	🟥	🟦	🟨 2 doses	🟨	🟨	🟨	🟨	🟨	🟨
RZV	⬜	🟨 2 doses at age ≥19 years	🟨	🟨	🟨 2 doses at age ≥50 years	🟨	🟨	🟨	🟨	🟨	🟨
HPV	🟥 Not Recommended*	🟨 3 doses through age 26 years	🟨	🟨	🟨 2 or 3 doses through age 26 years depending on age at initial vaccination or condition	🟨	🟨	🟨	🟨	🟨	🟨
Pneumococcal (PCV15, PCV20, PPSV23)	⬜	🟨 1 dose PCV15 followed by PPSV23 OR 1 dose PCV20 (see notes)	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟪	🟪
HepA	🟪	🟪	🟨	🟨	🟪 2 or 3 doses depending on vaccine	🟪	🟪	🟨	🟪	🟪	🟨
HepB	🟨 3 doses (see notes)	🟨 2, 3, or 4 doses depending on vaccine or condition	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
MenACWY	🟪 1 or 2 doses depending on indication, see notes for booster recommendations	🟪	🟨	🟨	🟨	🟪	🟪	🟪	🟪	🟪	🟪
MenB	🟧 Precaution	🟪 2 or 3 doses depending on vaccine and indication, see notes for booster recommendations	🟪	🟪	🟨	🟪	🟪	🟪	🟪	🟪	🟪
Hib	⬜	🟨 3 doses HSCT[3] recipients only	🟪	🟪	🟨 1 dose	🟪	🟪	🟪	🟪	🟪	🟪

🟨 Recommended vaccination for adults who meet age requirement, lack documentation of vaccination, or lack evidence of past infection
🟪 Recommended vaccination for adults with an additional risk factor or another indication
🟦 Recommended vaccination based on shared clinical decision-making
🟧 Precaution—vaccination might be indicated if benefit of protection outweighs risk of adverse reaction
🟥 Contraindicated or not recommended—vaccine should not be administered.
*Vaccinate after pregnancy.
⬜ No recommendation/ Not applicable

1. Precaution for LAIV4 does not apply to alcoholism. **2.** See notes for influenza; hepatitis B; measles, mumps, and rubella; and varicella vaccinations. **3.** Hematopoietic stem cell transplant.

NOTE: The above recommendations are reprinted from the website of the Centers for Disease Control and Prevention (CDC) and should be read along with the footnotes of this schedule available on the CDC website: https://cdc.gov/vaccines/schedules/.

immunity, but the acellular vaccine causes about one-tenth the rate of side effects (e.g., fever, injection site pain, erythema). Most developed countries now use acellular pertussis vaccine to reduce the reactivity profile, but many other countries continue to use the whole-cell vaccine successfully because the response is equally efficacious and more durable and the vaccine is economical.

Conjugated Bacterial Polysaccharide Vaccines

Haemophilus influenzae *Type B Vaccine.* *Haemophilus influenzae* is a major cause of life-threatening childhood bacterial diseases, including buccal, preseptal, and orbital cellulitis, epiglottitis, bacteremia with sepsis, and meningitis. Universal vaccination with the Hib vaccine has nearly eliminated these diseases in the U.S. The Hib vaccine is a polysaccharide-protein conjugate that confers immunity to the disease by inducing antibodies to the capsular polysaccharide polyribosylribitol phosphate (PRP). The Hib polysaccharide has been conjugated to diverse proteins, including the mutant diphtheria protein CRM$_{197}$ (a vaccine termed HbOC [Hib oligosaccharide conjugate]); the meningococcal group B outer membrane protein C (a vaccine termed PRP-OMPC [PRP outer membrane protein conjugate]); and tetanospasmin, which is a toxoid of the *C. tetani* neurotoxin (a vaccine termed PRP-T [PRP tetanus]). The vaccines all exhibit a high level of safety and immunogenicity. Interestingly, widespread immunization not only reduces disease in those vaccinated but also reduces nasal carriage of the bacterium, resulting in reduced transmission to even those not vaccinated and providing evidence of herd immunity.

Streptococcus pneumoniae *Vaccines.* The gram-positive encapsulated bacterium *S. pneumoniae* causes invasive diseases in infants and young children, including meningitis, bacteremia and sepsis, and pneumonia. There are myriad *S. pneumoniae* types, based on the capsular polysaccharide; thus, polyvalent vaccines are needed. Vaccines confer immunity by inducing type-specific antipolysaccharide antibodies. Two types of vaccines are available, *polysaccharide* and *conjugate* vaccines. The 23-valent polysaccharide vaccine contains long chains of capsular polysaccharides that are collected from inactivated bacteria. Polysaccharide vaccine is used in children older than 2 years and in at-risk adults. PCVs have been developed, and increasing numbers of serotypes have been incorporated over time. The combined 13 serotypes in PCV13 protect against most invasive disease in the U.S. Infants are given a primary series of PCV13 at ages 2, 4, and 6 months, with a booster at 12 to 15 months.

Neisseria meningitidis *Vaccines.* *Neisseria meningitidis* is a significant cause of invasive bacterial disease in childhood, causing sepsis and meningitis. As with *S. pneumoniae*, there are diverse types of polysaccharide; thus, type-specific anticapsular polysaccharide antibodies mediate protection against invasive disease. Therefore, multivalent vaccines are required for coverage against diverse strains. A licensed quadrivalent polysaccharide vaccine protects against four subtypes of meningococcus—designated A, C, Y, and W-135. The polysaccharide vaccine works only in children older than 2 years. A tetravalent meningococcal conjugate vaccine, also containing the A, C, Y, and W-135 subtypes, is available.

Four-component, protein-based meningococcal B vaccines (incorporating fHbp, NadA, NHBA, and PorA P1.4 proteins) have been developed to prevent septicemia and meningitis caused by serogroup B meningococcal strains. In the U.S., 11- to 12-year-old individuals should get a meningococcal conjugate vaccine, with a booster dose at 16 years old. Teens and young adults (16–23 years old) also may get a serogroup B meningococcal vaccine. The CDC also recommends meningococcal vaccination for other children and adults who are at increased risk for meningococcal disease.

Vaccines for Viruses

Poliovirus Vaccines

Polio is characterized by acute flaccid paralysis, against which the WHO and others are conducting a worldwide eradication campaign. There are two types of poliovirus vaccines in use. The first is a *live attenuated oral vaccine* in use since the early 1960s (the "Sabin vaccine"), containing attenuated poliovirus types I, II, and III, produced in monkey kidney cell tissue culture. The vaccine replicates in the intestine and induces systemic and mucosal immunity, but also is shed in the stool, sometimes transmitting to close contacts. Infection of most close contacts contributes to herd immunity in the human population. Rarely (about one case per million doses), partial revertant viruses occur that cause vaccine-associated paralytic poliomyelitis in contacts. In many parts of the world, live poliovirus vaccine is still used. The last known case of naturally acquired poliovirus disease acquired in the U.S. occurred in 1979; the U.S. discontinued use of the live vaccine in 2000. Live poliovirus vaccine is contraindicated in subjects with primary immunodeficiency. Pregnant women and children with symptomatic HIV infection should receive inactivated poliovirus vaccine (IPV).

The second type of vaccine is a *killed virus* preparation called *IPV* (the "Salk vaccine"). Killed vaccine induces mainly humoral immunity but still exhibits excellent efficacy against disease. IPV does not transmit virus to contacts and does not cause vaccine-associated paralysis. An enhanced-potency IPV vaccine has been available since 1998, and this IPV preparation is now a component of some combination vaccines.

Measles Virus Vaccines

The current measles vaccine is a live attenuated strain given subcutaneously. A live, "more attenuated" preparation of the Enders-Edmonston virus strain (designated the Moraten strain) is the MeV vaccine currently used in the U.S. Vaccination is initiated at 12 to 15 months of age in the U.S. because transplacentally acquired maternal antibodies inhibit immunogenicity of vaccine in the first year of life.

Mumps Virus Vaccine

Mumps virus causes a febrile illness most commonly associated with inflammation of the parotids and sometimes with more severe conditions, including aseptic meningitis. A live attenuated virus vaccine has been used exclusively since the 1970s. The Jeryl-Lynn vaccine (from a mixture of two strains) was isolated from the throat of the daughter of Maurice Hilleman, a noted vaccine developer. The vaccine is typically given as a component of the combination MMR (measles-mumps-rubella) or MMRV (measles-mumps-rubella-varicella) vaccine at 12 to 15 months of age and a second dose of MMR at 4 through 6 years of age. Although mumps outbreaks still occur even in vaccinated people in close-contact settings in the U.S., such as college dorms or close-knit religious communities, high vaccination coverage reduces the size and duration of those outbreaks.

Rubella Virus Vaccine

Rubella virus, the single member of the genus *Rubivirus* in the family *Togaviridae*, is spread by respiratory droplets and causes a mild infection with viremia. Rubella is harmful only to fetuses, and the effects can be devastating. A rubella infection during pregnancy can cause miscarriage, preterm birth, stillbirth, or various birth defects. The risks decrease as pregnancy progresses. The main goal of rubella immunization is to prevent congenital rubella syndrome. The live attenuated rubella virus vaccine is given subcutaneously, now usually as a component of MMR or MMRV vaccine, beginning between 12 and 15 months of age, and a second dose of MMR is given at 4 through 6 years of age. The live rubella virus vaccine strain RA 27/3 is grown in human diploid cell culture. In the U.S., universal immunization (both boys and girls) is used to reduce infection of pregnant women. As a result, rubella and congenital rubella syndrome have been eliminated in the U.S. Rubella vaccine is part of MMR or MMRV combination vaccines for universal immunization starting at 12 to 15 months, followed by a booster dose at school entry.

Varicella-Zoster Virus Vaccine

Varicella-zoster virus (VZV) is one of the most infectious agents that affect humans. It is spread by the respiratory route by small aerosol particles (cough, sneeze, etc.). Infection causes a febrile syndrome with vesicular rash, sometimes complicated by pneumonia or invasive bacterial skin disease. Congenital varicella syndrome can occur if varicella infection occurs during pregnancy. The vaccine used is the Oka strain of live attenuated VZV attenuated by sequential passage in cell monolayer cultures; it was licensed for universal immunization in the U.S. in 1995. The virus in the Oka/Merck vaccine in current use in the U.S. was further passaged in MRC-5 human diploid-cell cultures. The vaccine is often given as a part of the combination MMRV vaccine.

In 2017, the adjuvanted recombinant zoster vaccine (RZV or Shingrix), which is a two-dose, subunit vaccine containing recombinant VZV glycoprotein E in combination with a novel adjuvant (AS01B), was FDA approved for the prevention of herpes zoster in adults aged 50 years or older. The vaccine is given as two intramuscular doses, administered 2 to 6 months apart. The Advisory Committee on Immunization Practices (ACIP) recommends the recombinant zoster vaccine to prevent shingles in immunocompetent adults aged 50 years and older.

Hepatitis A Virus Vaccines

Hepatitis A virus infection causes acute liver disease after transmission by the fecal-oral route. An inactivated vaccine is recommended for all children, starting at 1 year of age. Two hepatitis A vaccines and one hepatitis A/hepatitis B combination vaccine are licensed in the U.S. The vaccine is given as a two-dose series.

Hepatitis B Virus Vaccines

Hepatitis B virus is transmitted between people by contact with blood or other bodily fluids, including by sexual contact and maternal transfer to fetus or infant. Hepatitis B virus can cause a life-threatening and sometimes chronic liver disease. All infants receive the hepatitis B vaccine. When the mother has active infection, the neonate is treated with both the vaccine and hepatitis B immune globulin. The vaccine is a recombinant protein produced in yeast that is the protective antigen, hepatitis B surface antigen. Chapter 63 covers the treatment of hepatitis B virus with nucleoside analogues and other agents.

Rotavirus Vaccines

Throughout the world, rotavirus is the most common cause of dehydrating diarrhea in infants. Four or five types (based on the surface proteins) cause severe disease. An early live attenuated vaccine (Rotashield) was withdrawn after association with intussusception (a segmental telescoping collapse of the intestine). Two similar vaccines are now used that are safe and immunogenic. One is an oral pentavalent human-bovine reassortant rotavirus vaccine (containing five reassortant rotaviruses developed from human and the Wistar Calf 3 bovine parent rotaviral strains) first licensed in the U.S. in 2006 (RotaTeq). This vaccine is administered in a three-dose schedule, at 2, 4, and 6 months of age. Another oral live attenuated rotavirus vaccine licensed in the U.S. is based on a single attenuated human strain (Rotarix) using a two-dose schedule beginning at 2 months of age. Rotavirus vaccines are used for universal immunization during infancy, with care to keep the initiation of the two- or three-dose series at a young age, as the rare rotavirus-associated intussusception risk with infection appears slightly higher at older ages.

Influenza Virus Vaccines

The orthomyxovirus influenza virus is a respiratory virus spread person-to-person by large-particle aerosols and fomites. The virus circulates in humans in two major serotypes (types A and B); two distinct A subtypes, designated H1N1 (hemagglutinin type 1 and neuraminidase type 1) and H3N2 (hemagglutinin type 3 and neuraminidase type 2), currently cause disease ("the flu") in humans. Current seasonal influenza vaccines are trivalent, including A/H1N1, A/H3N2, and B antigens, or quadrivalent with a second type B antigen. Experimental vaccines are being tested for some avian influenza viruses (e.g., A/H5N1 and A/H7N9) that have infected humans and have pandemic potential. During each annual seasonal epidemic, point mutations occur in genes encoding the hemagglutinin and neuraminidase proteins, which are the principal targets for protective antibodies. This antigenic drift in circulating influenza strains has led to a process in which regulatory officials and manufacturers adjust the virus antigens in influenza vaccines every year. Occasionally, the segmented virus genome reasserts during coinfection of an animal with a human and an avian virus, a new virus arises (antigenic shift), and a pandemic occurs. Major worldwide pandemics occurred in 1918 (H1N1), 1957 (H2N2 [hemagglutinin type 2 and neuraminidase type 2]), 1968 (H3N2), and 2009 (a novel H1N1). Major adjustments of vaccines must be made in such instances.

Three principal types of influenza vaccines are licensed at present: inactivated vaccine, recombinant protein vaccine, and live attenuated virus vaccine. The inactivated vaccine is prepared by treating wild-type viruses prepared in eggs or cell culture with an inactivating agent. Inactivated vaccine often prevents more than half of serious influenza-related disease when the viruses chosen for the seasonal vaccine antigenically match the eventual epidemic virus well. The vaccine is most effective at preventing severe respiratory disease and influenza-related hospitalizations.

All persons aged 6 months and older should be vaccinated. Those at most risk of severe disease and in most need of vaccine are infants, young children, people older than 65 years, pregnant women, and those with chronic health conditions or immunodeficiency. This vaccine is contraindicated in those who have had a life-threatening allergic reaction after a dose of influenza vaccine or have a severe allergy to any component of the vaccine, some of which contain a small amount of egg protein. Some people with a history of Guillain-Barré syndrome should not receive this vaccine. The vaccine is usually given as a single dose each year, although children 6 months through 8 years of age may need two doses during a single influenza season. Some inactivated influenza vaccines (IIVs) contain a small amount of the preservative thimerosal (see Preservatives, Including Thimerosal). Although any association with developmental disorders has been disproven, public concern about this topic has led to the development of thimerosal-free IIVs.

The second type of nonreplicating influenza vaccine is a purified recombinant hemagglutinin (HA) protein vaccine. A complementary DNA encoding the HA antigen of choice is used to make a recombinant baculovirus (a virus that infects invertebrates). The recombinant baculovirus is used to deliver the HA gene to an FDA-qualified insect cell line to produce large amounts of the HA antigen.

The third principal type of influenza vaccine is a trivalent or quadrivalent live attenuated virus vaccine that is administered topically by nasal spray. New vaccines are prepared each year to address antigenic drift by reasserting genes encoding the current HA and neuraminidase antigens with a virus genetic background containing internal viral genes with well-defined attenuating mutations. The vaccine is licensed in the U.S. for persons 2 to 49 years of age. In some pediatric studies, the live attenuated vaccine appeared to provide a higher level of protection than inactivated vaccine; however, CDC vaccine effectiveness data from the influenza seasons in 2013 to 2016 in the U.S. indicated that the quadrivalent live attenuated vaccine did not demonstrate statistically significant effectiveness in children 2 to 17 years of age. Therefore, the CDC provided an interim recommendation that the vaccine should not be used in any setting in the U.S. for the 2016 to 2017 influenza season. Live attenuated influenza vaccine was recommended again by the CDC in the 2018 to 2019 influenza season; due to the limited use of this vaccine in the U.S. since that time, there have been no recent estimates of effectiveness. Practitioners should check regularly for updated guidelines from the CDC on this point.

Human Papillomavirus Vaccines

Human papillomaviruses cause nearly all cases of cervical and anal cancer and most oropharyngeal cancers. Most such cancers are caused by just two of the many HPV serotypes, types 16 and 18. Remarkably, even though the virus cannot be grown efficiently in culture, effective HPV vaccines were developed using virus-like particles that are formed by HPV surface components. All licensed HPV vaccines protect against at least these two types and some protect against four or nine types of HPV, with effectiveness against vaginal and vulvar cancers in women, as well as most cases of anal cancer and genital warts in both females and males. HPV vaccines are recommended for all 11- and 12-year-olds to protect against HPV infection and for everyone through age 26 years, if not vaccinated already. The CDC recommends that some adults age 27 through 45 years, who are not already vaccinated, should receive the HPV vaccine depending on their risk for new HPV infections. HPV vaccination is also recommended for any man who has sex with a man. The vaccines are given in a three-dose regimen on a schedule of 0, 1 to 2, and 6 months.

Maternal Immunization

Maternal immunization during pregnancy can enhance newborn protection after birth by providing passive immunity to the neonate. Immunizing pregnant mothers is safe and protects the child from deadly infectious pathogens early in life when the immune system is not fully developed. One of the most successful maternal immunization protocols involves injection of tetanus toxoid to stimulate the production of IgG antibodies that have high neutralizing capacity and can cross the placenta. Vaccines for group B *Streptococcus*, Hib, RSV, *Streptococcus pneumoniae*, *Bordetella pertussis*, and trivalent IIVs have been tested in pregnant women. For a complete list of vaccines that may be used and those that are contraindicated, visit the CDC website on this topic: https://www.cdc.gov/vaccines/.

Vaccines for Travel

International travelers should ensure that their vaccination status is current for conventional vaccines, including diphtheria, tetanus, pertussis, hepatitis A and B, and poliovirus; exposures to these agents may be more common in some international settings. Listed below are additional vaccines that may be of benefit as preventive vaccines.

Japanese Encephalitis Virus Vaccine

Japanese encephalitis (JE) is a serious mosquito-borne flavivirus infection (not spread person to person) that can cause mild infections with fever and headache, serious neurological sequelae, and even death. Travelers who spend a month or longer in some rural parts of Korea, Japan, China, and eastern areas of Russia should consider vaccination. Two JE vaccines are licensed in the U.S.: an inactivated mouse brain–derived JE vaccine (JE-MB) for use in travelers aged 1 year or older and an inactivated Vero cell culture–derived JE vaccine (JE-VC) for persons aged 17 years or older.

Yellow Fever Virus Vaccine

Yellow fever is a mosquito-borne flaviviral disease with a wide range of systemic symptoms. In severe cases, the disease causes hepatitis, hemorrhagic fever, and death. The CDC recommends this vaccine for children older than 9 months and adults who will be traveling to high-risk areas. There is generally a requirement for documentation of vaccination for travel to and from infected areas. The vaccine is a live attenuated virus vaccine that has been used successfully for many decades. For international travel, yellow fever virus vaccine must be approved by WHO and must be administered by an approved yellow fever vaccination center that can provide both vaccination and a validated International Certificate of Vaccination. The vaccine should be given at least 10 days before travel to an endemic area. Generally, a single dose suffices. Major shortages of yellow fever vaccine have occurred in recent years due to manufacturing problems. To meet yellow fever vaccination needs, the FDA authorized the distribution of the yellow fever vaccine Stamaril (Sanofi Pasteur) through an expanded access program forecast to be effective through mid-2021 during the vaccine shortage. This vaccine is registered and distributed in over 70 countries but remains unlicensed in the U.S.

Typhoid Vaccine

Typhoid fever is an acute illness caused by the bacterium *Salmonella typhi*, which is transmitted by ingestion of contaminated water or food. Typhoid vaccination is recommended for international travelers who will visit rural areas or villages that have inadequate sanitation. Symptoms include fever, headache, anorexia, and abdominal discomfort; the disease can be fatal. Treatment is challenging, and there has been an increase in the number of drug-resistant strains of *S. typhi* over the last several decades. There are two vaccines available to prevent infection: a single-dose, injectable, inactivated typhoid vaccine and an oral live typhoid vaccine that is taken in a four-dose course.

Rabies Virus Vaccine

Rabies is caused by a lyssavirus transmitted to humans from the bite of infected mammals; the untreated infection is nearly always fatal in humans. Rabies vaccination is used in two ways, first as a preventive vaccine prior to exposure and second as a postexposure intervention to prevent progression to fatal disease. Candidates for preexposure vaccination are people at high risk of exposure to natural rabies (e.g., veterinarians, animal handlers, spelunkers) or to laboratory strains or tissues (e.g., those involved in production of rabies biologicals). Preventive vaccination should be offered to international travelers who are likely to encounter animals in parts of the world where rabies is common (see CDC website). The vaccine is given in a three-dose series on days 0, 7, and 28. For those who may be repeatedly exposed to rabies virus, periodic testing for immunity is recommended, and booster doses can be administered as needed to maintain immunity. Postexposure vaccination is used in emergency settings following a bite or close exposure to an animal that may be rabid. In this setting, the vaccine is given in a four-dose series on days 0, 3, 7, and 14, concomitant with two injections of rabies immune globulin on day 0, one locally into the bite site and a second in an intramuscular injection for systemic administration of antibodies. A bite victim who has been previously vaccinated should receive two doses of rabies vaccine on days 0 and 3 but does not need rabies immune globulin.

Specialty Vaccines

There are limited-use vaccines that are offered in special circumstances to at-risk persons.

Anthrax Vaccine

Anthrax vaccine is offered to certain at-risk adults 18 to 65 years of age, including some members of the U.S. military, laboratory workers who work with anthrax, and some veterinarians or other individuals who handle animals or animal products. Anthrax is a serious disease in animals and human caused by *Bacillus anthracis*. People can contract anthrax from contact with infected animals or animal products. Usually, the cutaneous infection causes ulcers on the skin and systemic symptoms, including fever and malaise; up to 20% of untreated cases are fatal. Inhaled spores of *B. anthracis* usually cause fatal infection. Anthrax vaccine adsorbed (AVA), given as multiple booster injections, protects against cutaneous and inhalation anthrax acquired by exposure on skin or by inhalation. The CDC recommends anthrax intramuscular booster shots at 1, 6, 12, and 18 months, and then annually.

Cholera Vaccines

In 2016, the FDA approved a live attenuated vaccine (Vaxchora) for the prevention of cholera caused by serogroup O1 for adults 18 through 64 years of age traveling to cholera-affected areas. The vaccine is taken as a single, oral liquid dose of approximately 3 fl oz at least 10 days before travel to a cholera-affected area. Efficacy was demonstrated in a randomized, placebo-controlled human challenge study of U.S. volunteers; efficacy was 90% among those challenged 10 days after vaccination and 80% among those challenged 3 months after vaccination. In late 2020, however, the manufacturer temporarily stopped making and selling the vaccine, which may therefore be in limited supply or unavailable. Three other oral inactivated or nonlive cholera vaccines are available: Dukoral, ShanChol, and Euvichol-Plus/Euvichol. These cholera vaccines are WHO prequalified but are not available in the U.S.

Vaccinia Virus (Smallpox Vaccine)

Vaccinia vaccine is a live attenuated *Orthopoxvirus* vaccine developed by multiple passages in cell culture to isolate viral variants that cause only limited infection in humans. The virus is produced as purified calf lymph and given percutaneously with a bifurcated needle. This vaccine was used in the first successful worldwide efforts to eradicate a human virus, variola or smallpox. Routine universal vaccinia immunization was discontinued around 1980, following the declaration by WHO that variola (smallpox) was eradicated, but the vaccine is still available. The nonemergency use of vaccinia vaccine includes vaccination of laboratory and healthcare workers exposed occupationally to vaccinia virus, to recombinant vaccinia viruses, or other orthopoxviruses that can infect humans, such as monkeypox virus and cowpox virus. Because there are still laboratory stocks of variola in research use in several countries, including the U.S., the U.S. ACIP has developed recommendations for the use of vaccinia vaccine if variola virus were used as an agent of biological terrorism or if a smallpox outbreak occurred accidentally. Large-scale use in the military and consideration of use in medical first responders in the U.S. has been implemented in recent decades. A derivative of conventional vaccinia virus vaccine has been developed that has desirable properties. Modified vaccinia Ankara (MVA) virus is a highly attenuated strain of vaccinia virus isolated after more than 500 passages in chicken embryo fibroblasts, during which the virus lost about 10% of the vaccinia genome and the ability to replicate productively in human and other primate cells.

Other Vaccines for Biodefense and Special Pathogens

There are several limited-use vaccines, such as those for workers in high-containment facilities conducting research on highly pathogenic agents that are emerging infectious diseases or potential agents for use in bioterrorism or biowarfare. Typically, these vaccines are used only under Investigational New Drug status. Examples include vaccines for Eastern equine encephalitis virus, Venezuelan equine encephalitis virus, Rift Valley fever virus, botulinum toxin, and others.

International Vaccines

There are additional vaccines pertinent to exposures in other countries that are licensed in some areas but not yet in the U.S.

Dengue Virus Vaccine

Dengue fever is another mosquito-borne flaviviral disease caused by four different viral serotypes and annually affecting about 400 million people worldwide. The disease can be a mild systemic febrile illness during primary infection but can cause severe dengue disease and death during a second infection with virus of a different serotype. It is thought that cross-reactive nonneutralizing antibodies induced by one infection enhance the disease caused by subsequent infection with a heterologous serotype virus. This antibody-dependent enhancement concern has been a significant barrier to vaccine development efforts. Nevertheless, much progress has been made recently in dengue vaccine development.

CYD-TDV developed by Sanofi Pasteur is a recombinant tetravalent (four-serotype) live attenuated virus vaccine that was first licensed in Mexico in December 2015 for use in individuals 9 to 45 years of age living in endemic areas. It is given as a three-dose series on a 0-, 6-, and 12-month schedule. The WHO recommends that the vaccine be given only to persons with confirmed previous dengue virus infection; the vaccine manufacturer announced in 2017 that people who receive the vaccine and have not been previously infected with a dengue virus may be at risk of developing severe dengue if they get dengue after being vaccinated. In May 2019, the vaccine was FDA-approved for use in children aged 9 to 16 years with laboratory-confirmed previous dengue virus infection and living in an area where dengue is endemic; in June 2021, the ACIP also recommended this use. Such areas in the U.S. include the territories of American Samoa, Puerto Rico, the U.S. Virgin Islands, and freely associated states, including the Federated States of Micronesia, the Republic of Marshall Islands, and the Republic of Palau. It should be noted that the vaccine is *not* approved for use in U.S. travelers who are visiting but not living in an area where dengue is endemic. Additional dengue vaccine candidates are in clinical development.

Malaria Vaccine

The RTS, S vaccine is a recombinant protein-based malaria vaccine with AS01 adjuvant against *Plasmodium falciparum* that was developed by a large international public-private consortium and is the first malaria vaccine to complete efficacy trial testing with a positive review of the outcome. It is relevant for *P. falciparum*, which is common in sub-Saharan Africa, but does not protect against *Plasmodium vivax* malaria, which is more common in many countries outside Africa. In July 2015, the EMA issued a "European Scientific Opinion" on the vaccine, and WHO and its Strategic Advisory Group of Experts have advocated its use in large-scale implementation pilot tests in Africa. Another candidate malaria vaccine is designated PfSPZ (*P. falciparum* and sporozoites). Sporozoites, the sexual form of the parasite extracted from mosquito salivary glands, are made noninfectious through irradiation and then injected or infused to immunize. Clinical trials have been promising, and phase III trials are underway in Africa. Chapter 66 covers the life cycle of malaria parasites and the pharmacotherapy of the disease.

BCG Vaccine

Bacille Calmette-Guérin (BCG) vaccine is used to prevent severe disease due to *Mycobacterium tuberculosis* (TB). BCG vaccine is produced using a live attenuated bovine bacillus strain, *Mycobacterium bovis*, that has lost its ability to cause severe disease in humans. The vaccine typically is given as a single intradermal dose, often to infants near the time of birth. The efficacy of BCG vaccine against TB is uncertain in many settings, but the consensus is that the vaccine does protect against the most severe forms of disseminated TB, such as miliary disease and TB meningitis. The vaccine is a WHO essential medicine for endemic areas but is not used for universal vaccination in the U.S. Chapter 65 covers agents used in the chemotherapy of tuberculosis.

SARS-CoV-2 and COVID-19

Coronavirus disease 2019 is caused by SARS-CoV-2, a new viral strain that has caused a global pandemic from 2019 to present. This novel coronavirus has led to widespread morbidity and mortality throughout the world. The SARS-CoV-2 virus infects through attachment to several host factors, especially the angiotensin-converting enzyme 2 (ACE-2) entry receptor that is expressed on the surface of airway epithelial cells. Infection can result in the upper and lower respiratory tracts, but multiple other organs also can be affected including the lung, pharynx, heart, liver, brain, gastrointestinal tract, and kidneys. A coordinated innate and adaptive immune response is necessary for effective clearance of the virus and development of memory B and T cells that protect the host from reinfection. SARS-CoV-2 subverts the immune response, and defects in the innate immune response can permit viral replication to proceed uninterrupted while delaying the adaptive immune response.

The adaptive immune response is critical in the resolution of SARS-CoV-2 infection. The presence of SARS-CoV-2–specific CD4$^+$ T cells is associated with mild COVID-19, while a delay or absence of these cells correlates with severe or fatal COVID-19 disease. The presence of virus-specific CD8$^+$ T cells is also associated with better COVID-19 patient outcomes. Antibodies are generated to most of the proteins of the virus, with protective neutralizing antibodies focused on the spike protein that mediates attachment and fusion to host cells. Antibodies alone cannot clear SARS-CoV-2 infection; T cells are required to achieve viral clearance.

Several risk factors for severe or fatal COVID-19 disease have been identified, with age being a major risk factor. In the U.S., when compared to a 18–29-year-old, a 65-year-old has about a 97-fold higher risk of death from COVID-19 (CDC, 2020). Additional risk factors for severe or fatal COVID-19 disease include obesity, diabetes, kidney disease, and thoracic malignancies, among others. Men have an increased risk of severe disease compared to women. While pediatric SARS-CoV-2 infection is not yet fully understood, young children usually have mild disease, although in rare cases, severe disease associated with multisystem inflammatory syndrome in children occurs. Socioeconomic risk factors increase the risk of COVID-19, principally due to close-contact settings at work or home. Inequality in access to healthcare and reduced quality of healthcare have contributed to increased COVID-19 prevalence in poorer communities, disproportionally impacting Black and Hispanic communities in the U.S. Some patients who have recovered from COVID-19 have persisting effects. These postinfection sequelae, termed "long COVID," include fatigue, decreased lung capacity, and inability to fully work or exercise. Other symptoms may include vision and cognitive problems.

SARS-CoV-2 Vaccines

Data from studies of a previous coronavirus outbreak caused by SARS-CoV indicated that B and T cells persist for years against that virus. SARS-CoV-2 infection elicits B- and T-cell memory; studies of durability of natural immunity for those previously infected with SARS-CoV-2 are ongoing. Studies suggest that SARS-CoV-2 infection can elicit B- and T-cell memory with estimated half-lives of 3 to 5 months.

The development of multiple vaccines with high efficacy against severe or fatal COVID-19 in record time has been an impressive achievement. The immunity from these vaccines has tended to fade with time, necessitating an additional "booster" dose at roughly 5 to 8 months after the initial dose(s). Current vaccines are delivered by injection; nasal spray formulations are forthcoming.

The panels of Figure 40–1 show some salient features of SARS-CoV-2 , its interaction with host cells, and the points of attack by antibodies produced in response to several available vaccines.

Several types of vaccines against SARS-CoV-2 are now available.

mRNA Vaccines

Three major mRNA-based SARS-CoV-2 vaccines have been tested at scale, and two, the Pfizer/BioNTech and Moderna vaccines, were remarkably efficacious and widely approved for use.

The Pfizer/BioNTech vaccine (BNT162b2) contains an mRNA encoding full-length SARS-CoV-2 spike protein, delivered in a lipid nanoparticle. Once injected, the nanoparticles are taken up by APCs that subsequently express spike protein, resulting in an immune response. The vaccine is safe and has a 95% efficacy in preventing severe or fatal COVID-19. It is injected in two doses, 21 days apart. The vaccine can be stored for 6 months at −70°C and 5 days at +2 to +8°C. The Moderna SARS-CoV-2 vaccine (mRNA-1273) is also a nanoparticle encapsulated mRNA that encodes a full-length SARS-CoV-2 spike protein that elicits 94% protective immunity against severe or fatal COVID-19. This vaccine also requires two doses, 28 days apart. The vaccine can be stored for 6 months at −20°C and 30 days at +2 to +8°C. A third two-dose mRNA SARS-CoV-2 vaccine (CVnCoV) was produced by CureVac, but the results of a 40,000-person efficacy trial announced in June 2021 showed that this vaccine was only 47% effective at preventing disease. The reason for reduced efficacy is under investigation. One plausible difference is that the BioNTech and Moderna vaccines use chemically modified nucleosides to avoid recognition by Toll-like receptors, while the CVnCoV candidate used unmodified nucleosides to produce mRNA.

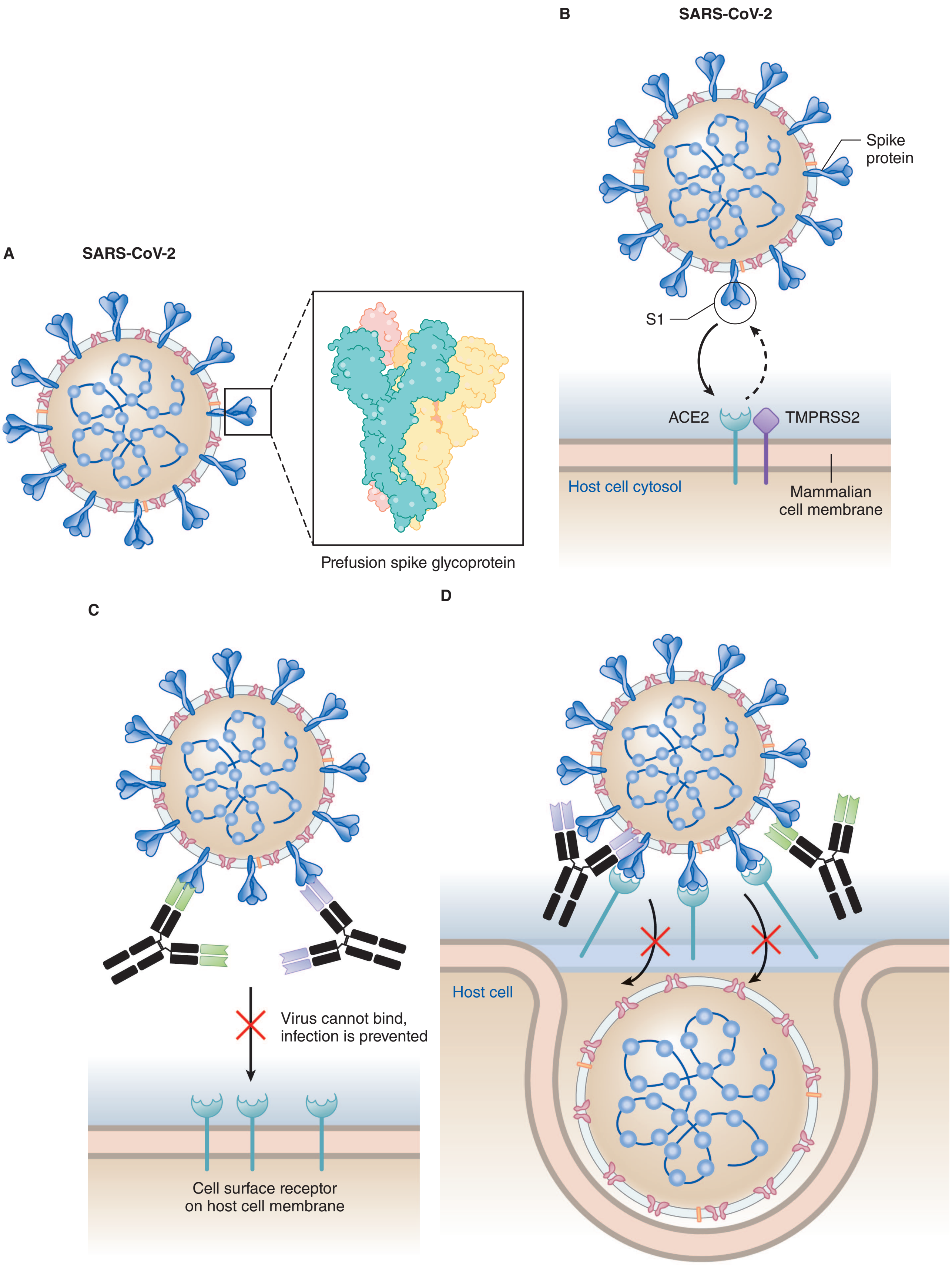

A
SARS-CoV-2
Prefusion spike glycoprotein
B
SARS-CoV-2
Spike protein
S1
ACE2
TMPRSS2
Host cell cytosol
Mammalian cell membrane
C
Virus cannot bind, infection is prevented
Cell surface receptor on host cell membrane
D
Host cell

Figure 40–1 **A.** *SARS-CoV-2 virus particles are decorated on the surface with trimeric spike (or "S") glycoproteins in a prefusion conformation.* **B.** The S1 segment of the spike protein of SARS-CoV-2 interacts with cell surface factors of mammalian host cells to facilitate attachment of virus to cells. These host attachment points include ACE2 (angiotensin-converting enzyme 2), a single-pass, transmembrane protease, and TMPRSS2 (transmembrane protease serine 2), a type II transmembrane-bound serine protease. **C.** Some spike protein–specific antibodies can block attachment of virus particles to host cell surface receptors, preventing infection. **D.** Other protein-specific antibodies can block fusion of virus and host cell membranes, even after the virus attaches to host cell surface factors. **E.** Some spike protein–specific antibodies that are bound to virus particles or to S protein on the surface of virus-infected cells activate Fc-receptor–bearing cells to promote virus clearance. Figure made with BioRender.com.

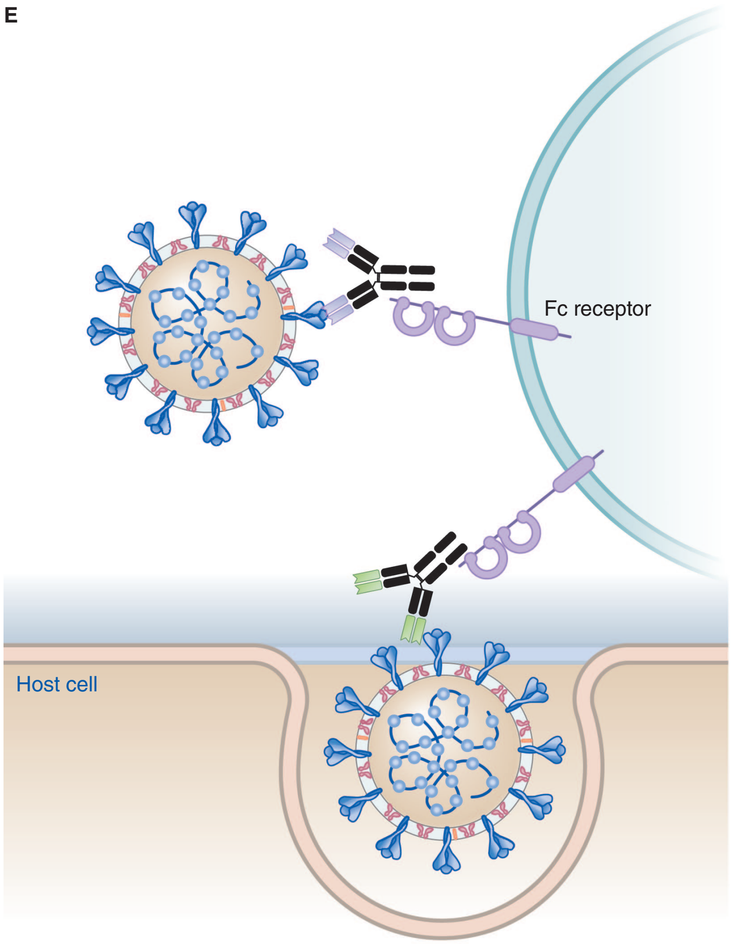


Figure 40–1 (*Continued*)

Adenovirus Vaccines

The Oxford/AstraZeneca vaccine (ChAdOx1/AZD1222) contains a safe adenovirus vector containing dsDNA encoding SARS-CoV-2 spike protein. Once injected, the adenovirus delivers dsDNA to antigen-presenting cells that then express spike proteins, resulting in an immune response. The vaccine, injected in two doses 12 weeks apart, is safe and has an 82% efficacy in preventing severe or fatal COVID-19. The vaccine can be stored for 6 months at +2 to +8°C. It has been authorized for use in Europe and other countries.

The Johnson & Johnson vaccine (JNJ-78436735/Ad26.COV2.S) contains a safe adenovirus vector containing dsDNA encoding SARS-CoV-2 spike protein. The vaccine, injected as a single dose, is safe and has a 72% efficacy in preventing severe or fatal COVID-19. The vaccine can be stored for 3 months at +2 to +8°C and for 2 years at −20°C. It has been authorized for use in the U.S., Europe, and other countries.

The Gamelaya vaccine (Sputnik V/Gam-Covid-Vac) contains a safe adenovirus vector containing dsDNA encoding SARS-CoV-2 spike protein. The vaccine, injected in two doses 21 days apart, is safe and has a 91% efficacy in preventing severe or fatal COVID-19. The vaccine can be stored for 6 months at +2 to +8°C and for 2 years at −20°C. It has been authorized for use in Russia and other countries.

Nanoparticle Vaccines

The Novavax vaccine (NVX-CoV2373) contains nanoparticles coated with synthetic spike proteins. Once injected with adjuvant, the particles are taken up by APCs that present spike proteins, resulting in an immune response. The vaccine, injected in two doses 21 days apart, is safe and has a 95% efficacy in preventing severe or fatal COVID-19. This vaccine can be stored for 6 months at +2 to +8°C and for 2 years at −20°C. The Novavax vaccine has been approved by the EMA and Drugs Controller General of India and has been granted emergency use status by the WHO.

Inactivated Virus Vaccine

The Sinopharm vaccine (BBIBP-CorV) contains inactivated coronavirus. The coronavirus is inactivated with β-propiolactone, which prevents the virus from replicating but allows all the proteins to remain intact. Once injected, the inactivated viral particles are taken up by APCs that present coronavirus proteins, resulting in an immune response. The vaccine, injected in two doses 21 days apart, has a 79% efficacy in preventing severe or fatal COVID-19. It can be stored at +2 to +8°C. This vaccine has been used in China.

The SinoVac vaccine (CoronaVac) also contains coronavirus inactivated with β-propiolactone. The vaccine, injected in two doses 14 days apart, has a 50% efficacy in preventing severe or fatal COVID-19. The vaccine can be stored at +2 to +8°C. It has been used in China.

The Bharat Biotech vaccine (BBV152/Covaxin) also contains coronavirus inactivated with β-propiolactone. This vaccine, injected in two doses 2 days apart, has an 81% efficacy in preventing severe or fatal COVID-19; it can be stored at +2 to +8°C; it has been used in India and other countries.

The Future of Vaccine Technology

Vaccination technology and improved methods to generate vaccines have led to the prevention of many infectious diseases. People no longer die at the high rates that prevailed before vaccines were developed. In the developing world, however, according to WHO reports, over 40% of deaths are due to infectious diseases, highlighting a continued need to improve existing vaccines, develop new vaccines, and improve delivery methods to increase efficacy. Viruses, bacteria, parasites, and antigens on cancerous cells are all future vaccine targets. New vaccines for pregnant mothers will be available to prevent diseases that can become chronic if the fetus becomes infected *in utero*, as is the case with malaria. Furthermore, an increasing elderly population will need access to better vaccines that can stimulate their aging immune systems, which are susceptible to infections like influenza and varicella viruses. Delivery methods are being explored to utilize nanoparticles and alternative adjuvants to improve vaccine immunogenicity so people will only need one vaccine dose rather than several. Needleless delivery is already possible, as in the cases of oral polio vaccine and nasal sprays for influenza. Investigation continues for developing new edible vaccines using plants, microneedles, and needle-free dermal patches.

Most vaccines work through preventing disease due to acute infections; the challenge remains to develop vaccines against chronic viral infections where the host is immunosuppressed. These pathogens evade the immune system and persist in the host's own cells. To overcome these chronic pathogens, vaccines need to elicit both antibody and T-cell responses, where B cells can neutralize the pathogen and T cells can actively kill and destroy infected cells. Vaccines against HPV and hepatitis B viruses protect not only from viral infection but also from developing infection-associated cancers.

There is a need for new vaccines for other viral pathogens that can cause further complications. For example, infection with group A *Streptococcus* can lead to rheumatic fever, *Helicobacter pylori* may result in stomach cancer, and chlamydia infection can cause blindness and infertility. Vaccines provide effective prophylaxis; however, the frontier in vaccine technology will involve vaccines as therapies for already-established disease. Vaccines can be used against pathogens that become chronic, as in shingles, and in conditions of autoimmunity and cancer, where the immune response is dysregulated. In the case of cancer, vaccines can be used to augment immunity to tumors to prevent their growth and metastasis. In the case of autoimmunity, the goal of this "negative vaccination" is to use vaccines to dampen immune function to prevent self-tissue destruction (Nossal, 2011).

Vaccine Safety: Myths, Truths, and Consequences

Vaccine Adjuvants and Safety

Adjuvants are substances added to vaccines to enhance the magnitude, quality, and duration of the protective immune response. Adjuvants are useful in vaccines because they stimulate the innate immune system that subsequently activates a strong adaptive immune response to ensure immune protection. Because many modern vaccines do not contain live pathogens, they must include adjuvants to ensure vaccine efficacy. Adjuvants are particularly useful in subunit protein vaccines, which often are inadequately immunogenic without enhancement.

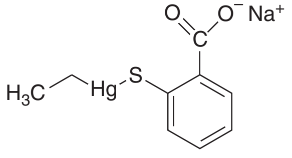
There is extensive experience in human vaccines with two adjuvants, aluminum and monophosphoryl lipid A. Aluminum, in the form of alum, has been used for nearly 90 years in vaccines; aluminum hydroxide [$Al(OH)_3$] and aluminum phosphate ($AlPO_4$) are currently used. Aluminum is used in many childhood vaccines in the U.S. targeted to diphtheria-tetanus-pertussis, Hib and pneumococcus, hepatitis A and B, and HPV. Monophosphoryl lipid A (isolated from bacteria) has been used in the HPV vaccine Cervarix since 2009. An influenza vaccine licensed for the 2016 to 2017 season included the adjuvant MF59, an oil-in-water emulsion of squalene oil. An influenza vaccine that is targeted to influenza H5N1 contains a new adjuvant termed AS03 (an "adjuvant system" containing α-tocopherol and squalene in an oil-in-water emulsion) and was licensed for inclusion in the U.S. pandemic influenza vaccine stockpile. Live attenuated virus vaccines do not contain adjuvants; thus, *adjuvant-free vaccines* include those directed against measles, mumps, rubella, chicken pox, rotavirus, polio, and live attenuated seasonal influenza virus.

Vaccines Do Not Cause Autism

Autism spectrum disorder (ASD) rates have increased in the U.S. and other parts of the world in parallel with expansion in the diagnostic criteria of autism that now include spectrum disorders with a broader array of symptoms (Hansen et al., 2015). The CDC found that 1 of 68 children in the U.S. has ASD. Patients with this disorder have developmental impairments that affect their communication, behavior, and social interactions. Even though some people have been concerned about a causal link between vaccines and autism, many large scientific studies have failed to detect any such link (Hviid et al., 2003; Madsen et al., 2002; Schechter and Grether, 2008; Taylor et al., 2014). The Institute of Medicine (IOM; now termed the National Academy of Medicine) conducted thorough reviews and concluded that current childhood and adult vaccines are very safe. In 2014, a CDC study added to reports around the world that vaccines do not cause ASD. They concluded that the total amount of antigen received from vaccines did not differ between children with ASD and those without the disorder. Vaccination with the MMR vaccine also is not associated with development of ASD in children.

Preservatives, Including Thimerosal

Preservatives added to vaccine preparations are designed to kill or inhibit the growth of bacteria and fungi that could contaminate a vaccine vial. There are historical reports of severe adverse events or death due to bacterial contamination of multidose vials lacking preservative. The highest risk of contamination is probably due to repetitive puncture of a multidose vaccine vial that is stored over time. Therefore, the U.S. *Code of Federal Regulations* requires the addition of a preservative to multidose vials of vaccines. Preservatives eliminate or reduce contamination in this setting. Several preservatives have been incorporated into licensed vaccines, including 2-phenoxyethanol, benzethonium chloride, phenol, and thimerosal.



thimerosal-Na$^+$

Thimerosal, known to many by the trade name Merthiolate, has been one of the most used preservatives; it is an organomercurial, an organic compound containing mercury. Thimerosal has been used safely since the early 20th century as a preservative in biologics, including many vaccines, and has a long history of use. Over time, concerns were raised about its safety because some organomercurials were increasingly associated with neurotoxicity, and children began receiving increasing numbers of licensed vaccines. The FDA chose to work with manufacturers toward reduction or elimination of thimerosal from childhood vaccines because of these *theoretical concerns*. As a result, *thiomersal has been eliminated or reduced to trace amounts in nearly all childhood vaccines except some IIVs.*

In terms of toxicity from mercury, most of the data in the field pertain to methylmercury, whereas thimerosal is a derivative of ethylmercury, which is cleared more rapidly. Thimerosal does not have significant toxic effects at the concentrations used in vaccine formulations. However, questions were raised about the potential association of thimerosal-containing vaccines in children and the occurrence of neurodevelopmental disorders, especially autism. A rather sordid history of fraud, conflict of interest, and other irregularities has been revealed pertaining to the now-debunked association studies of thimerosal and autism; decades of studies have been conducted in safety reviews around this matter.

The National Vaccine Advisory Committee, ACIP of the CDC, and the IOM's Immunization Safety Review Committee have all conducted extensive reviews of association studies, and the conclusion is that autism is not associated with the amount of thimerosal in childhood vaccines. In any event, recognizing public concern, between 2001 and 2003, thimerosal was eliminated from or reduced in childhood vaccines (except for influenza) for children under 6 years old in hopes of encouraging childhood vaccination. The CDC has compiled a thorough review and list of articles relating to this issue (CDC, 2015).

Adverse Events With Vaccines

For injectable vaccines, common adverse effects include minor *local reactions* to vaccines at the injection site (pain, swelling, and redness). More widespread effects, termed *systemic reactions*, may include fever, rash, irritability, drowsiness, and other symptoms, depending on the vaccine. The profile of reactions seen in large-scale trials is carefully documented in package inserts. During vaccine candidate testing, any occurrence of serious adverse events (SAEs) is examined carefully. SAEs are events following vaccination that involve hospitalization, life-threatening events, death, disability, permanent damage, congenital anomaly/birth defect, or other conditions requiring medical intervention. Vaccines with clear association with SAEs are typically not licensed. In some cases, to increase the likelihood of detecting rare SAEs, the FDA requires phase IV studies (postmarketing surveillance) to follow the performance of vaccines as use expands beyond the size of the trials leading to licensure. The government also collects data after licensure through the vaccine adverse event reporting system. Vaccines can be withdrawn from market if concerns arise. For example, licensure for use of the live oral rotavirus vaccine Rotashield, which was recommended for routine immunization of the U.S. infants in 1998, was withdrawn in 1999 when reports in the vaccine adverse event reporting system suggested an association between the vaccine and intussusception, a form of bowel obstruction.

Allergic Reactions

Allergy to components of vaccine formulations also can cause reactions. Trace amounts of antibiotics like *neomycin*, used to ensure sterility in some vaccines (e.g., MMR, trivalent IPV, and varicella vaccine), may cause adverse reactions. A history of anaphylactic reaction (but not local reaction) to *neomycin* is a contraindication to future immunization with those vaccines. Persons with a history of egg allergy should not be given an influenza vaccine prepared in eggs. Gelatin, which is used as a stabilizer in some virus vaccines like varicella and MMR vaccines, may cause allergic reaction in some.

Fainting

Fainting, or syncope, also has been reported in people after vaccination. Fainting is more common in adolescents than in children or adults and thus is more common after vaccination with HPV, meningococcal vaccine 4 (MCV4), and Tdap. Immediate fainting episodes following vaccination procedures are triggered by pain or anxiety, rather than the contents of the vaccines. While fainting is not serious, falling while fainting can cause injury, with head injuries the most serious. Clinicians can give patients drinks and snacks to prevent some fainting and can prevent falls by having patients lie down or sit during the procedure. Patients who faint after vaccination will recover after a few minutes, and clinicians should observe patients for at least 15 min after vaccination (a recommendation of the CDC).

Febrile Seizure

Fevers of 102°F (38.9°C) or higher can cause children to experience febrile seizures, which are characterized by body spasms and jerky movements that may last for up to 2 min. About 5% of children will experience a febrile seizure in their lifetime, with most occurring at 14 to 18 months of age. Children experiencing simple febrile seizures recover quickly without long-term harm. These common seizures also are caused by febrile illnesses associated with viral infections, especially roseola, ear infections, and other common childhood illnesses. Current vaccines sometimes induce fevers, usually low grade in nature, but rarely result in febrile seizures. Although fever following vaccination with most vaccines rarely causes febrile seizure, there is a small increase in risk after MMR and MMRV vaccines. The CDC also has reported a small increase in febrile seizures after a child receives the IIV together with PCV13 vaccine or in combination with diphtheria, tetanus, or DTaP vaccines. The increase of febrile seizures when combining these vaccines is small, and the CDC does not recommend delivering them on separate days. Importantly, vaccine usage can help prevent febrile seizures by providing vaccinated children protection against measles, mumps, rubella, chickenpox, influenza, and pneumococcal infectious pathogens that may result in febrile seizures.

Guillain-Barré Syndrome

Guillain-Barré syndrome (GBS) is a rare disease that affects the nervous system. Patients with GBS display muscle weakness and sometimes paralysis that results when their own immune system injures their neurons. GBS often occurs after an infection with bacteria or virus; most patients with GBS recover fully. However, some subjects can have permanent nerve damage. The incidence of GBS in the U.S. currently is about 3000 to 6000 cases per year; thus, it is rare in a population of about 350 million. GBS is more common in older adults, with people older than 50 years at greater risk. GBS may have several underlying causes, but scientists report that two-thirds of GBS cases occurred after patients were ill with gastroenteritis or respiratory tract infections. Infection with *Campylobacter jejuni* is the most common risk factor for the disease, but GBS also has been reported commonly after influenza virus, cytomegalovirus, or Epstein-Barr viral infection. GBS after vaccination is reported but rare.

An IOM study reported that widespread use of the 1976 swine influenza virus vaccine was associated with a small increase in risk for GBS, with an additional case of GBS per 100,000 people who were vaccinated, although later statistical review called this association into question. Current assessments are that there is no significant risk of GBS after obtaining a seasonal influenza vaccine, or if there is an association, the risk is approximately one case per million vaccinated individuals, a low rate that is difficult to detect with certainty. Studies have shown that a person is more likely to get GBS after influenza infection than after a vaccination. Importantly, severe morbidity and mortality are significant risks after influenza infection, and vaccination prevents complications and death.

Sudden Infant Death Syndrome

Sudden infant death syndrome (SIDS) peaks when babies are between 2 and 4 months old, and infants are also given many vaccines during this period. The temporal overlap of peak SIDS incidence and the period of initiation of childhood vaccination series led to questions about any causal relationship between vaccines and SIDS. Numerous studies have failed to detect a causative association for vaccines and SIDS (Silvers et al., 2001). The IOM 2003 report reviewed the relationship of SIDS and vaccines and concluded that vaccines do not cause SIDS. Infant death by SIDS has decreased dramatically due to the 1992 American Academy of Pediatrics recommendations to place infants on their backs to sleep and the 1994 National Institute of Child Health and Human Development campaign efforts.

Safety of Multiple Vaccinations

Children are exposed to many bacteria and viruses in their environment through food, teething of objects, and exposure to pets and to other humans. The typical viral infection results in exposure of the immune system to a dozen or more antigens; some bacteria express hundreds of antigens during infection. Each recommended childhood vaccine protects against 1 to 69 antigens. When a child is given the full recommended vaccines on the 2014 schedule, they are exposed to up to 315 antigens by age 2, which provides them critical protection against pathogens in the environment (CDC, 2016). Vaccinating patients against multiple antigens has been shown to be safe when they are delivered in combination at the same time. This strategy is advantageous for patients, especially children, because they lack immunity to most vaccine-preventable diseases, so receiving this protection during the relatively vulnerable period of early development is important. The patient also has fewer doctor visits with combination or multiple vaccinations, reducing cost in terms of money and time for parents and disruption for children. Numerous studies have shown that giving various vaccine combinations does not cause chronic disease. Furthermore, each time a combination vaccine or multiple vaccination schedule is licensed, that intervention already has been tested for safety and efficacy in combination with the vaccines previously recommended for that age group. The ACIP and the Academy of Pediatrics recommend receiving multiple vaccines at the same time (CDC, 2016).

Vaccine Myths and Their Public Health Consequences

The decreased rates of mortality and morbidity due to infectious diseases contracted in childhood and adulthood substantiate the success of vaccines. A dramatic example of success is the worldwide eradication of smallpox, a pathogen responsible for epidemics that killed 300 to 500 million people in the 20th century and disfigured many survivors. In the 20th century, poliovirus and MeV also incapacitated and killed infected individuals, especially young children. These scourges have been largely eliminated thanks to decades of successful public health vaccination strategies. Until the SARS-CoV-2 pandemic of 2020, younger generations had never seen the debilitating effects and mortality caused by such diseases. Infectious diseases, however, continue to affect the lives of many people in the developing world who have less access to healthcare or are affected by wars or famine. Recently, preventable diseases are arising again in the developed world because of vaccine myths that have reduced vaccination rates in these countries.

One of these myths concerns autism. A study that has been retracted and discredited claimed there was a link between vaccination in children and autism (Wakefield et al., 1998). Despite major shortcomings and incorrect interpretations, this study changed public perceptions regarding vaccine safety; the influence of this faulty article persists. Experimental studies in different parts of the world with large cohorts, statistical power, and rigor have found no evidence that vaccines cause autism (American Academy of Pediatrics, 2017; Madsen et al., 2002). Researchers have found that autism occurs in families, may have a genetic component, and may be affected by environmental triggers such as insecticides, certain drugs, and rubella virus. The exact causes of ASDs are unknown and continue to be investigated (Landrigan, 2010).

Nonetheless, the antivaccination movement has gained momentum, with celebrities, politicians, and social media continuing to propagate erroneous vaccine information and conspiracy theories. According to the CDC, vaccination rates have fallen in many parts of the U.S. In nine U.S. states, fewer than two-thirds of children ages 19 to 35 months have been vaccinated with the recommended seven-vaccination regimen. This dismissal of scientific evidence on vaccines can have deadly consequences. Infectious epidemics due to preventable agents like poliovirus and MeV can reemerge. Unvaccinated children will be more susceptible to infection, and many of them will not survive. Furthermore, unvaccinated subjects contribute to reducing the benefits of herd immunity that protects people who cannot be vaccinated for medical reasons, such as cancer, HIV infection, and other types of immunodeficiency.

Diseases due to pertussis, polio, measles, *H. influenzae*, and rubella virus once affected hundreds of thousands of people and killed thousands. Following the introduction of universal vaccinations, the rates of these diseases decreased to near-zero levels in the U.S. Some believe that because these diseases have been nearly eliminated in the U.S., vaccination is no longer needed. This thinking is incorrect. Vaccine-preventable diseases are communicable diseases, spreading from person to person, and the causative viruses and bacteria survive in nature. People, especially the unvaccinated, can be infected, and infected individuals will spread the disease to unvaccinated individuals. A greater fraction of vaccinated individuals in a population leads to fewer opportunities for the disease to spread (herd immunity).

Parental vaccine concerns should be taken seriously, and misconceptions should be thoroughly discussed by providers to ensure that patients have scientific information and are informed about the risks associated with failure to vaccinate. By providing parental education, pediatricians and other primary care medical providers can help reduce vaccine hesitancy.

In a similar vein, misinformation about the safety of SARS-CoV-2 vaccines has slowed vaccination efforts in the U.S. during the COVID-19 pandemic

Licensure and Monitoring of Vaccines

Immune Correlates and Mechanisms

During the process of vaccine development and testing, manufacturers seek to define laboratory tests and parameters that are associated with efficacy, which have been designated immune correlates of protection (CoPs). First, it is important theoretically to understand some features of the biological mechanism of protection to optimize development and use of vaccines. At a practical level, identification of a correlate allows monitoring of the reproducibility of vaccines during repetitive manufacture, monitoring the expected impact of new combinations of vaccine antigens on immunogenicity of existing vaccines, and other critical issues.

Plotkin and others have developed terminology for principal types of correlates (Plotkin and Gilbert, 2012). A *CoP* is a marker of immune function that statistically correlates with protection. Such markers can be simply associated with protection (termed nCoP) or alternatively may be known to directly measure the immune effectors that mediate protection (mCoP). From a practical standpoint, either an nCoP or an mCoP can enable monitoring and prediction of effective vaccination.

The ideal CoP is one that is quantitative and derives from a reproducible laboratory test that has been validated under good laboratory practice conditions. The type of protection suggested for a particular correlate may vary because vaccines may be designed to prevent differing classes of infection, such as local versus systemic infection or severe disease versus any disease. Examples of quantitative CoPs in use include a threshold of 10 mIU/mL in serum of hepatitis B antibodies detected in a standardized enzyme-linked immunosorbent assay, serum diphtheria toxin neutralization concentration of 0.01 to 0.1 IU/mL, a serum virus neutralization dilution titer of 1/5 for yellow fever virus, or a 1/40 dilution of serum in influenza hemagglutination inhibition titer.

Regulatory and Advisory Bodies

The Center for Biologics Evaluation and Research of the FDA regulates vaccine products in the U.S., with recommendations from its Vaccines and Related Biological Products Advisory Committee. The EMA regulates in Europe. Manufacturers conduct phase I studies (safety and immunogenicity studies) in a small number of closely monitored subjects; phase II studies (dose-ranging studies) typically in several hundred subjects; and then phase III trials (efficacy studies) typically in thousands of subjects. If the studies are successful, the sponsor submits a Biologics License Application to the FDA, which may lead to licensure. Licensure allows use, but decisions on whether vaccines are recommended for specific populations or for universal use are made by additional advisory bodies. The CDC hosts the ACIP, a committee of public health and medical experts that makes recommendations for use of vaccines in the U.S. Various professional medical societies also publish recommendations, for instance, the American Academy of Pediatrics publishes the *AAP Red Book*, or "Report of the Committee on Infectious Diseases of the American Academy of Pediatrics," which contains vaccine recommendations. Finally, third-party payers, such as insurance companies, affect usage through reimbursement policies; thus, issues of cost, benefit, and profitability become considerations, as examined in Chapter 1.

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Chapter 41

Lipid-Derived Autacoids: Eicosanoids and Platelet-Activating Factor

Emanuela Ricciotti, Tilo Grosser, and Garret A. FitzGerald

EICOSANOIDS

- Biosynthesis
- Inhibitors of Eicosanoid Biosynthesis
- Eicosanoid Degradation
- Pharmacological Properties
- Physiological Actions and Pharmacological Effects
- Therapeutic Uses

PLATELET-ACTIVATING FACTOR

- Chemistry and Biosynthesis
- Sites of PAF Synthesis
- Mechanism of Action of PAF
- Physiological and Pathological Functions of PAF
- PAF Receptor Antagonists

Membrane lipids supply the substrate for the synthesis of *eicosanoids* and *platelet-activating factor* (PAF). Arachidonic acid (AA) metabolites, including *PGs* (prostaglandins), *PGI_2* (prostacyclin), *TxA_2* (thromboxane A_2), *LTs* (leukotrienes), and *epoxygenase products* of cytochromes P450 (CYP)s, collectively the eicosanoids, are not stored but are produced by most cells when a variety of physical, chemical, and hormonal stimuli activate acyl hydrolases that make arachidonate available for further metabolism. *Membrane glycerophosphocholine* derivatives can be modified enzymatically to produce PAF. PAF is formed by a smaller number of cell types, principally leukocytes, platelets, and endothelial cells. Eicosanoids and PAF lipids function as signaling molecules in many biological processes, including the regulation of vascular tone, renal function, hemostasis, parturition, gastrointestinal (GI) mucosal integrity, and stem cell function. They are also important mediators of innate immunity and inflammation. Several classes of drugs, most notably NSAIDs (nonsteroidal anti-inflammatory drugs) (see Chapter 42), including *aspirin*, owe their principal therapeutic effects—relief of inflammatory pain and antipyresis—to blockade of PG formation.

Eicosanoids

Eicosanoids, from the Greek *eikosi* ("twenty"), are formed from precursor essential fatty acids that contain 20 carbons and 3, 4, or 5 double bonds: 8,11,14-eicosatrienoic acid (dihomo-γ-linolenic acid), 5,8,11,14-eicosatetraenoic acid (AA; Figure 41–1), and 5,8,11,14,17-eicosapentaenoic acid (EPA). AA is the most abundant precursor, derived from the dietary omega-6 fatty acid, linoleic acid (9,12-octadecadienoic acid), or ingested directly as a dietary constituent. EPA is a major constituent of oils from fatty fish such as salmon.

HISTORY

In 1930, American gynecologists Kurzrok and Lieb observed that strips of uterine myometrium relax or contract when exposed to semen. Subsequently, Goldblatt in England and von Euler in Sweden reported independently on smooth muscle contracting and vasodepressor activities in seminal fluid and accessory reproductive glands. In 1935, von Euler identified the active material as a lipid-soluble acid, which he named *prostaglandin*. Samuelsson, Bergström, and their colleagues elucidated the structures of PGE_1 and $\text{PGF}_{1\alpha}$ in 1962. In 1964, Bergström and coworkers and van Dorp and associates independently achieved biosynthesis of PGE_2 from AA. Discovery of TxA_2, PGI_2, and the LTs followed. Vane, Smith, and Willis in 1971 reported that *aspirin* and NSAIDs act by inhibiting PG biosynthesis. This remarkable period of discovery linked the Nobel Prize of von Euler in 1970 to that of Bergström, Samuelsson, and Vane in 1982.

Biosynthesis

Biosynthesis of eicosanoids is limited by the availability of AA and depends primarily on the release of esterified AA from membrane phospholipids or other complex lipids by acyl hydrolases, notably phospholipase A_2 (PLA_2). Once liberated, AA is metabolized rapidly to oxygenated products by cyclooxygenases (COXs), lipoxygenases (LOXs), and CYPs (see Figure 41–1).

Chemical and physical stimuli activate the Ca^{2+}-dependent translocation of group IV_A cytosolic phospholipase A_2 (cPLA_2) to the membrane, where it hydrolyzes the *sn*-2 ester bond of membrane phosphatidylcholine and phosphatidylethanolamine, releasing AA. Multiple additional PLA_2 isoforms (secretory [s] and Ca^{2+}-independent [i] forms) have been characterized. Under basal conditions, AA liberated by iPLA_2 (Ca^{2+}-independent PLA_2) is reincorporated into cell membranes by the action of arachidonoyl-CoA synthetase and lysophospholipid acyltransferases (Pérez-Chacón et al., 2009). During stimulation, cPLA_2 dominates the acute release of AA, while an inducible sPLA_2 contributes to AA release under conditions of sustained or intense stimulation. sPLA_2 contributes to platelet microparticle generation of eicosanoids that then direct microparticle internalization by neutrophils driving inflammation (Duchez et al., 2015).

Products of Cyclooxygenases (Prostaglandin G/H Synthases)

Prostaglandin endoperoxide G/H synthase is called *cyclooxygenase* or *COX* colloquially. Products of this pathway are PGs, PGI_2, and TxA_2, collectively termed *prostanoids*. The pathway is described by Figure 41–1 and its legend.

Prostanoids are distinguished by substitutions on their cyclopentane rings and the number of double bonds in their side chains, as indicated by numerical subscripts (dihomo-γ-linolenic acid is the precursor of *series*$_1$, AA for *series*$_2$, and EPA for *series*$_3$). Prostanoids derived from AA carry the subscript 2 and are the major series in mammals.

There are two distinct functional COX isoforms in humans, COX-1 and COX-2 (Rouzer and Marnett, 2009; Smith et al., 2011). COX-1, expressed constitutively in most cells, is the dominant source of prostanoids for housekeeping functions, such as cytoprotection of the gastric epithelium (see Chapter 53). COX-2, in contrast, is upregulated by cytokines, shear stress, and growth factors and is the principal source of prostanoid formation in inflammation and cancer. However, this distinction is

Abbreviations

15-PGDH: 15-hydroxyprostaglandin dehydrogenase
AA: arachidonic acid
ACTH: corticotropin (formerly adrenocorticotrophic hormone)
AERD: *aspirin*-exacerbated respiratory disease
AGEPC: acetyl glyceryl ether phosphorylcholine
APRL: antihypertensive polar renal lipid
BLT: leukotriene B$_4$ receptor
COX: cyclooxygenase
CYP: cytochrome P450
CysLT: cysteinyl leukotriene
CysLTr: cysteinyl leukotriene receptor
DPr: prostaglandin D$_2$ receptor
EDHF: endothelium-derived hyperpolarizing factor
EET: epoxyeicosatrienoic acid
EPA: 5,8,11,14,17-eicosapentaenoic acid
EPr: prostaglandin E$_2$ receptor
FLAP: 5-lipoxygenase-activating protein
fMLP: formyl-methionyl-leucyl-phenylalanine
FPr: prostaglandin F$_{2\alpha}$ receptor
GI: gastrointestinal
GPCR: G protein-coupled receptor
HETE: hydroxyeicosatetraenoic acid
H-PGDS: hematopoietic prostaglandin D synthase
HPETE: hydroperoxyeicosatetraenoic acid
IL: interleukin
IPr: prostacyclin receptor
LOX: lipoxygenase
LT: leukotriene
LX_: lipoxin, e.g., LXA, LXB
mPGES-1: microsomal prostaglandin E synthase-1
NO: nitric oxide
NSAID: nonsteroidal anti-inflammatory drug
PAF: platelet-activating factor
PAF-AH: platelet-activating factor acetylhydrolase
PAH: pulmonary arterial hypertension
PD-1: programmed cell death 1
PD-L1: programmed cell death 1 ligand
PG: prostaglandin
PGI$_2$: prostaglandin I$_2$ (prostacyclin)
PGT: prostaglandin transporter
PL: phospholipase, e.g., PLA, PLC
PMN: polymorphonuclear leukocyte
POX: peroxidase
oxPL: oxidized phospholipid
SARS-CoV-2: severe acute respiratory syndrome coronavirus 2
TNF: tumor necrosis factor
TPr: thromboxane A$_2$ receptor
TxA$_2$: thromboxane A$_2$

not absolute; both enzymes may contribute to the generation of autoregulatory and homeostatic prostanoids during physiological and pathophysiological processes.

With 61% amino acid identity, COX-1 and COX-2 have remarkably similar crystal structures. Both COXs are glycosylated on asparagine in all organisms and the *N*-glycosylation appears to be necessary for proper protein folding. Both isoforms are expressed as dimers, homotypically inserted into the endoplasmic reticular membrane. Each monomer exhibits half of the site's activity and binds a distinct ligand that may influence the catalytic activity of the other monomer (Smith and Malkowski, 2019). COX-2 is also found in the Golgi apparatus and associated with lipid droplets (Smith and Malkowski, 2019). Although the enzymatic proprieties of COX-1 and COX-2 are very similar, COX-2 is more catalytically effective in the presence of lower AA concentrations, because COX-1 exhibits negative allosterism in this setting, and peroxide concentrations. Through sequential COX and POX (peroxidase) activity, both COXs convert AA to two unstable intermediates that are then converted to the prostanoids by synthases, each expressed in a relatively cell-specific fashion. For example, COX-1–derived TxA$_2$ is the dominant product in platelets, whereas COX-2–derived PGE$_2$ and TxA$_2$ dominate in activated macrophages. Prostanoids are released from cells by diffusion, although transport may be facilitated through the multidrug resistance-associated protein transporter (Schuster, 2002). The prostaglandin transporter (PGT) transports extracellular PGs into the cytoplasm for degradation (Lu et al., 1996). Despite the structural similarities between the two COX isozymes, COX-2 has a broader substrate preference and is able also to oxygenate neutral derivatives of AA and other fatty acids (Smith and Malkowski, 2019). Moreover, there are variable levels of functional complementarity between COX-1 and COX-2 in cells and tissues. In the kidney, COX-1 cannot compensate for loss of COX-2 in renal development, while COX-2 can compensate for the loss of COX-1, independently from its prostaglandin-synthetic capacity under basal conditions (Li et al., 2018a). Similarly, in the female reproductive system, COX-1 function may be replaced by COX-2, but not vice versa (Li et al., 2018b).

Lipoxygenase Products

Major products of the LOX pathways are hydroxy fatty acid derivatives known as HETEs (hydroxyeicosatetraenoic acids), LTs, and LXs (lipoxins) (Figure 41–2) (Haeggström and Funk, 2011; Powell and Rokach, 2015). LTs play a major role in the development and persistence of the inflammatory response.

The LOXs are a family of enzymes containing nonheme iron; LOXs catalyze the oxygenation of polyenic fatty acids to corresponding lipid hydroperoxides. The enzymes require a fatty acid substrate with two cis double bonds separated by a methylene group. AA, which contains several double bonds in this configuration, is metabolized to hydroperoxyeicosatetraenoic acids (HPETEs), which vary in the site of insertion of the hydroperoxy group. HPETEs are converted to their corresponding HETEs either nonenzymatically or by a POX.

There are five active human LOXs—5(*S*)-LOX, 12(*S*)-LOX, 12(*R*)-LOX, 15(*S*)-LOX-1, and 15(*S*)-LOX-2—classified according to the site of hydroperoxy group insertion. Their expression is frequently cell specific; platelets have only 12(*S*)-LOX, whereas leukocytes contain both 5(*S*)- and 12(*S*)-LOX (see Figure 41–2). 12(*R*)-LOX is restricted in expression mostly to the skin. The epidermal LOXs, which constitute a distinct LOX subgroup, also include 15-LOX-2 and eLOX-3, the most recently identified family member. eLOX-3 has been reported to metabolize further 12(*R*)-HETE, the product of 12(*R*)-LOX, to a specific epoxyalcohol product.

The 5-LOX pathway leads to the synthesis of the LTs. When eosinophils, mast cells, polymorphonuclear leukocytes (PMNs), or monocytes are activated, 5-LOX translocates to the nuclear membrane and associates with 5-LOX–activating protein (FLAP), an integral membrane protein that facilitates AA to 5-LOX interaction (Evans et al., 2008). Drugs that inhibit FLAP block LT production. A two-step reaction is catalyzed by 5-LOX: oxygenation of AA to form 5-HPETE followed by dehydration to an unstable epoxide, LTA$_4$. LTA$_4$ is transformed by distinct enzymes to LTB$_4$ or LTC$_4$. Extracellular metabolism of the peptide moiety of LTC$_4$ generates LTD$_4$ and LTE$_4$ (Peters-Golden and Henderson, 2007). Collectively, LTC$_4$, LTD$_4$, and LTE$_4$ are cysteinyl leukotrienes (CysLT). LTB$_4$ and LTC$_4$ are actively transported out of the cell. LTA$_4$, the primary product of the 5-LOX pathway, is metabolized by 12-LOX to form LXA$_4$ and LXB$_4$. These mediators also can arise through 5-LOX metabolism of 15-HETE.

Products of CYPs

The CYP epoxygenases, primarily CYP2C and CYP2J, metabolize AA to EETs (epoxyeicosatrienoic acids) (Fleming, 2014). Initially described in endothelial cells as endothelial-derived hyperpolarizing factors (EDHFs) that promote vasodilation, particularly in the coronary circulation, EETs

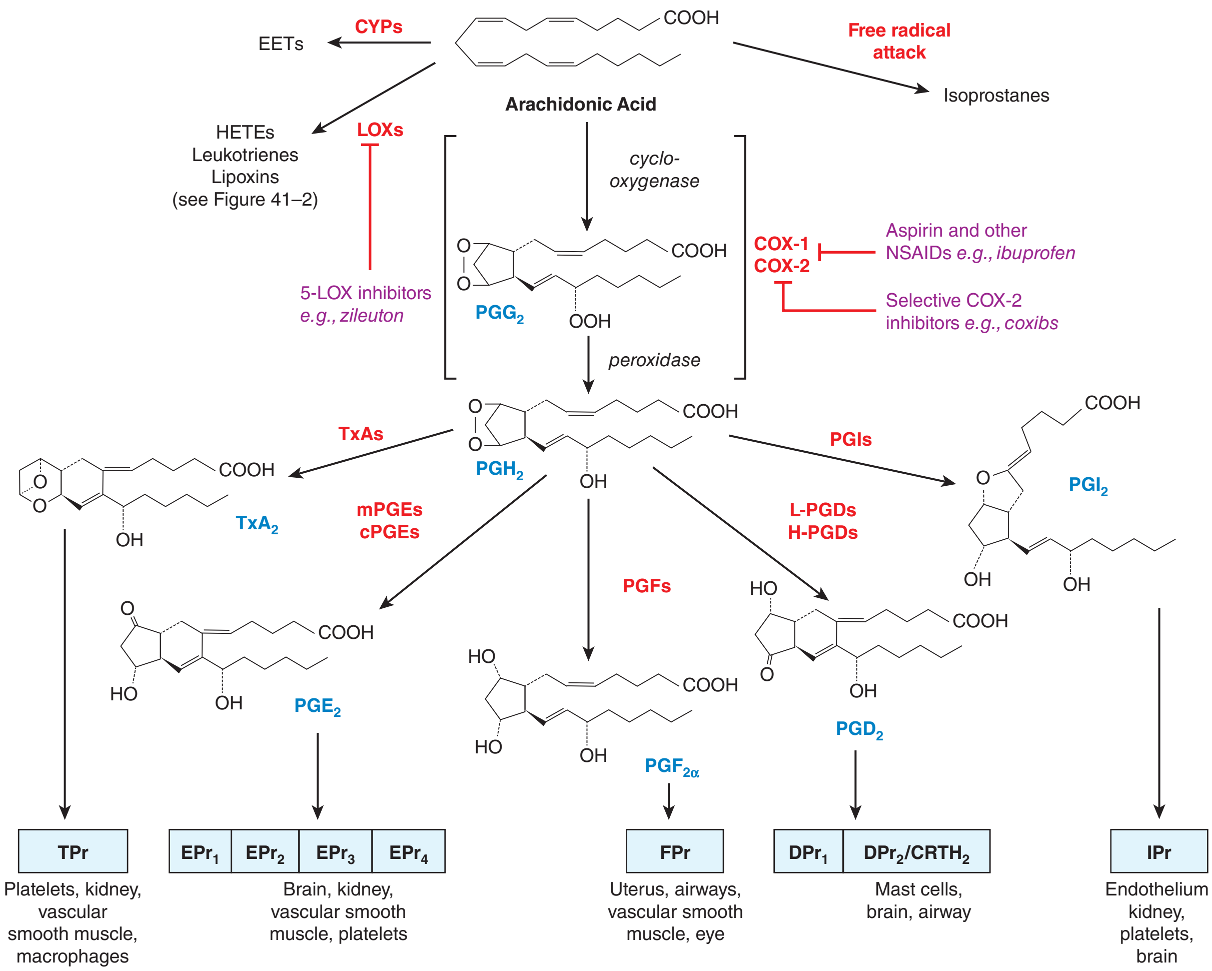

EETs
CYPs
COOH
Arachidonic Acid
Free radical attack
Isoprostanes
HETEs
Leukotrienes
Lipoxins
(see Figure 41–2)
LOXs
5-LOX inhibitors
e.g., zileuton
cyclo-oxygenase
COOH
PGG2
OOH
COX-1
COX-2
Aspirin and other NSAIDs e.g., ibuprofen
Selective COX-2 inhibitors e.g., coxibs
peroxidase
COOH
PGH2
OH
TxAs
COOH
TxA2
OH
mPGEs
cPGEs
PGIs
COOH
PGI2
OH
OH
L-PGDs
H-PGDs
PGFs
COOH
HO
OH
PGE2
HO
COOH
HO
OH
PGF2α
HO
COOH
OH
PGD2
TPr
EPr1
EPr2
EPr3
EPr4
FPr
DPr1
DPr2/CRTH2
IPr
Platelets, kidney, vascular smooth muscle, macrophages
Brain, kidney, vascular smooth muscle, platelets
Uterus, airways, vascular smooth muscle, eye
Mast cells, brain, airway
Endothelium kidney, platelets, brain

Figure 41–1 *Metabolism of AA.* Cyclic endoperoxides (PGG_2 and PGH_2) arise from the sequential COX and hydroperoxidase actions of COX-1 or COX-2 on AA released from membrane phospholipids. Subsequent products are generated by tissue-specific synthases and transduce their effects via membrane-bound receptors (blue boxes). EETs and isoprostanes are generated via CYP activity and nonenzymatic free radical attack, respectively. *Aspirin* and nonselective NSAIDs are nonselective inhibitors of COX-1 and COX-2 but do not affect LOX activity. See the text and the Abbreviations list for further definitions.

can be produced by any cell that expresses CYP epoxygenase, including astrocytes, and may play a protective role in cerebral ischemia (Wan et al., 2020; Zhang et al., 2017). EET biosynthesis can be altered by pharmacological, nutritional, and genetic factors that affect CYP expression.

Other Pathways

The isoeicosanoids, a family of eicosanoid isomers, are generated by nonenzymatic free radical catalyzed oxidation of AA. Unlike PGs, these compounds are initially formed esterified in phospholipids and released by PLs; the isoeicosanoids then circulate and are metabolized and excreted into urine. Their production is not inhibited *in vivo* by inhibitors of COX-1 or COX-2, but their formation is suppressed by antioxidants. Isoprostanes correlate with cardiovascular risk factors, and increased levels are found in many clinical conditions (Milne et al., 2015). Their relevance as biologically active mediators remains speculative. A series of compounds, *LXs, maresins,* and *resolvins*, when synthesized and administered to certain models of inflammation, hasten its resolution. It remains to be established whether the endogenous compounds are formed in quantities sufficient to exert this effect *in vivo* (Skarke et al., 2015).

Since COX, LOX, and CYP products are all formed from AA, the biosynthesis of these metabolites should be considered as part of the same metabolic network and should be assessed using broad-spectrum eicosanoid assays to uncover the interconnectivity between the different pathways and capture potential substrate rediversion in response to perturbations (Mazaleuskaya et al., 2016).

Inhibitors of Eicosanoid Biosynthesis

Inhibition of PLA_2 decreases the release of the precursor fatty acid and the synthesis of all its metabolites. PLA_2 may be inhibited by drugs that reduce the availability of Ca^{2+}. *Glucocorticoids* inhibit PLA_2 indirectly by inducing the synthesis of a group of proteins termed *annexins* that modulate PLA_2 activity. Glucocorticoids also downregulate induced expression of COX-2 but not of COX-1 (see Chapter 50). *Aspirin* and NSAIDs inhibit the COX, but not the POX, moiety of both COX enzymes and thus the formation of downstream prostanoids. These drugs do not inhibit LOXs and may cause increased formation of LTs by shunting of substrate to the LOX pathway. LTs may contribute to the adverse GI effects associated with NSAIDs (Janusz et al., 1998; Xu et al., 2009). Dual inhibitors of COX and 5-LOX have proven effective in some models of inflammation and tissue injury and may lessen the degree of adverse GI effects compared to traditional COX inhibitors (Minutoli et al., 2015; Oak et al., 2014; Shaaban et al., 2020).

Differences in the sensitivity of COX-1 and COX-2 to inhibition by anti-inflammatory drugs led to the development of selective inhibitors of COX-2, including the coxibs (Grosser et al., 2010) (see Chapter 42). These drugs were hypothesized to offer therapeutic advantages over older NSAIDs (many of which are nonselective COX inhibitors) because COX-2 was thought to be the predominant source of PGs in inflammation, whereas COX-1 is the major source of cytoprotective PGs in the GI tract and platelet TxA_2. Randomized trials of selective COX-2

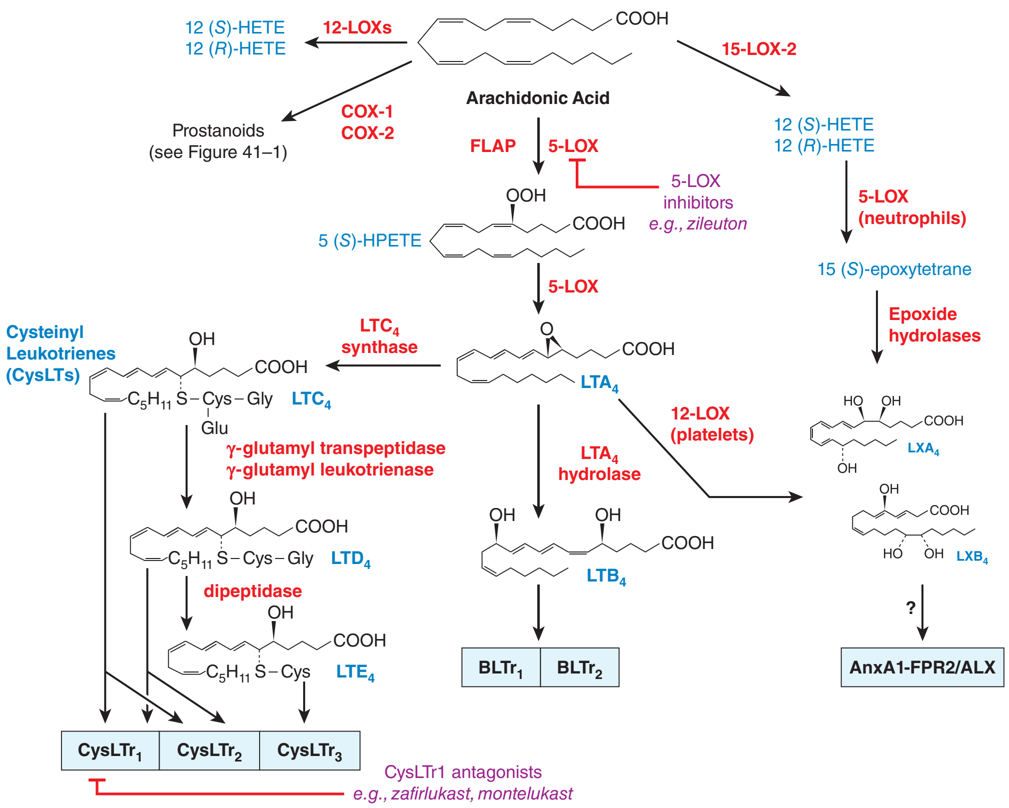

12 (S)-HETE
12 (R)-HETE
12-LOXs
COOH
15-LOX-2
Arachidonic Acid
COX-1
COX-2
Prostanoids
(see Figure 41–1)
FLAP
5-LOX
5-LOX inhibitors
e.g., zileuton
12 (S)-HETE
12 (R)-HETE
OOH
COOH
5 (S)-HPETE
5-LOX
(neutrophils)
15 (S)-epoxytetrane
5-LOX
Epoxide hydrolases
Cysteinyl Leukotrienes (CysLTs)
OH
COOH
LTC₄ synthase
O
COOH
LTA₄
C₅H₁₁ S – Cys – Gly LTC₄
Glu
12-LOX (platelets)
HO OH
COOH
LXA₄
OH
γ-glutamyl transpeptidase
γ-glutamyl leukotrienase
LTA₄ hydrolase
OH
COOH
OH
COOH
OH
OH
COOH
C₅H₁₁ S – Cys – Gly LTD₄
HO OH LXB₄
LTB₄
dipeptidase
OH
COOH
?
C₅H₁₁ S – Cys LTE₄
BLTr₁
BLTr₂
AnxA1-FPR2/ALX
CysLTr₁
CysLTr₂
CysLTr₃
CysLTr1 antagonists
e.g., zafirlukast, montelukast

Figure 41–2 *Lipoxygenase pathways of AA metabolism.* FLAP presents AA to 5-LOX, leading to the generation of the LTs. LXs are products of cellular interaction via a 5-LOX–12-LOX pathway or via a 15-LOX–5-LOX pathway. Biological effects are transduced via membrane-bound receptors (blue boxes). The biological relevance of LXs in vivo remains controversial (Schebb NH et al., 2022). LXA_4 can activate a GPCR also activated by annexin A1 and by the formyl peptide. This GPCR is termed the AnxA1-formyl peptide receptor 2/ALX receptor (AnxA1-FPR2/ALX) to reflect the range of its putative ligands. The existence of endogenous ligands for AnxA1-FPR2/ALX is disputable (Merlin J et al., 2022). LXB2 receptor has yet to be identified. *Zileuton* inhibits 5-LOX but not the COX pathways (expanded in Figure 41–1). CysLT antagonists prevent activation of $CysLTr_1$. See the text and the Abbreviations list for further definitions.

inhibitors reported their superiority in GI safety over nonselective NSAID comparators.

However, there now is compelling evidence that COX-2 inhibitors confer a spectrum of cardiovascular hazards (myocardial infarction, stroke, systemic and pulmonary hypertension, congestive heart failure, and sudden cardiac death) (Grosser et al., 2010). The hazards can be explained sufficiently by suppression of cardioprotective COX-2–derived PGs, especially PGI_2, and the unrestrained effects of endogenous stimuli, such as platelet COX-1–derived TxA_2, on platelet activation, vascular proliferation and remodeling, hypertension, and atherogenesis.

Because LTs mediate inflammation, efforts have focused on development of LT receptor antagonists and selective inhibitors of the LOXs. *Zileuton*, an inhibitor of 5-LOX, and selective $CysLTr_1$ antagonists (*zafirlukast, pranlukast*, and *montelukast*) have established efficacy in the treatment of mild-to-moderate asthma (see Chapter 44). However, these treatments remain less effective than inhaled corticosteroids. A common polymorphism in the gene for LTC_4 synthase that correlates with increased LTC_4 generation may be associated with higher asthma risk in some populations and with the efficacy of anti-LT therapy. Small studies have reported that polymorphisms in the genes encoding 5-LOX or FLAP may be linked to asthma and lung function (Bai et al., 2012; Holloway et al., 2008), in addition to myocardial infarction, stroke, and atherosclerosis (Peters-Golden and Henderson, 2007); thus, inhibition of LT biosynthesis may eventually prove to be useful in the prevention of cardiovascular disease.

Eicosanoid Degradation

Most eicosanoids are efficiently and rapidly inactivated (Figure 41–3). The enzymatic catabolic reactions are of two types:

- A rapid initial step, catalyzed by widely distributed PG-specific enzymes, wherein PGs lose most of their biological activity
- A second step in which these metabolites are oxidized, probably by enzymes identical to those responsible for the β and ω oxidation of fatty acids

The lung, kidney, and liver play prominent roles in the enzymatically catalyzed reactions. Metabolic clearance requires an energy-dependent cellular uptake by the PG transporter and possibly other transporters (Schuster, 2002). The initial step is the oxidation of the 15-OH group to the corresponding ketone by 15-hydroxyprostaglandin dehydrogenase (15-PGDH). PGI_2 and TxA_2, however, undergo spontaneous hydrolysis as a first degradative step. LTC_4 degradation occurs in the lungs, kidney, and liver but may also occur in myeloid tisuue via CYP4F enzymes. Inactivation of 15-PGDH, which elevates the capacity of tissues to form PGE_2, enhances tissue regeneration after hematopoietic stem cell transplantation and after hemihepatectomy (Zhang et al., 2015). Its enhanced expression with advancing age has been speculated to contribute to sarcopenia.

Pharmacological Properties

The eicosanoids function through activation of specific G protein-coupled receptors (GPCRs) (Table 41–1) that couple to intracellular second-messenger systems to modulate cellular activity (Figure 41–4).

Prostaglandin Receptors

The PGs activate membrane receptors locally near their sites of formation. Eicosanoid receptors interact with G_s, G_i, and G_q to modulate the activities of adenylyl cyclase and PLC (see Chapter 3). Single-gene products have been identified for the receptors for PGI_2 (the IPr), $PGF_{2\alpha}$ (the FPr), and TxA_2 (the TPr). Four distinct PGE_2 receptors (EPr_{1-4}) and two PGD_2 receptors (DPr_1 and DPr_2—also known as $CRTH_2$) have been cloned. Additional isoforms of the TPr (α and β), FPr (A and B), and EPr_3 (I–VI, e, f) receptors can arise through differential messenger RNA splicing (Smyth et al., 2009; Woodward et al., 2011). The prostanoid receptors

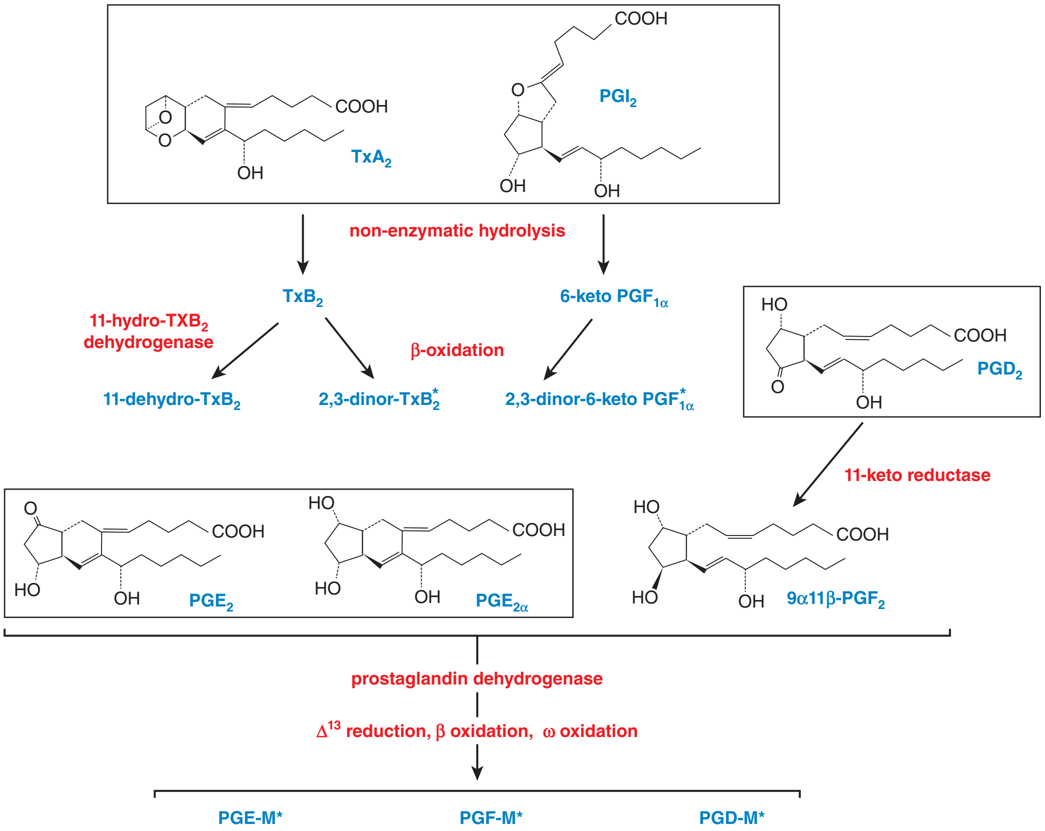


Figure 41–3 *Major pathways of prostanoid degradation.* Active metabolites are boxed. *Major urinary metabolites (M). See the text and the Abbreviations list for further definitions.

appear to derive from an ancestral EPr and share high homology. Phylogenetic comparison of this receptor family reveals three subclusters (see Figure 41–4):

- The relaxant receptors EPr_2, EPr_4, IPr, and DPr_1, which increase cellular cyclic AMP generation
- The contractile receptors EPr_1, FPr, and TPr, which increase cytosolic levels of Ca^{2+}
- EPr_3, which can couple to both elevation of cytosolic [Ca^{2+}] and inhibition of adenylyl cyclase

The DPr_2 receptor is an exception and is unrelated to the other prostanoid receptors; rather, it is a member of the fMLP (formyl-methionyl-leucyl-phenylalanine) receptor superfamily.

The structures of the active and inactive states of various human PG receptors, including DPr_2, EPr_3, EPr_4, and TPr, were recently resolved.

Leukotriene Receptors

Two receptors exist for LTB_4 (BLT_1 and BLT_2) and three for CysLTs ($CysLTr_1$ $CysLTr_2$ and $CysLTr_3$; Bäck et al., 2011, 2014; Kanaoka et al., 2013). The fMLP-2 receptor also binds LXA_4, but the functional importance of this ligand *in vivo* remains controversial. All are GPCRs and couple with G_q and other G proteins, depending on the cellular context. BLT_1 is expressed predominantly in leukocytes, thymus, and spleen, whereas BLT_2, the low-affinity receptor for LTB_4, is found in spleen, leukocytes, ovary, liver, and intestine.

$CysLTr_1$ binds LTD_4 with higher affinity than LTC_4, while $CysLTr_2$ shows equal affinity for both LTs. Both receptors bind LTE_4 with low affinity. $CysLTr_3$ (also known as OXGR1 or GPR99) appears to have high affinity only for LTE_4 (Kanaoka et al., 2013). Activation of G_q, leading to mobilization of intracellular Ca^{2+}, is the primary signaling pathway reported. Studies also have placed G_i downstream of $CysLTr_2$. $CysLTr_1$ is expressed in lung and intestinal smooth muscle, spleen, and peripheral blood leukocytes, whereas $CysLTr_2$ is found in heart, spleen, peripheral blood leukocytes, adrenal medulla, and brain. $CysLTr_3$ is expressed in kidney, trachea, lung, salivary glands, airway epithelial cells, and mast cells.

The crystal structures of $CysLTr_1$, $CysLTr_2$, and BLT_1 were recently resolved, advances that could facilitate development of therapeutic agents acting at these receptors.

Other Agents

Other AA-derived products (e.g., isoprostanes, EETs) have potent biological activities, and there is evidence for distinct receptors for some of these substances. An orphan receptor, GPR31, has been identified as a receptor for 12(S)-HETE (Powell and Rokach, 2015). Specific receptors for the HETEs and EETs have been proposed, and evidence that the orphan receptor GPR75 functions as a receptor for 20-HETE has been provided (Garcia et al., 2017).

Physiological Actions and Pharmacological Effects

The widespread biosynthesis and myriad pharmacological actions of eicosanoids are reflected in their complex physiology and pathophysiology. Knowledge of the distribution of the major eicosanoid receptors helps to put the complexity into perspective (see Figure 41–1). The development of mice with targeted disruptions of genes regulating eicosanoid biosynthesis and eicosanoid receptors has revealed unexpected roles for these autacoids and has clarified hypotheses about their functions (see Table 41–1).

Cardiovascular System

Because of their short $t_{1/2}$—seconds to minutes at physiological pH—the activities on vascular tone of endogenous prostanoids occur primarily locally at their sites of biosynthesis. Systemic effects of prostanoids on blood pressure involve renal and hormonal processes. PGI_2, the major arachidonate metabolite released from the vascular endothelium, is derived primarily from COX-2 in humans and is considered to be

TABLE 41–1 ■ HUMAN EICOSANOID RECEPTORS

RECEPTOR	LIGANDS 1° (2°)	PRIMARY COUPLING	MAJOR PHENOTYPE IN KNOCKOUT MICE
DPr_1	PGD_2	G_s	↓ Allergic asthma
DPr_2/$CHRT_2$	PGD_2 (15d-PGJ_2)	G_i	↑ or ↓ Allergic airway inflammation
EPr_1	PGE_2 (PGI_2)	G_q	↓ Response of colon to carcinogens
EPr_2	PGE_2	G_s	Impaired ovulation and fertilization Salt-sensitive hypertension
EPr_3 I–VI, e, f	PGE_2	G_i; G_s; G_q	Resistance to pyrogens ↓ Acute cutaneous inflammation
EPr_4	PGE_2	G_s	Patent ductus arteriosus ↓ Bone mass/density in aged mice ↑ Bowel inflammatory response ↓ Colon carcinogenesis
$FPr_{A,B}$	$PGF_{2\alpha}$ (IsoPs)	G_q	Failure of parturition
IPr	PGI_2 (PGE_2)	G_s	↑ Thrombotic response ↓ Response to vascular injury ↑ Atherosclerosis ↑ Cardiac fibrosis Salt-sensitive hypertension ↓ Joint inflammation
$TPr_{\alpha,\beta}$	TxA_2 (IsoPs)	G_q; G_i; $G_{12/13}$; G_{16}	↑ Bleeding time ↓ Response to vascular injury ↓ Atherosclerosis ↑ Survival after cardiac allograft
$BLTr_1$	LTB_4	G_{16}; G_i	Some suppression of inflammatory response
$BLTr_2$	LTB_4 [12(*S*)-HETE, 12(*R*)-HETE]	G_q-like; G_i-like; G_z-like	? (Reports of altered inflammatory processes)
$CysLTr_1$	LTD_4 (LTC_4/LTE_4)	G_q	↓ Innate and adaptive immune vascular permeability response ↑ Pulmonary inflammatory and fibrotic response
$CysLTr_2$	LTC_4/LTD_4 (LTE_4)	G_q	↓ Pulmonary inflammatory and fibrotic response

This table lists the major classes of eicosanoid receptors and their signaling characteristics. Splice variants for EPr_3, TPr, and FPr are indicated.

"atheroprotective." PGI_2 generation and release is regulated by shear stress and by both vasoconstrictor and vasodilator autacoids. In most vascular beds, PGE_2, PGI_2, and PGD_2 elicit vasodilation, and systemic infusion of high concentrations results in a drop in blood pressure; as mentioned above, physiologically, these responses are quite local because endogenous prostanoids are paracrine mediators that do not circulate (Smyth et al., 2009). $PGF_{2\alpha}$ is a potent constrictor of both pulmonary arteries and veins. TxA_2 is a potent vasoconstrictor and a mitogen in smooth muscle cells.

PGE_2 can also cause vasoconstriction through activation of EP_1 and EP_3. Infusion of PGD_2 in humans results in flushing, nasal congestion, and hypotension. Local subcutaneous release of PGD_2 contributes to dilation of the vasculature in the skin, which causes facial flushing associated with niacin treatment in humans. Subsequent formation of F-ring metabolites from PGD_2 may result in hypertension. PGI_2 relaxes vascular smooth muscle, causing hypotension and reflex tachycardia on intravenous administration. PGI_2 limits pulmonary hypertension induced by hypoxia and systemic hypertension induced by AngII and lowers pulmonary resistance in patients with pulmonary hypertension.

Cyclooxygenase 2–derived PGE_2, acting via the EPr_4, maintains the ductus arteriosus patent until birth, when reduced PGE_2 levels (a consequence of increased PGE_2 metabolism) permit closure. The traditional NSAIDs induce closure of a patent ductus in neonates (see Chapter 42). Contrary to expectation, animals lacking the EPr_4 during development die with a patent ductus during the perinatal period (see Table 41–1) because the mechanism for control of the ductus *in utero*, and its remodeling at birth, is absent.

Infusion of PGs of the E and F series generally increases cardiac output. Weak, direct inotropic effects have been noted in various isolated preparations. In the intact animal, however, increased force of contraction and increased heart rate are, in large measure, a reflex consequence of a fall in total peripheral resistance. PGI_2 and PGE_2, acting on the IPr or the EPr_3, respectively, protect against oxidative injury in cardiac tissue.

Studies suggest a role for COX-2 in cardiac function. PGI_2 and PGE_2, acting on the IPr or the EP_3, respectively, protect against oxidative injury in cardiac tissue. IPr deletion augments myocardial ischemia/reperfusion injury, and both microsomal PGE synthase-1 (mPGES-1) deletion and cardiomyocyte-specific deletion of the EPr_4 exacerbate the decline in cardiac function after experimental myocardial infarction. COX-2–derived TxA_2 contributed to oxidant stress, isoprostane generation, and activation of the TPr, and also possibly the FPr, to increase cardiomyocyte apoptosis and fibrosis in a model of heart failure. Selective deletion of COX-2 in cardiomyocytes results in mild heart failure and a predisposition to arrhythmogenesis in mice (Wang et al., 2009).

LTC_4 and LTD_4 can constrict or relax isolated vascular smooth muscle preparations, depending on the concentrations used and the vascular bed in question (Bäck et al., 2011). Although LTC_4 and LTD_4 have little effect on most large arteries or veins, nanomolar concentrations of these

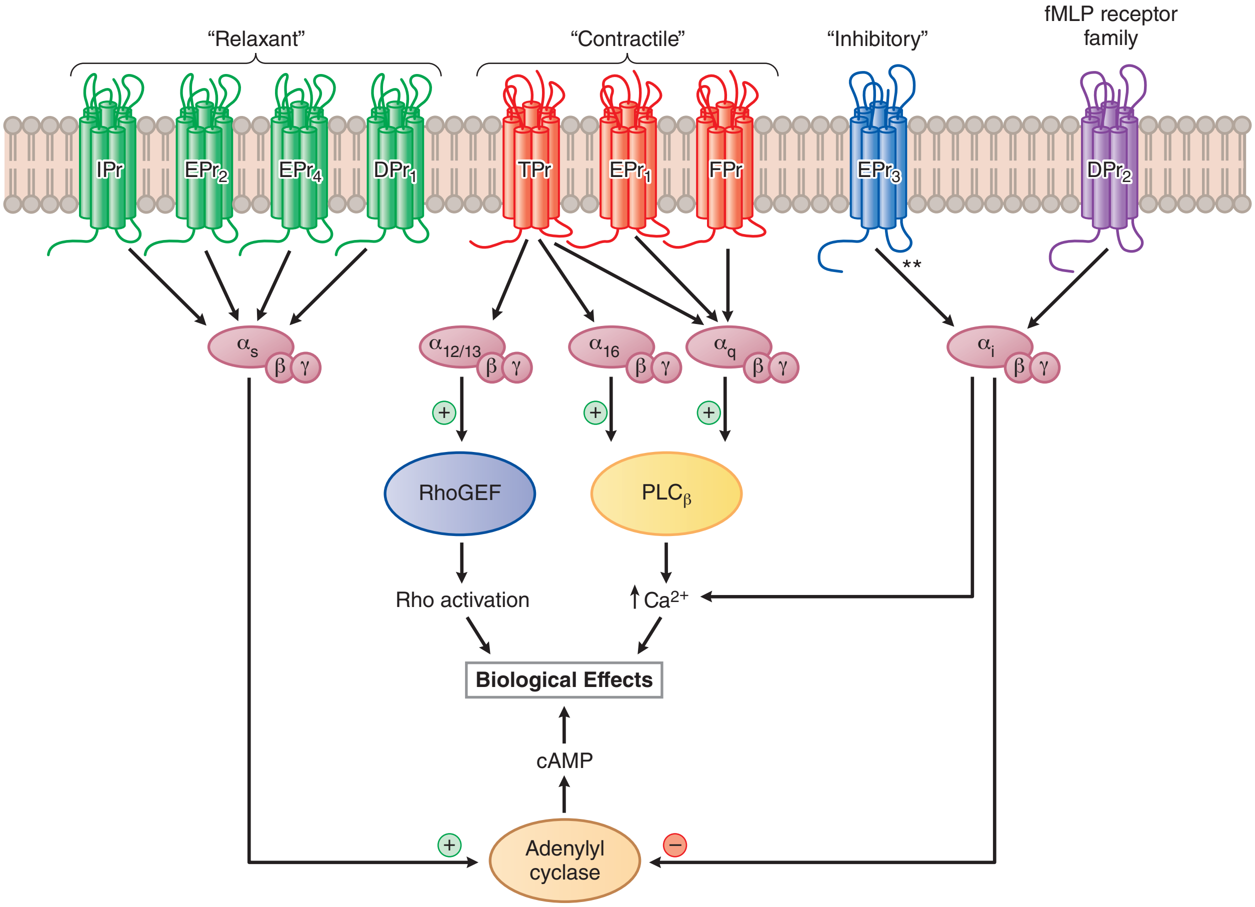


Figure 41–4 *Prostanoid receptors and their primary signaling pathways.* Prostanoid receptors are heptaspanning GPCRs. The terms *relaxant*, *contractile*, and *inhibitory* refer to the phylogenetic characterization of their primary effects. All EPr_3 isoforms couple through G_i; some can also activate G_s or $G_{12/13}$ pathways. RhoGEF, rho guanine nucleotide exchange factor.

agents contract coronary arteries and distal segments of the pulmonary artery. The renal vasculature is resistant to this constrictor action, but the mesenteric vasculature is not. LTC_4 and LTD_4 act in the microvasculature to increase permeability of postcapillary venules; they are about 1000-fold more potent than histamine in this regard. At higher concentrations, LTC_4 and LTD_4 can constrict arterioles and reduce exudation of plasma. There is evidence for a role of the LTs in cardiovascular disease (Peters-Golden and Henderson, 2007). Human genetic studies have indicated a link between cardiovascular disease and polymorphisms in the LT biosynthetic enzymes and FLAP.

The EETs cause vasodilation in several vascular beds by activating the large conductance Ca^{2+}-activated K^+ channels of smooth muscle cells, thereby hyperpolarizing the smooth muscle and causing relaxation. EETs also function as EDHFs, particularly in the coronary circulation. Endogenous biosynthesis of EETs is increased in human syndromes of hypertension.

Platelets

Platelet aggregation leads to activation of membrane phospholipases, with the release of AA and consequent eicosanoid biosynthesis. In human platelets, TxA_2 and 12-HETE are the two major eicosanoids formed, although eicosanoids from other sources (e.g., PGI_2 derived from vascular endothelium) also affect platelet function. Mature platelets express only COX-1. TxA_2, the major product of COX-1 in platelets, induces platelet aggregation and amplifies the signal for other more potent platelet agonists such as thrombin and ADP. The importance of the TxA_2 pathway is evident from the efficacy of platelet COX-1 inhibition with low-dose *aspirin* in the secondary prevention of myocardial infarction and ischemic stroke. The total biosynthesis of TxA_2, as determined by excretion of its urinary metabolites, is augmented in clinical syndromes of platelet activation, including unstable angina, myocardial infarction, and stroke. Deletion of the TPr in the mouse prolongs bleeding time, renders platelets unresponsive to TPr agonists, and blunts the response to vasopressors and the proliferative response to vascular injury (Smyth et al., 2009). TxA_2 induces platelet shape change, through G_{12}/G_{13}-mediated Rho/Rho kinase–dependent regulation of myosin light-chain phosphorylation, and aggregation through G_q-dependent activation of PKC. The actions of TxA_2 on platelets are restrained by its short $t_{1/2}$ (~30 sec), by rapid TPr desensitization, and by endogenous inhibitors of platelet function, including nitric oxide (NO) and PGI_2.

Low concentrations of PGE_2, via the EPr_3, enhance platelet aggregation. In contrast, higher concentrations of PGE_2, acting via the IPr or possibly EPr_2 or EPr_4, inhibit platelet aggregation. Both PGI_2 and PGD_2 inhibit the aggregation of platelets. PGI_2 limits platelet activation by TxA_2 and disaggregates preformed platelet clumps. The increased incidence of myocardial infarction and stroke in patients receiving selective inhibitors of COX-2, explained by inhibition of COX-2–dependent PGI_2 formation, supports this concept (Grosser et al., 2010).

Inflammation and Immunity

Eicosanoids play a major role in inflammatory and immune responses. LTs generally are proinflammatory and interact with PGs to promote and sustain inflammation (Ricciotti and FitzGerald, 2011), although there are some exceptions, such as the inhibitory actions of PGE_2 on mast cell activation. PGs and LXs and related compounds may also contribute to the resolution of inflammation (Buckley et al., 2014). COX-2 is the major source of prostanoids formed during and after an inflammatory response. Moreover, eicosanoids may modulate virus infection by regulating virus-host interactions and the host immune and inflammatory responses activated by viral pathogens. For example, eicosanoids may be involved in different stages of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and in the development of COVID-19. Eicosanoids may directly influence SARS-CoV-2 entry, replication, and clearance (Theken and FitzGerald, 2021). The nonstructural SARS-CoV-2 protein, Nsp7, may interact with microsomal prostaglandin E synthase-2 (Gordon et al., 2020). Infection caused by different viruses may impact the expression of genes for proteins in the arachidonic acid pathway and the biosynthesis of bioactive lipids. PGE_2, through its effects on type I interferon signaling, cytotoxic T-cell and Th17 responses, macrophage polarization, natural killer cell functions, neutrophil extracellular trap formation, inflammasome activation, and nuclear factor-κB activation, may contribute to the cytokine storm and hyperinflammation response observed in COVID-19 patients. CysLTs may regulate the immune and inflammatory response to SARS-CoV-2 infection by having a chemoattractant effect on neutrophils and lymphocytes and by causing macrophage activation and proinflammatory cytokine secretion. PGE_2 and PGI_2 are the predominant proinflammatory prostanoids that contribute to increased vascular permeability and blood flow in the inflamed region. TxA_2 can increase platelet-leukocyte interactions. PGD_2 may contribute to the resolution of inflammation. Lymphocytes have a minimal capacity to form PGs, yet they are a primary target of their action. PGs generally inhibit lymphocyte function and proliferation, suppressing the immune response. PGE_2 plays a complex role in the inflammatory process and depresses the humoral antibody response by inhibiting the differentiation of B lymphocytes into antibody-secreting plasma cells. PGE_2 can induce immunoglobulin class switching, resulting in increased IgE production (see Chapter 38). PGE_2 acts on T lymphocytes to inhibit mitogen-stimulated proliferation and lymphokine release by sensitized cells. PGE_2 and TxA_2 also may play a role in T-lymphocyte development by regulating apoptosis of immature thymocytes. PGE_2, acting via EPr_2 and EPr_4, can interact with the programmed cell death protein 1 (PD-1) (see Figure 38–7) to restrain cytotoxic T-cell function and survival during chronic infection in mice (Chen et al., 2015). The COX-2/mPGES-1/PGE_2 pathway can regulate PD ligand 1 (PD-L1) expression in tumor-infiltrating myeloid cells (Prima et al., 2017). Given the efficacy of blockade of this pathway in a range of cancers, the possibility that blockade of PGE_2 synthesis or action might augment this effect has been suggested.

The synthesis, release, and subsequent effects of eicosanoids are prominent components of the inflammatory response (see Figure 38–6). PGD_2 is a potent leukocyte chemoattractant, primarily through the DPr_2. The LTs are potent mediators of inflammation. Deletion of either 5-LOX or FLAP reduces inflammatory responses in model systems. LTB_4, a potent proinflammatory eicosanoid, acts as a chemotactic agent for neutrophils, T lymphocytes, eosinophils, monocytes, dendritic cells, and possibly also mast cells, recruiting them to sites of injury or inflammation (Bäck et al., 2011). LTB_4 stimulates the aggregation of eosinophils and promotes degranulation and generation of superoxide. It also promotes adhesion of neutrophils to vascular endothelial cells and their transendothelial migration and stimulates synthesis of proinflammatory cytokines from macrophages and lymphocytes.

The CysLTs are chemotaxins for eosinophils and monocytes. They also induce cytokine generation in eosinophils, mast cells, and dendritic cells. At higher concentrations, these LTs also promote eosinophil adherence, degranulation, cytokine or chemokine release, and oxygen radical formation. In addition, CysLTs contribute to inflammation by increasing endothelial permeability, thus promoting migration of inflammatory cells to the site of inflammation.

Bronchial and Tracheal Muscle

A complex mixture of autacoids is released when sensitized lung tissue is challenged by the appropriate antigen, including COX-derived bronchodilator and bronchoconstrictor substances. Among these, TxA_2, $PGF_{2\alpha}$, and PGD_2 contract, and PGE_2 and PGI_2 relax, bronchial and tracheal muscle. PGI_2 causes bronchodilation in most species; human bronchial tissue is particularly sensitive. PGI_2 antagonizes bronchoconstriction induced by other agents. PGI_2 suppresses allergic airway inflammation by promoting regulatory T-cell signaling and reprogramming (Norlander et al., 2021). PGD_2 appears to be the primary bronchoconstrictor prostanoid of relevance in humans. Polymorphisms in the genes for hematopoietic PGD_2 synthase (H-PGDS) and the TPr have been associated with asthma in humans.

Roughly 10% of people who take *aspirin* or NSAIDs develop bronchospasm, termed *aspirin*-exacerbated respiratory disease (AERD). This immune dysfunction involves a shift in AA metabolism to LT formation. This substrate diversion appears to involve COX-1, not COX-2. CysLTs are bronchoconstrictors that act principally on smooth muscle in the airways and are a thousand times more potent than histamine. They also stimulate bronchial mucus secretion and cause mucosal edema.

The CysLTs probably dominate during allergic constriction of the airway. Deficiency of 5-LOX leads to reduced influx of eosinophils in airways and attenuates bronchoconstriction. Furthermore, unlike COX inhibitors and histaminergic antagonists, CysLTr antagonists and 5-LOX inhibitors are effective in the treatment of human asthma (see Inhibitors of Eicosanoid Biosynthesis). The relatively slow LT metabolism in lung contributes to the long-lasting bronchoconstriction that follows challenge with antigen and may be a factor in the high bronchial tone that is observed in asthmatic patients in periods between acute attacks (see Chapter 44).

GI Smooth Muscle

PGE_2 and $PGF_{2\alpha}$ stimulate contraction of the main longitudinal muscle from stomach to colon. PG endoperoxides, TxA_2, and PGI_2 also produce contraction but are less active. Circular muscle generally relaxes in response to PGE_2 and contracts in response to $PGF_{2\alpha}$. The LTs have potent contractile effects. PGs reduce transit time in the small intestine and colon. Diarrhea, cramps, and reflux of bile have been noted in response to oral PGE analogues. PGE_2 and $PGF_{2\alpha}$ stimulate the movement of water and electrolytes into the intestinal lumen. Such effects may underlie the watery diarrhea that follows their oral or parenteral administration. PGE_2 appears to contribute to the water and electrolyte loss in cholera, a disease that is somewhat responsive to therapy with NSAIDs.

GI Secretion

In the stomach, PGE_2 and PGI_2 contribute to increased mucus secretion (*cytoprotection*), reduced acid secretion, and reduced pepsin content. PGE_2 and its analogues also inhibit gastric damage caused by a variety of ulcerogenic agents and promote healing of duodenal and gastric ulcers (see Chapter 53). While COX-1 may be the dominant source of such cytoprotective PGs under physiological conditions, both COX-1 and COX-2 may be contributing to ulcer healing through the regulation of angiogenesis (Jones et al., 1999). PGE_2 promotes healing of GI ulcers through the stimulation of angiogenesis via the upregulation of vascular endothelial growth factor (VEGF) expression mediated by EPr_4. Selective inhibitors of COX-2 and deletion of the enzyme delay ulcer healing in rodents, but the impact of COX-2 inhibitors in humans is unclear. CysLTs, by constricting gastric blood vessels and enhancing production of proinflammatory cytokines, may contribute to the gastric damage.

Uterus

Strips of nonpregnant human uterus are contracted by $PGF_{2\alpha}$ and TxA_2 but are relaxed by PGE_2. Sensitivity to the contractile response is most prominent before menstruation, whereas relaxation is greatest at midcycle. PGE_2, together with oxytocin, is essential for the onset of parturition. PGI_2 and high concentrations of PGE_2 produce uterine relaxation. The intravenous infusion of low concentrations of PGE_2 or $PGF_{2\alpha}$ to pregnant women produces a dose-dependent increase in uterine tone and in the frequency and intensity of rhythmic uterine contractions. PGE_2 and $PGF_{2\alpha}$ can be administered to terminate pregnancy. Uterine responsiveness to PGs increases as pregnancy progresses but remains smaller than the response to oxytocin.

Kidney

COX-1– and COX-2–derived eicosanoids regulate kidney blood flow and glomerular filtration rate. COX-2–derived PGE_2 and PGI_2 increase medullary blood flow, resulting in pressure diuresis, and inhibit tubular Na^+ reabsorption (Hao and Breyer, 2007). Expression of medullary COX-2 is increased during high salt intake. COX-1–derived products promote salt excretion in the collecting ducts. Cortical COX-2–derived PGE_2 and PGI_2 increase renal blood flow and glomerular filtration through their local vasodilating effects and as part of the tubuloglomerular feedback mechanism that controls renin release. Expression of COX-2 in macula densa cells increases in conditions of low distal tubular flow during low dietary salt intake or volume depletion. COX-2–derived PGE_2 and possibly PGI_2 result in increased renin release, leading to sodium retention and elevated blood pressure.

TxA_2, generated at low levels in the normal kidney, has potent vasoconstrictor effects that reduce renal blood flow and glomerular filtration rate. Infusion of $PGF_{2\alpha}$ causes both natriuresis and diuresis. Conversely, $PGF_{2\alpha}$ may activate the renin-angiotensin system, contributing to elevated blood pressure. CYP epoxygenase products may regulate renal function. Both 20-HETE and the EETs are generated in renal tissue; 20-HETE constricts the renal arteries, while EETs mediate vasodilation and natriuresis.

Bartter syndrome is an autosomal recessive trait that manifests as hypokalemic metabolic alkalosis. The antenatal variant of Bartter syndrome is due to dysfunctional ROMK2 (Kir1.1), the K^+ channel that recycles K^+ into the tubular fluid. This syndrome also is known as *hyper-PGE syndrome*. The relationship between dysfunctional ROMK2 and elevated PGE_2 synthesis is not clear; however, in patients with antenatal Bartter syndrome, inhibition of COX-2 ameliorates many of the clinical symptoms.

Eye

$PGF_{2\alpha}$ induces constriction of the iris sphincter muscle, but its overall effect in the eye is to decrease intraocular pressure by increasing the aqueous humor outflow. A variety of FPr agonists have proven effective in the treatment of open-angle glaucoma, a condition associated with the loss of COX-2 expression in the pigmented epithelium of the ciliary body (see Chapter 74). An EPr2 agonist, with a nonprostaglandin structure, has been recently approved for the treatment of glaucoma and ocular hypertension.

Central Nervous System

PGE_2 induces fever. The hypothalamus regulates the body temperature set point, which is elevated by endogenous pyrogens such as interleukin (IL)-1β, IL-6, TNFα (tumor necrosis factor α), and interferons (Morrison and Nakamura, 2011). The response is mediated by coordinate induction of COX-2 and mPGES-1 in the endothelium of blood vessels in the preoptic hypothalamic area to form PGE_2. PGE_2 can cross the blood-brain barrier and act on the EPr_3 (and perhaps EPr_1) on thermosensitive neurons, triggering the hypothalamus to elevate body temperature. Exogenous $PGF_{2\alpha}$ and PGI_2 induce fever but do not contribute to the endogenous pyretic response. PGE_2 can mediate age-associated maladaptive inflammation and cognitive decline through its myeloid EPr_2 by inducing an energy-deficient state in macrophages and microglia. EPr_2 blockade reduces the inflammatory response, restores the cell bioenergetics, and reverses cognitive aging (Minhas et al., 2021). PGD_2 appears to act on arachnoid trabecular cells in the basal forebrain to mediate an increase in extracellular adenosine that, in turn, facilitates induction of sleep. COX-2–derived prostanoids also have been implicated in the pathogenesis of several CNS degenerative disorders (e.g., Alzheimer's disease, Parkinson's disease; see Chapter 21).

Pain

Inflammatory mediators, including LTs and PGs, increase the sensitivity of nociceptors and potentiate pain perception. Centrally, both COX-1 and COX-2 are expressed in the spinal cord under basal conditions and release PGs in response to peripheral pain stimuli. Both PGE_2 (through EPr_1 and EPr_4) and PGI_2 (via IPr) reduce the threshold to stimulation of nociceptors, causing "peripheral sensitization." PGE_2, and perhaps PGD_2, PGI_2, and $PGF_{2\alpha}$, can increase excitability in neuronal pain transmission pathways in the spinal cord, causing hyperalgesia and allodynia. LTB_4 also produces hyperalgesia. The release of these eicosanoids during the inflammatory process thus serves as an amplification system for the pain mechanism. The role of PGE_2 and PGI_2 in inflammatory pain is discussed in more detail in Chapter 42.

Endocrine System

The systemic administration of PGE_2 increases circulating concentrations of ACTH (corticotropin), growth hormone, prolactin, and gonadotropins. Other effects include stimulation of steroid production by the adrenals, stimulation of insulin release, and thyrotropin-like effects on the thyroid. PGE_2 works as part of a positive-feedback loop to induce oocyte maturation required for fertilization during and after ovulation. PGE_2-dependent critical events for ovulation are regulated by the activity of the PGT, which modulates the extracellular levels of PGE_2 in granulosa cells (Yerushalmi et al., 2016). The critical role of $PGF_{2\alpha}$ in parturition relies on its capacity to induce an oxytocin-dependent decline in progesterone levels. LOX metabolites also have endocrine effects. 12-HETE stimulates the release of aldosterone from the adrenal cortex and mediates a portion of the aldosterone release stimulated by AngII, but not that which occurs in response to ACTH.

Bone

PGs are strong modulators of bone metabolism. COX-1 is expressed in normal bone, while COX-2 is upregulated in settings such as inflammation and during mechanical stress. PGE_2 stimulates bone formation by increasing osteoblastogenesis and bone resorption via activation of osteoclasts. Osteoblast-derived PGE_2 activates EPr_4 in sensory nerves to control bone homeostasis and regeneration (Chen et al., 2019).

Skeletal Muscle

PGs play an essential role in skeletal muscle biology. PGE_2 is the main PG produced in resting skeletal muscle. PGE_2 contributes to regeneration of damaged muscles and maintains mature muscle myofiber homeostasis. PGE_2, acting via EPr_4, drives muscle repair after injury by accelerating muscle stem cell expansion (Ho et al., 2017). COX-2 deletion or inhibition may impair skeletal muscle healing (Ho et al., 2017). In aging skeletal muscle, inactivation of 15-PGDH, which increases its expression with aging, restores PGE_2 and PGD_2 and increases muscle mass, strength, and exercise performance by impacting mitochondrial function, autophagy, and proteostasis (Palla et al., 2021). H-PGDS-derived PGD_2 is implicated in muscle necrosis and muscle tissue damage (Mohri et al., 2009). H-PGDS inhibition ameliorates myonecrosis in mouse models of Duchenne muscular dystrophy (Mohri et al., 2009), and urinary excretion of PGD_2 metabolites reflects the severity of this disease (Takeshita et al., 2018).

Cancer

Pharmacological inhibition or genetic deletion of COX-2 restrains tumor formation in models of colon, breast, lung, and other cancers. Large human epidemiological studies have reported that incidental use of NSAIDs is associated with significant reductions in relative risk for developing these and other cancers. PGE_2 has been implicated as the primary pro-oncogenic prostanoid in multiple studies. PGE_2, produced by the tumor cell, stromal cells, or cells in the tumor microenvironment, can promote cancer initiation and progression by controlling multiple biological processes. Fibroblast-derived PGE_2 may induce early tumor initiation by driving the proliferation of epithelial stem cells through EPr_4. Deletion or inhibition of EPr_4 reduces tumor formation in mouse models of colon cancer (Roulis et al., 2020). PGE_2-derived COX-2 can promote tumor progression by shaping the tumor microenvironment and favoring tumor escape. COX-2 deletion reduces tumor growth by restoring effector $CD8^+$ T-cell infiltration and reducing myeloid-derived suppressor cells, and COX-2 inhibition reverses tumor resistance to immunotherapy in mouse models of pancreatic adenocarcinoma (Markosyan et al., 2019). PGE_2 induces cytotoxic T-lymphocyte exhaustion by modulating inhibitory T-cell receptor function. PGE_2 can upregulate PD-1 expression on effector $CD8^+$ T-cells (Miao et al., 2017) and PD-L1 expression in tumor-associated macrophages and myeloid-derived suppressor cells (Prima et al., 2017). PGE_2 controls the suppressive activity of polymorphonuclear myeloid-derived suppressor cells, which dampen the antitumoral activity of T cells and other immune cells (Veglia et al., 2019). PGE_2 reduces the viability of natural killer cells, chemokine production, and chemokine receptor expression in conventional type 1 dendritic cells (Böttcher et al., 2018).

Therapeutic Uses

Inhibitors and Antagonists

The NSAIDs are used widely for their anti-inflammatory effects, whereas low-dose *aspirin* is used for cardioprotection (see Chapter 42). LT antagonists are useful clinically in the treatment of asthma, and FPr agonists are used in the treatment of open-angle glaucoma (see Chapter 74). EPr agonists are used to induce labor and to ameliorate gastric irritation owing to NSAIDs. DPr$_1$ antagonists have been explored for offsetting the facial flushing associated with niacin. Orally active antagonists of LTC$_4$ and LTD$_4$, which block CysLTr$_1$, are used in the treatment of asthma that is mild to moderately severe (see Chapter 44). They are also the mainstay of treatment for AERD, given the underlying dysregulation of LT production in that adverse response.

Prostanoids and Their Analogues

Prostanoids have a short $t_{1/2}$ in the circulation, and their systemic administration produces significant adverse effects (e.g., pain, flushing, and diarrhea). Nonetheless, several prostanoids are of clinical utility in the following situations.

Labor and Therapeutic Abortion. PGE$_2$, PGF$_{2\alpha}$, and their analogues are used to induce labor at term and terminate pregnancy by promoting uterine contractions. These agents facilitate labor by promoting ripening and dilation of the cervix. *Dinoprostone* or *misoprostol*, synthetic analogues of PGE$_2$ and PGE$_1$, are used for cervical ripening and induction of labor and as abortifacients in the second trimester of pregnancy. *Misoprostol*, in combination with the antiprogesterone *mifepristone* (RU486), is highly effective in the termination of pregnancy. An analogue of PGF$_{2\alpha}$, *carboprost tromethamine*, is used to induce second-trimester abortions and to control postpartum hemorrhage that does not respond to conventional methods.

Maintenance of Patent Ductus Arteriosus. The ductus arteriosus in neonates is highly sensitive to vasodilation by PGE$_1$. Maintenance of a patent ductus may be important hemodynamically in some neonates with congenital heart disease. PGE$_1$ (*alprostadil*) is highly effective for palliative therapy to maintain temporary patency until surgery can be performed. Apnea is observed in about 10% of neonates treated, particularly those who weigh less than 2 kg at birth.

Gastric Cytoprotection. Several PG analogues are used to suppress gastric ulceration. *Misoprostol*, a PGE$_1$ analogue, is approved for prevention of NSAID-induced gastric ulcers and is about as effective as the proton pump inhibitor *omeprazole* (see Chapter 53).

Impotence. PGE$_1$ (*alprostadil*), given as an intracavernous injection or urethral suppository, is a second-line treatment of erectile dysfunction. Phosphodiesterase 5 inhibitors (e.g., *sildenafil*, *tadalafil*, *vardenafil*, and *avanafil*; see Chapter 49) have superseded PGE$_1$ as the preferred treatment of this condition.

Pulmonary Arterial Hypertension. Long-term therapy with PGI$_2$ (*epoprostenol*), via continuous intravenous infusion, improves symptoms and hemodynamics and can delay or preclude the need for lung or heart-lung transplantation in a number of patients with pulmonary arterial hypertension (PAH). Several orally available PGI$_2$ analogues have also been used clinically for PAH. *Iloprost* specifically targets lung vasculature and can be inhaled or delivered by intravenous administration (injectable form is not available in the U.S.). *Treprostinil* ($t_{1/2}$ ~4 h) may be delivered by continuous subcutaneous infusion, intravenous infusion, or orally. *Selexipag* ($t_{1/2}$ ~0.8–2.5 h; $t_{1/2}$ active metabolite ~6.2–13.5 h) is the first approved PGI$_2$ analogue with a nonprostanoid structure. Chapter 35 presents a comprehensive picture of the treatment of PAH.

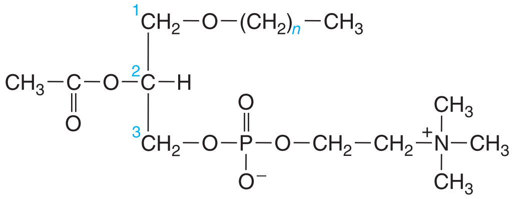
Glaucoma. *Latanoprost*, a stable, long-acting PGF$_{2\alpha}$ derivative, was the first prostanoid used for glaucoma. Similar prostanoids with ocular hypotensive effects include *bimatoprost*, *tafluprost*, and *travoprost*. These drugs act as agonists at the FPr and are administered as ophthalmic drops. Recently, *latanoprost* has been combined with other moieties to treat glaucoma: *Latanoprostene bunod* is metabolized into *latanoprost* and *butanediol mononitrate* (a source of NO); *latanoprost* has also been combined with Rho kinase inhibitors (Tanna and Johnson, 2018). Chapter 74 covers the clinical pharmacology of these drugs in the treatment of glaucoma.

Platelet-Activating Factor

In 1971, Henson demonstrated that a soluble factor released from leukocytes caused platelets to aggregate. Benveniste and his coworkers characterized the factor as a polar lipid and named it PAF. During this period, Muirhead described an antihypertensive polar renal lipid (APRL) produced by interstitial cells of the renal medulla that proved to be identical to PAF. Hanahan and coworkers then synthesized acetyl glyceryl ether phosphorylcholine (AGEPC) and determined that this phospholipid had chemical and biological properties identical to those of PAF. Independent determination of the structures of PAF and APRL showed them to be structurally identical to AGEPC. The commonly accepted name for this substance is PAF; however, its actions extend far beyond platelets.

Chemistry and Biosynthesis

PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) represents a family of phospholipids because the alkyl group at position 1 can vary in length from 12 to 18 carbon atoms (Prescott et al., 2000). In human neutrophils, PAF consists predominantly of a mixture of the 16- and 18-carbon ethers, but its composition may change when cells are stimulated.



PLATELET-ACTIVATING FACTOR (n = 11 to 17)

Biosynthesis of eicosanoids and PAF depends on PLA$_2$ activity. The major biosynthetic pathway for PAF, the remodeling pathway, involves the precursor 1-*O*-alkyl-2-acyl-glycerophosphocholine, a membrane lipid; the 2-acyl substituents include AA. PAF is synthesized from this substrate in two steps (Figure 41–5). The rate-limiting step is the second

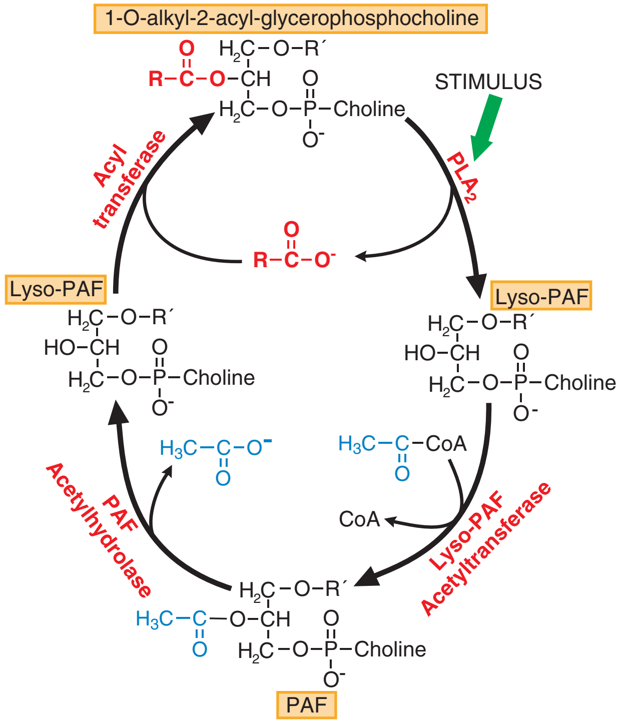


Figure 41–5 *Synthesis and degradation of PAF.* RCOO$^-$ is a mixture of fatty acids but is enriched in AA that may be metabolized to eicosanoids.

one, acetyl-coenzyme-A-lyso-PAF acetyltransferase. The synthesis of PAF may be stimulated during antigen-antibody reactions or by a variety of agents, including chemotactic peptides, thrombin, collagen, and other autacoids; PAF also can stimulate its own formation. Both the PL and acetyltransferase are Ca^{2+}-dependent enzymes; thus, PAF synthesis is regulated by the availability of Ca^{2+}. PAF-AHs (AHs, acetylhydrolases) inactivate PAH by catalyzing the hydrolysis of the acetyl group, generating Lyso-PAF, which is then converted to a 1-*O*-alkyl-2-acyl-glycerophosphocholine by an acyltransferase (McIntyre et al., 2009; Stafforini et al., 2003).

Synthesis of PAF also can occur *de novo* by transfer of a phosphocholine substituent to alkyl acetyl glycerol by a lyso-glycerophosphate acetyl–coenzyme A transferase. This pathway may contribute to physiological levels of PAF for normal cellular functions. PAF-like molecules can be formed from oxidized phospholipids (oxPLs) (Stafforini et al., 2003). These compounds are increased in settings of oxidant stress, such as cigarette smoking, and differ structurally from PAF in that they contain a fatty acid at the *sn*-1 position of glycerol joined through an ester bond and various short-chain acyl groups at the *sn*-2 position. oxPLs mimic the structure of PAF, bind to its receptor, and elicit the same responses. Unlike the highly controlled synthesis of PAF, oxPL production is unregulated. Degradation of oxPLs by PAF-AH is necessary to suppress toxicity. Increased levels of plasma PAF-AH have been reported in colon cancer, cardiovascular disease, and stroke.

Sites of PAF Synthesis

PAF is not stored in cells but is synthesized in response to stimulation. PAF is synthesized by platelets, neutrophils, monocytes, mast cells, eosinophils, renal mesangial cells, renal medullary cells, and vascular endothelial cells. Depending on cell type, PAF can either remain cell-associated or be secreted. For example, PAF is released from monocytes but retained by leukocytes and endothelial cells. In endothelial cells, PAF is displayed on the surface for juxtacrine signaling and stimulates adherent leukocytes.

Mechanism of Action of PAF

Extracellular PAF exerts its actions by stimulating a specific GPCR (Honda et al., 2002). The PAF receptor couples to G_q (to activate the phospholipase/inositol 1,4,5-trisphosphate/Ca^{2+} pathway) and to G_i (to inhibit adenylyl cyclase). Consequent activation of PLA_2, PLC, and PLD gives rise to second messengers, including AA-derived PGs, TxA_2, or LTs, which may function as mediators of the effects of PAF.

In addition, p38 mitogen-activated protein kinase is activated downstream of the PAF receptor–G_q interaction, while extracellular signal–regulated kinase activation can occur via interaction of activated PAF receptor with G_q, G_o, or their βγ subunits, or via transactivation of the epidermal growth factor receptor, leading to nuclear factor-κB activation. The crystal structure of the PAF receptor was recently resolved (Cao et al., 2018). PAF exerts many of its important proinflammatory actions without leaving its cell of origin. For example, PAF is synthesized in a regulated fashion by endothelial cells stimulated by inflammatory mediators. This PAF is presented on the surface of the endothelium, where it activates the PAF receptor on juxtaposed cells, including platelets, PMNs, and monocytes, and acts cooperatively with P-selectin to promote adhesion. This proadhesive function of PAF is important for orchestrating the interaction of platelets and circulating inflammatory cells with the inflamed endothelium.

Physiological and Pathological Functions of PAF

PAF is a potent lipid mediator of pathological events and has been implicated in allergic asthma, endotoxic shock, acute pancreatitis, certain cancers, dermal inflammation, and inflammatory cardiovascular diseases such as atherosclerosis.

Inflammatory and Allergic Responses

Experimental administration of PAF reproduces many of the signs and symptoms in anaphylactic shock. However, the effects of PAF antagonists in the treatment of inflammatory and allergic disorders have been disappointing. In patients with asthma, PAF antagonists partially inhibit the bronchoconstriction induced by antigen challenge but not by challenges by methacholine, exercise, or inhalation of cold air. These results may reflect the complexity of these pathological conditions and the likelihood that other mediators contribute to the inflammation associated with these disorders.

Cardiovascular System

PAF is a potent vasodilator in most vascular beds; when administered intravenously, it causes hypotension. PAF-induced vasodilation is independent of effects on sympathetic innervation, the renin-angiotensin system, or AA metabolism and likely results from a combination of direct and indirect actions. PAF may, alternatively, induce vasoconstriction depending on the concentration, vascular bed, and involvement of platelets or leukocytes. For example, the intracoronary administration of very low concentrations of PAF increases coronary blood flow by a mechanism that involves the release of a platelet-derived vasodilator. Coronary blood flow is decreased at higher doses by the formation of intravascular aggregates of platelets or the formation of TxA_2. The pulmonary vasculature also is constricted by PAF, and a similar mechanism is thought to be involved.

Intradermal injection of PAF causes an initial vasoconstriction followed by a typical wheal and flare. PAF increases vascular permeability and edema by causing contraction of venular endothelial cells. In this respect, the actions of PAF resemble those of histamine and bradykinin, but PAF is more potent than histamine or bradykinin by three orders of magnitude.

Platelets

The PAF receptor is constitutively expressed on the surface of platelets. PAF potently stimulates platelet aggregation. The intravenous injection of PAF causes formation of intravascular platelet aggregates and thrombocytopenia. Although this is accompanied by the release of TxA_2 and the granular contents of the platelet, PAF does not require the presence of TxA_2 or other aggregating agents to produce this effect. PAF antagonists fail to block thrombin-induced aggregation, even though they prolong bleeding time and prevent thrombus formation in some experimental models. Thus, PAF may contribute to thrombus formation, but it does not function as an independent mediator of platelet aggregation.

Leukocytes

Platelet-activating factor is a potent and common activator of inflammatory cells and stimulates a variety of responses in PMNs (eosinophils, neutrophils, and basophils). PAF stimulates PMNs to aggregate, degranulate, and generate free radicals and LTs. PAF is also a potent chemotactic factor for eosinophils, neutrophils, and monocytes and promotes PMN-endothelial adhesion, contributing, along with other adhesion molecular systems, to leukocyte rolling, tight adhesion, and migration through the endothelial monolayer. PAF also stimulates basophils to release histamine, activates mast cells, and induces cytokine release from monocytes. In addition, PAF promotes aggregation of monocytes and degranulation of eosinophils.

Smooth Muscle

PAF contracts GI, uterine, and pulmonary smooth muscle. PAF enhances the amplitude of spontaneous uterine contractions; these contractions are inhibited by inhibitors of PG synthesis. PAF does not affect tracheal smooth muscle but contracts airway smooth muscle. When given by aerosol, PAF increases airway resistance as well as the responsiveness to other bronchoconstrictors. PAF also increases mucus secretion and the permeability of pulmonary microvessels.

Stomach

In addition to contracting the fundus of the stomach, PAF is the most potent known ulcerogen. When given intravenously, it causes hemorrhagic erosions of the gastric mucosa that extend into the submucosa.

Kidney

PAF decreases renal blood flow, glomerular filtration rate, urine volume, and excretion of Na^+ without changes in systemic hemodynamics. PAF exerts a receptor-mediated biphasic effect on afferent arterioles, dilating them at low concentrations and constricting them at higher concentrations. The vasoconstrictor effect appears to be mediated, at least in part, by COX products, whereas vasodilation is a consequence of the stimulation of NO production by endothelium.

Other

PAF, a potent mediator of angiogenesis, has been implicated in many cancers such as breast, prostate, esophageal, lung, pancreatic, and skin. Cigarette smoke exposure has been found to upregulate PAF signaling (Kispert et al., 2019). Lordan and colleagues (2019) have recently reviewed the breadth of actions ascribed to PAF. PAF-AH deficiency has been associated with small increases in a range of cardiovascular and thrombotic diseases in some human populations.

PAF Receptor Antagonists

Several experimental PAF receptor antagonists exist that selectively inhibit the actions of PAF *in vivo* and *in vitro*. None has proven clinically useful. Inhibition of PAF-AH, which also functions as a lipoprotein-associated PLA$_2$, was studied as a maneuver that might elevate endogenous levels of PAF. Trials of an inhibitor *darapladib* failed to establish either clinical efficacy attributable to eicosanoid suppression or an adverse effect profile attributable to increased levels of PAF (O'Donoghue et al., 2014).

Acknowledgment: *Emer M. Smyth contributed to this chapter in the previous edition of this book. We have retained some of her text in the current edition.*

Drug Facts for Your Personal Formulary: *Eicosanoids*

Drug	Therapeutic Uses	Clinical Pharmacology and Tips
Prostanoids and Prostanoid Analogues: PGE$_1$/PGE$_2$		
Alprostadil (PGE$_1$)	• Erectile dysfunction • Temporary maintenance of patent ductus arteriosus in neonates	• Rapidly metabolized • Prolonged erection (4–6 h) in 4% of patients • Apnea in 10%–12% of neonates with congenital heart defects; ventilator assistance should be available during treatment
Misoprostol (PGE$_1$ analogue)	• Protection from NSAID-induced gastric toxicity	• Contraindicated for use in pregnant women; women who may become pregnant must use birth control when taking misoprostol • Combined with mifepristone to terminate early pregnancy
Dinoprostone (PGE$_2$)	• Labor induction	• Rapidly metabolized
Prostanoids and Prostanoid Analogues: PGI$_2$ (Prostacyclin)		
Epoprostenol (PGI$_2$)	• Pulmonary arterial hypertension	• Rapidly metabolized • Administered by intravenous infusion • Most common dose-limiting adverse effects are nausea, vomiting, headache, hypotension, and flushing
Iloprost (PGI$_2$ analogue)	• Pulmonary arterial hypertension	• Administered by inhalation • Synthetic PGI$_2$ analogue with longer $t_{1/2}$ • May increase risk of bleeding when used with anticoagulants or platelet inhibitors
Treprostinil (PGI$_2$ analogue)	• Pulmonary arterial hypertension	• May be administered by subcutaneous/intravenous infusion or by inhalation or orally • Adverse events similar to iloprost
Selexipag (PGI$_2$ analogue)	• Pulmonary arterial hypertension	• Oral selective IPr agonist • Active metabolite $t_{1/2}$ 6.2–13.5 h • Adverse effects similar to epoprostenol • Contraindicated with strong CYP2C8 inhibitors
Prostanoids and Prostanoid Analogues: PGF$_{2\alpha}$		
Carboprost tromethamine	• Abortifacient (second trimester) • Postpartum hemorrhage	• Common adverse effects are vomiting, diarrhea, nausea, fever, flushing
Bimatoprost	• Ocular hypertension • Open-angle glaucoma • Hypotrichosis of the eyelashes	• Upper respiratory tract infections in about 10% of patients • May cause changes in pigmentation and hair growth
Latanoprost	• Ocular hypertension • Open-angle glaucoma	• Increased iris pigmentation with time
Tafluprost	• Ocular hypertension • Open-angle glaucoma	• Metabolized to active drug in the eye • May cause increased iris pigmentation
Travoprost	• Ocular hypertension • Open-angle glaucoma	• May cause increased iris pigmentation
Nonsteroidal Anti-Inflammatory Drugs		
Listed in Chapter 42		
Cysteinyl Leukotriene Receptor Antagonists/5-Lipoxygenase Inhibitors		
Listed in Chapter 44		

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Chapter 42

Pharmacotherapy of Inflammation, Fever, Pain, and Gout

Tilo Grosser, Emanuela Ricciotti, and Garret A. FitzGerald

INFLAMMATION, PAIN, AND FEVER
- Inflammation
- Pain
- Fever

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS
- Mechanism of Action
- Therapeutic Uses
- Adverse Effects of NSAID Therapy
- Drug Interactions
- Pediatric and Geriatric Use

SPECIFIC PROPERTIES OF INDIVIDUAL NSAIDs
- Aspirin and Other Salicylates
- Acetaminophen
- Acetic Acid Derivatives
- Propionic Acid Derivatives
- Fenamates
- Enolic Acids (Oxicams)
- Purpose-Developed COX-2–Selective NSAIDs

DISEASE-MODIFYING ANTIRHEUMATIC DRUGS

PHARMACOTHERAPY OF GOUT
- Colchicine
- Allopurinol
- Febuxostat
- Uricase
- Uricosuric Agents

This chapter describes the nonsteroidal anti-inflammatory drugs (NSAIDs) used to treat inflammation, pain, and fever and the drugs used for hyperuricemia and gout. The NSAIDs are first considered by class, then by groups of chemically similar agents described in more detail. Many of the basic properties of these drugs are summarized in Tables 42–1, 42–2, and 42–3.

The NSAIDs act by inhibiting the prostaglandin (PG) G/H synthase enzymes, colloquially known as the cyclooxygenases (COXs) (see Chapter 41). There are two forms, COX-1 and COX-2. The inhibition of COX-2 is thought to mediate, in large part, the antipyretic, analgesic, and anti-inflammatory actions of NSAIDs. Adverse reactions are largely caused by the inhibition of COX-1 and COX-2 in tissues in which they fulfill physiological functions, such as the GI tract, the kidney, and the cardiovascular system. *Aspirin* is the only irreversible inhibitor of the COX enzymes in clinical use. All other NSAIDs bind the COXs reversibly and act either by competing directly with arachidonic acid (AA) at the active site of COX-1 and COX-2 or by changing their steric conformation in a way that alters their ability to bind arachidonic acid. *Acetaminophen* (*paracetamol*) is effective as an antipyretic and analgesic agent at typical doses that partly inhibit COXs and has only weak anti-inflammatory activity. Purposefully designed selective inhibitors of COX-2 (*celecoxib*, *etoricoxib*) are a subclass of NSAIDs; several of the older traditional NSAIDs, such as *diclofenac* and *meloxicam* (Figure 42–1) also selectively inhibit COX-2 at therapeutic doses.

HISTORICAL PERSPECTIVE

The history of *aspirin* provides an interesting example of the translation of a compound from the realm of herbal folklore to contemporary therapeutics. The use of willow bark and leaves to relieve fever has been attributed to Hippocrates but was most clearly documented by Edmund Stone in a 1763 letter to the president of the Royal Society. Similar properties were attributed to potions from meadowsweet (*Spiraea ulmaria*), from which the name *aspirin* is derived. Salicin was crystallized in 1829 by Leroux, and Pina isolated salicylic acid in 1836. In 1859, Kolbe synthesized salicylic acid, and by 1874 it was being produced industrially. It soon was being used for rheumatic fever and gout and as a general antipyretic. However, its unpleasant taste and adverse GI effects made it difficult to tolerate for more than short periods. In 1899, Hoffmann, a chemist at Bayer Laboratories, sought to improve the adverse effect profile of salicylic acid (which his father was taking with difficulty for arthritis). Hoffmann came across the earlier work of the French chemist Gerhardt, who had acetylated salicylic acid in 1853, apparently ameliorating its adverse effect profile, but without improving its efficacy, and therefore abandoned the project. Hoffmann resumed the quest, and Bayer began testing acetylsalicylic acid (ASA) in animals by 1899 and proceeded soon thereafter to human studies and the marketing of *aspirin*.

Acetaminophen was first used in medicine by von Mering in 1893. However, it gained popularity only after 1949, when it was recognized as the major active metabolite of both *acetanilide* and *phenacetin*. *Acetanilide* is the parent member of this group of drugs. It was introduced into medicine in 1886 under the name *antifebrin* by Cahn and Hepp, who had discovered its antipyretic action accidentally. However, *acetanilide* proved to be excessively toxic. A number of chemical derivatives were developed and tested. One of the more satisfactory of these was *phenacetin*. It was introduced into therapy in 1887 and was extensively employed in analgesic mixtures until it was implicated in analgesic abuse nephropathy, hemolytic anemia, and bladder cancer; it was withdrawn in the 1980s.

Inflammation, Pain, and Fever

Inflammation

The inflammatory process is the immune system's protective response to an injurious stimulus. It can be evoked by noxious agents, infections, and physical injuries, which release damage- and pathogen-associated molecules that are recognized by cells charged with immune surveillance (Tang et al., 2012). The ability to mount an inflammatory response is essential for survival in the face of environmental pathogens and injury. Sometimes, inflammation may be exaggerated and sustained without apparent benefit and even with severe adverse consequences

Abbreviations

AA: arachidonic acid
Ab: antibody
ABCG2: ATP Binding Cassette Subfamily G Member 2
ACE: angiotensin-converting enzyme
ASA: acetylsalicylic acid/aspirin
AUC: area under the curve
CABG: coronary artery bypass graft
CNS: central nervous system
COX: cyclooxygenase
CSF: cerebrospinal fluid
DMARD: disease-modifying antirheumatic drug
G6PD: glucose-6-phosphate dehydrogenase
GI: gastrointestinal
GLUT: glucose transporter
GSH: glutathione
FAP: familial adenomatous polyposis
FDA: U.S. Food and Drug Administration
15(*R*)-HETE: 15(*R*)-hydroxyeicosatetraenoic acid
5-HIAA: 5-hydroxyindoleacetic acid
5HT: 5-hydroxytryptamine/serotonin
Ig: immunoglobulin
IL: interleukin
IM: intramuscular
IV: intravenous
LOX: lipoxygenase
LT: leukotriene
MI: myocardial infarction
MRP: multidrug resistance-associated protein
NAC: *N*-acetylcysteine
NAPQI: *N*-acetyl-*p*-benzoquinone imine
NERD: NSAID-exacerbated respiratory disease
NPT: sodium-dependent phosphate transporter
NSAID: nonsteroidal anti-inflammatory drug
OAT: organic anion transporter
OTC: over the counter
PAF: platelet-activating factor
PG: prostaglandin
PGI_2: prostacyclin
PPI: proton pump inhibitor
r: receptor
TNF: tumor necrosis factor
Tx: thromboxane
SLC5A8: Solute Carrier Family 5 Member 8
SLC5A12: Solute Carrier Family 5 Member 12
UGT: uridine diphosphate glucuronosyltransferase
URAT: urate transporter
XO: xanthine oxidase

(e.g., hypersensitivity, autoimmune diseases, chronic inflammation). The inflammatory response is characterized mechanistically by:

- Transient local vasodilation and increased capillary permeability
- Infiltration of leukocytes and phagocytic cells
- Resolution with or without tissue degeneration and fibrosis

Many molecules are involved in the promotion and resolution of the inflammatory process. Histamine, bradykinin, 5HT, prostanoids, LTs, PAF, and an array of cytokines are important mediators (see Chapters 38, 41, and 43). Prostanoid biosynthesis is significantly increased in inflamed tissue. PGE_2 and prostacyclin (PGI_2) are the primary prostanoids that mediate inflammation. They increase local blood flow, vascular permeability, and leukocyte infiltration through activation of their respective receptors, EPr and IPr. PGD_2, a major product of mast cells, contributes to inflammation in allergic responses, particularly in the lung.

Activation of endothelial cells plays a key role in recruiting circulating cells to inflammatory sites (Muller, 2011). Endothelial activation results in leukocyte rolling and adhesion as the leukocytes recognize newly expressed selectins, integrins, and adhesion molecules. PGE_2 and TxA_2 enhance leukocyte chemoattraction and endothelial adhesion.

The recruitment of inflammatory cells to sites of injury also involves the concerted interactions of the complement factors, PAF, and eicosanoids such as LTB_4 (see Chapter 41). All can act as chemotactic agonists. Cytokines play essential roles in orchestrating the inflammatory process, especially TNF and IL-1. Several biological anti-inflammatory therapeutics target these cytokines or their signaling pathways (see Chapter 39). Other cytokines and growth factors (e.g., IL-2, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor) contribute to manifestations of the inflammatory response. The concentrations of many of these factors are increased in the synovia of patients with inflammatory arthritis. Glucocorticoids interfere with the synthesis and actions of cytokines, such as IL-1 or TNF-α (see Chapter 39). Although some of the actions of these cytokines are accompanied by the release of PGs and TxA_2, COX inhibitors appear to block primarily their pyrogenic effects.

Pain

Nociceptors, peripheral terminals of primary afferent fibers that sense pain, can be activated by various stimuli, such as heat, acids, or pressure. Inflammatory mediators released from nonneuronal cells during tissue injury increase the sensitivity of nociceptors and potentiate pain perception. Among these mediators are bradykinin, H^+, 5HT, ATP, neurotrophins (nerve growth factor), LTs, and PGs. PGE_2 and PGI_2 reduce the threshold to stimulation of nociceptors, causing *peripheral sensitization*. Reversal of peripheral sensitization is thought to represent the mechanistic basis for the peripheral component of the analgesic activity of NSAIDs. NSAIDs may also have important central actions in the spinal cord and brain. Both COX-1 and COX-2 are expressed in the spinal cord under basal conditions and release PGs in response to peripheral pain stimuli.

Centrally active PGE_2 and perhaps also PGD_2, PGI_2, and $PGF_{2\alpha}$ contribute to *central sensitization*, an increase in excitability of spinal dorsal horn neurons that causes hyperalgesia and allodynia in part by disinhibition of glycinergic pathways (Grosser et al., 2017a). Central sensitization reflects the plasticity of the nociceptive system that is invoked by injury. This usually is reversible within hours to days following adequate responses of the nociceptive system (e.g., in postoperative pain). However, chronic inflammatory diseases may cause persistent modification of the architecture of the nociceptive system, which may lead to long-lasting changes in its responsiveness. These mechanisms contribute to chronic pain.

Fever

The hypothalamus regulates the set point at which body temperature is maintained. This set point is elevated in fever, reflecting an infection, or resulting from tissue damage, inflammation, graft rejection, or malignancy. These conditions all enhance formation of cytokines such as IL-1β, IL-6, TNF-α, and interferons, which act as endogenous pyrogens. The initial phase of the thermoregulatory response to such pyrogens may be mediated by ceramide release in neurons of the preoptic area in the anterior hypothalamus (Sanchez-Alavez et al., 2006). The second phase is mediated by coordinate induction of COX-2 and formation of PGE_2 (Engblom et al., 2003). PGE_2 can cross the blood-brain barrier and act on EPr_3 and perhaps EPr_1 receptors on thermosensitive neurons. This triggers the hypothalamus to elevate body temperature by promoting an increase in heat generation and a decrease in heat loss. NSAIDs suppress this response by inhibiting COX-2–dependent PGE_2 synthesis.

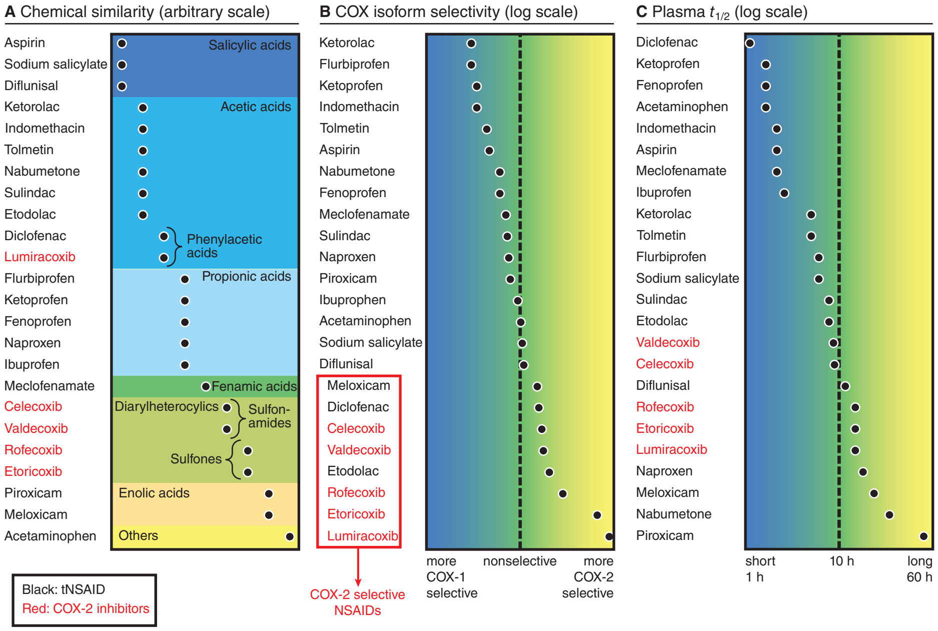


Figure 42–1 *Classification of NSAIDs by chemical similarity* (**A**), *COX isoform selectivity* (**B**), *and plasma* $t_{1/2}$ (**C**). The COX selectivity chart is plotted from data published in Warner T., et al. Nonsteroid drug selectivities for cyclooxygenase-1 rather than cyclooxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. *Proc Natl Acad Sci USA*, **1999**, *96*:7563–7568; and FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. *N Engl J Med*, **2001**, *345*:433–442.

Nonsteroidal Anti-inflammatory Drugs

The NSAIDs are mechanistically classified as *isoform nonselective NSAIDs*, which inhibit both COX-1 and COX-2, and *COX-2–selective NSAIDs* (FitzGerald and Patrono, 2001). Most NSAIDs are competitive, noncompetitive, or mixed reversible inhibitors of the COX enzymes. *Aspirin* (ASA) is a noncompetitive, irreversible inhibitor because it acetylates the isozymes in the hydrophobic AA-binding channel by which the lipid substrate gains access to the COX catalytic site deep within the enzyme. *Acetaminophen*, which is antipyretic and analgesic but largely devoid of anti-inflammatory activity at commonly used doses, acts as a noncompetitive reversible inhibitor by reducing the peroxide site of the enzymes.

The majority of NSAIDs are organic acids with relatively low pK_a values. As organic acids, the compounds generally are well absorbed orally, highly bound to plasma proteins, and excreted either by glomerular filtration or by tubular secretion. They also accumulate in sites of inflammation, where the pH is lower, potentially confounding the relationship between plasma concentrations and duration of drug effect. Most COX-2–selective NSAIDs have a relatively bulky side group, which aligns with a large side pocket in the AA-binding channel of COX-2 but hinders its optimal orientation in the smaller binding channel of COX-1 (Smith et al., 2011). Both isoform nonselective NSAIDs and the COX-2–selective NSAIDs generally are hydrophobic drugs, a feature that allows them to access the AA-binding channel and results in shared pharmacokinetic characteristics. Again, *aspirin* and *acetaminophen* are exceptions to this rule.

Mechanism of Action

Cyclooxygenase Inhibition

The principal therapeutic effects of NSAIDs derive from their ability to inhibit PG production. The first enzyme in the PG synthetic pathway is COX, also known as PG G/H synthase. This enzyme converts AA to the unstable intermediates PGG_2 and PGH_2 and leads to the production of the prostanoids, TxA_2, and a variety of PGs (see Chapter 41). COX-1, expressed constitutively in most cells, is the dominant source of prostanoids for housekeeping functions, such as hemostasis. Conversely, COX-2, induced by cytokines, shear stress, and tumor promoters, is the more important source of prostanoid formation in inflammation and perhaps in cancer (see Chapter 41). However, both enzymes contribute to the generation of autoregulatory and homeostatic prostanoids with important functions in normal physiology (see Chapter 41). The indiscriminate inhibition of both inflammatory and homeostatic prostanoids by NSAIDs explains mechanistically most adverse reactions to this drug class. For example, inhibition of COX-1 accounts largely for the gastric adverse events and bleeding that complicate therapy because COX-1 is the dominant cytoprotective isoform in gastric epithelial cells and forms TxA_2 in platelets, which amplifies platelet activation and constricts blood vessels at the site of injury. Similarly, COX-2–derived products play important roles in blood pressure regulation and act as endogenous inhibitors of hemostasis. Inhibition of COX-2 can cause or exacerbate hypertension and increase the likelihood of thrombotic events.

While the functional COX enzymes are sequence homodimers, they are configured as conformational heterodimers in which one of the monomers functions as the catalytic subunit with heme bound and the other, without heme, serves as the allosteric subunit. Most NSAIDs inhibit the catalytic subunits of COX-1 and COX-2. However, COX-2 inhibition by *naproxen* and *flurbiprofen* occurs primarily on the allosteric subunit (Dong et al., 2011; Zou et al., 2012).

Irreversible Cyclooxygenase Inhibition by Aspirin

Aspirin covalently acetylates the catalytic subunits of the COX-1 and COX-2 dimers, irreversibly inhibiting COX activity. This is an important distinction from all the other NSAIDs because the duration of *aspirin*'s effects is related to the turnover rate of the COXs in different target tissues.

The importance of enzyme turnover in recovery from *aspirin* action is most notable in platelets, which, being anucleate, have a markedly limited capacity for protein synthesis. Thus, the consequences of inhibition of platelet COX-1 last for the lifetime of the platelet. Inhibition of platelet COX-1–dependent TxA_2 formation therefore is cumulative with repeated doses of *aspirin* (at least as low as 30 mg/day) and takes 8 to 12 days (the platelet turnover time) to recover fully once therapy has been stopped. Importantly, even a partially recovered platelet pool—just a few days after the last *aspirin* dose—may afford recovery of sufficient hemostatic integrity for some types of elective surgery to be performed.

However, such a partial platelet function also may predispose non-adherent patients on low-dose *aspirin* for antiplatelet therapy to thrombotic events. The unique sensitivity of platelets to inhibition by low doses of *aspirin* is related to their presystemic inhibition in the portal circulation before *aspirin* is deacetylated to salicylate on first pass through the liver (Pedersen and FitzGerald, 1984). In contrast to *aspirin*, salicylic acid has no acetylating capacity. It is a relatively weak, reversible inhibitor of COX. Salicylic acid derivates, rather than the acid, are available for clinical use.

The COXs are configured such that the active site is accessed by the AA substrate via a hydrophobic channel. *Aspirin* acetylates serine 529 of COX-1, located high up in the hydrophobic channel. Interposition of the bulky acetyl residue prevents the binding of AA to the active site of the enzyme and thus impedes the ability of the enzyme to make PGs. *Aspirin* acetylates a homologous serine at position 516 in COX-2. Although covalent modification of COX-2 by *aspirin* also blocks the COX activity of this isoform, an interesting property not shared by COX-1 is that acetylated COX-2 synthesizes 15(*R*)-HETE. This may be metabolized, at least *in vitro*, by 5-LOX to yield 15-epi-lipoxin A_4, which has anti-inflammatory properties in model systems (see Chapter 41).

Selective Inhibition of Cyclooxygenase 2

The chronic use of the NSAIDs is limited by their poor GI tolerability. Selective inhibitors of COX-2 were developed to afford efficacy like traditional NSAIDs with better GI tolerability (FitzGerald and Patrono, 2001). Six such COX-2 inhibitors, the coxibs, were approved for clinical use: *celecoxib, rofecoxib, valdecoxib* (initially approved in the U.S.) and its prodrug *parecoxib*, *etoricoxib*, and *lumiracoxib*. Most coxibs (including *valdecoxib*) have been either restricted in their use or withdrawn from the market in view of their adverse cardiovascular risk profile (Grosser et al., 2010). *Celecoxib* currently is the only COX-2 inhibitor licensed for use in the U.S. Some older NSAID compounds—*diclofenac*, *etodolac*, *meloxicam*, and *nimesulide* (not available in the U.S.)—exhibit selectivity for COX-2 similar to *celecoxib* (see Figure 42–1).

ADME

Absorption. The NSAIDs are rapidly absorbed following oral ingestion, and peak plasma concentrations are reached within 2 to 3 h. The poor aqueous solubility of most NSAIDs often is reflected by a less-than-proportional increase in the AUC of plasma concentration–time curves, due to incomplete dissolution, when the dose is increased. Food intake may delay absorption and systemic availability (i.e., *fenoprofen*, *sulindac*). Antacids, commonly prescribed to patients on NSAID therapy, variably delay absorption. Some compounds (e.g., *diclofenac*, *nabumetone*) undergo first-pass or presystemic elimination. *Aspirin* begins to acetylate platelets within minutes of reaching the presystemic circulation (Pedersen and FitzGerald, 1984).

Distribution. Most NSAIDs are extensively bound (95%–99%) to plasma proteins, usually albumin. Conditions that alter plasma protein concentration may result in an increased free drug fraction with potential toxic effects. Highly protein bound NSAIDs have the potential to displace other drugs if they compete for the same binding sites. Most NSAIDs are distributed widely throughout the body and readily penetrate arthritic joints, yielding synovial fluid concentrations in the range of half the plasma concentration (i.e., *ibuprofen, naproxen, piroxicam*) (Day et al., 1999). Most NSAIDs achieve sufficient concentrations in the CNS to have a central analgesic effect. *Celecoxib* is particularly lipophilic and moves readily into the CNS. Multiple NSAIDs are marketed in formulations for topical application on inflamed or injured joints. However, direct transport of topically applied NSAIDs into inflamed tissues and joints appears to be minimal, and detectable concentrations in synovial fluid of some agents (i.e., *diclofenac*) following topical use are primarily attained via dermal absorption and systemic circulation. Methods designed to enhance transdermal delivery into inflamed joints, such as iontophoresis, electroporation, or chemical penetration enhancers and nanoparticulate formulations, are under investigation. Other targeted applications (e.g., to macrophages) are also being explored as a delivery route for NSAIDs (Mazaleuskaya et al., 2021).

Metabolism and Excretion. Hepatic biotransformation, often via cytochrome P450 isoforms (CYPs) 2C9, 1A2, and 3A4, and renal excretion are the principal routes of metabolism and elimination of the majority of NSAIDs. Plasma $t_{1/2}$ varies considerably among NSAIDs. *Ibuprofen*, *diclofenac*, and *acetaminophen* have a $t_{1/2}$ of 1 to 4 h, while *piroxicam* has a $t_{1/2}$ of about 50 h at steady state. *Naproxen* has a comparatively long but highly variable $t_{1/2}$ ranging from 9 to 25 h. Elimination pathways frequently involve oxidation or hydroxylation. *Acetaminophen*, at therapeutic doses, is oxidized only to a small degree to form traces of the highly reactive metabolite NAPQI. Following overdose, however, the principal metabolic pathways are saturated, and hepatotoxic NAPQI concentrations can be formed (see Figure 9–4).

Several NSAIDs or their metabolites are glucuronidated or otherwise conjugated. In some cases, such as the propionic acid derivatives *naproxen* and *ketoprofen*, the glucuronide metabolites can hydrolyze back to form the active parent drug; this may prolong elimination of the NSAID significantly. In general, NSAIDs are not recommended in the setting of advanced hepatic or renal disease due to their adverse pharmacodynamic effects. NSAIDs usually are not removed by hemodialysis due to their extensive plasma protein binding; salicylic acid is an exception to this rule. The activity of the major metabolizing enzymes is influenced by genetic variation, age, sex, circadian variation, disease, social behavior such as smoking or drinking, and interacting drugs that are CYP substrates, inhibitors, or inducers. Variation in the composition of intestinal microbiota may also contribute to variability in metabolism and elimination (Liang et al., 2015). Clinical evidence linking genetic variation in metabolizing enzymes to an increased rate of adverse events with NSAIDs use is limited. However, an association between *CYP2C9* alleles that decrease enzymatic function and elevated NSAID plasma concentrations has been established. Carriers of these variants should consider reductions in the starting NSAID dose or selection of NSAIDs with shorter half-lives to reduce the likelihood of adverse effects (Theken et al., 2020).

Therapeutic Uses

The NSAIDs are antipyretic, analgesic, and anti-inflammatory, with the exception of *acetaminophen*, which is antipyretic and analgesic but is largely devoid of anti-inflammatory activity at therapeutic doses.

Inflammation

The NSAIDs provide mostly symptomatic relief from pain and inflammation associated with musculoskeletal disorders, such as rheumatoid arthritis and osteoarthritis. Some NSAIDs are approved for the treatment of ankylosing spondylitis and gout. Patients with more debilitating disease may not respond adequately to full therapeutic doses of NSAIDs and may require aggressive therapy with second-line agents.

Pain

The NSAIDs are effective against inflammatory pain of low to moderate intensity. Although their maximal efficacy is generally less than that of opioids, NSAIDs lack the unwanted adverse effects of opiates in the CNS, including respiratory depression and the potential for development of physical dependence. Coadministration of NSAIDs can reduce the opioid dose needed for sufficient pain control with the objective of reducing the adverse effects of opioids. NSAIDs, unlike opioids, are devoid of addictive potential. NSAIDs do not change the perception of sensory modalities other than pain. NSAIDs are particularly effective when inflammation has caused sensitization of pain perception (see other discussion in this section on inflammation, pain, and fever). Thus, postoperative pain or pain arising from inflammation, such as arthritic pain, is controlled well by NSAIDs, whereas pain arising from the hollow viscera usually is not relieved. An exception to this is menstrual pain. Treatment of menstrual pain with NSAIDs has been met with considerable success because cramps and other symptoms of primary dysmenorrhea are caused by the release of PGs by the endometrium during menstruation. NSAIDs are commonly used to treat migraine attacks and can be combined with drugs such as the triptans or antiemetics to aid relief from the associated nausea. NSAIDs generally lack efficacy in neuropathic pain.

Fever

Antipyretic therapy is reserved for patients in whom fever may be deleterious and for those who experience considerable relief when fever is lowered. NSAIDs reduce fever in most situations, but not the circadian variation in temperature or the rise in response to exercise or increased ambient temperature.

Fetal Circulatory System

The PGs are implicated in the maintenance of patency of the ductus arteriosus; *indomethacin*, *ibuprofen*, and other NSAIDs have been used in neonates to close an inappropriately patent ductus. Conversely, infusion of prostanoid analogues maintains ductal patency after birth in settings of ductus arteriosus–dependent congenital heart disease as a bridge to definitive repair (see Chapter 41).

Cardioprotection

Ingestion of *aspirin* prolongs bleeding time. This effect is due to irreversible acetylation of platelet COX and the consequent inhibition of platelet function. It is the permanent suppression of platelet TxA_2 formation that is thought to underlie the cardioprotective effect of *aspirin*.

Aspirin reduces the risk of serious vascular events in high-risk patients (e.g., those with previous myocardial infarction) by 20% to 25%. The reduction of subsequent thrombotic strokes is somewhat less, roughly 10% to 15% (Antithrombotic Trialists' Collaboration et al., 2009). Low-dose *aspirin* (≤100 mg/day) is associated with a lower risk for GI adverse events than higher doses (e.g., 325 mg/day) and is often used following percutaneous coronary intervention (Xian et al., 2015). Low doses of *aspirin* are associated with a small (roughly 2-fold) but detectable increase in the incidence of serious GI bleeds and intracranial bleeds in placebo-controlled trials. The benefit from *aspirin*, however, outweighs these risks in the case of secondary prevention of cardiovascular disease. The issue is much more nuanced in patients who have never had a serious atherothrombotic event (primary prevention); here, prevention of myocardial infarction by *aspirin* is numerically balanced by the serious GI bleeds it precipitates (Patrono, 2015). Recent randomized clinical trials of *aspirin* in primary cardioprevention failed to detect a beneficial effect, perhaps obscured by other cardioprotective treatments, while the GI bleeding risk remains apparent (Ricciotti and FitzGerald, 2021). Given their relatively short $t_{1/2}$ and reversible COX inhibition, most other NSAIDs are not thought likely to afford cardioprotection. Cardioprotection is lost when combining low-dose *aspirin* with other short acting NSAIDs like *ibuprofen* through a drug-drug interaction at the *aspirin* target site in platelet COX-1 (Catella-Lawson et al., 2001; Farkouh et al., 2004; Li et al., 2014). COX-2–selective NSAIDs are devoid of antiplatelet activity, as mature platelets do not express COX-2.

Other Clinical Uses

Systemic Mastocytosis. Systemic mastocytosis is a condition in which there are excessive mast cells in the bone marrow, reticuloendothelial system, GI system, bones, and skin (Theoharides et al., 2015). In patients with systemic mastocytosis, PGD_2, released from mast cells, is the major mediator of severe episodes of flushing, vasodilation, and hypotension; this effect of PGD_2 is resistant to antihistamines. The addition of *aspirin* or *ketoprofen* (off-label use) may be beneficial in patients with high levels of urinary PGD metabolites who have flushing and angioedema. However, NSAIDs can cause degranulation of mast cells, so blockade with histamine receptor antagonists should be established before NSAIDs are initiated.

Niacin Tolerability. Large doses of niacin (nicotinic acid) effectively lower serum cholesterol levels, reduce low-density lipoprotein, and raise high-density lipoprotein (see Chapter 37). However, niacin induces intense facial flushing mediated largely by release of PGD_2 from the skin, which can be inhibited by treatment with *aspirin* (Song et al., 2012).

Bartter Syndrome. Bartter syndrome includes a series of rare disorders (frequency ≤1/100,000 persons) characterized by hypokalemic, hypochloremic metabolic alkalosis with normal blood pressure, and hyperplasia of the juxtaglomerular complex. Fatigue, muscle weakness, diarrhea, and dehydration are the main symptoms. Distinct variants are caused by mutations in a Na^+-K^+-$2Cl^-$ cotransporter, an apical ATP-regulated K^+ channel, a basolateral Cl^- channel, a protein (barttin) involved in cotransporter trafficking, and the extracellular Ca^{2+}-sensing receptor. Renal COX-2 is induced, and biosynthesis of PGE_2 is increased. Treatment with *indomethacin*, combined with potassium repletion and *spironolactone*, is associated with improvement in the biochemical derangements and symptoms. Selective COX-2 inhibitors also have been used (Nusing et al., 2001).

Preeclampsia. A syndrome characterized by hypertension and proteinuria during pregnancy, preeclampsia is a leading cause of maternal and perinatal morbidity and mortality. Low-dose *aspirin* (81 or 100 mg) reduces the incidence of preterm preeclampsia effectively when administered early to women at increased risk (Rolnik et al., 2020). Platelet activation through endothelial inflammation and renal dysregulation of blood pressure are proposed targets of low-dose *aspirin* in preeclampsia (Burton et al., 2019). The antihypertensive and antiplatelet effects of *aspirin* may be most pronounced when dosed in the evening (Bonten et al., 2015; Chen et al., 2018; Hermida et al., 2003).

Cancer Chemoprevention. Epidemiological studies suggested that daily use of *aspirin* is associated with a 24% decrease in the incidence of and a 35% decrease in mortality from sporadic colon cancer (Rothwell et al., 2010). Randomized controlled trials provide direct evidence for a beneficial effect of *aspirin* in the primary prevention of colorectal cancer in patients at high risk due to the Lynch syndrome, but only weak evidence in patients with familial adenomatous polyposis (FAP; Ricciotti and FitzGerald, 2021). COX-2–selective NSAIDs have shown efficacy in patients with FAP but are no longer approved for this indication due to cardiovascular safety concerns.

Adverse Effects of NSAID Therapy

Adverse events common to *aspirin* and NSAIDs are outlined in Table 42–1. To minimize potential adverse events of NSAIDs, the lowest effective dose should be used for the shortest feasible length of time. Age generally is correlated with an increased probability of developing serious adverse reactions to NSAIDs, and caution is warranted in choosing a lower starting dose for elderly patients. NSAIDs are labeled with a black-box warning related to cardiovascular risks and are specifically contraindicated following coronary artery bypass graft (CABG) surgery.

Gastrointestinal

The most common symptoms associated with these drugs are GI (~40% of patients), including dyspepsia, abdominal pain, anorexia, nausea, and diarrhea. However, these symptoms are not predictive of gastric or intestinal lesions such as subepithelial hemorrhages, erosions, and ulcers, which can be endoscopically detected in about 30% to 50% of NSAID users but are often asymptomatic and tend to heal spontaneously. Serious complications—bleeding, perforation, or obstruction—occur at an annual rate of 1% to 2% in regular NSAID users. Many patients who develop a serious upper GI adverse event while receiving NSAID therapy are asymptomatic prior to diagnosis. The risk is particularly high in those with *Helicobacter pylori* infection, heavy alcohol consumption, or other risk factors for mucosal injury, including the concurrent use of glucocorticoids. All selective COX-2 inhibitors are less prone to induce gastric ulcers than equally efficacious doses of isoform nonselective NSAIDs (Sostres et al., 2013).

Several mechanisms contribute to NSAID-induced GI complications (see Chapter 41). Inhibition of COX-1 in gastric and intestinal epithelial cells depresses mucosal cytoprotective PGs, especially PGI_2 and PGE_2. These eicosanoids inhibit acid secretion by the stomach, enhance mucosal blood flow, and promote the secretion of cytoprotective mucus in the intestine. COX-2 also contributes to constitutive formation of these PGs by human gastric epithelium, and products of COX-2 may contribute to ulcer healing. Another factor that may play a part in the formation of ulcers is the local irritation from contact of orally

TABLE 42–1 ■ SOME SHARED ADVERSE EFFECTS OF NSAIDs[a]

SYSTEM	MANIFESTATIONS
Gastrointestinal	Abdominal pain, bleeding, constipation, diarrhea, dyspepsia, dysphagia, eructation,[b] esophageal stricture/ulceration, esophagitis, flatulence, gastritis, hematemesis,[b] melena,[b] nausea, odynophagia, perforation, pyrosis, stomatitis, ulcers, vomiting, xerostomia[b]
Platelets	Inhibited platelet activation,[b] propensity for bruising,[b] increased risk of hemorrhage,[b] platelet dysfunction,[b] thrombocytopenia[b]
Renal	Azotemia,[b] cystitis,[b] dysuria,[b] hematuria, hyponatremia, interstitial nephritis, nephrotic syndrome,[b] oliguria,[b] polyuria,[b] renal failure, renal papillary necrosis, proteinuria, salt and water retention, hypertension, worsening of renal function in renal/cardiac/cirrhotic patients, effectiveness of antihypertensives and diuretics, hyperkalemia,[b] ↓ urate excretion (especially with aspirin)
Cardiovascular	Edema,[b] heart failure,[c] hypertension, MI,[c] palpitations,[b] premature closure of ductus arteriosus, sinus tachycardia,[b] stroke,[c] thrombosis,[c] vasculitis[b]
Neurologic	Anorexia,[b] anxiety,[b] aseptic meningitis, confusion,[b] depression, dizziness, drowsiness,[b] headache, insomnia,[b] malaise,[b] paresthesias, tinnitus, seizures,[b] syncope,[b] vertigo[b]
Reproductive	Prolongation of gestation, inhibition of labor, delayed ovulation
Hypersensitivity	Anaphylactoid reactions, angioedema, severe bronchospasm, urticaria, flushing, hypotension, shock
Hematologic	Anemia, agranulocytosis, aplastic anemia,[b] hemolytic anemia,[b] leukopenia[b]
Hepatic	Elevated enzymes, hepatitis, hepatic failure,[b] jaundice
Dermatologic	Diaphoresis,[b] exfoliative dermatitis, photosensitivity,[b] pruritus, purpura,[b] rash, Stevens-Johnson syndrome, toxic epidermal necrolysis, urticaria
Respiratory	Dyspnea,[b] hyperventilation (salicylates)
Other	Alopecia,[b] blurred vision,[b] conjunctivitis,[b] epistaxis,[b] fever,[b] hearing loss,[b] pancreatitis,[b] paresthesias, visual disturbance,[b] weight gain[b]

[a] Refer to product label for specific information.
[b] Reported for most, but not all, NSAIDs.
[c] With the exception of low-dose aspirin.

administered NSAIDs—most of which are organic acids—with the cell surface phospholipid bilayer of the gastric and intestinal mucosa. Such irritation may compromise the hydrophobic lining of the gastroduodenal mucosa, making it vulnerable to subsequent damage from gastric acid. NSAIDs may also cause mitochondrial and endoplasmic reticulum damage in epithelial cells (Bjarnason et al., 2018). However, the incidence of serious GI adverse events is not significantly reduced by formulations devised to limit drug contact with the gastric mucosa, such as enteric coating or efferent solutions, suggesting that the contribution of direct irritation to the overall risk is temporary. Platelet inhibition by NSAIDs increases the likelihood of bleeds when mucosal damage has occurred. In the small intestine, NSAIDs can change the composition and activity of the gut microbiota. These changes may alter the disposition and the pharmacokinetic proprieties of the NSAIDs and their efficacy and toxicity profiles (Liang et al., 2015). NSAID-gut microbiota interactions may contribute to the development of NSAID-induced enteropathy and, to lesser extent, to NSAID exacerbation of *Clostridium difficile* infection. There is less evidence that the gut microbiota modulates *aspirin*'s antiplatelet and anticancer effects (Bjarnason et al., 2018).

Coadministration of proton pump inhibitors or H$_2$ antagonists in conjunction with NSAIDs reduces the rate of duodenal and gastric ulceration (see Chapter 53, Figure 53–1). However, proton pump inhibitors, which are also metabolized by CYP2C9 and liable to interact with NSAIDs, may increase the risk of damage in the small intestine by inducing dysbiosis (Washio et al., 2016). Approaches to preventing or mitigating NSAID-induced small intestinal damage, for example by administration of poorly absorbable antibiotics, mucosal protective agents, or supplementation with probiotics, are under investigation.

Cardiovascular

The COX-2–selective NSAIDs were developed to improve GI safety. However, COX-2 inhibitors depress formation of PGI$_2$ but do not inhibit the COX-1–catalyzed formation of platelet TxA$_2$. PGI$_2$ inhibits platelet aggregation and constrains the effect of prothrombotic and atherogenic stimuli by TxA$_2$ (Grosser et al., 2006, 2010, 2017a), and renal PGI$_2$ and PGE$_2$ formed by COX-2 contribute to arterial pressure homeostasis (see Chapter 41). Genetic deletion of the PGI$_2$ receptor (IPr) in mice augments the thrombotic response to endothelial injury, accelerates experimental atherogenesis, increases vascular proliferation, and adds to the effect of hypertensive stimuli (Cheng et al., 2002, 2006; Egan et al., 2004; Kobayashi et al., 2004). Tissue-specific genetic deletion of COX-2 in the vasculature accelerates the response to thrombotic stimuli and raises blood pressure (Yu et al., 2012). Together, these mechanisms would be expected to increase the cardiovascular risk of humans by shifting to a prothrombotic environment on endothelial surfaces, as COX-2 inhibition in humans depresses PGI$_2$ synthesis (Catella-Lawson et al., 1999; McAdam et al., 1999). Indeed, a human mutation of the IPr that disrupts its signaling may be associated with increased cardiovascular risk (Arehart et al., 2008).

Clinical trials—with *celecoxib*, *valdecoxib* (withdrawn), and *rofecoxib* (withdrawn)—revealed an increase in the incidence of myocardial infarction, stroke, and vascular death by approximately 1.4-fold (Coxib and Traditional NSAID Trialists' Collaboration et al., 2013). The risk extends to *diclofenac*, which is almost as COX-2 selective as *celecoxib*, and to some of the other older NSAIDs. An exception in some individuals may be *naproxen*. There is considerable between-person variation in the $t_{1/2}$ of *naproxen*, and platelet inhibition might be anticipated throughout the dosing interval in some, but not all, individuals on *naproxen* (Capone et al., 2005; Grosser et al., 2017b). While this is supported by randomized controlled trials (Coxib and Traditional NSAID Trialists' Collaboration et al., 2013), identifying individuals who fall into the long-acting group is currently not practical in clinical routine. The FDA has determined that the data differentiating the risk between distinct NSAIDs is not sufficient to distinguish between drugs on the regulatory level; thus, a cardiovascular risk warning is included on the label of all NSAIDs (FDA, 2015). The Standard Care versus Celecoxib Outcome Trial (SCOT) (MacDonald et al., 2017) and the Prospective Randomized Evaluation of Celecoxib Integrated Safety versus Ibuprofen or Naproxen (PRECISION; Nissen et al., 2016) trial, mandated by the European Medicines Agency and the FDA, respectively, to compare the cardiovascular profile of *celecoxib* to traditional NSAIDs, failed to resolve this issue due to the limitations in their study designs (FitzGerald, 2017; Grosser et al., 2017b). Similarly, all prescription NSAIDs share a black-box warning contraindicating their use for the treatment of perioperative pain in the setting of CABG surgery.

The NSAIDs with selectivity for COX-2 should be reserved for patients at high risk for GI complications. The cardiovascular risk appears to be conditioned by factors influencing drug exposure, such as dose, $t_{1/2}$, degree of COX-2 selectivity, potency, and treatment duration. Thus, the lowest possible dose should be prescribed for the shortest possible period.

NSAIDs increase the risk of heart failure and may exacerbate preexisting heart failure, particularly at high doses (Arfé et al., 2016). Mechanisms may involve fluid retention and blood pressure increases caused by renal effects of NSAIDs and potentially direct effects on the myocardium and vasculature.

Blood Pressure and Renal Adverse Events

All NSAIDs have been associated with renal and renovascular adverse events including renal failure. Up to 5% of regular NSAID users can be expected to develop hypertension. Hypertensive complications may occur more commonly in patients treated with COX-2–selective than with nonselective NSAIDs. Heart failure risk is roughly doubled.

The NSAIDs have little effect on renal function or blood pressure in healthy human subjects because of the redundancy of systems that regulate renal function. In situations that challenge the regulatory systems, such as dehydration, hypovolemia, volume depletion from diuretics, congestive heart failure, hepatic cirrhosis, chronic kidney disease, and other states of activation of the sympathoadrenal or renin-angiotensin systems, regulation of renal function by PG formation becomes crucial (see Chapter 41). NSAIDs impair the PG-induced inhibition of both the reabsorption of Cl^- and the action of antidiuretic hormone, which may result in the retention of salt and water resulting in edema. Inhibition of COX-2–derived PGs that contribute to the regulation of renal medullary blood flow may lead to a rise in blood pressure, increasing the risk of cardiovascular thrombotic events and heart failure. NSAIDs promote reabsorption of K^+ because of decreased availability of Na^+ at distal tubular sites and suppression of the PG-induced secretion of renin. While the last effect may account in part for the usefulness of NSAIDs in the treatment of Bartter syndrome (see Bartter Syndrome section, above), hyperkalemia is a potential complication of NSAIDs, particularly with concurrent use of other drugs that raise potassium levels.

Renal Failure. Depression of afferent and medullary renal blood flow by inhibition of vasodilator PGs can lead to ischemic episodes and acute kidney injury including acute tubular necrosis. NSAID-induced acute kidney injury can occur during chronic use, but it is typically characterized by an increase in plasma creatinine in the absence of major proteinuria following initiation of therapy (Zhang et al., 2017). This has proven a particular risk in older individuals and professional athletes. If recognized early, discontinuation of NSAIDs permits recovery of renal function. A form of more subacute nephrotoxicity induced by NSAID-triggered immune mechanisms is acute interstitial nephritis. Analgesic nephropathy is a condition of slowly progressive renal failure characterized by papillary necrosis and chronic interstitial nephritis. It has become rare since *phenacetin* was removed from the market.

Pregnancy

Myometrial COX-2 expression and levels of PGE_2 and $PGF_{2\alpha}$ increase markedly in the myometrium during labor. Prolongation of gestation by NSAIDs has been demonstrated in humans. Some NSAIDs, particularly *indomethacin*, have been used off label to stop preterm labor. However, this use is associated with closure of the ductus arteriosus and impaired fetal circulation *in utero*, particularly in fetuses older than 32 weeks of gestation. COX-2–selective inhibitors have been used off label as tocolytic agents; this use has been associated with stenosis of the ductus arteriosus and oligohydramnios.

Hypersensitivity

Pseudo-allergic and allergic reactions to NSAIDs are among the most frequent hypersensitivity drug reactions observed in clinical practice. Pseudo-allergic reactions are thought to involve inhibition of PGE_2 formation and diversion of eicosanoid precursors to alternative biosynthetic pathways—particularly the 5-lipoxygenase pathway resulting in the formation of cysteinyl leukotrienes—when COX-1 is inhibited (Cahill and Boyce, 2017). Ensuing mast cell and eosinophil activation underlie various phenotypes in susceptible individuals (Blanca-Lopez et al., 2019). NSAID-exacerbated respiratory disease (NERD) is characterized by symptoms ranging from vasomotor rhinitis and bronchial constriction to angioedema and generalized urticaria. Patients with asthma and chronic rhinosinusitis often including nasal polyps are predisposed to developing NERD shortly after initiation of NSAID treatment. Urticaria and angioedema are the major symptoms in NSAID-exacerbated cutaneous disease in patients with underlying chronic urticaria and NSAID-induced urticaria/angioedema in patients without chronic urticaria. Pseudo-allergic hypersensitivity is a contraindication to therapy with any other NSAID because of a high probability of cross sensitivity. Allergic hypersensitivity reactions to NSAIDs are induced by specific IgE- or T-cell–dependent immunologic mechanisms and are more restricted to individual or chemically similar NSAIDs. They may present with cutaneous and respiratory symptoms, hypotension, and anaphylaxis. Although less common in children, hypersensitivity reactions to NSAIDs may occur in 10% to 25% of patients with asthma, nasal polyps, or chronic urticaria and in 1% of apparently healthy individuals. Hypersensitivity can be provoked by even low doses (<80 mg) of *aspirin*. Treatment is like that of other hypersensitivity reactions, including support of vital organ function and administration of *epinephrine* in severe cases.

Aspirin Resistance

All forms of treatment failure with *aspirin* have been collectively called *aspirin resistance*, but pharmacological resistance to *aspirin* is rare. Pseudo-resistance, reflecting delayed and reduced drug absorption, complicates enteric-coated but not immediate-release *aspirin* administration (Grosser et al., 2013).

Hepatotoxicity

Liver injury occurs in 17% of adults with unintentional *acetaminophen* overdose (Blieden et al., 2014). Liver toxicity from therapeutic doses of *acetaminophen* is extremely rare (see Acetaminophen section). By contrast, therapeutic dosing of *diclofenac* (or *lumiracoxib*, a *diclofenac* analogue) may be complicated by hepatotoxicity. While the entire class of NSAIDs has a rate of less than 1 liver injury per 100,000 patients on average, chronic consumption of *diclofenac* is associated with a risk of 6 to 11 liver injuries per 100,000 users (Bjornsson et al., 2013; de Abajo et al., 2004) (see Diclofenac section). Mechanisms of hepatotoxicity include hypersensitivity reactions and metabolic aberrations, which are likely influenced by genetic susceptibility. NSAIDs are not recommended in advanced hepatic or renal disease.

Reye Syndrome

Due to the possible association with Reye syndrome, *aspirin* and other salicylates are contraindicated in children and young adults less than 20 years of age with viral illness–associated fever (Schrör, 2007). Reye syndrome, a severe and often fatal disease, is characterized by the acute onset of encephalopathy, liver dysfunction, and fatty infiltration of the liver and other viscera. Although a mechanistic understanding is lacking, the epidemiologic association between *aspirin* use and Reye syndrome is sufficiently strong that *aspirin* and *bismuth subsalicylate* labels indicate the risk. As the use of *aspirin* in children has declined dramatically, so has the incidence of Reye syndrome. *Acetaminophen* and *ibuprofen* have not been implicated in Reye syndrome and are the agents of choice for antipyresis in children and youths.

Vaccination

NSAIDs may be taken to prevent or treat local or systemic vaccine reactions including pain or fever, headache, myalgia, or malaise associated with immunization. However, contrasting data have been reported on a potential interference with the desired vaccination-induced immune response (Saleh et al., 2016). Currently, the World Health Organization advises against the prophylactic use of NSAIDs at the time of vaccination due to lack of evidence about their impact on the vaccine response (World Health Organization, 2016).

Drug Interactions

Refer to the individual product labels for a comprehensive listing of NSAID drug-drug interactions.

Concomitant NSAIDs and Low-Dose Aspirin

Many patients consume both an NSAID for chronic pain and low-dose *aspirin* for cardioprevention. Epidemiological studies suggest that this combination therapy increases significantly the likelihood of GI adverse events over either class of NSAID alone. In addition, prior occupancy of platelet COX-1 by the NSAID can impede access of *aspirin* to its acetylation target Ser^{529} and prevents irreversible inhibition of platelet function (Catella-Lawson et al., 2001). This occurs with *ibuprofen* and *naproxen* and may also affect other isoform nonselective NSAIDs (Li et al., 2014). This drug-drug interaction may undermine the cardioprotective effect of *aspirin*. *Celecoxib* is unlikely to cause this drug-drug interaction *in vivo* but confers a direct cardiovascular hazard (Grosser et al., 2017a). Thus, pain management in patients with preexisting cardiovascular disease remains a particular challenge because of the cardiovascular adverse effects of NSAIDs and the risk of drug-drug interactions that might undermine the antiplatelet effects of *aspirin*.

Other Drug-Drug Interactions

ACE Inhibitors. ACE inhibitors act, at least partly, by preventing the breakdown of kinins that stimulate PG production (see Figure 43–4). Thus, NSAIDs may attenuate the effectiveness of ACE inhibitors by blocking the production of vasodilator and natriuretic PGs. The combination of NSAIDs and ACE inhibitors also can produce marked hyperkalemia, leading to cardiac arrhythmia, especially in the elderly and in patients with hypertension, diabetes mellitus, or ischemic heart disease. Corticosteroids and selective serotonin reuptake inhibitors may increase the frequency or severity of GI complications when combined with NSAIDs.

Warfarin. The NSAIDs may augment the risk of bleeding in patients receiving *warfarin* because almost all NSAIDs suppress normal platelet function temporarily during the dosing interval and because some NSAIDs also increase *warfarin* levels by interfering with its metabolism. Thus, concurrent administration should be avoided.

Miscellaneous. Many NSAIDs are highly bound to plasma proteins, so they may displace other drugs from their binding sites. Such interactions can occur in patients given salicylates or other NSAIDs together with *warfarin*, sulfonylurea hypoglycemic agents, or *methotrexate*; the dosage of such agents may require adjustment to prevent toxicity. Patients taking *lithium* should be monitored because NSAIDs, including *aspirin*, reduce the renal excretion of this drug and can lead to toxicity.

Pediatric and Geriatric Use

Therapeutic Uses in Children

Therapeutic uses for NSAIDs in children include fever (*acetaminophen, ibuprofen*); pain (*acetaminophen, ibuprofen*); postoperative pain (*ketorolac* injection [single-dose only]); inflammatory disorders, such as juvenile arthritis (*celecoxib, etodolac, meloxicam, naproxen, oxaprozin, tolmetin*) and Kawasaki disease (off-label high-dose *aspirin*); and relief of ocular itching due to seasonal allergic rhinitis and postoperative inflammation after cataract extraction (*ketorolac* ophthalmic solution).

Kawasaki Disease. *Aspirin* generally is avoided in pediatric populations due to its potential association with Reye syndrome. However, in Kawasaki disease, children are treated with high doses of *aspirin* (30–100 mg/kg per day) during the acute phase, followed by low-dose antiplatelet therapy in the subacute phase until normalization of inflammatory markers.

Pharmacokinetics in Children

NSAID dosing recommendations frequently are based on extrapolation of pharmacokinetic data from adults or children older than 2 years, and there are often insufficient data for dose selection in younger infants. For example, the pharmacokinetics of the most used NSAID in children, *acetaminophen*, differ substantially between the neonatal period and older children or adults. The systemic bioavailability of rectal *acetaminophen* formulations in neonates and preterm babies is higher than in older patients. *Acetaminophen* clearance is reduced in preterm neonates, probably due to their immature glucuronide conjugation system (sulfation is the principal route of biotransformation at this age). Therefore, *acetaminophen* dosing intervals need to be extended (8–12 h) or daily doses reduced to avoid accumulation and liver toxicity.

Aspirin elimination also is delayed in neonates and young infants compared to adults, raising the risk of accumulation. Disease also may affect NSAID disposition in children. For example, *ibuprofen* plasma concentrations are reduced, and clearance is increased (~80%) in children with cystic fibrosis. This is probably related to the GI and hepatic pathologies associated with this disease. *Aspirin*'s kinetics are markedly altered during the febrile phase of rheumatic fever or Kawasaki vasculitis. The reduction in serum albumin associated with these conditions causes an elevation of the free salicylate concentration, which may saturate renal excretion and result in salicylate accumulation to toxic levels. In addition to dose reduction, monitoring of the free drug may be warranted in these situations.

Pharmacokinetics in the Elderly

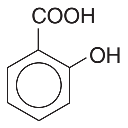
The clearance of many NSAIDs is reduced in the elderly due to changes in hepatic metabolism and creatine clearance. NSAIDs with a long $t_{1/2}$ and primarily oxidative metabolism (i.e., *piroxicam, tenoxicam, celecoxib*) have elevated plasma concentrations in elderly patients. For example, plasma concentrations after the same dose of *celecoxib* may rise 2-fold in patients older than 65 years compared to patients younger than 50 years of age, warranting dose adjustment. The capacity of plasma albumin to bind drugs is diminished in older patients and may result in higher concentrations of unbound NSAIDs. For example, free *naproxen* concentrations are markedly increased in older patients, and the higher susceptibility of older patients to GI complications may be due in part to elevated total or free NSAID concentrations. Generally, it is advisable to start most NSAIDs at a low dosage in the elderly and increase the dosage only if the therapeutic efficacy is insufficient.

Specific Properties of Individual NSAIDs

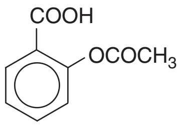
In this section, important characteristics of individual substances are discussed. NSAIDs are grouped by their chemical similarity, as in Figure 42–1.

Aspirin and Other Salicylates

The salicylates include *aspirin, salicylic acid, methyl salicylate, diflunisal, salsalate* (an unapproved marketed drug in the U.S.), *olsalazine, sulfasalazine, balsalazide, choline magnesium trisalicylate* (an unapproved marketed drug in the U.S.), *magnesium salicylate, mesalamine*, and *salicylamide* (a carboxamide derivative of *salicylic acid* contained as an ingredient in some OTC combination pain relievers). Salicylic acid is so irritating to the GI tract that it can only be used externally; therefore, the various derivatives of this acid have been synthesized for systemic use. For example, *aspirin* is the acetate ester of salicylic acid. *Aspirin* is widely consumed most often at low doses for cardioprotection and at higher doses as an analgesic, antipyretic, and anti-inflammatory agent. Its possibility of misuse and serious toxicity is underappreciated, and it remains a cause of fatal poisoning in children.



SALICYLIC ACID



ASPIRIN

Table 42–2 summarizes the clinical pharmacokinetic properties of two salicylates, *aspirin* and *diflunisal*.

TABLE 42–2 ■ NSAIDs: SALICYLATES, ACETAMINOPHEN, AND ACETIC ACID DERIVATIVES

CLASS/DRUG	PHARMACOKINETICS	DOSING	COMMENTS	COMPARED TO ASPIRIN
Salicylates				
Aspirin	Peak C_p, 1 h Protein binding, 80%–90% Metabolite, salicyluric acid $t_{1/2}$, therapeutic, 2–3 h $t_{1/2}$, toxic dose, 15–30 h	Antiplatelet, 40–80 mg/day Pain/fever, 325–650 mg every 4–6 h Rheumatic fever, children 1 g every 4–6 h or 10 mg/kg every 4–6 h	Permanent platelet COX-1 inhibition Adverse effects: GI, ↑clotting time hypersensitivity Avoid in children with acute febrile illness (Reye syndrome)	
Diflunisal	Peak C_p, 2–3 h Protein binding, 99% Metabolite, glucuronide $t_{1/2}$, 8–12 h	250–500 mg every 8–12 h (maximum = 1 g/dose and 4 g/day); children <12 y: 10–15 mg/kg every 4 h (maximum 5 doses/24 h) IV (>50 kg): 1000 mg every 6 h or 650 mg every 4 h; (<50 kg): 15 mg/kg every 6 h or 12.5 mg/kg every 4 h	Not metabolized to salicylate, competitive COX inhibitor, excreted into breast milk. Not marketed for IV administration in the U.S.	Analgesic and anti-inflammatory, 4–5× more potent Antipyretic, weaker Fewer platelet and GI side effects
Para-aminophenol derivative				
Acetaminophen	Peak C_p, 30–60 min Protein binding, 20%–50% Metabolites, glucuronides (60%); sulfates (35%) $t_{1/2}$, 2 h	650 mg or less every 4 h (maximum of 4000 mg/24 h)	Weak nonspecific COX inhibitor at common doses Potency may be modulated by peroxidase Overdose → toxic metabolite, (NAPQI) liver necrosis	Analgesic/antipyretic, equivalent Anti-inflammatory, GI, and platelet effects < aspirin at 1000 mg/day
Acetic acid derivatives				
Indomethacin	Peak C_p, 1–2 h Protein binding, 99% Metabolites, *O*-demethyl (50%); unchanged (20%) $t_{1/2}$, 2 h	25 mg 2–3 times/day; 75–100 mg at night	Side effects (3%–50%); frontal headache, neutropenia, thrombocytopenia; 20% discontinue	10–40× more potent; intolerance typically limits dose
Sulindac (sulfoxide prodrug)	Peak C_p 1–2 h; active metabolite 8 h, extensive enterohepatic circulation Metabolites, sulfone/conjugates (30%); sulindac/conjugate (25%) $t_{1/2}$, 7 h; 18 h for active sulfone metabolite	150–200 mg twice/day orally	20% GI side effects; 10% CNS side effects (headache, dizziness, rash)	Efficacy comparable
Etodolac	Peak C_p, 1 h Protein binding, 99% Metabolism, hepatic $t_{1/2}$, 7 h	200–400 mg 3–4 times/day, max: 1200 mg/day or 1000 mg/day (extended release) >6 years (extended release): 400 mg/day (20–30 kg); add 200 mg/15 kg more weight	Some COX-2 selectivity *in vitro* Adverse effects similar to sulindac, but ~ half as frequent	100 mg etodolac efficacy ≈ 650 mg of aspirin; may be better tolerated
Tolmetin	Peak C_p, 20–60 min Protein binding, 99% Metabolites, carboxylate conjugates $t_{1/2}$, 5 h	Adults: 400–600 mg 3 times/day Children >2 y: 20 mg/kg/day in 3–4 divided doses	Food delays and decreases peak absorption May persist in synovial fluid ⇒ biological efficacy > plasma $t_{1/2}$	Efficacy similar; 25%–40% develop side effects; 5%–10% discontinue drug
Ketorolac	Peak C_p, 30–60 min Protein binding, 99% Metabolite, glucuronide (90%) $t_{1/2}$, 4–6 h	See FDA package insert	Parenterally (60 mg IM, then 30 mg every 6 h, or 30 mg IV every 6 h) Available as ocular prep	Potent analgesic, poor anti-inflammatory

(Continued)

TABLE 42–2 ■ NSAIDs: SALICYLATES, ACETAMINOPHEN, AND ACETIC ACID DERIVATIVES (*CONTINUED*)

CLASS/DRUG	PHARMACOKINETICS	DOSING	COMMENTS	COMPARED TO ASPIRIN
Diclofenac	Peak C_p, 1 h; extended release, 5 h Protein binding, 99% Metabolites, glucuronide and sulfide (renal 65%, bile 35%) $t_{1/2}$, 1.2–2 h (immediate-release tabs); 12 h (topical epolamine patch)	50 mg 3 times/day or 75 mg twice/day	As topical gel, ocular solution, oral tablets combined with misoprostol First-pass effect; oral bioavailability, 50%	More potent; 20%, side effects; 2%, discontinue; 15%, elevated liver enzymes Substrate for CYPs 2C9 and 3A4
Nabumetone (6-methoxy-2-napthylacetic acid prodrug)	Peak C_p, ~3 h Protein binding, 99% Metabolites, conjugates $t_{1/2}$, 19–26 h; 22–38 h (elderly)	500–1000 mg 1–2 times/day (maximum 2000 mg/day) Patients <50 kg less likely to require >1000 mg/day	First-pass effects, 35% conversion of prodrug to active metabolite; preferential COX-2 inhibition at low doses; adverse effects (13%): GI upset, abdominal pain	Less fecal blood loss during short-term therapy

Time to peak plasma drug concentration (C_p) is after a single dose. In general, food delays absorption but does not decrease peak concentration. The majority of NSAIDs undergo hepatic metabolism, and the metabolites are excreted in the urine. Major metabolites or disposal pathways are listed. Typical $t_{1/2}$ is listed for therapeutic doses; if $t_{1/2}$ is much different with the toxic dose, this is also given. Typical adult oral doses are listed unless otherwise noted. Refer to the current product labeling for complete prescribing information, including current labeled pediatric indications.

GI, gastrointestinal.

Mechanism of Action

The effects of *aspirin* are largely caused by its capacity to acetylate proteins and inhibit cyclooxygenase irreversibly. Other salicylates generally act by virtue of their content of salicylic acid, which is a relatively weak inhibitor of the purified COX enzymes. Salicylic acid may also suppress inflammatory upregulation of COX-2 by interfering with transcription factor binding to the COX-2 promoter.

ADME

Absorption. Orally ingested salicylates are absorbed rapidly, partly from the stomach, but mostly from the upper small intestine. The peak plasma level is reached in about 1 h. The rate of absorption is determined by disintegration and dissolution rates of the tablets administered, the pH at the mucosal surface, and gastric emptying time. Even though salicylate is more ionized as the pH is increased, a rise in pH also increases the solubility of salicylate and thus dissolution of the tablets. The overall effect is to enhance absorption. The presence of food delays absorption of salicylates. Rectal absorption of salicylate usually is slower than oral absorption and is incomplete and inconsistent.

Salicylic acid is absorbed rapidly from the intact skin, especially when applied in oily liniments or ointments, and systemic poisoning has occurred from its application to large areas of skin. Methyl salicylate likewise is speedily absorbed when applied cutaneously; however, its GI absorption may be delayed many hours, making gastric lavage effective for removal even in poisonings that present late after oral ingestion.

Enteric coating delays and reduces the bioavailability of *aspirin* by roughly half and renders absorption more variable in the presence of food (Bogentoft et al., 1978), which is likely the cause of “pseudo-resistance” to *aspirin* (see Aspirin Resistance).

Distribution. After absorption, salicylates are distributed throughout most body tissues and transcellular fluids, primarily by pH-dependent processes. Salicylates are transported actively out of the CSF across the choroid plexus. The drugs readily cross the placental barrier. Roughly 80% to 90% of the salicylate in plasma is bound to proteins, especially albumin; the proportion of the total that is bound declines as plasma concentrations increase. Hypoalbuminemia is associated with a proportionately higher level of free salicylate in the plasma. Salicylate competes with a variety of compounds for plasma protein-binding sites; these include thyroxine, triiodothyronine, *penicillin*, *phenytoin*, *sulfinpyrazone*, bilirubin, uric acid, and other NSAIDs, such as *naproxen*. *Aspirin* is plasma protein bound to a more limited extent; however, it acetylates albumin *in vivo* by reaction with the ε-amino group of lysine and may change the binding of other drugs to albumin. *Aspirin* also acetylates other plasma and tissue proteins, but there is no evidence that this contributes to clinical efficacy or adverse events.

Metabolism and Excretion. *Aspirin* is rapidly deacetylated to form salicylic acid by spontaneous hydrolysis or esterases located in the intestinal wall, red blood cells, and liver. The three chief metabolic products are salicyluric acid (the glycine conjugate), the ether or phenolic glucuronide, and the ester or acyl glucuronide. Salicylates and their metabolites are excreted in the urine. The excretion of free salicylates is variable and depends on the dose and the urinary pH. For example, the clearance of salicylate is about four times as great at pH 8 as at pH 6, and it is well above the glomerular filtration rate at pH 8. High rates of urine flow decrease tubular reabsorption, whereas the opposite is true in oliguria. The plasma $t_{1/2}$ for *aspirin* is about 20 min, and for salicylate, it is 2 to 3 h at antiplatelet doses, rising to 12 h at usual anti-inflammatory doses. The $t_{1/2}$ of salicylate may rise to 15 to 30 h at high therapeutic doses or when there is intoxication. This dose-dependent elimination is the result of the limited capacity of the liver to form salicyluric acid and the phenolic glucuronide, resulting in a larger proportion of unchanged drug being excreted in the urine at higher doses. Salicylate metabolism shows high intersubject variability due to the variable contribution of different metabolic pathways. Women frequently exhibit higher plasma concentrations, perhaps due to lower intrinsic esterase activity and gender differences in hepatic metabolism. In the elderly, salicylate clearance is reduced and salicylate exposure is significantly increased. The plasma concentration of salicylate is increased by conditions that decrease the glomerular filtration rate or reduce proximal tubule secretion, such as renal disease, or the presence of inhibitors that compete for the transport system (e.g., *probenecid*). In case of an overdose, hemodialysis and hemofiltration techniques remove salicylic acid effectively from the circulation.

Monitoring of Plasma Salicylate Concentrations. *Aspirin* is one of the NSAIDs for which plasma salicylate can provide a means to monitor therapy and toxicity. Intermittent analgesic-antipyretic doses of *aspirin* typically produce plasma *aspirin* levels of less than 20 μg/mL and plasma salicylate levels of less than 60 μg/mL. The *daily* ingestion of anti-inflammatory doses of 4 to 5 g of *aspirin* produces plasma salicylate levels in the range of 120 to 350 μg/mL. Optimal anti-inflammatory effects for patients with rheumatic diseases require plasma salicylate concentrations of 150 to 300 μg/mL. Significant adverse effects can be seen at levels greater than 300 μg/mL. At lower concentrations, the drug clearance is nearly constant (despite saturation of metabolic capacity being approached) because the fraction of drug that is free, and thus available for metabolism or excretion, increases as binding sites on plasma proteins are saturated. The total concentration of salicylate in plasma is therefore a relatively linear function of dose at lower concentrations. At higher concentrations, however, as metabolic pathways of disposition become saturated, small increments in dose can disproportionately increase plasma salicylate concentration. Failure to anticipate this phenomenon can lead to toxicity.

Therapeutic Uses

Systemic Uses. The *analgesic-antipyretic* dose of *aspirin* for adults is 325 to 1000 mg orally every 4 to 6 h. It is only rarely used for inflammatory diseases such as *arthritis, spondyloarthropathies*, and *systemic lupus erythematosus*; NSAIDs with an assumed better GI safety profile (though weakly supported by evidence from comparisons in controlled trials) are preferred. The anti-inflammatory doses of *aspirin*, as might be given in rheumatic fever, range from 4 to 8 g/day in divided doses. The maximum recommended daily dose of *aspirin* for adults and children 12 years or older is 4 g. The rectal administration of *aspirin* suppositories may be preferred in infants or when the oral route is unavailable. *Aspirin* suppresses clinical signs and improves tissue inflammation in acute rheumatic fever. Other salicylates available for systemic use include *salsalate* (salicylsalicylic acid), *magnesium salicylate*, *diflunisal*, and a combination of choline salicylate and magnesium salicylate (*choline magnesium trisalicylate*).

Diflunisal is a difluorophenyl derivative of salicylic acid that is not converted to salicylic acid *in vivo*. It is a competitive inhibitor of COX and a potent anti-inflammatory drug but is largely devoid of antipyretic effects, perhaps because of poor penetration into the CNS. The drug has been used primarily as an analgesic in the treatment of osteoarthritis and musculoskeletal strains or sprains; in these circumstances, it is about three to four times more potent than *aspirin*. *Diflunisal* may produce fewer auditory side effects (see Ototoxic Effects) and appears to cause fewer and less-intense GI and antiplatelet effects than does *aspirin*.

Local Uses. *Mesalamine* (5-aminosalicylic acid) is a salicylate that is used for its local effects in the treatment of *inflammatory bowel disease* (see Figure 55–4). Oral formulations that deliver drug to the lower intestine are efficacious in the treatment of inflammatory bowel disease (in particular, ulcerative colitis). These preparations rely on pH-sensitive coatings and other delayed-release mechanisms such as linkage to another moiety to create a poorly absorbed parent compound that must be cleaved by bacteria in the colon to form the active drug. *Mesalamine* is available as a rectal enema for treatment of mild-to-moderate ulcerative colitis, proctitis, and proctosigmoiditis and as a rectal suppository for the treatment of active ulcerative proctitis. *Mesalamine* derivatives in clinical use include *balasalazide*, *sulfasalazine*, and *olsalazine*. *Sulfasalazine* (salicylazosulfapyridine) contains *mesalamine* linked covalently to *sulfapyridine*, and *balsalazide* contains *mesalamine* linked to the carrier molecule 4-aminobenzoyl-β-alanine (see Figure 55-3). *Sulfasalazine* and *olsalazine* have been used in the treatment of rheumatoid arthritis and ankylosing spondylitis. Some OTC medications to relieve indigestion and some diarrhea agents contain bismuth subsalicylate and have the potential to cause salicylate intoxication, particularly in children.

The keratolytic action of free salicylic acid is employed for the local treatment of warts, corns, fungal infections, and certain types of eczematous dermatitis. After treatment with salicylic acid, cells swell, soften, and desquamate. *Methyl salicylate* (oil of wintergreen) is a common ingredient of ointments and deep-heating liniments used in the management of musculoskeletal pain; it also is available in herbal medicines and as a flavoring agent. The cutaneous application of *methyl salicylate* can result in pharmacologically active, and even toxic, systemic salicylate concentrations and has been reported to increase prothrombin time in patients receiving *warfarin*.

Adverse Effects and Toxicity

Respiration. Salicylates increase O_2 consumption and CO_2 production (especially in skeletal muscle) at anti-inflammatory doses, a result of uncoupling oxidative phosphorylation. The increased production of CO_2 stimulates respiration. Salicylates also stimulate the respiratory center directly in the medulla. Respiratory rate and depth increase, the P_{CO_2} falls, and respiratory alkalosis ensues.

Acid-Base and Electrolyte Balance and Renal Effects. Therapeutic doses of salicylate produce definite changes in the acid-base balance and electrolyte pattern. Compensation for the initial event, respiratory alkalosis, is achieved by increased renal excretion of bicarbonate, which is accompanied by increased Na^+ and K^+ excretion; plasma bicarbonate is thus lowered, and blood pH returns toward normal. This stage of compensatory renal acidosis was often seen in adults given intensive salicylate therapy before the development of safer alternatives. Today, it is an indicator of ensuing intoxication (see Salicylate Intoxication). Salicylates can cause retention of salt and water, as well as acute reduction of renal function in patients with congestive heart failure, renal disease, or hypovolemia. Although long-term use of salicylates alone rarely is associated with nephrotoxicity, the prolonged and excessive ingestion of analgesic mixtures containing salicylates in combination with other NSAIDs can produce papillary necrosis and interstitial nephritis (see Analgesic Nephropathy).

Cardiovascular Effects. Low-dose *aspirin* (≤100 mg daily) lowers cardiovascular risk and is recommended for the prevention of myocardial infarction and stroke in patients at elevated risk (see Cardioprotection section) (Patrono, 2015). At high therapeutic doses (≥3 g daily), salt and water retention can lead to an increase (≤20%) in circulating plasma volume and decreased hematocrit (via a dilutional effect). There is a tendency for the peripheral vessels to dilate because of a direct effect on vascular smooth muscle. Cardiac output and work are increased. Those with carditis or compromised cardiac function may not have sufficient cardiac reserve to meet the increased demands, and congestive cardiac failure and pulmonary edema can occur. High doses of salicylates can produce noncardiogenic pulmonary edema, particularly in older patients who ingest salicylates regularly over a prolonged period.

GI Effects. Ingestion of salicylates may result in epigastric distress, heartburn, dyspepsia, nausea, and vomiting. Salicylates also may cause erosive and reactive gastropathies, GI ulceration, and hemorrhage.

Aspirin-induced gastric bleeding sometimes is painless and, if unrecognized, may lead to iron-deficiency anemia. The daily ingestion of anti-inflammatory doses of *aspirin* (3–4 g) results in an average fecal blood loss of between 3 and 8 mL/day, as compared with about 0.6 mL/day in untreated subjects. Gastroscopic examination of *aspirin*-treated subjects often reveals discrete ulcerative and hemorrhagic lesions of the gastric mucosa.

Hepatic Effects. Salicylates can cause hepatic injury, usually after high doses that result in plasma salicylate concentrations greater than 150 μg/mL. The injury is not an acute effect; rather, the onset characteristically occurs after several months of high-dose treatment. The majority of cases occur in patients with connective tissue disorders. There usually are no symptoms, simply an increase in serum levels of hepatic transaminases, but some patients note right upper quadrant abdominal discomfort and tenderness. Overt jaundice is uncommon. The injury usually is reversible on discontinuation of salicylates. However, the use of salicylates is contraindicated in patients with chronic liver disease. Large doses of salicylates may cause hyperglycemia, glycosuria, and depletion of liver and muscle glycogen.

Uricosuric Effects. The effects of salicylates on uric acid excretion are markedly dependent on dose. Low doses (1 or 2 g/day) may decrease urate excretion and elevate plasma urate concentrations; intermediate doses (2 or 3 g/day) usually do not alter urate excretion. Larger-than-recommended doses (>5 g/day) induce uricosuria and lower plasma urate levels; however, such large doses are tolerated poorly. Even small doses of salicylate can block the effects of *probenecid* and other uricosuric agents that decrease tubular reabsorption of uric acid.

Hematologic Effects. Irreversible inhibition of platelet function underlies the cardioprotective effect of *aspirin*. If possible, *aspirin* therapy should be stopped at least 1 week before surgery; however, preoperative *aspirin* often is recommended prior to cardiovascular surgery and percutaneous interventions. Patients with severe hepatic damage, hypoprothrombinemia, vitamin K deficiency, or hemophilia should avoid *aspirin* because the inhibition of platelet hemostasis can result in hemorrhage. Salicylates ordinarily do not alter the leukocyte or platelet count, the hematocrit, or the hemoglobin content. However, doses of 3 to 4 g/day markedly decrease plasma iron concentration and shorten erythrocyte survival time. *Aspirin* can cause a mild degree of hemolysis in individuals with a deficiency of G6PD.

Endocrine Effects. Long-term administration of salicylates decreases thyroidal uptake and clearance of iodine but increases O_2 consumption and the rate of disappearance of thyroxine and triiodothyronine from the circulation. These effects probably are caused by the competitive displacement by salicylate of thyroxine and triiodothyronine from transthyretin and the thyroxine-binding globulin in plasma (see Chapter 47).

Ototoxic Effects. Hearing impairment, alterations of perceived sounds, and tinnitus commonly occur during high-dose salicylate therapy and are sometimes observed at low doses. Ototoxic symptoms are caused by increased labyrinthine pressure or an effect on the hair cells of the cochlea, perhaps secondary to vasoconstriction in the auditory microvasculature. Symptoms usually resolve within 2 or 3 days after withdrawal of the drug. As most competitive COX inhibitors are not associated with hearing loss or tinnitus, a direct effect of salicylic acid rather than suppression of PG synthesis is likely.

Salicylates and Pregnancy. Infants born to women who ingest salicylates for long periods may have significantly reduced birth weights. When administered during the third trimester, there also is an increase in perinatal mortality, anemia, antepartum and postpartum hemorrhage, prolonged gestation, and complicated deliveries; thus, its use during this period should be avoided. NSAIDs during the third trimester of pregnancy also can cause premature closure of the ductus arteriosus and should be avoided.

Local Irritant Effects. Salicylic acid is irritating to skin and mucosa and destroys epithelial cells.

Salicylate Intoxication. Salicylate poisoning or serious intoxication most often occurs in children and sometimes is fatal. CNS effects, intense hyperpnea, and hyperpyrexia are prominent symptoms. Death has followed use of 10 to 30 g of *sodium salicylate* or *aspirin* in adults, but much larger amounts (130 g of *aspirin* in one case) have been ingested without a fatal outcome. The lethal dose of *methyl salicylate* (also known as oil of wintergreen, sweet birch oil, gaultheria oil, or betula oil) is considerably less than that of sodium salicylate. As little as a 4 mL (4.7 g) of *methyl salicylate* may cause severe systemic toxicity in children. Mild chronic salicylate intoxication is called *salicylism*. When fully developed, the syndrome includes headache, dizziness, tinnitus, difficulty hearing, dimness of vision, mental confusion, lassitude, drowsiness, sweating, thirst, hyperventilation, nausea, vomiting, and occasionally diarrhea.

Neurological Effects. In high doses, salicylates have toxic effects on the CNS, consisting of stimulation (including convulsions) followed by depression. Confusion, dizziness, tinnitus, high-tone deafness, delirium, psychosis, stupor, and coma may occur. Salicylates induce nausea and vomiting, which result from stimulation of sites that are accessible from the CSF, probably in the medullary chemoreceptor trigger zone.

Respiration. The respiratory effects of salicylates contribute to the serious acid-base balance disturbances that characterize poisoning by this class of compounds. Salicylates stimulate respiration indirectly by uncoupling of oxidative phosphorylation and directly by stimulation of the respiratory center in the medulla. Uncoupling of oxidative phosphorylation also leads to excessive heat production, and salicylate toxicity is associated with hyperthermia, particularly in children. Prolonged exposure to high doses of salicylates leads to depression of the medulla, with central respiratory depression and circulatory collapse, secondary to vasomotor depression. Because enhanced CO_2 production continues, respiratory acidosis ensues. Respiratory failure is the usual cause of death in fatal cases of salicylate poisoning. Elderly patients with chronic salicylate intoxication often develop noncardiogenic pulmonary edema, which is considered an indication for hemodialysis.

Acid-Base Balance and Electrolytes. High therapeutic doses of salicylate are associated with a primary respiratory alkalosis and compensatory metabolic acidosis. The phase of primary respiratory alkalosis rarely is recognized in children with salicylate toxicity. They usually present in a state of mixed respiratory and metabolic acidosis, characterized by a decrease in blood pH, a low plasma bicarbonate concentration, and normal or nearly normal plasma $P\text{CO}_2$. Direct salicylate-induced depression of respiration prevents adequate respiratory hyperventilation to match the increased peripheral production of CO_2. Consequently, plasma $P\text{CO}_2$ increases and blood pH decreases. Because the concentration of bicarbonate in plasma already is low due to increased renal bicarbonate excretion, the acid-base status at this stage essentially is an uncompensated respiratory acidosis.

Superimposed, however, is a true metabolic acidosis caused by accumulation of acids because of three processes. First, toxic concentrations of salicylates displace plasma bicarbonate. Second, vasomotor depression caused by toxic doses of salicylates impairs renal function, with consequent accumulation of sulfuric and phosphoric acids; renal failure can ensue. Third, salicylates in toxic doses may decrease aerobic metabolism because of inhibition of various enzymes. This derangement of carbohydrate metabolism leads to the accumulation of organic acids, especially pyruvic, lactic, and acetoacetic acids.

The same series of events also causes alterations of water and electrolyte balance. The low plasma $P\text{CO}_2$ leads to decreased renal tubular reabsorption of bicarbonate and increased renal excretion of Na^+, K^+, and water. Water also is lost by salicylate-induced sweating (especially in the presence of hyperthermia) and hyperventilation. Dehydration, which can be profound, particularly in children, rapidly occurs. Because more water than electrolyte is lost through the lungs and by sweating, the dehydration is associated with hypernatremia.

Cardiovascular Effects. Toxic doses of salicylates lead to an exaggeration of the unfavorable cardiovascular responses seen at high therapeutic doses, and central vasomotor paralysis occurs. Petechiae may be seen due to defective platelet function.

Metabolic Effects. Large doses of salicylates may cause hyperglycemia and glycosuria and deplete liver and muscle glycogen; these effects are partly explained by the release of epinephrine. Such doses also reduce aerobic metabolism of glucose, increase glucose-6-phosphatase activity, and promote the secretion of glucocorticoids. There is a greater risk of hypoglycemia and subsequent permanent brain injury in children. Salicylates in toxic doses cause a significant negative nitrogen balance, characterized by an aminoaciduria. Adrenocortical activation may contribute to the negative nitrogen balance by enhancing protein catabolism. Salicylates reduce lipogenesis by partially blocking incorporation of acetate into fatty acids; they also inhibit epinephrine-stimulated lipolysis in fat cells and displace long-chain fatty acids from binding sites on human plasma proteins. The combination of these effects leads to increased entry and enhanced oxidation of fatty acids in muscle, liver, and other tissues and to decreased plasma concentrations of free fatty acids, phospholipid, and cholesterol; the oxidation of ketone bodies also is increased.

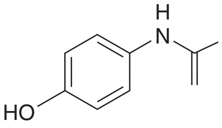
Management of Salicylate Overdose. Salicylate poisoning represents an acute medical emergency, and death may result despite maximal therapy. Monitoring of salicylate levels is a useful guide to therapy but must occur in conjunction with an assessment of the patient's overall clinical condition, acid-base balance, formulation of salicylate ingested, timing, and dose. There is no specific antidote for salicylate poisoning. Treatment is supportive and may include alkalinization with intravenous sodium bicarbonate or hemodialysis.

Drug Interactions

The plasma concentration of salicylates generally is little affected by other drugs, but concurrent administration of *aspirin* lowers the concentrations of *indomethacin*, *naproxen*, *ketoprofen*, and *fenoprofen*, at least in part by displacement from plasma proteins. Important adverse interactions of *aspirin* with *warfarin*, sulfonylureas, and *methotrexate* were mentioned previously (in Drug Interactions). Other interactions of *aspirin* include the antagonism of *spironolactone*-induced natriuresis and the blockade of the active transport of *penicillin* from CSF to blood. Magnesium-aluminum hydroxide antacids can alkalize the urine enough to increase salicylic acid clearance significantly and reduce steady-state concentrations. Conversely, discontinuation of antacid therapy can increase plasma concentrations to toxic levels.

Acetaminophen

Acetaminophen (paracetamol; *N*-acetyl-*p*-aminophenol) is the active metabolite of phenacetin.



ACETAMINOPHEN

Acetaminophen raises the threshold to painful stimuli, thus exerting an analgesic effect against pain due to a variety of etiologies. *Acetaminophen* is available without a prescription and is used as a common household analgesic by children and adults. It also is available in fixed-dose combinations containing narcotic and nonnarcotic analgesics (including *aspirin* and other salicylates), barbiturates, caffeine, vascular headache remedies, sleep aids, toothache remedies, antihistamines, antitussives, decongestants, expectorants, cold and flu preparations, and sore throat treatments. *Acetaminophen* is well tolerated; however, overdosage—two-thirds of which are intentionally induced—can cause severe hepatic damage (see Figure 9–4); it leads to nearly 80,000 emergency department visits and 30,000 hospitalizations annually in the U.S. (Blieden et al., 2014). The maximum FDA-recommended dose of *acetaminophen* is 4 g/day.

Mechanism of Action

Acetaminophen has analgesic and antipyretic effects like those of *aspirin*, but only weak anti-inflammatory effects at commonly used doses (1000 mg/day). It is a nonselective COX inhibitor (Catella-Lawson et al., 2001), which acts at the peroxide site of the enzyme and is thus distinct among NSAIDs. The presence of high concentrations of peroxides, as occur at sites of inflammation, reduces its COX-inhibitory activity.

ADME

Oral *acetaminophen* has excellent bioavailability. Peak plasma concentrations occur within 30 to 60 min, and the $t_{1/2}$ in plasma is about 2 h. *Acetaminophen* is relatively uniformly distributed throughout most body fluids. Binding of the drug to plasma proteins is variable, but less than with other NSAIDs. Some 90% to 100% of drug may be recovered in the urine within the first day at therapeutic dosing, primarily after hepatic conjugation with glucuronic acid (~60%), sulfuric acid (~35%), or cysteine (~3%); small amounts of hydroxylated and deacetylated metabolites also have been detected (see Table 42–2). Children have less capacity for glucuronidation of the drug than do adults. A small proportion of *acetaminophen* undergoes CYP-mediated *N*-hydroxylation to form NAPQI, a highly reactive intermediate. This metabolite normally reacts with sulfhydryl groups in GSH and thereby is rendered harmless. However, after ingestion of large doses of *acetaminophen*, the metabolite is formed in amounts sufficient to deplete hepatic GSH and contributes significantly to the toxic effects of overdose (see Management of Acetaminophen Intoxication).

Therapeutic Uses

Acetaminophen is suitable for analgesic or antipyretic uses; it is particularly valuable for patients in whom *aspirin* is contraindicated (e.g., those with aspirin hypersensitivity, children with a febrile illness, patients with bleeding disorders). The conventional oral dose of *acetaminophen* is 325 to 650 mg every 4 to 6 h; total daily doses should not exceed 4 g (2 g/day for persons with a history of heavy alcohol use). Single doses for children 2 to 11 years old depend on age and weight (~10–15 mg/kg); no more than five doses should be administered in 24 h. An injectable preparation is typically used in combination with narcotic analgesics for its opioid-sparing effect. Particular attention is warranted due to the availability of a wide variety of prescription and nonprescription multi-ingredient medications that represent potentially toxic overlapping sources of *acetaminophen*.

Adverse Effects and Toxicity

Acetaminophen usually is well tolerated. Therapeutic doses of *acetaminophen* have no clinically relevant effects on the cardiovascular and respiratory systems, platelets, or coagulation. The GI adverse effects are less common than with therapeutic doses of NSAIDs at 1000 mg, the most commonly used daily dose. This corresponds to approximately 50% inhibition of COXs at time of peak drug action. Common therapeutic doses of other NSAIDs (e.g., *ibuprofen*) achieve 100% inhibition. Doses of 3000 to 4000 mg/day *acetaminophen* have been associated with GI and hypertensive adverse effects seen at therapeutic doses of other NSAIDs (García-Rodríguez and Hernández-Díaz, 2001; Sudano et al., 2010). Rash and other allergic reactions occur occasionally, but sometimes these are more serious and may be accompanied by drug fever and mucosal lesions. Patients who show hypersensitivity reactions to the salicylates only rarely exhibit sensitivity to *acetaminophen*. The most serious acute adverse effect of overdosage of *acetaminophen* is a potentially fatal hepatic necrosis. Hepatic injury with *acetaminophen* involves its conversion to the toxic metabolite NAPQI (see Figure 9–4). The glucuronide and sulfate conjugation pathways become saturated, and increasing amounts undergo CYP-mediated *N*-hydroxylation to form NAPQI. This is eliminated rapidly by conjugation with GSH and then further metabolized to a mercapturic acid and excreted into the urine. In the setting of *acetaminophen* overdose, hepatocellular levels of GSH become depleted. The highly reactive NAPQI metabolite binds covalently to cell macromolecules, leading to dysfunction of enzymatic systems and structural and metabolic disarray. Furthermore, depletion of intracellular GSH renders the hepatocytes highly susceptible to oxidative stress and apoptosis. Renal tubular necrosis and hypoglycemic coma also may occur.

In adults, hepatotoxicity may occur after ingestion of a single dose of 10 to 15 g (150–250 mg/kg) of *acetaminophen* in adults and greater than 150 mg/kg in children; doses of 20 to 25 g or more are potentially fatal. Conditions of CYP induction (e.g., chronic heavy alcohol consumption) or GSH depletion (e.g., fasting or malnutrition) increase the susceptibility to hepatic injury, which has been documented, albeit uncommonly, with doses in the therapeutic range. Plasma transaminases become elevated, sometimes markedly so, beginning about 12 to 36 h after ingestion. Symptoms that occur during the first 2 days of acute poisoning by *acetaminophen* reflect gastric distress (e.g., nausea, abdominal pain, anorexia) and belie the potential seriousness of the intoxication. Clinical indications of hepatic damage manifest within 2 to 4 days of ingestion of toxic doses, with right subcostal pain, tender hepatomegaly, jaundice, and coagulopathy. Renal impairment or frank renal failure may occur. Liver enzyme abnormalities typically peak 72 to 96 h after ingestion and may be accompanied by hepatic encephalopathy. Biopsy of the liver reveals centrilobular necrosis with sparing of the periportal area. In nonfatal cases, the hepatic lesions are reversible over a period of weeks or months. The hepatotoxicity of *acetaminophen* has led to the promotion of much lower doses for clinical use, resulting in lower maximal inhibition of COXs than other NSAIDs.

Management of Acetaminophen Intoxication. Severe liver damage occurs in 90% of patients with plasma concentrations of *acetaminophen* greater than 300 μg/mL at 4 h or 45 μg/mL at 15 h after the ingestion of the drug. Activated charcoal, if given within 4 h of ingestion, decreases *acetaminophen* absorption by 50% to 90% and should be administered if the ingested dose is suspected to exceed 7.5 g. *N*-acetylcysteine (NAC) is the accepted antidote for *acetaminophen* overdose and is indicated for those at risk of hepatic injury. NAC functions by detoxifying NAPQI. It both repletes GSH stores and may conjugate directly with NAPQI by serving as a GSH substitute. If given within 8 to 16 h of *acetaminophen* overdose, the otherwise high mortality plummets to less than 1%. In addition to NAC therapy, aggressive supportive care is warranted. This includes management of hepatic and renal failure, if they occur, and intubation if the patient becomes obtunded. Hypoglycemia can result from liver failure, and plasma glucose should be monitored closely. Fulminant hepatic failure is an indication for liver transplantation.

Acetic Acid Derivatives

Diclofenac

Diclofenac, a phenylacetic acid derivative, is the most frequently used NSAID in Europe. *Diclofenac* has analgesic, antipyretic, and anti-inflammatory activities. Its potency is substantially greater than that of other NSAIDs. Although it was not developed to be a COX-2–selective drug, the selectivity of *diclofenac* for COX-2 resembles that of *celecoxib* (see Figure 42–1).

ADME. *Diclofenac* displays rapid absorption, extensive protein binding, and a $t_{1/2}$ of 1 to 2 h (see Table 42–2). The short $t_{1/2}$ makes it necessary to give doses of *diclofenac* considerably higher than would be required to inhibit COX-2 fully at peak plasma concentrations to afford sustained COX inhibition throughout the dosing interval. Thus, both COX isoforms are inhibited for the first phase of the dosing interval. However, as plasma levels decrease, *diclofenac* behaves like a COX-2 inhibitor in the later phase of the dosing interval. There is a substantial first-pass effect, such that only about 50% of *diclofenac* is available systemically. The drug accumulates in synovial fluid after oral administration, which may explain why its duration of therapeutic effect is considerably longer than its plasma $t_{1/2}$. *Diclofenac* is metabolized in the liver by a member of the CYP2C subfamily to 4-hydroxydiclofenac, the principal metabolite, and other hydroxylated forms; after glucuronidation and sulfation, the metabolites are excreted in the urine (65%) and bile (35%).

Therapeutic Uses. *Diclofenac* is approved in the U.S. for the long-term symptomatic treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, pain, primary dysmenorrhea, and acute migraine. Multiple oral formulations are available, providing a range of release times; the usual daily oral dosage is 50 to 150 mg, given in several divided doses. For acute pain such as migraine, a powdered form for dissolution in water and a solution for intravenous injection are available. *Diclofenac* also is available in combination with *misoprostol*, a PGE_1 analogue. This combination retains the efficacy of *diclofenac* while reducing the frequency of GI ulcers and erosions. A 1% topical gel, a topical solution, and a transdermal patch are available for short-term treatment of pain due to minor strains, sprains, and bruises. A 3% gel formulation is indicated for topical treatment of actinic keratosis. In addition, an ophthalmic solution of *diclofenac* is available for treatment of postoperative inflammation following cataract extraction and for the temporary relief of pain and photophobia in patients undergoing corneal refractive surgery.

Adverse Effects. *Diclofenac* produces side effects (particularly GI) in about 20% of patients. The incidence of serious GI adverse effects, hypertension, and myocardial infarction is similar to the COX-2–selective inhibitors (Cannon et al., 2006). Hypersensitivity reactions have occurred following topical application and systemic administration. Severe liver injury occurs in 6 to 11 per 100,000 regular users annually (Bjornsson et al., 2013; de Abajo et al., 2004). Elevation of hepatic transaminases in plasma by more than three times the upper normal limit, indicating significant liver damage, occurs in about 4% of patients (Rostom et al., 2005). Transaminases should be monitored during the first 8 weeks of therapy with *diclofenac*. Other untoward responses to *diclofenac* include CNS effects, rashes, fluid retention, edema, and renal function impairment. The drug is not recommended for children, nursing mothers, or pregnant women.

Diclofenac is extensively metabolized. One metabolite, 4′-hydroxy diclofenac, can form reactive benzoquinone imines (similar to *acetaminophen*'s metabolite NAPQI) that deplete hepatic GSH. UGT2B7 is the primary catalyst in the formation of another highly reactive metabolite, diclofenac acyl glucuronide (King et al., 2001). Genetic variation that causes higher catalytic activity of UGT2B7 is associated with an increased risk of hepatotoxicity among patients taking *diclofenac* (Daly et al., 2007).

Indomethacin

Indomethacin is a methylated indole derivative indicated for the treatment of moderate-to-severe rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis; acute gouty arthritis; and acute painful shoulder. Although *indomethacin* is still used clinically, mainly as a steroid-sparing agent, toxicity and the availability of safer alternatives have limited its use.

Indomethacin is a potent nonselective inhibitor of the COXs. It also inhibits the motility of polymorphonuclear leukocytes, depresses the biosynthesis of mucopolysaccharides, and may have a direct, COX-independent vasoconstrictor effect. *Indomethacin* has prominent anti-inflammatory and analgesic-antipyretic properties like those of the salicylates.

ADME. Oral *indomethacin* has excellent bioavailability. Peak concentrations occur 1 to 2 h after dosing (see Table 42–2). The concentration of the drug in the CSF is low, but its concentration in synovial fluid is equal to that in plasma within 5 h of administration. There is enterohepatic cycling of the *indomethacin* metabolites and probably of *indomethacin* itself. The $t_{1/2}$ in plasma is variable, perhaps because of enterohepatic cycling, but averages about 2.5 h.

Therapeutic Uses. While *indomethacin* is estimated to be about 20 times more potent than *aspirin*, a high rate of intolerance limits its use. An intravenous formulation of *indomethacin* is approved for closure of persistent patent ductus arteriosus in premature infants. The regimen involves intravenous administration of 0.1 to 0.25 mg/kg every 12 h for three doses, with the course repeated one time if necessary. Successful closure can be expected in more than 70% of neonates treated. The principal limitation of treating neonates is renal toxicity, and therapy is interrupted if the output of urine falls significantly (<0.6 mL/kg/h). An injectable formulation of *ibuprofen* is an alternative for the treatment of patent ductus arteriosus.

Adverse Effects. A very high percentage (35%–50%) of patients receiving *indomethacin* experience adverse drug reactions. GI adverse events are common and can be fatal; elderly patients are at significantly greater risk. Diarrhea may occur and sometimes is associated with ulcerative lesions of the bowel. Acute pancreatitis has been reported, as have rare, but potentially fatal, cases of hepatitis. The most frequent CNS effect is severe frontal headache. Dizziness, vertigo, light-headedness, and mental confusion may occur. Seizures have been reported, as have severe depression, psychosis, hallucinations, and suicide. Caution is advised when administering *indomethacin* to elderly patients or to those with underlying epilepsy, psychiatric disorders, or Parkinson's disease because they are at greater risk for the development of serious CNS adverse effects. Hematopoietic reactions include neutropenia, thrombocytopenia, and, rarely, aplastic anemia.

Concurrent administration of *probenecid* increases the total plasma concentration of *indomethacin* and its inactive metabolites. *Indomethacin* antagonizes the natriuretic and antihypertensive effects of *furosemide* and thiazide diuretics and blunts the antihypertensive effect of β receptor antagonists, AT_1 receptor antagonists, and ACE inhibitors.

Sulindac

Sulindac is a congener of *indomethacin*. *Sulindac* is a prodrug whose anti-inflammatory activity resides in its sulfide metabolite, which is more than 500 times more potent than *sulindac* as an inhibitor of COX but less than half as potent as *indomethacin* (see Figure 42–1). ADME data are summarized in Table 42–2. *Sulindac* is used for the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, painful shoulder, and gouty arthritis. Its analgesic and anti-inflammatory effects are comparable to those achieved with *aspirin*. The most common dosage for adults is 150 to 200 mg twice a day. Although the incidence of toxicity is lower than with *indomethacin*, adverse reactions to *sulindac* are common. The typical NSAID GI side effects are seen in nearly 20% of patients. CNS side effects as described for *indomethacin* are seen in 10% or fewer of patients. Rash occurs in 3% to 9% of patients, and pruritus occurs in 1% to 3% of patients. Transient elevations of hepatic transaminases in plasma are less common. The same precautions that apply to other NSAIDs regarding patients at risk for GI toxicity, cardiovascular risk, and renal impairment also apply to *sulindac*.

Etodolac

Etodolac is an acetic acid derivative with some degree of COX-2 selectivity (see Table 42–2, Figure 42–1). A single oral dose (200–400 mg) of *etodolac* provides postoperative analgesia that lasts for 6 to 8 h. *Etodolac* also is effective in the treatment of osteoarthritis, rheumatoid arthritis, and mild-to-moderate pain, and the drug appears to be uricosuric. Sustained-release preparations are available. *Etodolac* is relatively well tolerated. About 5% of patients who have taken the drug for 1 year or less discontinue treatment because of GI side effects, rashes, and CNS effects.

Tolmetin

Tolmetin is approved for the treatment of osteoarthritis, rheumatoid arthritis, and juvenile rheumatoid arthritis and has been used in the treatment of ankylosing spondylitis. ADME and comparison to *aspirin* are provided in Table 42–2. Recommended doses of *tolmetin* for adults (200–600 mg three times/day) are typically given with meals, milk, or antacids to lessen abdominal discomfort. However, peak plasma concentrations and bioavailability are reduced when the drug is taken with food. Side effects occur in 25% to 40% of patients who take tolmetin. GI side effects are the most common (~15%), and gastric ulceration has been observed. CNS side effects similar to those seen with *indomethacin* and *aspirin* occur, but they are less common and less severe.

Ketorolac

Ketorolac is a potent analgesic but only a moderately effective anti-inflammatory drug. The use of *ketorolac* is limited to 5 days or less for acute pain; the drug can be administered orally, intravenously, intramuscularly, or intranasally. Typical doses are 30 to 60 mg (intramuscular), 15 to 30 mg (intravenous), 10 to 20 mg (oral), and 31.5 mg (intranasal). Pediatric patients aged between 2 and 16 years may receive a single intramuscular (1 mg/kg up to 30 mg) or intravenous (0.5 mg/kg up to 15 mg) dose of *ketorolac* for severe acute pain. *Ketorolac* has a rapid onset of action and a short duration of action (see Table 42–2).

Topical (ophthalmic) *ketorolac* is approved for the treatment of seasonal allergic conjunctivitis and postoperative ocular inflammation. *Ketorolac* in a fixed-dose combination with *phenylephrine* is indicated as an irrigation during cataract or intraocular lens replacement surgery to maintain pupil size, prevent miosis, and reduce postoperative pain. Side effects of systemic *ketorolac* include somnolence (6%), dizziness (7%), headache (17%), GI pain (13%), dyspepsia (12%), nausea (12%), and pain at the site of injection (2%). Serious adverse GI, renal, bleeding, and hypersensitivity reactions to *ketorolac* may occur. Patients receiving greater than recommended doses or concomitant NSAID therapy and the elderly appear to be particularly at risk. Although *ketorolac* is widely used in postoperative patients, it should not be used for routine obstetric analgesia, in patients with severe kidney disease, for perioperative pain after CABG, or in combination with *probenecid* (*probenecid* triples AUC and doubles $t_{1/2}$ of *ketorolac*, thereby promoting adverse effects).

Nabumetone

Nabumetone is the prodrug of 6-methoxy-2-naphthylacetic acid. *Nabumetone* is approved for the treatment of rheumatoid arthritis and osteoarthritis. Its comparative pharmacokinetic properties are summarized in Table 42–2. *Nabumetone* is associated with crampy lower abdominal pain (12%) and diarrhea (14%). Other side effects include rash (3%–9%); headache (3%–9%); dizziness (3%–9%); and heartburn, tinnitus, and pruritus (3%–9%).

Propionic Acid Derivatives

The propionic acid derivatives *ibuprofen, naproxen, flurbiprofen, fenoprofen, ketoprofen*, and *oxaprozin* are available in the U.S. (see Table 42–3). *Ibuprofen* is the most used NSAID in the U.S. and is available with or without a prescription. *Naproxen*, also available with or without a prescription, has a longer but variable $t_{1/2}$. *Oxaprozin* also has a long $t_{1/2}$ and may be given once daily.

Mechanism of Action

Propionic acid derivatives are nonselective COX inhibitors with the effects and side effects common to other NSAIDs. Some of the propionic acid derivatives, particularly *naproxen*, have inhibitory effects on leukocyte function, and some evidence suggests that *naproxen* may have slightly better efficacy regarding analgesia and relief of morning stiffness. This suggestion of benefit accords with the longer $t_{1/2}$ of *naproxen* in comparison to other propionic acid derivatives.

Therapeutic Uses

Propionic acid derivatives are approved for use in the symptomatic treatment of rheumatoid arthritis, juvenile arthritis, and osteoarthritis. Some also are approved for pain, ankylosing spondylitis, acute gouty arthritis, tendinitis, bursitis, headache, postoperative dental pain and swelling, and primary dysmenorrhea. These agents may be comparable in efficacy to *aspirin* for the control of the signs and symptoms of rheumatoid arthritis and osteoarthritis.

Drug Interactions

Ibuprofen and *naproxen* have been shown to interfere with the antiplatelet effects of *aspirin* (Catella-Lawson et al., 2001; Li et al., 2014). Propionic acid derivatives have not been shown to alter the pharmacokinetics of the oral hypoglycemic drugs or *warfarin*. Refer to the full product labeling for a comprehensive listing of other drug interactions.

Ibuprofen

ADME. Table 42–3 summarizes the comparative pharmacokinetics of *ibuprofen*. *Ibuprofen* is absorbed rapidly, bound avidly to protein, and undergoes hepatic metabolism (90% is metabolized to hydroxylate or carboxylate derivatives) and renal excretion of metabolites. The $t_{1/2}$ is about 2 h. Slow equilibration with the synovial space means that its antiarthritic effects may persist after plasma levels decline. In experimental animals, *ibuprofen* and its metabolites readily cross the placenta.

Therapeutic Uses. *Ibuprofen* is supplied as tablets, chewable tablets, capsules, caplets, and gelcaps containing 50 to 600 mg; as oral drops; and as an oral suspension. An injectable formulation of *ibuprofen* is approved to close patent ductus arteriosus in premature infants. Solid oral dosage forms containing 200 mg or less are available without a prescription. *Ibuprofen* is licensed for marketing alone and in fixed-dose combinations with antihistamines, decongestants, *famotidine*, *oxycodone*, and *hydrocodone*. It is short acting, with a $t_{1/2}$ of about 2 h. The usual dose for mild-to-moderate pain is 400 mg every 4 to 6 h as needed.

Adverse Effects. *Ibuprofen* is better tolerated than *aspirin* and *indomethacin* and has been used in patients with a history of GI intolerance to other NSAIDs. Nevertheless, 5% to 15% of patients experience GI side effects. Less-frequent adverse effects of *ibuprofen* include rashes (3%–9%), thrombocytopenia (<1%), headache (1%–3%), dizziness (3%–9%), blurred vision (<1%), and, in a few cases, toxic amblyopia (<1%), fluid retention (1%–3%), and edema (1%–3%). Patients who develop ocular disturbances should discontinue the use of *ibuprofen* and have an ophthalmic evaluation. *Ibuprofen* can be used occasionally by pregnant women; however, the concerns apply regarding third-trimester effects, including delay of parturition. Excretion into breast milk is thought to be minimal, so *ibuprofen* also can be used with caution by women who are breastfeeding.

Naproxen

Naproxen is supplied as tablets, delayed-release tablets, extended-release tablets, gelcaps, and caplets containing 200 to 500 mg of *naproxen* or *naproxen sodium* and as oral suspensions and suppositories. Solid oral dosage forms containing 200 mg or less are available without a prescription. *Naproxen* is licensed for marketing alone and in fixed-dose combinations with *pseudoephedrine*, *diphenhydramine*, *esomeprazole*, and *sumatriptan*; it is packaged with *lansoprazole*. *Naproxen* is indicated for juvenile and rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, pain, primary dysmenorrhea, tendonitis, bursitis, and acute gout.

TABLE 42–3 ■ COMPARISON OF REPRESENTATIVE NSAIDs: FENAMATES AND PROPIONIC ACID DERIVATIVES

CLASS/DRUG	PHARMACOKINETICS	DOSING	COMMENTS	COMPARED TO ASPIRIN
Fenamates				
Mefenamic acid	Peak C_p, 2–4 h Protein binding, >90% Metabolism, CYP2C9 oxidation; glucuronidation of parent drug and metabolites $t_{1/2}$, 2–4 h	500 mg load, then 250 mg every 6 h	Therapy usually should not exceed 7 days or 2–3 days (dysmenorrhea); 15% elevated liver enzymes; excreted in breast milk	Efficacy similar
Meclofenamate	Peak C_p, 0.5–2 h; 3–4 h (with food) Protein binding, 99% Metabolism, oxidation to 3-OH (~20% activity of parent) $t_{1/2}$, 0.8–2.1 h (parent); 0.5–4 h (active metabolite)	50–100 mg 4–6 times/day (maximum 400 mg/day)	Side effects: CNS, GI, and rash (all >10%); administration with food ↓ rate/extent of absorption	Efficacy similar
Propionic acid derivatives				
Ibuprofen	Peak C_p, 2 h (tablets), 1 h (chewable tablets), 0.75 h (liquid) Protein binding, 99% Metabolites, CYP2C9 oxidation to 2- and 3-hydroxylates; conjugation to acyl glucuronides $t_{1/2}$, 2–4 h (adults); 23–75 h (premature infants); 0.9–2.3 h (children)	200–800 mg 3–6 times/day with food (maximum 3.2 g/day); Canadian and U.S. pediatric max, 2.4 g/day Children: 4–10 mg/kg/dose, 3–4 times/day	10%–15% discontinue; may increase risk of aseptic meningitis; excreted in breast milk Racemate: 60% of R-enantiomer converts to S-ibuprofen	Equipotent
Naproxen	Peak C_p, 2–4 h (base tabs); 1–4 h (liquid); 1–2 h (sodium salt); 4–12 h (delayed-release tabs) Protein binding, 99% (↑ free fraction in elderly) Metabolism, CYPs 2C9, 1A2, 2C8 oxidation to 6-O-desmethyl and other metabolites $t_{1/2}$, 9–25 h	250 mg 3–4 times/day; 250–550 mg 2 times/day; 750–1000 mg daily (extended release) Children: 5 mg/kg 2 times/day (max 15 mg/kg/day)	Peak anti-inflammatory effects after 2–4 weeks; ↑ free fraction and ↓ excretion ⇒ ↑ risk of toxicity in elderly; may increase risk of aseptic meningitis; excreted in breast milk; variably prolonged $t_{1/2}$ may afford cardioprotection in some individuals	Usually better tolerated
Fenoprofen	Peak C_p, 2 h Protein binding, 99% Metabolites, 4-OH metabolite; glucuronide conjugates $t_{1/2}$, 2.5–3 h	200 mg 4–6 times/day or 300–600 mg 3–4 times/day (max 3.2 g/day)	Peak anti-inflammatory effects after 2–3 weeks; 15% experience side effects; few discontinue use; excreted in breast milk	Generally better tolerated
Ketoprofen	Peak C_p, 1.2 h; 6.8 h (extended release) Protein binding, 99% Metabolites, glucuronide conjugates; enterohepatic recirculation? $t_{1/2}$, 0.9–3.3 h	25–50 mg 3–4 times/day; 75 mg 3 times/day; 200 mg daily (extended release); max 300 mg/day Anti-inflammatory, 50–75 mg 3–4 times/day	30% develop side effects (usually GI, usually mild); ~13% liver function abnormalities; unbound fraction, systemic exposure, and $t_{1/2}$ ↑ with age in elderly; excreted in breast milk	Generally better tolerated; biological efficacy > plasma $t_{1/2}$
Flurbiprofen	Peak C_p, ~2 h Protein binding, 99% Metabolism, CYP2C9 oxidation, UGTB7 glucuronidation of parent and 4′-OH metabolite $t_{1/2}$, 7.5 h	200–300 mg/day in 2–4 divided doses (maximum 100 mg/dose)	Racemate; excreted in breast milk; available for ophthalmic use	Generally better tolerated

(*Continued*)

TABLE 42–3 ■ COMPARISON OF REPRESENTATIVE NSAIDs: FENAMATES AND PROPIONIC ACID DERIVATIVES (*CONTINUED*)

CLASS/DRUG	PHARMACOKINETICS	DOSING	COMMENTS	COMPARED TO ASPIRIN
Oxaprozin	Peak C_p, 2.4–3 h Protein binding, 99% Metabolism, 65% oxidates, 35% glucuronides $t_{1/2}$, 41–55 h	600–1200 mg daily (maximum 1800 mg); children >21 kg: 600–1200 mg daily based on weight (maximum 1200 mg)	Slow onset, not indicated for fever or acute pain; dose in elderly adjusted on the basis of weight; expected to be excreted in breast milk	Generally better tolerated

Time to peak plasma drug concentration (C_p) is after a single dose. In general, food delays absorption but does not decrease peak concentration. The majority of NSAIDs undergo hepatic metabolism, and the metabolites are excreted in the urine. Major metabolites or disposal pathways are listed. Typical $t_{1/2}$ is listed for therapeutic doses; if $t_{1/2}$ is much different with the toxic dose, this is also given. Typical adult oral doses are listed unless otherwise noted. Refer to the current product labeling for complete prescribing information, including current labeled pediatric indications.

ADME. Naproxen is absorbed fully after oral administration. *Naproxen* also is absorbed rectally but more slowly than after oral administration. *Naproxen* is almost completely (99%) bound to plasma proteins after normal therapeutic doses. The $t_{1/2}$ of naproxen in plasma is variable, 9 to 25 h. Age plays a role in the variability of the $t_{1/2}$ because of the age-related decline in renal function (and consequently longer $t_{1/2}$) (see Table 42–3). Low doses should be prescribed in the elderly. *Naproxen* is extensively metabolized in the liver. About 30% of the drug undergoes 6-desmethylation, and most of this metabolite and *naproxen*, itself, are excreted in the urine as the glucuronide or other conjugates. *Naproxen* crosses the placenta and appears in the milk of lactating women at about 1% of the maternal plasma concentration.

Adverse Effects. Although the best available data were consistent with the suggestion that *naproxen* is an NSAID that is not associated with an increase in myocardial infarction (Coxib and Traditional NSAID Trialists' Collaboration et al., 2013), in 2015, based on the advisory committee recommendations, the FDA issued a warning that NSAIDs can cause heart attacks and strokes and that there is inconclusive evidence regarding whether the particular risk of any NSAID is definitively higher or lower than another NSAID (https://www.fda.gov/Drugs/DrugSafety/ucm451800.htm).

About 1% to 10% of patients taking *naproxen* experience GI adverse effects that include heartburn, abdominal pain, constipation, diarrhea, nausea, dyspepsia, and stomatitis. Adverse effects with *naproxen* occur at approximately the same frequency as with *indomethacin* and other NSAIDs (see Table 42–1). CNS side effects include drowsiness (3%–9%), headache (3%–9%), dizziness (≤9%), vertigo (<3%), and depression (<1%). Other common reactions include pruritus (3%–9%) and diaphoresis (<3%). Rare instances of jaundice, impairment of renal function, angioedema, thrombocytopenia, and agranulocytosis have been reported.

Fenamates

The fenamates (anthranilic acids) include *mefenamic acid*, *meclofenamate*, and *flufenamic acid*. The pharmacological properties of the fenamates are those of typical NSAIDs, and therapeutically, they have no advantages over others in the class (see Table 42–3). *Mefenamic acid* and *meclofenamate sodium* are used in the short-term treatment of pain in soft-tissue injuries, dysmenorrhea, and rheumatoid arthritis and osteoarthritis. These drugs are not recommended for use in children or pregnant women. Roughly 5% of patients develop a reversible elevation of hepatic transaminases. Diarrhea, which may be severe and associated with steatorrhea and inflammation of the bowel, also is relatively common. Autoimmune hemolytic anemia is a potentially serious but rare side effect.

Enolic Acids (Oxicams)

The oxicam derivative *piroxicam* is the nonselective COX inhibitor with the longest $t_{1/2}$. *Meloxicam* shows COX-2 selectivity comparable to celecoxib (see Figure 42–1) and was approved as a COX-2–selective NSAID in some countries. These agents are similar in efficacy to *aspirin*, *indomethacin*, or *naproxen* for the long-term treatment of rheumatoid arthritis or osteoarthritis. The main advantage suggested for these compounds is their long $t_{1/2}$, which permits once-a-day dosing (see comparative pharmacokinetic and dosing data in Table 42–4).

Piroxicam

Piroxicam may inhibit activation and aggregation of neutrophils, apparently independently of its ability to inhibit COX; hence, additional modes of anti-inflammatory action have been proposed, including inhibition of proteoglycanase and collagenase in cartilage and decreasing proinflammatory cytokine levels.

Piroxicam is approved for the treatment of rheumatoid arthritis and osteoarthritis. Due to its slow onset of action and delayed attainment of steady state, it is less suited for acute analgesia but has been used to treat acute gout.

ADME. The pharmacokinetics of *piroxicam* are described in Table 42–4. The usual daily dose is 20 mg. *Piroxicam* is absorbed completely after oral administration and undergoes enterohepatic recirculation. Estimates of the $t_{1/2}$ in plasma have been variable; the average is about 50 h in adults. Steady-state blood levels are reached in 7 to 12 days. Less than 5% of the drug is excreted into the urine unchanged. The major metabolic transformation in humans is hydroxylation of the pyridyl ring (predominantly by an isozyme of the CYP2C subfamily), and this inactive metabolite and its glucuronide conjugate account for about 60% of the drug excreted in the urine and feces.

Adverse Effects. Approximately 20% of patients experience side effects with *piroxicam*, and about 5% of patients discontinue use because of these effects. *Piroxicam* may be associated with more GI and serious skin reactions than other nonselective NSAIDs. In 2007, the European Medicines Agency reviewed the safety of orally administered *piroxicam* and concluded that its benefits outweigh its risks but advised that it should no longer be considered a first-line agent or used for the treatment of acute (short-term) pain and inflammation.

Meloxicam

Meloxicam is approved for use in osteoarthritis, rheumatoid arthritis, and juvenile rheumatoid arthritis. The recommended adult dose of *meloxicam* is 7.5 to 15 mg once daily. *Meloxicam* demonstrates some COX-2 selectivity (see Figure 42–1). There is significantly less gastric injury compared to *piroxicam* (20 mg/day) in subjects treated with 7.5 mg/day of *meloxicam*, but the advantage is lost with a dosage of 15 mg/day (Patoia et al., 1996).

Purpose-Developed COX-2–Selective NSAIDs

Selective inhibitors of COX-2 are molecules with side chains that fit within its hydrophobic pocket but are too large to block COX-1 with equally high affinity. *Celecoxib* is the only purposefully developed COX-2 inhibitor still approved in the U.S. (see its clinical pharmacokinetic properties and precautions in Table 42–4). As mentioned, other, older compounds (*diclofenac*, *etodolac*, *meloxicam*, *nimesulide*) have been retrospectively found to have similar selectivity for COX-2 (see Figure 42–1). *Etoricoxib* is approved in several countries but restricted in its indications; *rofecoxib*, *valdecoxib*, and *lumiracoxib* were withdrawn worldwide because of the

TABLE 42–4 ■ REPRESENTATIVE NSAIDs: ENOLIC ACID DERIVATIVES AND COXIBS

CLASS/DRUG	PHARMACOKINETICS	DOSING	COMMENTS	COMPARED TO ASPIRIN
Enolic acid derivatives				
Piroxicam	Peak C_p, 3–5 h Protein binding, 99% Metabolism, CYP2C9 hydroxylation, conjugation, *N*-demethylation $t_{1/2}$, ~50 h	20 mg daily	20% side effects; 5% discontinue; slow onset, not indicated for fever or acute pain; excreted in breast milk	Equipotent with lower incidence of minor GI effects
Meloxicam	Peak C_p, 4–5 h (and 12–14 h due to biliary recycling) Protein binding, 99% Metabolism, hydroxylation $t_{1/2}$, 15–20 h	7.5 mg daily (maximum 15 mg/day); children ≥2: lowest effective dose, 0.125 mg/kg daily (maximum 7.5 mg daily)	Some COX-2 selectivity, especially at lower doses; elderly females have higher systemic exposure and peak plasma concentrations than men and young women; excretion in breast milk unknown	—
Diaryl heterocyclic NSAIDs (COX-2 selective)				
			Evidence for cardiovascular adverse events	Decrease in GI side effects and in platelet effects
Celecoxib	Peak C_p, ~3 h Protein binding, 97% Metabolism, CYPs 2C9 (major) and 3A4 (minor), glucuronidation $t_{1/2}$, 11.2 h	100–200 mg 1–2 times/day; 400 mg followed by 200 mg if needed on first day (acute pain); maximum, 800 mg/day. Children >2 y: 50 mg (10–25 kg) or 100 mg (>25 kg) 2 times/day	CYP2D6 and CYP2D8 inhibitor; adverse effects: GI complaints (5%); aseptic meningitis and methemoglobinemia have been reported; disseminated intravascular coagulation risk in pediatric patients; 40% higher systemic exposure in blacks and elderly females; excreted in breast milk	Usually better tolerated; does not usually prolong bleeding time

Time to peak plasma drug concentration C_p is after a single dose. In general, food delays absorption but does not decrease peak concentration. The majority of NSAIDs undergo hepatic metabolism, and the metabolites are excreted in the urine. Major metabolites or disposal pathways are listed. Typical $t_{1/2}$ is listed for therapeutic doses; if $t_{1/2}$ is much different with the toxic dose, this is also given. Typical adult oral doses are listed unless otherwise noted. Refer to the current product labeling for complete prescribing information, including current labeled pediatric indications.

cardiovascular complications caused by suppression of cardioprotective COX-2–derived PGs, especially PGI_2, and the unrestrained effects of endogenous stimuli, such as platelet COX-1–derived TxA_2, on platelet activation, vascular proliferation and remodeling, hypertension, and atherogenesis. COX-2–selective NSAIDs should be avoided in patients prone to cardiovascular or cerebrovascular disease. While the purposefully developed COX-2 inhibitors have generally been shown to reduce severe GI complications when compared to isoform nonselective compounds, none of the COX-2–selective NSAIDs has established superior efficacy.

Celecoxib

ADME. The bioavailability of oral *celecoxib* is not known; peak plasma levels occur at 2 to 4 h after administration. The elderly (≥65 years of age) may have up to 2-fold higher peak concentrations and AUC values than younger patients (≤55 years of age). *Celecoxib* is bound extensively to plasma proteins. Most is excreted as carboxylic acid and glucuronide metabolites in the urine and feces. The elimination $t_{1/2}$ is approximately 11 h. The drug commonly is given once or twice daily during chronic treatment. Plasma concentrations are increased in patients with mild and moderate hepatic impairment, requiring reduction in dose. *Celecoxib* is metabolized predominantly by CYP2C9 and inhibits CYP2D6. Thus, vigilance is necessary during coadministration of drugs that are known to inhibit CYP2C9 and drugs that are metabolized by CYP2D6.

Therapeutic Uses. *Celecoxib* is used for the management of acute pain for the treatment of osteoarthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, ankylosing spondylitis, and primary dysmenorrhea. The recommended dose for treating osteoarthritis is 200 mg/day as a single dose or divided as two doses. In the treatment of rheumatoid arthritis, the recommended dose is 100 to 200 mg twice daily. Due to cardiovascular hazard, physicians should use the lowest possible dose for the shortest possible duration.

Adverse Effects. *Celecoxib* confers a risk of myocardial infarction and stroke, and this appears to relate to dose and the underlying risk of cardiovascular disease. Effects attributed to inhibition of PG production in the kidney—hypertension and edema—occur with nonselective COX inhibitors and also with *celecoxib*. Selective COX-2 inhibitors lose their GI advantage over other NSAIDs alone when used in conjunction with aspirin. Chronic use of *celecoxib* may decrease bone mineral density, particularly in older male patients. There is some suggestion that *celecoxib* may slow fracture healing and tendon-to-bone healing.

Etoricoxib

Etoricoxib is a COX-2–selective inhibitor with selectivity second only to that of *lumiracoxib* (see Figure 42–1). *Etoricoxib* is incompletely (~80%) absorbed and has a $t_{1/2}$ of 20 to 26 h. It is extensively metabolized before excretion. Patients with hepatic impairment are prone to drug accumulation. Renal insufficiency does not affect drug clearance. *Etoricoxib* is used for symptomatic relief in the treatment of osteoarthritis, rheumatoid arthritis, and acute gouty arthritis, as well as for the short-term treatment of musculoskeletal pain, postoperative pain, and primary dysmenorrhea. The drug is associated with the increased risk of heart attack and stroke. *Etoricoxib* is not available in the U.S.

Disease-Modifying Antirheumatic Drugs

Rheumatoid arthritis is an autoimmune disease that affects about 1% of the population. The pharmacological management of rheumatoid arthritis includes symptomatic relief through the use of NSAIDs. However, although they have anti-inflammatory effects, NSAIDs have minimal, if any, effect on progression of joint deformity. Disease-modifying

TABLE 42–5 ■ DISEASE-MODIFYING ANTIRHEUMATIC DRUGS

DRUG	CLASS OR ACTION	CHAPTER NUMBER
Small molecules		
Methotrexate	Antifolate	70
Leflunomide	Pyrimidine synthase inhibitor	70
Hydroxychloroquine	Antimalarial	66
Minocycline	5-Lipoxygenase inhibitor, tetracycline antibiotic	41, 60
Sulfasalazine	Salicylate	42, 55
Azathioprine	Purine synthase inhibitor	70
Cyclosporine	Calcineurin inhibitor	39
Cyclophosphamide	Alkylating agent	70
Penicillamine	Chelating agent	76
Auranofin	Gold compound	76
Biologicals		
Adalimumab Golimumab Etanercept Infliximab Certolizumab	Ab, TNF-α antagonist Ab, TNF-α antagonist Ab, TNF-α antagonist IgG-TNF receptor fusion protein (anti-TNF) Fab fragment toward TNF-α	38, 39
Abatacept	T-cell costimulation inhibitor (binds B7 protein on antigen-presenting cell)	38, 39
Rituximab	Ab toward CD20 (cytotoxic toward B cells)	72
Anakinra	IL-1 receptor antagonist	39, 72
Tocilizumab	IL-6 receptor antagonist	39, 72
Tofacitinib	Janus kinase inhibitor	39, 71

antirheumatic drugs (DMARDs), on the other hand, reduce the disease activity of rheumatoid arthritis and retard the progression of arthritic tissue destruction. DMARDs include a diverse group of small-molecule nonbiological and biological agents (mainly antibodies or binding proteins), as summarized in Table 42–5.

Biological DMARDs remain reserved for patients with persistent moderate or high disease activity and indicators of poor prognosis. Therapy is tailored to the individual patient, and the use of these agents must be weighed against their potentially serious adverse effects. The combination of NSAIDs with these agents is common.

Pharmacotherapy of Gout

Gout results from the precipitation of urate crystals in the tissues and the subsequent inflammatory response. Acute gout usually causes painful distal monoarthritis and can cause joint destruction, subcutaneous deposits (tophi), and renal calculi and damage. Gout affects 3% of the adult population of Western countries. Risk factors include male sex, diuretic use, alcohol intake, obesity, hypertension, and consumption of sweetened beverages, red meat, and certain types of seafood (Hainer et al., 2014).

The pathophysiology of gout is incompletely understood. Hyperuricemia, while a prerequisite, does not inevitably lead to gout. Uric acid, the end product of purine metabolism, is relatively insoluble compared to its hypoxanthine and xanthine precursors, and normal serum urate levels (~5 mg/dL, or 0.3 mM) approach the limit of solubility. In most patients with gout, hyperuricemia arises from underexcretion rather than overproduction of urate. Mutations of one of the renal URATs, URAT-1, are associated with hypouricemia. Urate tends to crystallize as monosodium urate in colder or more acidic conditions. Monosodium urate crystals activate monocytes/macrophages via the toll-like receptor pathway, mounting an innate immune response. This results in the activation of the NLRP3 inflammasome; the secretion of cytokines, including IL-1β and TNF-α; endothelial activation; and attraction of neutrophils to the site of inflammation. Neutrophils secrete inflammatory mediators that lower the local pH and lead to further urate precipitation. The aims of treatment are to:

- Decrease the symptoms of an acute attack
- Decrease the risk of recurrent attacks
- Lower serum urate levels

The following therapeutic strategies are available for these purposes:

- Drugs that relieve inflammation and pain (NSAIDs, *colchicine*, glucocorticoids)
- Drugs that prevent inflammatory responses to crystals (*colchicine* and NSAIDs)
- Drugs that act by inhibition of urate formation (e.g., *allopurinol*, *febuxostat*) or augmentation of urate excretion (*probenecid*)

The NSAIDs have been discussed previously. Glucocorticoids are discussed in Chapter 50. This section focuses on *colchicine, allopurinol, febuxostat, pegloticase, rasburicase*, and the uricosuric agents *probenecid* and *benzbromarone*. Some other drugs used off label to reduce uric acid levels or treat gout include *losartan*, *fenofibrate*, and *canakinumab*. *Canakinumab*, a recombinant human monoclonal antibody to the proinflammatory cytokine IL-1β, is approved by the European Medicines Agency for the treatment of gout when management with *colchicine*, corticosteroids, or NSAIDs is ineffective or when a patient experiences frequent attacks. In the U.S., the agent is not FDA-approved for gout but is approved for use in treating several autoinflammatory syndromes and systemic idiopathic juvenile arthritis.

Colchicine

Colchicine is one of the oldest available therapies for acute gout. The botanical source of *colchicine*, the autumn crocus, *Colchicum autumnale*, was known to the Greeks and Egyptians as a purgative and antiarthritic agent by 1500 BCE and described as a treatment for gout in the 1st century CE (Škubnik et al., 2020). Extracts and corms containing *colchicine* were being used for gout and joint pain around 550 CE. *Colchicine* is considered second-line therapy because it has a narrow therapeutic window and a high rate of side effects, particularly at higher doses.

Mechanism of Action

Colchicine exerts a variety of pharmacological effects; how these relate to its activity in gout is partially understood (Leung et al., 2015). *Colchicine* has antimitotic effects, arresting cell division in G_1 by interfering with microtubule and spindle formation (an effect shared with vinca alkaloids). This effect is greatest on cells with rapid turnover (e.g., neutrophils, GI epithelium). Depolymerization of microtubules by *colchicine* reduces neutrophil recruitment to inflamed tissue and neutrophil adhesion. *Colchicine* may alter neutrophil motility and decreases the secretion of chemotactic factors and superoxide anions by activated neutrophils. *Colchicine* limits monosodium urate crystal–induced NLRP3 inflammasome activation and subsequent formation of IL-1β and IL-18. This mechanism may explain its therapeutic activity in familial Mediterranean fever and other inflammatory diseases. *Colchicine* inhibits the release of histamine-containing granules from mast cells, the secretion of insulin from pancreatic β cells, and the movement of melanin granules in melanophores.

Colchicine also exhibits a variety of other pharmacological effects. It lowers body temperature, increases the sensitivity to central depressants, depresses the respiratory center, enhances the response to sympathomimetic agents, constricts blood vessels, and induces hypertension by central vasomotor stimulation. It enhances GI activity by neurogenic stimulation but depresses it by a direct effect and alters neuromuscular function.

ADME

Absorption of oral *colchicine* is rapid but variable. Peak plasma concentrations occur 0.5 to 2 h after dosing. Food does not affect the rate or extent of *colchicine* absorption. In plasma, 39% of *colchicine* is protein bound, primarily to albumin. The formation of *colchicine*-tubulin complexes in many tissues contributes to its large volume of distribution. There is significant enterohepatic circulation. The exact metabolism of *colchicine* in humans is unknown; *in vitro* studies indicate that it may undergo oxidative demethylation by CYP3A4 and possibly glucuronidation. In healthy volunteers, 40% to 65% of the total absorbed oral dose of *colchicine* is recovered unchanged in the urine. The kidney, liver, and spleen also contain high concentrations of *colchicine*, but it apparently is largely excluded from heart, skeletal muscle, and brain. *Colchicine* is a substrate of the P-glycoprotein exporter (see Chapter 4). The plasma $t_{1/2}$ of *colchicine* is approximately 31 h. The drug is contraindicated in patients with hepatic or renal impairment requiring concomitant therapy with CYP3A4 or P-glycoprotein inhibitors. *Colchicine* is not removed by hemodialysis.

Therapeutic Uses

The dosing regimen for *colchicine* must be individualized based on age, renal and hepatic function, concomitant use of other medications, and disease severity. A minimum of 3 days, but preferably 7 to 14 days, should elapse between courses of gout treatment with *colchicine* to avoid cumulative toxicity. Patients with hepatic or renal disease and dialysis patients should receive reduced doses or less-frequent therapy. For elderly patients, adjust the dose for renal function. For those with cardiac, renal, hepatic, or GI disease, NSAIDs or glucocorticoids may be preferred.

Acute Gout. *Colchicine* dramatically relieves acute attacks of gout. It is effective in roughly two-thirds of patients if given within 24 h of attack onset. Pain, swelling, and redness abate within 12 h and are completely gone within 48 to 72 h. The regimen approved for adults recommends a total of two doses taken 1 h apart: 1.2 mg (2 tablets) at the first sign of a gout flare followed by 0.6 mg (1 tablet) 1 h later. Patients with severe renal or hepatic dysfunction and patients receiving dialysis should not receive repeat courses of therapy more frequently than every 2 weeks.

Prevention of Acute Gout. *Colchicine* is used in the prevention of recurrent gout, particularly in the early stages of antihyperuricemic therapy. The typical dose for prophylaxis in patients with normal renal and hepatic function is 0.6 mg taken orally 3 or 4 days/week for patients who have less than one attack per year, 0.6 mg daily for patients who have more than one attack per year, and 0.6 mg up to two times daily for patients who have severe attacks.

Adverse Effects

Exposure of the GI tract to large amounts of *colchicine* and its metabolites via enterohepatic circulation and the rapid rate of turnover of the GI mucosa may explain why the GI tract is particularly susceptible to *colchicine* toxicity. Nausea, vomiting, diarrhea, and abdominal pain are the most common untoward effects and the earliest signs of impending *colchicine* toxicity. Drug administration should be discontinued as soon as these symptoms occur. There is a latent period, which is not altered by dose, of several hours or more between the administration of the drug and the onset of symptoms. A dosing study demonstrated that one dose initially and a single additional dose after 1 h was much less toxic than the traditional hourly dosing regimen for acute gout flares. Acute intoxication causes hemorrhagic gastropathy.

Other serious side effects of *colchicine* therapy include myelosuppression, leukopenia, granulocytopenia, thrombopenia, aplastic anemia, and rhabdomyolysis. Rarely, it can cause reversible axonal neuromyopathy.

Life-threatening toxicities are associated with administration of concomitant therapy with P-glycoprotein or CYP3A4 inhibitors. The FDA suspended the U.S. marketing of all injectable dosage forms of *colchicine* in 2008. *Colchicine* is marketed in a fixed-dose combination with *probenecid* for the management of frequent recurrent gout attacks.

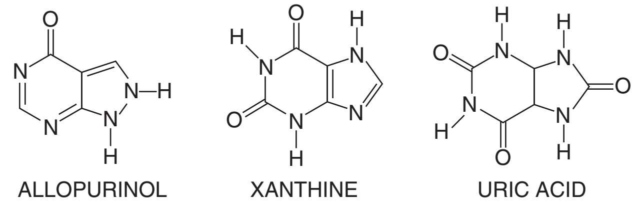
Allopurinol

History

Allopurinol initially was synthesized as a candidate antineoplastic agent but was found to lack antineoplastic activity. Subsequent testing showed it to be an inhibitor of xanthine oxidase (XO) that was useful clinically for the treatment of gout.

Allopurinol inhibits XO and prevents the synthesis of urate from hypoxanthine and xanthine. *Allopurinol* is used for treating hyperuricemia in patients with gout and for prevention in those with hematological malignancies about to undergo chemotherapy (acute tumor lysis syndrome). Even though underexcretion rather than overproduction of urate is the underlying defect in most gout patients, *allopurinol* remains effective therapy.

Allopurinol is an analogue of hypoxanthine. Its active metabolite, oxypurinol, is an analogue of xanthine.



Mechanism of Action

Both *allopurinol* and its primary metabolite, oxypurinol (alloxanthine), reduce urate production by inhibiting XO, the enzyme that converts xanthine to uric acid. *Allopurinol* competitively inhibits XO at low concentrations and is a noncompetitive inhibitor at high concentrations. *Allopurinol* also is a substrate for XO; the product of this reaction, oxypurinol, also is a noncompetitive inhibitor of the enzyme. The formation of oxypurinol, together with its long persistence in tissues, is responsible for much of the pharmacological activity of *allopurinol*.

In the absence of *allopurinol*, the dominant urinary purine is uric acid. During *allopurinol* treatment, the urinary purines include hypoxanthine, xanthine, and uric acid. Because each has its independent solubility, the concentration of uric acid in plasma is reduced and purine excretion is increased, without exposing the urinary tract to an excessive load of uric acid. Despite their increased concentrations during *allopurinol* therapy, hypoxanthine and xanthine are efficiently excreted, and tissue deposition does not occur. There is a small risk of xanthine stones in patients whose urate load before *allopurinol* therapy is very high; this risk can be minimized by liberal fluid intake and urinary alkalization.

Allopurinol facilitates the dissolution of tophi and prevents the development or progression of chronic gouty arthritis by lowering the uric acid concentration in plasma below the limit of its solubility. The formation of uric acid stones virtually disappears with therapy, which prevents the development of nephropathy. Once significant renal injury has occurred, *allopurinol* cannot restore renal function but may delay disease progression. The incidence of acute attacks of gouty arthritis may increase during the early months of *allopurinol* therapy because of mobilization of tissue stores of uric acid. Coadministration of *colchicine* helps suppress such acute attacks. In some patients, the *allopurinol*-induced increase in excretion of oxypurines is less than the reduction in uric acid excretion; this disparity primarily is a result of reutilization of oxypurines and feedback inhibition of *de novo* purine biosynthesis.

ADME

Allopurinol is absorbed relatively rapidly after oral ingestion, and peak plasma concentrations are reached within 60 to 90 min. About 20% is excreted in the feces in 48 to 72 h, presumably as unabsorbed drug, and 10% to 30% is excreted unchanged in the urine. The remainder undergoes metabolism, mostly to oxypurinol, an active metabolite. Oxypurinol is excreted slowly in the urine by glomerular filtration, counterbalanced by some tubular reabsorption. The plasma $t_{1/2}$ of *allopurinol* is approximately 1 to 2 h, and that of oxypurinol is approximately 18 to 30 h (longer in individuals with renal impairment). This allows for once-daily dosing and makes *allopurinol* the most commonly used antihyperuricemic agent. *Allopurinol* and its active metabolite oxypurinol are distributed in total tissue water, with the exception of brain, where their concentrations are about one-third of those in other tissues. Neither compound is bound to plasma proteins. The plasma concentrations of the two compounds do not correlate well with therapeutic or toxic effects.

Therapeutic Uses

Allopurinol is available for oral and intravenous use. Oral therapy provides effective therapy for primary and secondary gout, hyperuricemia secondary to malignancies, and calcium oxalate calculi. The goal of therapy is to reduce the plasma uric acid concentration to less than 6 mg/dL (<360 μmol/L) and typically less than 5 mg/dL (<297 μmol/L) in patients with tophi to accelerate the clearance of monosodium urate. Historically, it was customary to antecede *allopurinol* therapy with *colchicine* as it was thought *allopurinol* could worsen acute attacks. It is now considered safe and routine to initiate *allopurinol* during acute attacks as long as NSAIDs and *colchicine* are given concomitantly. Fluid intake should be sufficient to maintain daily urinary volume greater than 2 L; slightly alkaline urine is preferred. An initial daily dose of 100 mg in patients with estimated glomerular filtration rates greater than 40 mg/min is increased by 100-mg increments at weekly intervals. Most patients can be maintained on 300 mg/day. Patients with reduced glomerular filtration require a lower dose to achieve the targeted uric acid concentration. With frequent monitoring for adverse effects, daily doses may exceed 300 mg, even in renal impairment. Those with hematological malignancies may need up to 800 mg/day beginning 2 to 3 days before the start of chemotherapy.

The usual dose in children with secondary hyperuricemia associated with malignancies is 150 to 300 mg/day, depending on age. *Allopurinol* also is useful in lowering the high plasma concentrations of uric acid in patients with Lesch-Nyhan syndrome, thereby preventing the complications resulting from hyperuricemia; however, there is no evidence that it alters the progressive neurological and behavioral abnormalities that are characteristic of the disease. Other orphan uses for *allopurinol* include Chagas disease and the *ex vivo* preservation of cadaveric kidneys prior to transplantation.

Adverse Effects

Allopurinol generally is well tolerated. The most common adverse effects are hypersensitivity reactions that may manifest after months or years of therapy. If indicated, desensitization to *allopurinol* can be carried out starting at 10 to 25 μg/day, with the drug diluted in oral suspension and doubled every 3 to 14 days until the desired dose is reached; the success rate is approximately 50%.

Serious hypersensitivity reactions preclude further use of the drug. The cutaneous reaction caused by *allopurinol* is predominantly a pruritic, erythematous, or maculopapular eruption, but occasionally, the lesion is urticarial or purpuric. Rarely, toxic epidermal necrolysis or Stevens-Johnson syndrome occurs, which can be fatal. The risk for Stevens-Johnson syndrome is limited primarily to the first 2 months of treatment. Because the rash may precede severe hypersensitivity reactions, patients who develop a rash should discontinue *allopurinol*. Among polymorphic genetic factors that appear to contribute to such delayed hypersensitivity reactions is the human leucocyte antigen B*5801 allele (HLA-B*5801) (Li et al., 2021). Its presence may help to identify those at risk among susceptible populations (e.g., Korean, Thai, Sardinian Italian, and Han Chinese) (Yu et al., 2017).

Oxypurinol has orphan drug status and is available for compassionate use in the U.S. for patients who are intolerant of *allopurinol*. Fever, malaise, and myalgias also may occur in about 3% of patients, more frequently in those with renal impairment. Transient leukopenia or leukocytosis and eosinophilia are rare reactions that may require cessation of therapy. Hepatomegaly and elevated levels of transaminases in plasma and progressive renal insufficiency also may occur.

Allopurinol is contraindicated in patients who have exhibited serious adverse effects or hypersensitivity reactions to the medication and in nursing mothers and children, except those with malignancy or certain inborn errors of purine metabolism (e.g., Lesch-Nyhan syndrome). *Allopurinol* generally is used in patients with hyperuricemia posttransplantation. It can be used in conjunction with a uricosuric agent.

Drug Interactions

Allopurinol increases the $t_{1/2}$ of *probenecid* and enhances its uricosuric effect, while *probenecid* increases the clearance of oxypurinol, thereby increasing dose requirements of *allopurinol*. *Allopurinol* inhibits the enzymatic inactivation of *mercaptopurine* and its derivative *azathioprine* by XO. Thus, when *allopurinol* is used concomitantly with oral *mercaptopurine* or *azathioprine*, dosage of the antineoplastic agent must be reduced to 25% to 33% of the usual dose (see Chapters 39 and 70). This is important when treating gout in a transplant recipient. The risk of bone marrow suppression also is increased when *allopurinol* is administered with cytotoxic agents that are not metabolized by XO, particularly *cyclophosphamide*. *Allopurinol* may interfere with the hepatic inactivation of other drugs, including *warfarin*. Although the effect is variable, increased monitoring of prothrombin activity is recommended in patients receiving these medications concomitantly.

Whether the increased incidence of rash in patients receiving concurrent *allopurinol* and *ampicillin* can be ascribed to *allopurinol* or to hyperuricemia is not known. Hypersensitivity reactions have been reported in patients with compromised renal function, especially those who are receiving a combination of *allopurinol* and a thiazide diuretic. The concomitant administration of *allopurinol* and *theophylline* leads to increased accumulation of an active metabolite of *theophylline*, 1-methylxanthine; the concentration of *theophylline* in plasma also may be increased (see Chapter 44).

Febuxostat

Febuxostat is an XO inhibitor approved for treatment of hyperuricemia in patients with gout.

Mechanism of Action

Febuxostat is a nonpurine inhibitor of XO. Unlike oxypurinol, the active metabolite of *allopurinol* that inhibits the reduced form of XO, *febuxostat* forms a stable complex with both the reduced and oxidized enzymes and inhibits catalytic function in both states.

ADME

Febuxostat is rapidly absorbed, reaching C_{Pmax} at 1 to 1.5 h after dose. The absolute bioavailability is unknown. Magnesium hydroxide and aluminum hydroxide delay absorption by about 1 h; food reduces absorption slightly. *Febuxostat* ($t_{1/2}$ of 5–8 h) is extensively metabolized by both conjugation via UGT enzymes, including UGTs 1A1, 1A3, 1A9, and 2B7, and oxidation by CYPs 1A2, 2C8, and 2C9 and non-CYP enzymes; elimination occurs via hepatic and renal pathways. Mild-to-moderate renal or hepatic impairment does not produce relevant effects on its elimination kinetics.

Therapeutic Use

Febuxostat is approved for hyperuric patients with gout attacks but is not recommended for treatment of asymptomatic hyperuricemia. It is available in 40- and 80-mg oral tablets. A dose of 40 mg/day *febuxostat* lowers serum uric acid to similar *levels* as 300 mg/day *allopurinol*. More patients reach the target urate concentration of 6.0 mg/dL (360 μmol/L) on 80 mg/day *febuxostat* than on 300 mg/day *allopurinol*. Thus, therapy should be initiated with 40 mg/day and the dose increased if the target serum uric acid concentration is not reached within 2 weeks.

Adverse Events

In clinical studies, the most common adverse reactions noted were liver function abnormalities, nausea, joint pain, and rash. Liver function should be monitored periodically. An increase in gout flares was frequently observed after initiation of therapy, due to reduction in serum uric acid levels resulting in mobilization of urate from tissue deposits. Concurrent prophylactic treatment with an NSAID or *colchicine* is usually required. There was a higher rate of myocardial infarction and stroke in patients taking *febuxostat* compared to *allopurinol*. Although a causal relationship between the cardiovascular events and *febuxostat* therapy is not clear, *febuxostat* carries a boxed warning about possible increased risk of cardiovascular death. Patients should be monitored for cardiovascular complications.

Drug Interactions

Plasma levels of drugs metabolized by XO (e.g., *mercaptopurine*, *azathioprine*) will increase when administered concurrently with *febuxostat*. Thus, *febuxostat* is contraindicated in patients on *azathioprine* or *mercaptopurine*. *Febuxostat* does inhibit the XO-catalyzed hydroxylation of 1-methylxanthine, the metabolite of *theophylline*, to 1-methylurate. While this elevates the urinary excretion of 1-methylxanthine by 400-fold, *febuxostat* (80 mg/day) does not significantly alter the C_{Pmax} and AUC for *theophylline* (Tsai et al., 2012).

Uricase

Pegloticase is a pegylated uricase (urate oxidase) that catalyzes the enzymatic oxidation of uric acid into allantoin, a more soluble and inactive metabolite. The recombinant enzyme, based on the porcine uricase, is administered by infusion every 2 weeks. *Pegloticase* is used for the treatment of severe, treatment-refractory, chronic gout or when use of other urate-lowering therapies is contraindicated.

The drug's efficacy may be hampered by the production of antibodies against the drug. *Pegloticase* antibodies develop in nearly 90% of people, and high titers are associated with loss of the urate-lowering effect and with an elevated risk for infusion reactions. Anaphylactic reactions and hemolysis in G6PD-deficient patients have been associated with the use of *pegloticase*. Other frequently observed adverse reactions include vomiting, nausea, chest pain, constipation, diarrhea, and erythema, pruritus, and urticaria.

Rasburicase is a recombinant uricase that has been shown to lower urate levels more effectively than *allopurinol*. It is indicated for the initial management of elevated plasma uric acid levels in pediatric and adult patients with leukemia, lymphoma, and solid tumor malignancies who are receiving anticancer therapy expected to result in tumor lysis and significant hyperuricemia. The experience with *rasburicase* for treatment of gout is limited because of the formation of activity-limiting antibodies against the drug. Hemolysis in G6PD-deficient patients, methemoglobinemia, acute renal failure, and anaphylaxis have been associated with the use of *rasburicase*. Other frequently observed adverse reactions include vomiting, fever, nausea, headache, abdominal pain, constipation, diarrhea, and mucositis. *Rasburicase* causes enzymatic degradation of the uric acid in blood samples, and special handling is required to prevent spuriously low values for plasma uric acid in patients receiving the drug.

Uricosuric Agents

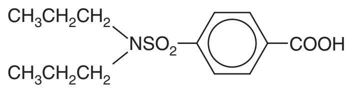
Uricosuric agents increase the excretion of uric acid. These agents are typically reserved for patients who underexcrete uric acid relative to their plasma levels. In humans, urate is filtered, secreted, and reabsorbed by the kidneys. Reabsorption is robust, such that the net amount excreted usually is about 10% of that filtered. Reabsorption is mediated by an OAT family member, URAT-1, encoded by the *SLC22A12* gene, which is expressed at the apical brush border of the proximal nephron.

Urate is exchanged by URAT-1 for either an organic anion such as lactate or nicotinate or less potently for an inorganic anion such as chloride. Uricosuric drugs such as *probenecid*, *benzbromarone* (not available in the U.S.), and *losartan* compete with urate for the transporter from the apical side of tubular epithelial cells, thereby inhibiting its reabsorption via the urate–anion exchanger system. Increased levels of antiuricosuric metabolites such as nicotinate (a metabolite of niacin), pyrazinoate (a metabolite of *pyrazinamide*), hydroxybutyrate and acetoacetate (e.g., in diabetic ketoacidosis), and lactate (e.g., in alcohol intoxication) enhance reabsorption of urate by stimulating URAT-1–dependent anion exchange. Depending on dosage, substrates of URAT-1 may, via competition, decrease transport of urate into the tubular cell or, via promotion of anion exchange, increase the excretion of urate out of the cell. Transport of urate through tubular cells is bidirectional, resulting in the secretion of urate, although the quantitative relevance of the efflux mechanism remains unclear. Therefore, there are two mechanisms by which a drug may nullify the uricosuric action of another. First, the drug may inhibit the secretion of the uricosuric agent, thereby denying it access to its site of action, the luminal aspect of the brush border. Second, the inhibition of urate secretion by one drug may counterbalance the inhibition of urate reabsorption by the other.

Urate homeostasis and the characteristics of the multiple renal transporters participating in urate secretion and reabsorption (see Figure 42–2) and their modulation by pharmacological agents have recently been reviewed (Tátrai et al., 2021).

Probenecid

Probenecid is a highly lipid-soluble benzoic acid derivative (pK_a = 3.4).



PROBENECID

Mechanism of Action. ***Inhibition of Organic Acid Transport.*** The actions of *probenecid* are confined largely to inhibition of the transport of organic acids across epithelial barriers. *Probenecid* inhibits the reabsorption of uric acid by OATs, principally URAT-1. Uric acid is the only important endogenous compound whose excretion is known to be increased by *probenecid*. The uricosuric action of *probenecid* is blunted by the coadministration of salicylates.

Inhibition of Transport of Miscellaneous Substances. *Probenecid* decreases the tubular secretion of several drugs by inhibition of OAT-1 and -3, which are expressed at the basolateral surface of proximal tubular cells. OAT-1/3 inhibition reduces renal secretion and increases plasma levels of drugs such as *methotrexate* and the active metabolite of *clofibrate*. It inhibits renal secretion of the inactive glucuronide metabolites of NSAIDs such as *naproxen*, *ketoprofen*, and *indomethacin* and thereby can increase their plasma concentrations. *Probenecid* inhibits the transport of

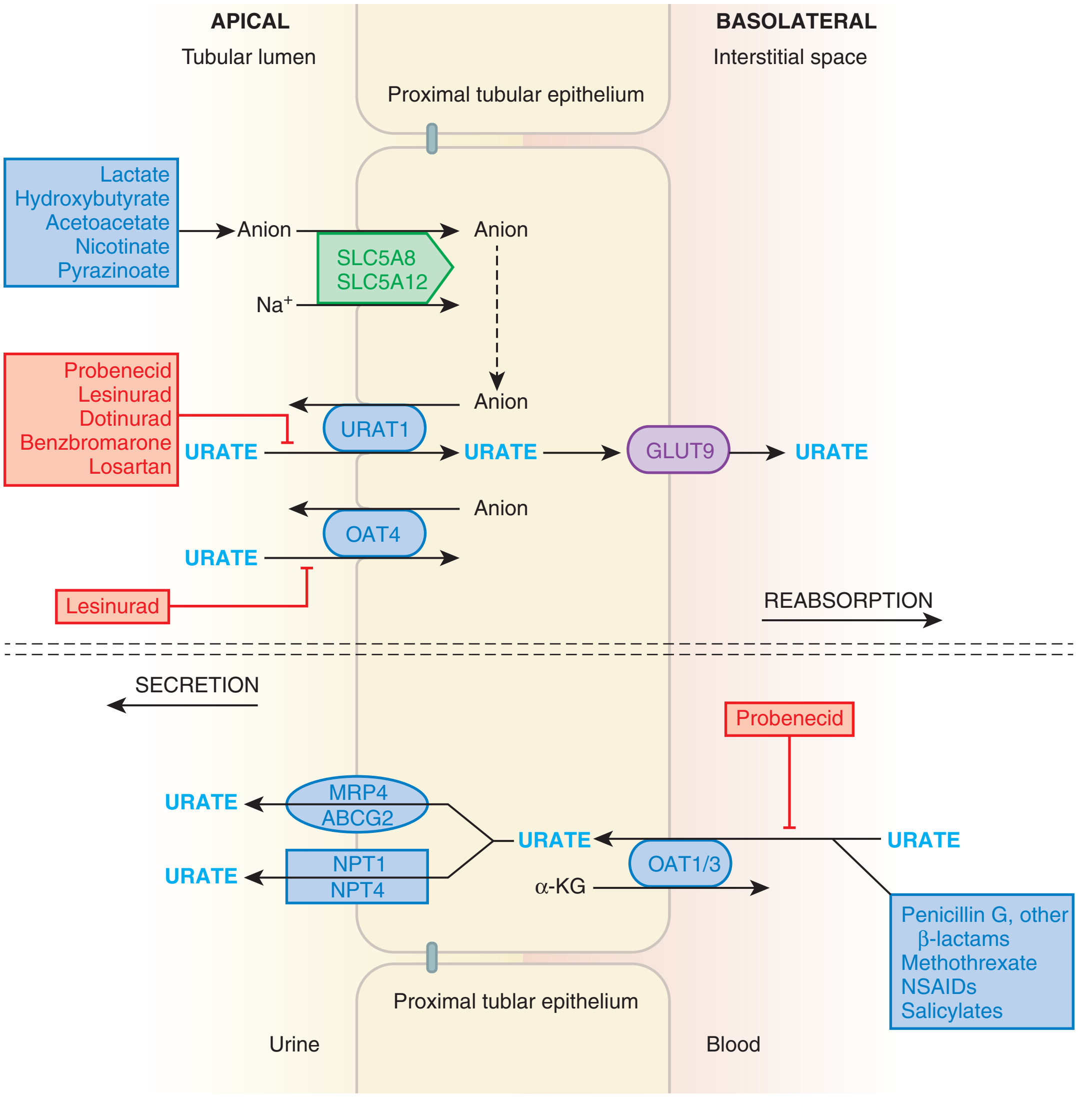

APICAL
Tubular lumen
BASOLATERAL
Interstitial space
Proximal tubular epithelium
Lactate
Hydroxybutyrate
Acetoacetate
Nicotinate
Pyrazinoate
Anion
Anion
SLC5A8
SLC5A12
Na+
Probenecid
Lesinurad
Dotinurad
Benzbromarone
Losartan
Anion
URAT1
URATE
URATE
GLUT9
URATE
Anion
OAT4
URATE
Lesinurad
REABSORPTION
SECRETION
Probenecid
URATE
MRP4
ABCG2
URATE
URATE
NPT1
NPT4
OAT1/3
URATE
α-KG
Penicillin G, other β-lactams
Methothrexate
NSAIDs
Salicylates
Proximal tublar epithelium
Urine
Blood

Figure 42–2 *A pharmacologist's view of urate transport in the proximal tubule and its inhibition by drugs.* URAT1 is the primary anion exchange transporter for urate from the tubular lumen into the proximal tubular epithelial cell. OAT4 does likewise but to a much lesser degree. Na^+-coupled anion transport by SLC5A8 and SLC5A12 provides intracellular anions to URAT. High luminal concentrations of these organic anions support URAT1 activity. GLUT9 is a major contributor to basolateral transport of urate (reabsorption). Several polymorphisms of GLUT9 are associated with altered risk for gout. The basolateral urate transporters OAT1 and OAT3 play a role in the secretion of urate into the tubular lumen, which involves MRP4, ABCG2, NTP1, and NTP4 at the apical cell membrane. In gout, *probenecid* generally leads to a net increase in urate excretion. Drug-drug interactions occur when drugs compete for transport via OAT3 and OAT4, leading to increase in plasma levels. *Probenecid*'s inhibition of the secretion of *penicillin* and other β-lactam antibiotics is therapeutically exploited to prolong the dwell time of these drugs in the body. α-KG, α-ketoglutarate.

5-HIAA and other acidic metabolites of cerebral monoamines from the CSF to the plasma. The transport of drugs such as *penicillin G* also may be affected, and *probenecid* is used therapeutically to elevate and prolong plasma β-lactam levels. *Probenecid* depresses the biliary secretion of certain compounds, including the diagnostic agents indocyanine green and bromosulfophthalein. It also decreases the biliary secretion of *rifampin*, leading to higher plasma concentrations.

ADME. *Probenecid* is absorbed completely after oral administration. Peak plasma concentrations are reached in 2 to 4 h. The $t_{1/2}$ of the drug in plasma is dose dependent and varies from less than 5 to more than 8 h. Between 85% and 95% of the drug is bound to plasma albumin; the 5% to 15% of unbound drug is cleared by glomerular filtration and active secretion by the proximal tubule. A small amount of probenecid glucuronide appears in the urine. It also is hydroxylated to metabolites that retain their carboxyl function and have uricosuric activity.

Therapeutic Uses. *Probenecid* is marketed for oral administration, alone and in combination with *colchicine*. The starting dose is 250 mg twice daily, increasing over 1 to 2 weeks to 500 to 1000 mg twice daily. *Probenecid* increases urinary urate levels. Liberal fluid intake therefore should be maintained throughout therapy to minimize the risk of renal stones. *Probenecid* should not be used in gouty patients with nephrolithiasis or with overproduction of uric acid. Concomitant *colchicine* or NSAIDs are indicated early during therapy to avoid precipitating an attack of gout, which may occur in 20% or fewer of gouty patients treated with *probenecid* alone. After 6 months, if serum uric acid levels are within normal limits and there have been no gout attacks, the dose of *probenecid* may be tapered off by 500 mg every 6 months.

Combination with Penicillin. Higher doses of *probenecid* (1–2 g/day) are used as an adjuvant to prolong the dwell time of *penicillin* and other β-lactam antibiotics in the body (see Chapter 58).

Adverse Effects. *Probenecid* is well tolerated. Approximately 2% of patients develop mild GI irritation. The risk is increased at higher doses. It is ineffective in patients with renal insufficiency and should be avoided in those with creatinine clearance of less than 50 mL/min. Hypersensitivity reactions usually are mild and occur in 2% to 4% of patients. Substantial overdosage with *probenecid* results in CNS stimulation, convulsions, and death from respiratory failure.

Dotinurad

Dotinurad is a novel inhibitor of renal uric acid reabsorption currently approved in Japan (Kuriyama, 2020).

Mechanism of Action. *Dotinurad*, which is structurally derived from benzbromarone, is a potent inhibitor of URAT-1 (Uda et al., 2020) localized in the brush-border membrane of the renal proximal tubules. Like other uricosuric agents, the drug is thought to inhibit the transporter from the luminal side in the renal tubules.

ADME. Mass balance calculations suggest that more than 90% of an orally administered *dotinurad* dose is absorbed (Omura et al., 2020). C_{Pmax} is reached after approximately 3 h. *Dotinurad* is highly plasma bound (>99%) and eliminated with a $t_{1/2}$ of approximately 10 h (clearance ~1 L/h). Glucuronidation and sulfation are the major routes of metabolism.

Therapeutic Uses. Clinical trials demonstrated effective reduction in uric acid levels at doses of 1, 2, and 4 mg/day.

Adverse Effects. *Dotinurad* was well tolerated in clinical trials. Adverse reactions reported in an open-label study of 8 to 12 months of treatment with daily administration of 2 to 4 mg *dotinurad* included nasopharyngitis (17.9%) and gouty arthritis (13.0%). Renal calculi were reported in 1.5% of patients, suggesting that, as with *lesinurad*, a potential impact on renal function needs to be considered during chronic treatment.

Lesinurad

Lesinurad was FDA-approved for combination therapy with an XO inhibitor in treating hyperuricemia. However, the drug has recently been discontinued in the U.S. and withdrawn in the European Union.

Mechanism of Action. *Lesinurad* inhibits the URAT-1 and OAT-4 transporters, thereby reducing renal uric acid reabsorption.

ADME. *Lesinurad* is rapidly absorbed after oral administration and has bioavailability of about 100%. *Lesinurad* is largely bound to plasma albumin and other plasma proteins (<98%). The elimination $t_{1/2}$ is about 5 h (clearance ~6 L/h). CYP2C9 is the major metabolizing enzyme. *Lesinurad* (30% unchanged) and its metabolites are excreted in the urine (>60% of dose) and feces. Renal impairment increases exposure, and *lesinurad* should not be used when renal function is severely reduced (estimated creatinine clearance <45 mL/min).

Therapeutic Uses. *Lesinurad* (200 mg/day) is marketed for the treatment of gout in patients who have not achieved the target serum uric acid levels with an XO inhibitor alone. It should not be used for the treatment of asymptomatic hyperuricemia or as monotherapy.

Adverse Effects. *Lesinurad* has been labeled with a boxed warning because of a risk of acute renal failure that is more common when it is used without an XO inhibitor. Increases in blood creatinine levels (1.5- to 2-fold) were observed with a frequency of approximately 4% during combination therapy and 8% during monotherapy. Renal failure occurred in less than 1% of patients during combination therapy but approximately 9% during monotherapy. Similarly, the risk of nephrolithiasis is increased when *lesinurad* is given alone. Thus, if treatment with the XO inhibitor is interrupted, *lesinurad* dosing should also be interrupted. Other adverse reactions reported by patients during clinical trials include headache (~5%), influenza-like symptoms (~5%), and gastroesophageal reflux (~3%).

Benzbromarone

Benzbromarone, a potent uricosuric agent, is a reversible inhibitor of the urate-anion exchanger, URAT-1, in the proximal tubule. Hepatotoxicity reported in conjunction with its use has limited its availability, and it is not approved for use in the U.S. or the European Union. The drug is absorbed readily after oral ingestion; peak plasma levels are achieved in about 4 h. It is metabolized to monobrominated and dehalogenated derivatives, both of which have uricosuric activity, and is excreted primarily in the bile. *Benzbromarone* metabolism involves predominantly CYP2C9, with contributions by CYP3A4 and CYP1A1. The drug's potent inhibition of CYP2C9 predisposes to drug-drug interactions, and patients homozygous for the CYP2C9*3/*3 polymorphism metabolize the drug more slowly to 6-OH-benzbromarone (Uchida et al., 2010); the clinical significance of this finding is unclear. In addition to the formation of hepatotoxic metabolites, inhibition of mitochondrial function may play a role in the development of a fulminant hepatitis in some patients.

As the micronized powder, it is effective in a single daily dose ranging from 25 to 100 mg. It is effective in patients with renal insufficiency and is prescribed to patients who are either allergic to or refractory to other drugs used for the treatment of gout. Preparations that combine *allopurinol* and *benzbromarone* are more effective than either drug alone in lowering serum uric acid levels, despite the fact that *benzbromarone* lowers plasma levels of oxypurinol, the active metabolite of *allopurinol*. The uricosuric action is blunted by *aspirin* or *sulfinpyrazone*.

Acknowledgment: *Emer Smyth contributed to this chapter in earlier editions of this book. We have retained some of her text in the current edition.*

Drug Facts for Your Personal Formulary: *NSAIDs* (see also Tables 42–1, 42–2, and 42–3)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Salicylates • Used to treat pain, fever, inflammation • Adverse Effects: Primarily GI and cardiovascular, salicylate intoxication		
Aspirin	• Vascular indications • Pain/fever • Rheumatoid disease/rheumatic fever	• Irreversible COX inhibitor ⇒ long-acting inhibition of platelet function at low doses • At higher concentrations, small increments in dose disproportionately ↑ C_p and toxicity • Use in children: limited due to Reye syndrome association • Reduces the risk of recurrent adenomas in persons with a history of colorectal cancer or adenomas • Prolongs bleeding time for ~36 h after a dose
Salsalate	• Arthritis • Rheumatic disorders	• Prodrug of salicylic acid • Marketed but not approved in the U.S.
Diflunisal	• Mild to moderate pain • Osteoarthritis/rheumatoid arthritis	• Salicylic acid derivative • Largely devoid of antipyretic effects • $t_{1/2}$ prolonged with renal impairment

Drug Facts for Your Personal Formulary: *NSAIDs* (see also Tables 42–1, 42–2, and 42–3) (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Mesalamine (5-aminosalicylic acid)	• Inflammatory bowel disease (see Chapter 55)	• Oral formulation delivers 5-aminosalicylic acid to lower GI tract; relative bowel specificity reduces side effects • May cause an acute intolerance syndrome (difficult to discern from an exacerbation)
Sulfasalazine	• Rheumatoid arthritis • Inflammatory bowel disease (see Chapter 55)	• Active metabolite, 5-aminosalicylic acid (see mesalamine), is released by colonic bacteria • With G6PD deficiency: susceptibility to hemolytic anemia • Urine and skin discolorations • Incidence of side effects increased in slow acetylators
Olsalazine	• Inflammatory bowel disease (see Chapter 55)	• Active metabolite, 5-aminosalicylic acid (see mesalamine), is released by colonic bacteria • Photosensitivity
Balsalazide	• Inflammatory bowel disease (see Chapter 55)	• Active metabolite, 5-aminosalicylic acid (see mesalamine), is released by colonic bacteria
Para-Aminophenol Derivative • Only acetaminophen remains on the market		
Acetaminophen	• Pain • Fever	• Weak nonspecific COX inhibitor at common doses • Low anti-inflammatory activity • Little effect on platelets • Overdose results in formation of hepatotoxic metabolite (NAPQI) • Toxicity risk ↑ with liver impairment, ethanol consumption ≥3 drinks/day, or malnutrition
Acetic Acid Derivatives		
Indomethacin	• Acute pain • Arthritis, inflammatory conditions • Patent ductus arteriosus	• Potent anti-inflammatory with frequent adverse events (20% discontinue) • High-risk medication in patients ≥65 years
Sulindac	• Inflammatory diseases including osteoarthritis, rheumatoid arthritis, acute gouty arthritis, ankylosing spondylitis, acute painful shoulder	• Sulfoxide prodrug
Etodolac	• Pain, osteoarthritis, rheumatoid arthritis, juvenile arthritis	• Some COX-2 selectivity
Tolmetin	• Osteoarthritis, rheumatoid arthritis, juvenile arthritis	• ~33% of patients experience side effects
Ketorolac	• Moderate-to-severe acute pain • Off label: pericarditis, migraine • Ocular pain, seasonal allergic conjunctivitis	• Potent analgesic, poor anti-inflammatory • Limit systemic therapy to 5 days (risk of GI bleeding) • Do not use postoperatively for pain from CABG or in advanced renal disease • Avoid administration with other NSAIDs or probenecid (probenecid increases ketorolac AUC [3×] and $t_{1/2}$ [2×])
Diclofenac	• Pain • Dysmenorrhea • Migraine (oral solution) • Osteoarthritis, rheumatoid arthritis • Ankylosing spondylitis	• Some COX-2 selectivity • Short $t_{1/2}$ requires relatively high doses to extend dosing interval • Rate of cardiovascular toxicity similar to that of COX-2 inhibitors • Liver toxicity (4%); severe liver injury in ~8 per 100,000 regular users annually
Nabumetone	• Osteoarthritis, rheumatoid arthritis	• Some COX-2 selectivity • 6-Methoxy-2-napthylacetic acid prodrug
Fenamates • Anthranilic acids; Nonselective COX inhibitors with effects similar to other NSAIDs		
Mefenamic acid	• Pain • Dysmenorrhea	• For patients ≥14 years and ≤7 days of treatment • ↑ Hepatic enzymes in 5%
Meclofenamate	• Pain/fever, dysmenorrhea • Osteoarthritis, rheumatoid arthritis, juvenile arthritis • Ankylosing spondylitis, acute gouty arthritis, acute painful shoulder	• For patients ≥14 years • ↑ Hepatic enzymes in 5%
Propionic Acid Derivatives • Nonselective COX inhibitors with the effects and side effects common to other NSAIDs		
Ibuprofen	• Pain/fever, dysmenorrhea • Osteoarthritis, rheumatoid arthritis • Inflammatory diseases • Patent ductus arteriosus	• Over-the-counter NSAID and by prescription • Injectable solution available • $t_{1/2}$: 2–4 h (adults); 23–75 h (premature infants); 0.9–2.3 h (children) • Interacts with aspirin's antiplatelet effect
Naproxen	• Pain, dysmenorrhea • Osteoarthritis, rheumatoid arthritis, ankylosing spondylitis; gout; juvenile arthritis, inflammatory diseases • Patent ductus arteriosus	• Over-the-counter NSAID and by prescription • $t_{1/2}$ variable (9–25 h), age-related • FDA warning: naproxen may not have a lower risk of cardiovascular side effects compared to other NSAIDs • Interacts with aspirin's antiplatelet effect
Fenoprofen	• Pain • Osteoarthritis, rheumatoid arthritis	

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Drug Facts for Your Personal Formulary: *NSAIDs* (see also Tables 42–1, 42–2, and 42–3) (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Ketoprofen	• Pain, dysmenorrhea • Osteoarthritis, rheumatoid arthritis	• 30% develop side effects (usually GI, usually mild) • ↑ Hepatic enzymes ~1%
Flurbiprofen	• Osteoarthritis, rheumatoid arthritis	• ↑ Hepatic enzymes >1%
Oxaprozin	• Osteoarthritis, rheumatoid arthritis, juvenile arthritis	• $t_{1/2}$: 41–55 h • Slow onset, not indicated for fever or acute pain
Enolic Acid Derivatives		
Piroxicam	• Osteoarthritis, rheumatoid arthritis	• Nonselective COX inhibitor with the longest $t_{1/2}$ (~50 h) • Slow onset, not indicated for fever or acute pain • Adverse effects, 20%; 5% of patients discontinue; more GI and serious skin reactions than other NSAIDs
Meloxicam	• Osteoarthritis, rheumatoid arthritis, juvenile arthritis	• Some COX-2 selectivity • $t_{1/2}$: 15–20 h
Diaryl Heterocyclic NSAIDs		
Celecoxib	• Pain • Dysmenorrhea • Osteoarthritis, rheumatoid arthritis, juvenile arthritis • Ankylosing spondylitis • Off-label use: gout	• COX-2 selective • Sulfonamide • Risk of myocardial infarction observed in randomized placebo-controlled trials
Drugs That Relieve Inflammation and Pain		
NSAIDs	• *See* NSAIDs, above	• *See* NSAIDs, above
Glucocorticoids	• *See* Chapter 50	• *See* Chapter 50
Colchicine	• Prophylaxis and the treatment of acute gout flares	• Depolymerizes microtubules ⇒ ↓ neutrophil migration into inflamed area • Narrow therapeutic index; toxic effects related to antimitotic activity • $t_{1/2}$: 31 h (21–50 h) • Individualize dose on the basis of age and hepatic and renal function • Contraindicated in patients with GI, renal, hepatic, or cardiac disorders • Adverse effects: primarily GI • Drug interactions with P-glycoprotein and CYP3A4 inhibitors
Xanthine Oxidase (XO) Inhibitors • Inhibit urate synthesis		
Allopurinol	• Hyperuricemia in patients with gout • Calcium oxalate calculi • Hyperuricemia associated with cancer treatment	• Reduce dose in renal impairment • Rash, diarrhea, nausea frequent; discontinue if rash develops • Beware of Stevens-Johnson syndrome/toxic epidermal necrosis (high risk of mortality); test for genetic factors (e.g., HLA-B*5801) in susceptible populations (see text) • N.B.: Risk of gout attacks in early months of therapy (tissue urate mobilization)
Febuxostat	• Hyperuricemia	• Nonpurine • More selective for XO than allopurinol • Liver function abnormalities (5%–7%)
Uricase • Oxidizes uric acid to allantoin (more soluble and inactive metabolite)		
Pegloticase	• Chronic gout refractory to conventional therapy	• ↓ Blood urate within hours of initial administration • Antibody development against drug may limit efficacy, cause hypersensitivity reactions • Adverse effects: bruising (11%), urticaria (11%), nausea (11%), gout flare during early therapy (74%), chest pain (6%)
Rasburicase	• Hyperuricemia associated with malignancy (pediatric and adult patients)	• ↓ Uric acid levels within hours of initial administration • Not suitable for chronic gout; activity-limiting antibodies form against the drug
Uricosuric Drugs • Inhibit of reabsorption of uric acid by organic anion transporters, thereby increasing excretion of uric acid		
Probenecid	• Hyperuricemia associated with gout (but not for acute attacks) • Prolongation and elevation of β-lactam plasma levels	• Promotes net tubular secretion of urate; alters tubular handling of organic acids (Figure 42–2) • Risk of gout attacks during the early months of therapy (tissue urate mobilization) • Ineffective in patients with renal insufficiency
Lesinurad	• Gout in patients who have not achieved the target serum uric acid levels with XO inhibitor alone	• Discontinued in the U.S., withdrawn in European Union • Substrate of the highly polymorphic CYP2C9; caution needed in CYP2C9 poor metabolizers • Must be used together with XO inhibitor due to renal failure risk

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Chapter 43

Histamine, Bradykinin, and Their Antagonists

Bruce L. Zuraw and Sandra C. Christiansen

HISTAMINE

- Distribution and Biosynthesis
- Release and Functions of Endogenous Histamine
- Physiological and Pharmacological Effects

HISTAMINE RECEPTOR ANTAGONISTS

- H_1 Receptor Antagonists
- H_2 Receptor Antagonists
- H_3 Receptor Antagonists
- H_4 Receptor Antagonists

BRADYKININ, KALLIDIN, AND THEIR ANTAGONISTS

- The Endogenous Kallikrein-Kininogen-Kinin System
- Physiological and Pharmacological Effects of Kallikrein-Kinin Pathways
- Physiological Effects of Kinins
- Pathological Effects of Kinins

DRUGS ACTING ON THE KALLIKREIN-KININ SYSTEM

- Kallikrein Inhibitors
- Kinin Receptor Antagonists
- FXII Inhibitors
- ACE Inhibitors

Histamine is an endogenous biogenic amine that plays a role in the immediate allergic response and is an important regulator of gastric acid secretion. More recently, a role for histamine as a modulator of neurotransmitter release in the central and peripheral nervous systems has emerged. The cloning of four receptors for histamine and the development of subtype-specific receptor antagonists have enhanced our understanding of the physiological and pathophysiological roles of histamine. Competitive antagonists of H_1 receptors are used therapeutically in treating allergies, urticaria, anaphylactic reactions, nausea, motion sickness, and insomnia. Antagonists of the H_2 receptor are effective in reducing gastric acid secretion.

The peptides bradykinin and kallidin, released after activation of the kallikrein-kinin system, have cardiovascular effects similar to those of histamine and play prominent roles in inflammation and nociception. *Icatibant*, a competitive antagonist of the bradykinin B_2 receptor, and *ecallantide*, a specific plasma kallikrein inhibitor, are approved for the treatment of acute episodes of edema in patients with hereditary angioedema.

Histamine

Histamine is a hydrophilic molecule consisting of an imidazole ring and an amino group connected by an ethylene group; histamine is biosynthesized from histidine by decarboxylation (Figure 43–1). Histamine acts through four classes of receptors, designated H_1 through H_4. The four histamine receptors, all G protein-coupled receptors (GPCRs), can be differentially activated by analogues of histamine (Figure 43–2) and inhibited by specific antagonists (Table 43–1).

Distribution and Biosynthesis

Distribution

Almost all mammalian tissues contain histamine in amounts ranging from less than 1 to more than 100 μg/g. Concentrations in plasma and other body fluids are generally very low, but they are significant in human CSF (cerebrospinal fluid). The concentration of histamine is particularly high in tissues that contain large numbers of mast cells, such as skin, bronchial mucosa, and intestinal mucosa.

EARLY HISTORY OF HISTAMINE

Histamine was first prepared synthetically in 1907 and isolated from ergot extracts in 1910. It was identified as a natural constituent of mammalian tissues in 1927 by Best and colleagues and named *histamine* after the Greek word for tissue, *histos*. Dale and Laidlaw made the crucial observation that histamine injection into mammals caused a shock-like reaction and proposed its role in mediating symptoms of anaphylaxis (Emanuel, 1999).

Synthesis, Storage, and Metabolism

Histamine is formed by the decarboxylation of histidine by the enzyme *L-histidine decarboxylase* (Figure 43–1). Mast cells and basophils synthesize histamine and store it in secretory granules. At the secretory granule pH of about 5.5, histamine is positively charged and ionically complexed with negatively charged acidic groups on other granule constituents, primarily proteases and heparin or chondroitin sulfate proteoglycans. The turnover rate of histamine in secretory granules is slow (days to weeks). Non–mast cell sites of histamine formation include the epidermis, enterochromaffin-like cells of the gastric mucosa, neurons within the central nervous system (CNS), and cells in regenerating or rapidly growing tissues. Turnover is rapid at these non–mast cell sites because the histamine is released continuously rather than stored. Non–mast cell sites of histamine production contribute significantly to the daily excretion of histamine metabolites in the urine. Because L-histidine decarboxylase is an inducible enzyme, the histamine-forming capacity at such sites is subject to regulation. Histamine that is released or ingested is rapidly metabolized by either ring methylation catalyzed by *histamine-*N*-methyltransferase* or oxidative deamination catalyzed by *diamine oxidase* (Figure 43–1), and the metabolites are eliminated in the urine.

Release and Functions of Endogenous Histamine

Histamine is released from storage granules as a result of the interaction of antigen with immunoglobulin E (IgE) antibodies on the mast cell surface. Histamine plays a central role in immediate hypersensitivity and allergic responses. The actions of histamine on bronchial smooth muscle and

Abbreviations

ACE: angiotensin I–converting enzyme
ADHD: attention-deficit/hyperactivity disorder
Ang: angiotensin
ARNI: angiotensin receptor–neprilysin inhibitor
AT: angiotensin receptor (e.g., AT_1 and AT_2 receptors)
AV: atrioventricular
C1: the C1 esterase of the complement system
C1-INH: inhibitor of the activated C1 component of complement
CSF: cerebrospinal fluid
EET: epoxyeicosatrienoic acid
eNOS: endothelial nitric oxide synthase
GABA: γ-aminobutyric acid
GI: gastrointestinal
GPCR: G protein-coupled receptor
HMW: high molecular weight
5HT: serotonin (5-hydroxytryptamine)
IgE: immunoglobulin E
IL-1: interleukin-1
iNOS: inducible nitric oxide synthase
IP_3: inositol trisphosphate
LMW: low molecular weight
NO: nitric oxide
PKC: protein kinase C
TNFα: tumor necrosis factor α

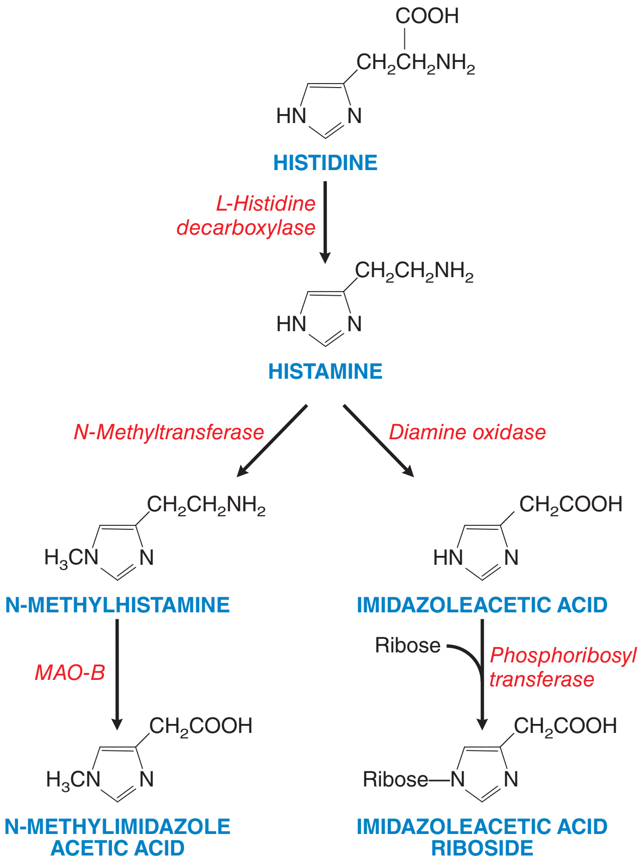


Figure 43–1 *Pathways of histamine synthesis and metabolism in humans.* Histamine is synthesized from histidine by decarboxylation. Histamine is metabolized via two pathways, predominantly by methylation of the ring followed by oxidative deamination (left side of figure) and secondarily by oxidative deamination and then conjugation with ribose.

blood vessels account for many of the symptoms of the allergic response. Histamine is a leukocyte chemoattractant, plays a major role in regulating gastric acid secretion, and modulates neurotransmitter release. In addition, some drugs act directly on mast cells to release histamine, causing untoward effects (see below).

Role in Allergic Responses

The principal target cells of immediate hypersensitivity reactions are mast cells and basophils. As part of the allergic response to an antigen, IgE antibodies are generated and bind to the surfaces of mast cells and basophils via specific high-affinity Fc receptors. This receptor, FcεRI, consists of α, β, and two γ chains (see Chapter 38). Antigen bridges the IgE molecules and via FcεRI activates signaling pathways in mast cells or basophils involving tyrosine kinases and subsequent phosphorylation of multiple protein substrates within 5 to 15 sec of contact with antigen. These events trigger the exocytosis of the contents of secretory granules that, in addition to histamine, includes serotonin, proteases, lysosomal enzymes, cytokines, and proteoglycans.

Release of Other Autacoids

Stimulation of IgE receptors also activates PLA_2 (phospholipase A_2), leading to the production of a host of mediators, including PAF (platelet-activating factor) and metabolites of arachidonic acid such as leukotrienes C_4 and D_4, which contract bronchial smooth muscle (see Chapters 41 and 44). Kinins also are generated during some allergic responses. Thus, the mast cell secretes a variety of inflammatory mediators in addition to histamine, each contributing to aspects of the allergic response (see discussion that follows).

Histamine Release by Drugs, Peptides, Venoms, and Other Agents

Mechanical injury and many compounds, including a large number of therapeutic agents, stimulate the release of histamine from mast cells directly and without prior sensitization (McNeil, 2021a). Responses of this sort are most likely to occur following intravenous injections of certain categories of substances, particularly organic bases. *Tubocurarine*, *succinylcholine*, *morphine*, some antibiotics, radiocontrast media, and certain carbohydrate plasma expanders also may elicit the response. The phenomenon is one of clinical concern and may account for unexpected anaphylactoid reactions. Basic polypeptides often are effective histamine releasers, and over a limited range, their potency generally increases with the number of basic groups. For example, bradykinin is a poor histamine releaser, whereas kallidin (Lys-bradykinin) and substance P, with more positively charged amino acids, are more active (Johnson and Erdos, 1973). Some venoms, such as that of the wasp, contain potent histamine-releasing peptides. Basic polypeptides released on tissue injury constitute pathophysiological stimuli for secretion from mast cells and basophils.

The mechanism by which basic secretagogues release histamine likely involves their direct interaction with MRGPRX2, a mast cell–specific member of the Mas-related G protein-coupled receptor (MRGPR) superfamily of eight proteins. Two of these proteins in the X family, MRGPRX1 and MRGPRX2, interact with a variety of positively charged compounds, transducing signals that result in itch and pain. Activation of MRGPRX2 on mast cells appears to provide a route for histamine release that is independent of the traditional IgE pathway and likely contribute to some adverse drug reactions (McNeil, 2021b). Inhibitors of MRGPRX2 may have therapeutic potential for the treatment of pseudoallergic and inflammatory diseases.

Within seconds of the intravenous injection of a histamine liberator, human subjects experience a burning, itching sensation. This effect, which is most marked in the palms of the hand and in the face, scalp, and ears, is soon followed by a feeling of intense warmth. The skin reddens, and the color rapidly spreads over the trunk. Blood pressure falls, the heart rate accelerates, and the subject usually complains of headache. After a few minutes, blood pressure recovers, and crops of hives usually appear on the skin. Colic, nausea, hypersecretion of acid, and moderate

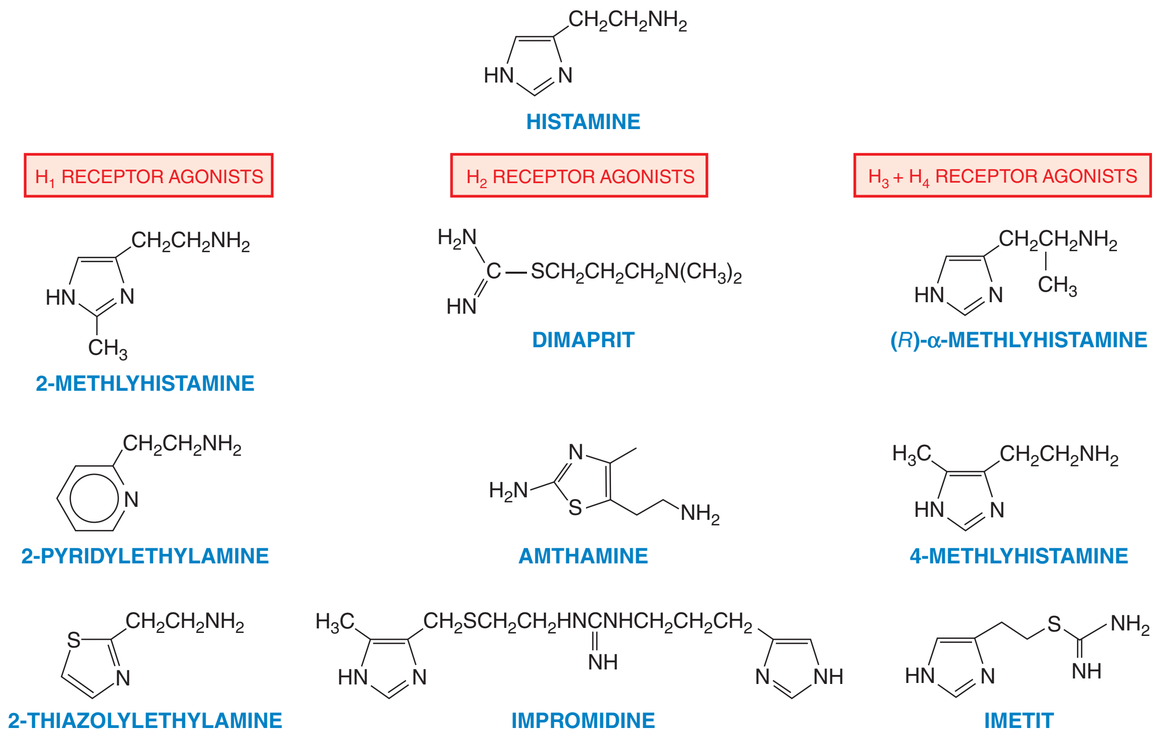


Figure 43–2 *Structure of histamine and some H_1, H_2, H_3, and H_4 agonists.* *Dimaprit* and *4-methylhistamine*, originally identified as specific H_2 agonists, have a much higher affinity for the H_4 receptor; *4-methylhistamine* is the most specific available H_4 agonist, with about 10-fold higher affinity than *dimaprit*, a partial H_4 agonist. *Impromidine* not only is among the most potent H_2 agonists but also is an antagonist at H_1 and H_3 receptors and a partial agonist at H_4 receptors. (*R*)-*α-Methylhistamine* and *imetit* are high-affinity agonists of H_3 receptors and lower-affinity full agonists at H_4 receptors.

bronchospasm also frequently occur. The effect becomes less intense with successive administration of the secretagogue as mast cell stores of histamine are depleted. Histamine liberators do not deplete histamine from non–mast cell sites.

Increased Proliferation of Mast Cells and Basophils; Gastric Carcinoid Tumors

In urticaria pigmentosa (cutaneous mastocytosis), mast cells aggregate in the upper corium and give rise to pigmented cutaneous lesions that sting when stroked. In systemic mastocytosis, overproliferation of mast cells is also found in other organs. Patients with these syndromes suffer a constellation of signs and symptoms attributable to excessive histamine release, including urticaria, dermographism, pruritus, headache, weakness, hypotension, flushing of the face, and a variety of gastrointestinal (GI) effects, such as diarrhea or peptic ulceration. A variety of stimuli, including exertion, insect stings, exposure to heat, and allergens (including drugs to which a patient is allergic), can activate mast cells and cause histamine release, as can organic bases (many drugs) that cause histamine release directly. In myelogenous leukemia, elevation of blood basophils can result in histamine content high enough to cause flushing, pruritus, and hypotension. Patients with a history of anaphylaxis to venoms often have elevated baseline tryptase levels, suggesting either an increase in the mast cell burden or hereditary alpha tryptasemia. Management of these patients can be complicated by a large release of histamine after cytolysis, causing shock. Gastric carcinoid tumors secrete histamine, which is responsible for episodes of vasodilation as part of the patchy "geographical" flush.

Gastric Acid Secretion

Histamine acting at H_2 receptors is a powerful gastric secretagogue, evoking copious secretion of acid from parietal cells (see Figure 53–1); it also increases the output of pepsin and intrinsic factor. The secretion of gastric acid from parietal cells also is caused by stimulation of the vagus nerve and by the enteric hormone gastrin. However, histamine is the dominant

TABLE 43–1 ■ CHARACTERISTICS OF HISTAMINE RECEPTORS

	H_1	H_2	H_3[a]	H_4
Size (amino acids)	487	359	329–445	390
G protein coupling (second messengers)	$G_{q/11}$ (↑ Ca^{2+}; ↑ NO and ↑ cGMP)	G_s (↑ cAMP)	$G_{i/o}$ (↓ cAMP; ↑ MAP kinase)	$G_{i/o}$ (↓ cAMP; ↑ Ca^{2+})
Distribution	Smooth muscle, endothelial cells, CNS	Gastric parietal cells, cardiac muscle, mast cells, CNS	CNS: pre- and postsynaptic	Cells of hematopoietic origin
Representative agonist	2-CH_3-histamine	Amthamine	(*R*)-α-CH_3-histamine	4-CH_3-histamine
Representative antagonist	Chlorpheniramine	Ranitidine	Pitolisant	JNJ7777120

cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; MAP, mitogen-activated protein; NO, nitric oxide.

[a] At least 20 alternately spliced H_3 isoforms have been detected at the mRNA level. Eight of these isoforms, ranging in size from 329 to 445 residues, were found to be functionally competent by binding or signaling assays (see Esbenshade et al., 2008).

physiological mediator of acid secretion; blockade of H_2 receptors not only antagonizes acid secretion in response to histamine but also inhibits responses to gastrin and vagal stimulation (see Chapter 53).

CNS

Histamine-containing neurons affect both homeostatic and higher brain functions, including regulation of the sleep-wake cycle, circadian and feeding rhythms, immunity, learning, memory, drinking, and body temperature. However, no human disease has yet been directly linked to dysfunction of the brain histamine system. Histamine, histidine decarboxylase, enzymes that metabolize histamine, and H_1, H_2, and H_3 receptors are distributed widely but nonuniformly in the CNS. H_1 receptors are associated with both neuronal and nonneuronal cells and are concentrated in regions that control neuroendocrine function, behavior, and nutritional state. Distribution of H_2 receptors is more consistent with histaminergic projections than that of H_1 receptors, suggesting that they mediate many of the postsynaptic actions of histamine. H_3 receptors are concentrated in areas known to receive histaminergic projections, consistent with their function as presynaptic autoreceptors. Histamine inhibits appetite and increases wakefulness via H_1 receptors.

Physiological and Pharmacological Effects

Receptor-Effector Coupling and Mechanisms of Action

Histamine receptors are GPCRs, coupling to second-messenger systems and producing effects (Simons, 2004) as noted in Table 43–1. H_1 receptors couple to $G_{q/11}$ and activate the PLC-IP_3-Ca^{2+} pathway and its myriad sequelae, including activation of PKC (protein kinase C), PLA_2, eNOS (endothelial nitric oxide synthase), and other Ca^{2+}-calmodulin–dependent enzymes. H_2 receptors link to G_s to activate the adenylyl cyclase–cyclic AMP–PKA pathway; H_3 and H_4 receptors couple to $G_i/_o$ to inhibit adenylyl cyclase and decrease cellular cyclic AMP. Activation of H_3 receptors also can activate MAP kinase and inhibit the Na^+/H^+ exchanger; activation of H_4 receptors can mobilize stored Ca^{2+} (Simons and Simons, 2011). H_3 and H_4 receptors have about 1000-fold higher affinity for histamine (low nanomolar range) than do H_1 and H_2 receptors (low micromolar range). Activation of H_1 receptors on vascular endothelium stimulates eNOS to produce nitric oxide (NO), which diffuses to nearby smooth muscle cells to increase cyclic GMP and cause relaxation. Stimulation of H_1 receptors on smooth muscle will mobilize Ca^{2+} and cause contraction, whereas activation of H_2 receptors on the same smooth muscle cell will link via G_s to enhanced cyclic AMP accumulation, activation of PKA, and then to relaxation.

Pharmacological definition of H_1, H_2, and H_3 receptors was possible through the use of relatively specific agonists and antagonists. Because the H_4 receptor exhibits 35% to 40% homology to isoforms of the H_3 receptor, the two were initially harder to distinguish pharmacologically, but this has been resolved by the development of several H_3- and H_4-selective antagonists (Sander et al., 2008; Thurmond, 2015). *4-Methylhistamine* and *dimaprit*, previously identified as specific H_2 agonists, are actually more potent H_4 agonists.

H_1 and H_2 Receptors

H_1 and H_2 receptors are distributed widely in the periphery and in the CNS, and their activation by histamine can exert local or widespread effects (Simons and Simons, 2011). For example, histamine causes itching and stimulates secretion from nasal mucosa. It contracts many smooth muscles, such as those of the bronchi and gut, but markedly relaxes others, including those in small blood vessels. H_2-receptor–mediated cyclic AMP signaling is a crucial step in acid secretion by gastric parietal cells. Other, less-prominent effects include formation of edema and stimulation of sensory nerve endings. Bronchoconstriction and contraction of the gut are mediated, in part, by H_1 receptors. In the CNS, H_1 activation inhibits appetite and increases wakefulness. Gastric secretion results from the activation of H_2 receptors. Some responses, such as vascular dilation, are mediated by both H_1 and H_2 receptor stimulation.

H_3 and H_4 Receptors

The H_3 receptors are expressed mainly in the CNS, especially in the basal ganglia, hippocampus, and cortex (Haas et al., 2008). Presynaptic H_3 receptors function as autoreceptors on histaminergic neurons, inhibiting histamine release, and as heteroreceptors on nonhistaminergic neurons (i.e., for serotoninergic, noradrenergic, dopaminergic, γ-aminobutyric acid [GABA]-ergic, glutamatergic, and cholinergic neurons), modulating the release of other neurotransmitters. H_3 receptors are also found postsynaptically, especially in the basal ganglia, but their function is still being unraveled (Ellenbroek and Ghiabi, 2014). H_3 agonists promote sleep, and H_3 antagonists promote wakefulness. The H_4 receptors primarily are found in eosinophils, dendritic cells, mast cells, monocytes, basophils, and T cells but have also been detected in the GI tract, dermal fibroblasts, CNS, and primary sensory afferent neurons (Thurmond, 2015). H_4 gene and/or protein expression is reduced in some human tumor cell types including breast, colorectal, oral squamous cell, bladder, and gastric cancer and melanoma (Massari et al., 2020).

Feedback Regulation of Release

Stimulation of H_2 receptor increases cyclic AMP and leads to feedback inhibition of histamine release from mast cells and basophils, whereas activation of H_3 and H_4 receptors has the opposite effect by decreasing cellular cyclic AMP. Activation of presynaptic H_3 receptors inhibits histamine release from histaminergic neurons. Because H_3 receptors have high constitutive activity, histamine release is tonically inhibited. H_3 inverse agonists thus reduce receptor activation and increase histamine release from histaminergic neurons.

Cardiovascular System

Histamine dilates resistance vessels, increases capillary permeability, and lowers systemic blood pressure. In some vascular beds, histamine constricts veins, contributing to the extravasation of fluid and edema formation upstream in capillaries and postcapillary venules.

Vasodilation. Vasodilation is the most important vascular effect of histamine in humans and can result from activation of either the H_1 or H_2 receptor. H_1 receptors have a higher affinity for histamine and cause Ca^{2+}-dependent activation of eNOS in endothelial cells; NO diffuses to vascular smooth muscle, increasing cyclic GMP (see Table 43–1) and causing rapid and short-lived vasodilation. By contrast, activation of H_2 receptors on vascular smooth muscle stimulates the cyclic AMP–PKA pathway, causing dilation that develops more slowly and is more sustained. As a result, H_1 antagonists effectively counter small dilator responses to low concentrations of histamine but blunt only the initial phase of larger responses to higher concentrations of the amine.

Increased Capillary Permeability. Histamine's effect on small vessels results in efflux of plasma protein and fluid into the extracellular spaces and an increase in lymph flow, causing edema. H_1 receptor activation on endothelial cells is the major mediator of this response, leading to G_q-mediated activation of RhoA and ROCK, which stimulates the contractile machinery of the cells and disrupts interendothelial junctions (Mikelis et al., 2015). The gaps between endothelial cells also may permit passage of circulating cells recruited to tissues during the mast cell response. Recruitment of circulating leukocytes is enhanced by H_1 receptor–mediated expression of adhesion molecules (e.g., P-selectin) on endothelial cells.

Triple Response of Lewis. If histamine is injected intradermally, it elicits a characteristic phenomenon known as the *triple response*. This consists of the following:

- A localized "reddening" around the injection site, appearing within a few seconds and maximal at about 1 min
- A "flare" or red flush extending about 1 cm beyond the original red spot and developing more slowly
- A "wheal" or swelling that is discernible in 1–2 min at the injection site

The initial red spot (a few millimeters) results from the direct vasodilating effect of histamine (H_1 receptor–mediated NO production).

The flare is due to histamine-induced stimulation of axonal reflexes that cause vasodilation indirectly, and the wheal reflects histamine's capacity to increase capillary permeability (edema formation).

Heart. Histamine affects both cardiac contractility and electrical events directly. It increases the force of contraction of both atrial and ventricular muscle by promoting the influx of Ca^{2+}, and it speeds heart rate by hastening diastolic depolarization in the sinoatrial node. It also directly slows atrioventricular (AV) conduction to increase automaticity and, in high doses, can elicit arrhythmias. The slowed AV conduction involves mainly H_1 receptors, while the other effects are largely attributable to H_2 receptors and cyclic AMP accumulation. The direct cardiac effects of histamine given intravenously are overshadowed by baroreceptor reflexes stimulated by reduced blood pressure.

Histamine Shock. Histamine given in large doses or released during systemic anaphylaxis causes a profound and progressive fall in blood pressure. As the small blood vessels dilate, they trap large amounts of blood, their permeability increases, and plasma escapes from the circulation. These effects, resembling surgical or traumatic shock, diminish effective blood volume, reduce venous return, and greatly lower cardiac output.

Extravascular Smooth Muscle

Histamine directly contracts or, more rarely, relaxes various extravascular smooth muscles. Contraction is due to activation of H_1 receptors on smooth muscle to increase intracellular Ca^{2+}, and relaxation is mainly due to activation of H_2 receptors. Although the spasmogenic influence of H_1 receptors is dominant in human bronchial muscle, H_2 receptors with dilator function also are present. Thus, histamine-induced bronchospasm *in vitro* is potentiated slightly by H_2 blockade. Patients with bronchial asthma and certain other pulmonary diseases are much more sensitive to the bronchoconstrictor effects of histamine.

Peripheral Nerve Endings

Histamine stimulates various nerve endings, causing sensory effects. In the epidermis, it causes itch; in the dermis, it evokes pain, sometimes accompanied by itching. Stimulant actions on nerve endings, including autonomic afferents and efferents, contribute to the "flare" component of the triple response and to indirect effects of histamine on the bronchi and other organs.

Central Nervous System

Histaminergic neurons originate in the tuberomammillary nucleus of the posterior hypothalamus, projecting to virtually all regions of the brain. Neuronal histamine has both stimulatory and inhibitory functions in the CNS. Stimulatory functions promote wakefulness, cognition, locomotion, energy metabolism, and nociception. Inhibitory functions suppress appetite, convulsions, stress-induced excitation, and denervation-induced super sensitivity.

Immune System

Activation of H_4 receptors has been associated with induction of cellular shape change, chemotaxis, secretion of cytokines, and upregulation of adhesion molecules (Thurmond, 2015). Because of the unique localization and function of H_4 receptors, H_4 antagonists are promising candidates to treat inflammatory conditions such as allergic rhinitis, asthma, rheumatoid arthritis, atopic dermatitis, and possibly pruritus and neuropathic pain. None has yet been FDA approved.

Histamine Shock

Histamine given in large doses or released during systemic anaphylaxis causes a profound and progressive fall in blood pressure. As the small blood vessels dilate, they trap large amounts of blood, their permeability increases, and plasma escapes from the circulation. These effects, resembling surgical or traumatic shock, diminish effective blood volume, reduce venous return, and greatly lower cardiac output.

Histamine Toxicity From Ingestion

Histamine is the toxin in food poisoning from spoiled scombroid fish such as tuna. Symptoms include severe nausea, vomiting, headache, flushing, and sweating. Histamine toxicity also can follow red wine consumption in persons with a diminished ability to degrade histamine. The symptoms of histamine poisoning can be suppressed by H_1 antagonists.

HISTORY

Antihistamine activity was first demonstrated by Bovet and Staub in 1937 with one of a series of amines with a phenolic ether moiety. The substance, 2-isopropyl-5-methylphenoxy-ethyldiethyl-amine, protected guinea pigs against several lethal doses of histamine but was too toxic for clinical use. By 1944, Bovet and his colleagues had described *pyrilamine maleate*, an effective histamine antagonist of this category. The discovery of highly effective *diphenhydramine* and *tripelennamine* soon followed. In the 1980s, nonsedating H_1 histamine receptor antagonists were developed for treatment of allergic diseases. Despite success in blocking allergic responses to histamine, the H_1 antihistamines failed to inhibit a number of other responses, notably gastric acid secretion. The discovery of H_2 receptors and H_2 antagonists by James Black and colleagues provided a new class of agents that antagonized histamine-induced acid secretion (Black et al., 1972); the pharmacology of these drugs (e.g., *cimetidine*, *famotidine*) is described in Chapter 53. Black shared the 1988 Nobel Prize in Medicine/Physiology with Gertrude Elion and George Hitchings, awarded to the trio "for their discoveries of important principles for drug treatment."

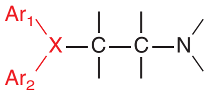
Histamine Receptor Antagonists

H_1 Receptor Antagonists

Pharmacological Properties

All the available H_1 receptor "antagonists" are actually inverse agonists (see Chapter 3) that reduce constitutive activity of the receptor and compete with histamine binding to the receptor (Simons, 2004). The pharmacological actions and therapeutic applications of these antagonists can be largely predicted from knowledge of the location and mode of signaling of the histamine receptors.

Chemistry. Like histamine, many H_1 antagonists contain a substituted ethylamine moiety (the black portion on the figure that follows). Unlike histamine, which has a primary amino group and a single aromatic ring, most H_1 antagonists have a tertiary amino group linked by a two- or three-atom chain to two aromatic substituents (in red) and conform to the general formula:

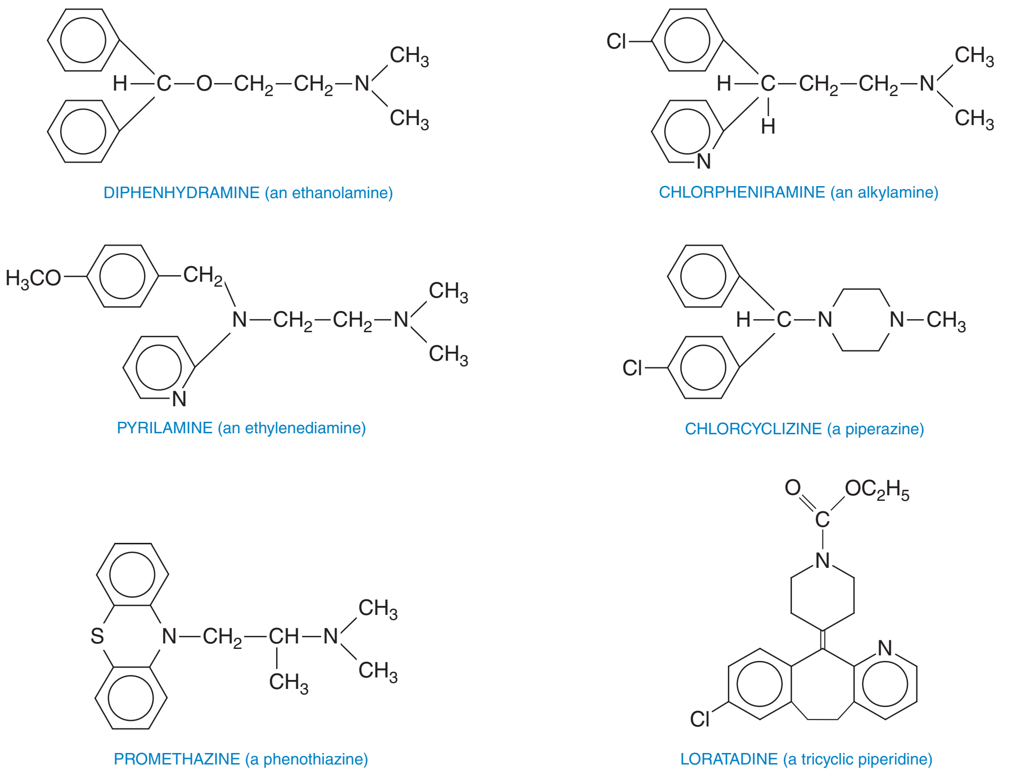


where Ar is aryl and X is a nitrogen or carbon atom or a —C—O— ether linkage to the β-aminoethyl side chain. Sometimes, the two aromatic rings are bridged, as in the tricyclic derivatives, or the ethylamine may be part of a ring structure. Figure 43–3 shows the varied structures of representative H_1 antagonists built around this framework and that constitute the several generations of compounds.

Effects on Physiological Systems

Smooth Muscle. The H_1 antagonists inhibit most of the effects of histamine on smooth muscles, especially the constriction of respiratory smooth muscle. H_1 antagonists inhibit the more rapid vasodilator effects mediated by activation of H_1 receptors on endothelial cells (synthesis/release of NO and other mediators) at lower doses of histamine. They also inhibit venous constriction seen in some vascular beds.

Capillary Permeability. H_1 antagonists strongly block the increased capillary permeability and formation of edema and wheal caused by histamine.



DIPHENHYDRAMINE (an ethanolamine)

CHLORPHENIRAMINE (an alkylamine)

PYRILAMINE (an ethylenediamine)

CHLORCYCLIZINE (a piperazine)

PROMETHAZINE (a phenothiazine)

LORATADINE (a tricyclic piperidine)

Figure 43–3 *Representative H_1 antagonists.*

Flare and Itch. H_1 antagonists suppress the action of histamine on nerve endings, including the flare component of the triple response and the itching caused by intradermal injection.

Exocrine Glands. H_1 antagonists do not suppress gastric secretion. However, the antimuscarinic properties of many H_1 antagonists may contribute to lessened secretion in cholinergically innervated glands and reduce ongoing secretion in, for example, the respiratory tree.

Immediate Hypersensitivity Reactions: Anaphylaxis and Allergy. During hypersensitivity reactions, histamine is one of the many potent autacoids released, and its relative contribution to the ensuing symptoms varies widely with species and tissue. The protection afforded by H_1 antagonists varies accordingly. In humans, edema formation and itch are effectively suppressed. Other effects, such as hypotension, are less well antagonized. H_1 antagonists are ineffective in blocking bronchoconstriction due to asthma.

Mast Cell–Stabilizing and Anti-inflammatory Properties. Many second-generation H_1 antagonists (e.g., *cetirizine*, *desloratadine*, *fexofenadine*, *olopatadine*, *ketotifen*, *alcaftadine*, and others) exhibit mast cell–stabilizing effects, resulting in reduced release of mast cell mediators during an allergic response (Levi-Schaffer and Eliashar, 2009). These agents also have anti-inflammatory properties, which can include reduced cytokine secretion, decreased adhesion molecule expression, and inhibition of eosinophil infiltration. These effects can be both H_1 receptor dependent and independent, but precise mechanisms are still unclear, and it is unknown what role they play at normal therapeutic doses of these drugs. There is some evidence that H_1 antagonists with these additional properties may be more effective in the topical treatment of allergic conjunctivitis (Abelson et al., 2015).

CNS. The first-generation H_1 antagonists can both stimulate and depress the CNS (Simons and Simons, 2011). Stimulation is occasionally encountered in patients given conventional doses; the patients become restless, nervous, and unable to sleep. Central excitation also is a striking feature of overdose, which commonly results in convulsions, particularly in infants. Central depression, on the other hand, usually accompanies therapeutic doses of the older H_1 antagonists. Diminished alertness, slowed reaction times, and somnolence are common manifestations. Patients vary in their susceptibility and responses to individual drugs. The ethanolamines (e.g., *diphenhydramine*) are particularly prone to causing sedation. Because of the sedation that occurs with first-generation antihistamines, these drugs cannot be tolerated or used safely by many patients except at bedtime. Even then, patients may experience an antihistamine "hangover" in the morning, resulting in sedation with or without psychomotor impairment. Second-generation H_1 antagonists are termed *nonsedating* because they do not cross the blood-brain barrier appreciably. This is due to their decreased lipophilicity and because they are substrates of P-glycoprotein, which pumps them out of the blood-brain barrier capillary endothelial cells and back into the capillary lumen (see Chapter 4 and Simons and Simons, 2011).

Many antipsychotic agents are H_1 and H_2 receptor antagonists, but it is unclear whether this property plays a role in the antipsychotic effects of these agents. In test systems, the atypical antipsychotic agent *clozapine* is an effective H_1 antagonist, a weak H_3 antagonist, and an H_4 receptor agonist. The H_1 antagonist activity of typical and atypical antipsychotic drugs is responsible for the propensity of these agents to cause weight gain.

Anticholinergic Effects. Many of the first-generation H_1 antagonists tend to inhibit muscarinic cholinergic responses and may be manifest during clinical use (Simons and Simons, 2011). Some H_1 antagonists also can be used to treat motion sickness (see Chapters 11 and 54), probably as a result of their anticholinergic properties. Indeed, *promethazine* has perhaps the strongest muscarinic-blocking activity among these agents and is the most effective H_1 antagonist in combating motion sickness. The second-generation H_1 antagonists have no effect on muscarinic receptors (Simons and Simons, 2011).

Local Anesthetic Effect. Some H_1 antagonists have local anesthetic activity, and a few are more potent than *procaine*. *Promethazine* is especially active. However, the concentrations required for this effect are much higher than those that antagonize histamine's interactions with its receptors.

ADME. The H_1 antagonists are well absorbed from the GI tract. Following oral administration, peak plasma concentrations are achieved in 1 to

3 h, and effects usually last 4 to 6 h for first-generation agents; however, some of the drugs are much longer acting, as are most second-generation H_1 antagonists (del Cuvillo et al., 2006; Simons, 2004) (Table 43–2). These agents are distributed widely throughout the body, including the CNS for the first-generation agents. Peak concentrations of these drugs in the skin may persist long after plasma levels have declined. Thus, inhibition of "wheal-and-flare" responses to the intradermal injection of histamine or allergen can persist for 36 h or more after initial treatment and up to 7 days after discontinuation of treatment in patients who regularly use an H_1 antagonist for 1 week or more (del Cuvillo et al., 2006).

All first-generation and most second-generation H_1 antagonists are metabolized by CYPs, and little, if any, is excreted unchanged in the urine; most appears there as metabolites (Bartra et al., 2006; Simons, 2004). Exceptions are *cetirizine* and *acrivastine* (<40% metabolized) and *fexofenadine*, *levocetirizine*, and *epinastine* (<10% metabolized). *Cetirizine*, *levocetirizine*, and *acrivastine* are excreted primarily into the urine; *fexofenadine* is mainly excreted in the feces; and *epinastine* is excreted in both urine (55%) and feces (30%).

The H_1 antagonists that are metabolized are eliminated more rapidly by children than by adults and more slowly in those with severe liver disease. These antagonists also have higher potential for drug interactions. For example, plasma levels of H_1 antagonists may be reduced when coadministered with drugs that induce CYP synthesis (e.g., benzodiazepines) or elevated when taken with drugs that compete with or inhibit the same CYP isoform (e.g., *erythromycin*, *ketoconazole*, antidepressants) (Bartra et al., 2006; Simons, 2004). Clinically relevant interactions are more likely with first-generation than second-generation drugs, which have a higher therapeutic index. However, two second-generation H_1 antagonists marketed previously, *terfenadine* and *astemizole*, were found in rare cases to prolong the QTc interval and induce torsade de pointes, a potentially fatal arrhythmia, due to their capacity to inhibit a cardiac K^+ channel, I_{Kr}, when their metabolism was impaired and their plasma concentrations rose too high, due, for instance, to liver disease or to drugs that inhibited the CYP3A family (Bartra et al., 2006; Simons, 2004) (see Chapter 30). This led to the withdrawal of *terfenadine* and *astemizole* from the market. *Astemizole* and an active hydroxylated metabolite naturally have very long half-lives. *Terfenadine* is a prodrug, metabolized by hepatic CYP3A4 to fexofenadine, which is its replacement and lacks noticeable cardiotoxicity. *In vitro* testing for a new drug's capacity to inhibit I_{Kr} is now available.

Therapeutic Uses

The H_1 antagonists are used for treatment of various immediate hypersensitivity reactions. The central properties of some of the drugs also are of therapeutic value for suppressing motion sickness or for sedation.

Allergic Diseases. H_1 antagonists are useful in acute types of allergy that present with symptoms of rhinitis, urticaria, and conjunctivitis (Simons and Simons, 2011). Their effect is confined to the suppression of symptoms attributable to the histamine released by the antigen-antibody reaction. In bronchial asthma, histamine antagonists have limited efficacy and are not used as sole therapy (see Chapters 42 and 44). In the treatment of systemic anaphylaxis, where autacoids other than histamine are important, the mainstay of therapy is *epinephrine*; histamine antagonists have only a subordinate and adjuvant role. The same is true for severe angioedema, in which laryngeal swelling constitutes a threat to life (see Chapter 14).

Certain allergic dermatoses respond favorably to H_1 antagonists. The benefit is most striking in acute urticaria. H_1 antagonists are also first-line therapy for chronic urticaria but may require doses up to four times higher than that approved for treating rhinitis; patients refractory to high-dose H_1 antagonists should be switched to drugs targeting the immune response (Viegas et al., 2014). H_1 antagonists have a place in the treatment of pruritus. Some relief may be obtained in many patients with atopic and contact dermatitis (although topical corticosteroids are more effective) and in such diverse conditions as insect bites and poison ivy. The urticarial and edematous lesions of serum sickness respond to H_1 antagonists, but fever and arthralgia often do not.

Common Cold. H_1 antagonists are without value in combating the common cold. The weak anticholinergic effects of the older agents may tend to lessen rhinorrhea, but this drying effect may do more harm than good, as may their tendency to induce somnolence.

Motion Sickness, Vertigo, and Sedation. *Scopolamine*, the muscarinic antagonist, given orally, parenterally, or transdermally, is the most effective drug for the prophylaxis and treatment of motion sickness. Some H_1 antagonists are useful for milder cases and have fewer adverse effects. These drugs include *dimenhydrinate* and the piperazines (e.g., *cyclizine*, *meclizine*). *Promethazine*, a phenothiazine, is more potent and more effective, and its additional antiemetic properties may be of value in reducing vomiting; however, its pronounced sedative action usually is disadvantageous. Whenever possible, the various drugs should be administered about 1 h before the anticipated motion. Treatment after the onset of nausea and vomiting rarely is beneficial. Some H_1 antagonists, notably *dimenhydrinate* and *meclizine*, often are of benefit in vestibular disturbances such as Ménière disease and other types of true vertigo. Only *promethazine* is useful in treating the nausea and vomiting subsequent to chemotherapy or radiation therapy for malignancies; however, other, more effective, antiemetic drugs (e.g., serotonin $5HT_3$ antagonists) are available (see Chapter 54). *Diphenhydramine* can reverse the extrapyramidal side effects caused by antipsychotics (see Chapter 19). The tendency of some H_1 receptor antagonists to produce somnolence has led to their use as hypnotics. H_1 antagonists, principally *diphenhydramine*, often are present in various proprietary over-the-counter remedies for insomnia. The sedative and mild antianxiety activities of *hydroxyzine* contribute to its use as an anxiolytic.

Adverse Effects

The most frequent side effect of first-generation H_1 antagonists is sedation. Concurrent ingestion of alcohol or other CNS depressants produces an additive effect that impairs motor skills. Other untoward central actions include dizziness, tinnitus, lassitude, incoordination, fatigue, blurred vision, diplopia, euphoria, nervousness, insomnia, and tremors. Additional potential side effects, including loss of appetite, nausea, vomiting, epigastric distress, and constipation or diarrhea, may be reduced by taking the drug with meals. H_1 antagonists such as *cyproheptadine* may increase appetite and cause weight gain. Other side effects, owing to the antimuscarinic actions of some first-generation H_1 antagonists, include dryness of the mouth and respiratory passages (sometimes inducing cough), urinary retention or frequency, and dysuria. These effects are not observed with second-generation H_1 antagonists. Allergic dermatitis is not uncommon; other hypersensitivity reactions include drug fever and photosensitization. Hematological complications, such as leukopenia, agranulocytosis, and hemolytic anemia, are very rare.

Because H_1 antihistamines cross the placenta, caution is advised for women who are or may become pregnant (Simons and Simons, 2011). Several antihistamines (e.g., *azelastine*, *hydroxyzine*, *fexofenadine*) had teratogenic effects in animal studies, whereas others (e.g., *chlorpheniramine*, *diphenhydramine*, *cetirizine*, *loratadine*) did not. A recent systematic review concluded that antihistamines are unlikely to be strong risk factors for major birth defects (Gilboa et al., 2014). A combination drug consisting of the H_1 antagonist *doxylamine* and vitamin B_6 (*pyridoxine*) was approved in 1956 for treating the nausea and vomiting of pregnancy and then voluntarily removed in 1983 due to concerns over birth defects. Subsequent analyses showed the drug caused no increased risk of birth defects, and in 2013, it was reapproved for the same indication in a fixed-dose, delayed-release formulation. Antihistamines can be excreted in small amounts in breast milk, and first-generation antihistamines taken by lactating mothers may cause symptoms such as irritability, drowsiness, or respiratory depression in the nursing infant.

In acute poisoning with first-generation H_1 antagonists, their central excitatory effects constitute the greatest danger. The syndrome includes hallucinations, excitement, ataxia, incoordination, athetosis, convulsions, and fixed, dilated pupils with a flushed face, together with sinus tachycardia, urinary retention, dry mouth, and fever. The syndrome exhibits a remarkable similarity to that of *atropine* poisoning. Terminally, there is

TABLE 43–2 ■ PREPARATIONS AND DOSAGE OF REPRESENTATIVE H_1 RECEPTOR ANTAGONISTS[a]

CLASS *Generic Name*	DURATION OF ACTION (h)[b]	PREPARATIONS[c]	SINGLE DOSE (adult)
First-generation agents			
Tricyclic dibenzoxepins			
Doxepin HCl	6–24	O, L, T	10–150 mg; insomnia: 6 mg (O) Pruritus: thin film 4 times/day (T)
Ethanolamines			
Carbinoxamine maleate	3–6	O, L	4–8 mg; 6–16 mg (SR)
Clemastine fumarate	12	O, L	1.34–2.68 mg
Diphenhydramine HCl	12	O, L, I, T	25–50 mg (O/L/I)
Dimenhydrinate[d]	4–6	O, I	50–100 mg
Ethylenediamines			
Pyrilamine maleate (only in combination products)	4–6	O, L	7.5–30 mg
Alkylamines			
Chlorpheniramine maleate	24	O, L, I, SR	4 mg, 12 mg (SR)
Brompheniramine maleate	4–6	O, L, I, SR	2 mg
Piperazines			
Hydroxyzine HCl	6–24	O, L, I	25–100 mg
Hydroxyzine pamoate	6–24	O, L (not in the U.S.)	25–100 mg
Cyclizine HCl	4–6	O	50 mg
Cyclizine lactate (not in the U.S.)	4–6	I	50 mg
Meclizine HCl	12–24	O	25–50 mg
Phenothiazines			
Promethazine HCl	4–6	O, L, I, S	12.5–50 mg
Piperidines			
Cyproheptadine HCl[e]	4–6	O, L	1–6.5 mg
Second-generation agents			
Tricyclic dibenzoxepins			
Olopatadine HCl	6–12	T	2 sprays/nostril; 1 drop/eye
Alkylamines			
Acrivastine[f]	6–8	O	8 mg
Piperazines			
Cetirizine HCl[f]	12–24	O, L	5–10 mg
Levocetirizine HCl	12–24	O, L	2.5–5 mg
Piperidines			
Alcaftadine	16–24	T	1 drop/eye
Bepotastine besilate	8	T	1 drop/eye
Desloratadine	24	O, L	5 mg
Fexofenadine HCl	12–24	O, L	60–180 mg
Ketotifen fumarate	8–12	T	1 drop/eye
Loratadine	24	O, L	10 mg
Other second-generation drugs			
Azelastine HCl[f]	12–24	T	2 sprays/nostril; 1 drop/eye
Emedastine	8–12	T	1 drop/eye
Epinastine	8–12	T	1 drop/eye

[a] For a discussion of phenothiazines, see Chapter 19.

[b] Duration of action of H_1 antihistamines by objective assessment of suppression of histamine- or allergen-induced symptoms is longer than expected from measurement of plasma concentrations or terminal elimination $t_{1/2}$ values.

[c] Preparations are designated as follows: O, oral solids; L, oral liquids; I, injection; S, suppository; SR, sustained release; T, topical. Many H_1 receptor antagonists also are available in preparations that contain multiple drugs. SR forms dissuade pseudoephedrine extraction for methamphetamine production.

[d] Dimenhydrinate is a combination of diphenhydramine and 8-chlorotheophylline in equal molecular proportions.

[e] Also has antiserotonin properties.

[f] Has mild sedating effects.

deepening coma with cardiorespiratory collapse and death usually within 2 to 18 h. Treatment is along general symptomatic and supportive lines. Overdoses of second-generation H_1 antagonists have not been associated with significant toxicity (Simons and Simons, 2011).

Pediatric and Geriatric Indications and Problems. Although little clinical testing has been done, second-generation antihistamines are preferred for elderly patients (>65 years of age), especially those with impaired cognitive function, because they lack the sedative and anticholinergic effects of first-generation drugs (Simons, 2004). In addition, a recent prospective study in participants 65 years old and older without dementia showed a significant 10-year cumulative dose-response relationship between use of anticholinergics (first-generation H_1 antagonists among the most common) and risk of dementia, primarily Alzheimer's disease (Gray et al., 2015).

First-generation antihistamines are not recommended for use in children because their sedative effects can impair learning and school performance. The second-generation drugs have been approved by the FDA for use in children and are available in appropriate lower-dose formulations (e.g., chewable or rapidly dissolving tablets, syrup). Use of over-the-counter cough and cold medicines (containing mixtures of antihistamines, decongestants, antitussives, and expectorants) in young children has been associated with serious side effects and deaths. In 2008, the FDA recommended that they should not be used in children less than 2 years of age, and drug manufacturers affiliated with the Consumer Healthcare Products Association voluntarily relabeled products "do not use" for children less than 4 years of age.

Available H_1 Antagonists

Summarized next are notable properties of a number of H_1 antagonists, grouped by their chemical structures. Representative preparations are listed in Table 43–2. Second-generation H_1 antagonists cause fewer adverse effects and are superior in safety compared to older first-generation H_1 antagonists (Fein et al., 2019).

First-Generation Dibenzoxepin Tricyclic (Doxepin). *Doxepin* is marketed as a tricyclic antidepressant (see Chapter 18). It also is one of the most potent H_1 antagonists and has significant H_2 antagonist activity, but this does not translate into greater clinical effectiveness. It can cause drowsiness and is associated with anticholinergic effects. *Doxepin* is better tolerated by patients with depression than by those who are not depressed, for whom even small doses may cause disorientation and confusion.

Second-Generation Dibenzoxepin Tricyclic (Olopatadine). Olopatadine is a topical H_1 antagonist with additional mast cell–stabilizing and anti-inflammatory properties. In drop form, it is an effective treatment of allergic conjunctivitis and as a spray helps reduce the nasal symptoms of allergic rhinitis.

Ethanolamines (Prototype: Diphenhydramine). The ethanolamines possess significant antimuscarinic activity and have a pronounced tendency to induce sedation. About half of those treated acutely with conventional doses experience somnolence. The incidence of GI side effects, however, is low with this group.

Ethylenediamine (Prototype: Pyrilamine). *Pyrilamine* is among the most specific H_1 antagonists. Although its central effects are relatively feeble, somnolence occurs in a fair proportion of patients. GI side effects are common.

First-Generation Alkylamines (Prototype: Chlorpheniramine). The first-generation alkylamines are among the most potent H_1 antagonists. The drugs are less prone to produce drowsiness and are more suitable for daytime use, but a significant proportion of patients still experience sedation. Side effects involving CNS stimulation are more common than with other groups.

Second-Generation Alkylamine (Acrivastine). The second-generation alkylamine is a derivative of the first-generation alkylamine *triprolidine* and may exhibit a somewhat higher incidence of mild sedation than other second-generation H_1 antagonists.

First-Generation Piperazines. *Hydroxyzine* is a long-acting compound that is used widely for skin allergies; its considerable CNS-depressant activity may contribute to its prominent antipruritic action, and it is also used as a sedative and antianxiety agent. *Cyclizine* and *meclizine* have been used primarily to counter motion sickness, although *promethazine* and *diphenhydramine* are more effective (as is the antimuscarinic *scopolamine*).

Second-Generation Piperazines (Cetirizine). *Cetirizine* has minimal anticholinergic effects. It also has negligible penetration into the brain but is associated with a somewhat higher incidence of drowsiness than most other second-generation H_1 antagonists. The active enantiomer *levocetirizine* has slightly greater potency and may be used at half the dose with less resultant sedation. *Cetirizine* and *levocetirizine* have additional mast cell–stabilizing and anti-inflammatory properties.

Phenothiazines (Prototype: Promethazine). *Promethazine*, which has prominent sedative and considerable anticholinergic effects, and its many congeners are used primarily for their antiemetic effects (see Chapter 54).

First-Generation Piperidine (Cyproheptadine). *Cyproheptadine* uniquely has both antihistamine and antiserotonin activity by antagonizing the $5HT_{2A}$ receptor. *Cyproheptadine* causes drowsiness; it also has significant anticholinergic effects and can increase appetite.

Second-Generation Piperidines (Prototype: Loratadine). *Terfenadine* and *astemizole*, early second-generation drugs, are no longer marketed because of their potential for causing a rare, but potentially fatal, arrhythmia, torsade de pointes (see previous discussion). *Terfenadine* was replaced by *fexofenadine*, an active metabolite that lacks the toxic side effects of *terfenadine*, is not sedating, and retains the antiallergic properties of the parent compound. Another antihistamine of this class developed using this strategy is *desloratadine*, an active metabolite of *loratadine*. These agents lack significant anticholinergic actions and penetrate poorly into the CNS. Taken together, these properties appear to account for the low incidence of side effects of piperidine antihistamines. All members of this class have mast cell–stabilizing and anti-inflammatory properties. Although the therapeutic significance of these additional effects is unclear for the drugs administered orally, they appear to provide additional benefit when used in topical formulations to treat allergic conjunctivitis. *Alcaftadine* has additional antagonist activity on H_4 receptors, which likely explains its superiority to other topical H_1 antagonists in reducing the ocular itch of allergic conjunctivitis (Thurmond, 2015).

Other Second-Generation H_1 Antagonists. Drugs in this group (*azelastine*, *emedastine*, and *epinastine*) have divergent structures with therapeutic efficacy and side effects similar to other second-generation H_1 antagonists. They all are marketed as topical eye drops for the treatment of allergic conjunctivitis; *azelastine* is also available as a nasal spray for treating symptoms of allergic or vasomotor rhinitis. *Epinastine* has both H_1 and H_2 antagonist activity, which may help reduce eyelid edema. *Epinastine* and *azelastine* exhibit mast cell–stabilizing and anti-inflammatory properties. *Emedastine* is a highly selective H_1 antagonist without these additional actions.

H_2 Receptor Antagonists

The pharmacology and clinical utility of H_2 antagonists (e.g., *cimetidine*, *ranitidine*) for inhibiting gastric acid secretion in the treatment of GI disorders are described in Chapter 53.

H_3 Receptor Antagonists

The H_3 receptors are presynaptic autoreceptors on histaminergic neurons that originate in the tuberomammillary nucleus in the hypothalamus and project throughout the CNS, most prominently to the hippocampus, amygdala, nucleus accumbens, globus pallidus, striatum, hypothalamus, and cortex (Haas et al., 2008; Sander et al., 2008). The activated H_3 receptor depresses neuronal firing at the level of cell bodies/dendrites and decreases histamine release from depolarized terminals. Thus, H_3 agonists decrease histaminergic transmission, and antagonists increase it.

The H_3 receptors also are presynaptic heteroreceptors on a variety of neurons in brain and peripheral tissues, and their activation inhibits transmitter release from noradrenergic, serotoninergic, GABAergic, cholinergic, and glutamatergic neurons, as well as pain-sensitive C fibers. H_3 receptors in the brain have significant constitutive activity in the absence of agonist; consequently, inverse agonists reduce this constitutive activity, withdraw inhibition of transmitter release, and thereby promote transmitter release (activation of these neurons).

The H_3 antagonists/inverse agonists have a wide range of central effects; for example, they promote wakefulness, improve cognitive function (e.g., enhance memory, learning, and attention), and reduce food intake. As a result, there is considerable interest in developing H_3 antagonists for possible treatment of sleeping disorders, ADHD (attention-deficit/hyperactivity disorder), epilepsy, cognitive impairment, schizophrenia, obesity, neuropathic pain, and Alzheimer's disease (Haas et al., 2008; Sander et al., 2008).

Thioperamide was the first "specific" H_3 antagonist/inverse agonist available experimentally, but it was equally effective at the H_4 receptor. A number of other imidazole derivatives have been developed as H_3 antagonists, including *clobenpropit*, *ciproxifan*, and *proxyfan*, but the imidazole ring enhances binding to the H_4 receptor and CYPs. Because of this, more selective nonimidazole H_3 antagonists/inverse agonists (*thioperamide*, *ciproxifan*, *pitolisant*) were developed (Haas et al., 2008; Sander et al., 2008).

Based on the functions of H_3 receptors in the CNS, H_3 antagonists have potential in the treatment of sleeping disorders, ADHD, epilepsy, cognitive impairment, schizophrenia, obesity, neuropathic pain, and Alzheimer's disease. The selective H_3 receptor antagonist/inverse agonist *pitolisant* demonstrated efficacy in the treatment of excessive daytime sleepiness or cataplexy in adults with narcolepsy (Wang, 2021) and is now FDA-approved. *Pitolisant* was also shown to be effective for the treatment of daytime sleepiness in patients with obstructive sleep apnea. *Pitolisant* binds with high affinity to H_3 receptors (K_i = 1 nM), without appreciable affinity for H_1, H_2, or H_4 receptors (K_i > 10 μM).

H_4 Receptor Antagonists

The H_4 receptors are expressed on cells with inflammatory or immune functions and can mediate histamine-induced chemotaxis, induction of cell shape change, secretion of cytokines, and upregulation of adhesion molecules (Thurmond et al., 2008). The H_4 receptors also have a role in pruritus and neuropathic pain. Because of the unique localization and function of H_4 receptors, H_4 antagonists are promising candidates to treat inflammatory conditions and possibly pruritus and neuropathic pain (Thurmond, 2015).

The H_4 receptor has the highest homology with the H_3 receptor and binds many H_3 ligands, especially those with imidazole rings, although sometimes with different effects (Thurmond et al., 2008). For example, *thioperamide* is an effective inverse agonist at both H_3 and H_4 receptors, whereas the H_3 inverse agonist *clobenpropit* is a partial agonist of the H_4 receptor; *impentamine* (an H_3 agonist) and *iodophenpropit* (an H_3 inverse agonist) are both neutral H_4 antagonists.

H_4-specific antagonists have been tested in phase I and II clinical trials for treatment of persistent asthma, pruritis, dermatitis, and rheumatoid arthritis. JNJ39758979 showed benefit for the treatment of eosinophilic asthma but did not meet the study primary endpoint. Another H_4 receptor antagonist, ZPL-3893787, effected significant improvement in atopic dermatitis scores in patients with moderate-to-severe disease. No H_4 receptor antagonist has yet been FDA-approved.

Bradykinin, Kallidin, and Their Antagonists

In the 1920s and 1930s, Frey, Kraut, and Werle characterized a hypotensive substance in urine, which was also found in other fluids and tissues, and named this material *kallikrein* after a Greek synonym for the pancreas, an especially rich source (Werle, 1970). It was established that kallikrein generates a pharmacologically active substance from an inactive precursor present in plasma; the active substance, *kallidin*, proved to be a polypeptide cleaved from a plasma globulin (Werle, 1970). Rocha e Silva, Beraldo, and associates later reported that trypsin and certain snake venoms acted on plasma globulin to produce a substance that lowered blood pressure and caused a slowly developing contraction of the gut (Rocha e Silva et al., 1949); they named it *bradykinin*, derived from the Greek words *bradys*, meaning "slow," and *kinein*, meaning "to move." In 1960, bradykinin, a nine amino acid peptide, was isolated and synthesized; shortly thereafter, kallidin was identified as bradykinin with an additional N-terminal Lys residue. The kinins have short half-lives because they are destroyed by plasma and tissue peptidases (Erdös and Skidgel, 1997). Two types of kinin receptors, B_1 and B_2, were identified based on the rank order of potency of kinin analogues and later validated by cloning (Leeb-Lundberg et al., 2005). The development of receptor-specific antagonists and receptor knockout mice has furthered our understanding of the role of kinins in the regulation of cardiovascular homeostasis and inflammatory processes (Leeb-Lundberg et al., 2005).

Tissue damage, allergic reactions, viral infections, and other inflammatory events activate a series of proteolytic reactions that generate bradykinin and kallidin in tissues. These peptides contribute to inflammatory responses as autacoids that act locally to produce pain, vasodilation, and increased vascular permeability but can also have beneficial effects, for example, in the heart, kidney, and circulation (Bhoola et al., 1992). Much of their activity is due to stimulation of the release of potent mediators such as prostaglandins, NO, or EDHF (endothelial-derived hyperpolarizing factor).

The Endogenous Kallikrein-Kininogen-Kinin Systems

The nonapeptide bradykinin and decapeptide kallidin (*lysyl-bradykinin*) (Table 43–3) are cleaved from α_2 globulins termed *kininogens* (Figure 43–4). There are two kininogens: high molecular weight (HMW) kininogen and low molecular weight (LMW) kininogen. A number of serine proteases will generate kinins, but the two highly specific proteases that release bradykinin and kallidin from the kininogens are termed *kallikreins*. Bradykinin and kallidin are the natural ligands for the bradykinin B_2 receptor (BDKRB2), while the carboxypeptidase-cleaved

TABLE 43–3 ■ STRUCTURE OF KININ AGONISTS AND ANTAGONISTS

NAME	STRUCTURE	FUNCTION
Bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Agonist, B_2
Kallidin	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Agonist, B_2
[des-Arg^9]-Bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe	Agonist, B_1
[des-Arg^{10}]-Kallidin	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe	Agonist, B_1
des-Arg^{10}-[Leu^9]-Kallidin	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu	Antagonist, B_1
NPC-349	[D-Arg]-Arg-Pro-Hyp-Gly-Thi-Ser-D-Phe-Thi-Arg	Antagonist, B_2
HOE-140	[D-Arg]-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Oic-Arg	Antagonist, B_2
[des-Arg^{10}]-HOE-140	[D-Arg]-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Oic	Antagonist, B_1
FR173657	See Table 32–3 of the 12th edition	Antagonist, B_2
FR190997		Agonist, B_2
SSR240612		Antagonist, B_1

Hyp, trans-4-hydroxy-Pro; Thi, β-(2-thienyl)-Ala; Tic, [D]-1,2,3,4-tetrahydroisoquinolin-3-yl-carbonyl; Oic, (3aS, 7aS)-octahydroindol-2-yl-carbonyl.

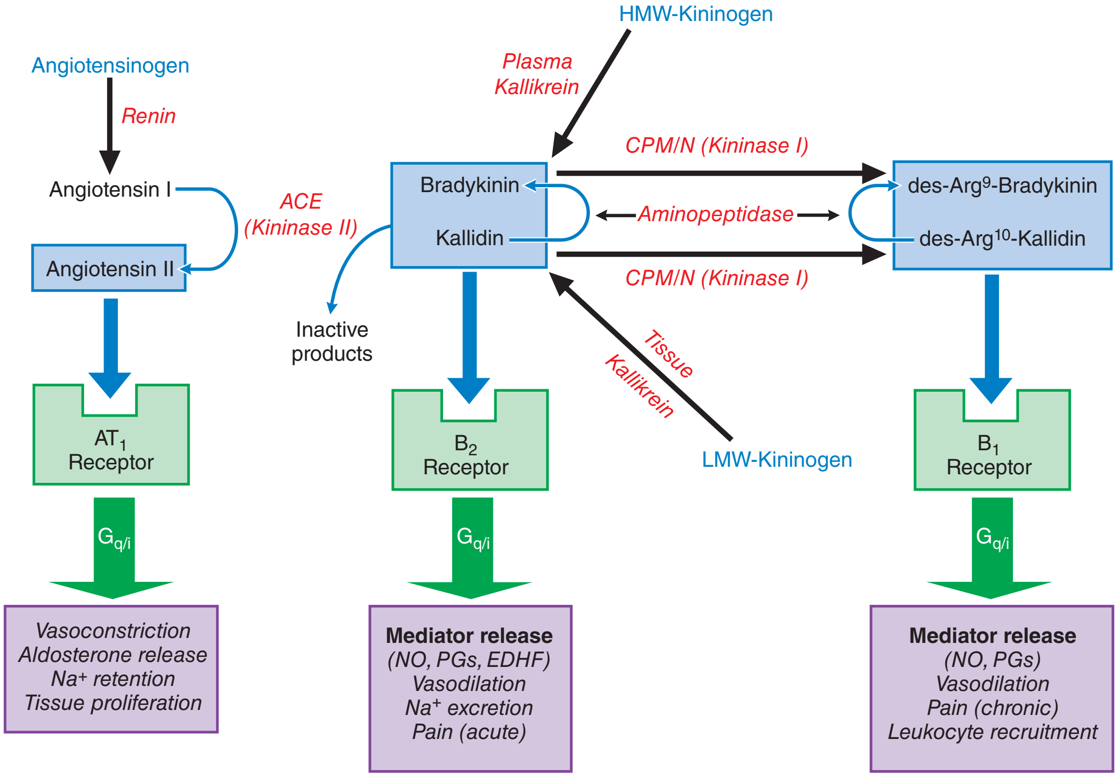


Figure 43–4 *Synthesis and receptor interactions of active peptides generated by the kallikrein-kinin and renin-angiotensin systems.* Bradykinin is generated by the action of plasma kallikrein on HMW kininogen, whereas kallidin (Lys1-bradykinin) is released by the hydrolysis of LMW kininogen by tissue kallikrein. Kallidin and bradykinin are the natural ligands of the B_2 receptor but can be converted to corresponding agonists of the B_1 receptor by removal of the C-terminal Arg by kininase I–type enzymes: the plasma membrane–bound CPM or soluble plasma CPN. Kallidin or [des-Arg^{10}]-kallidin can be converted to the active peptides bradykinin or to [des-Arg^9]-bradykinin by aminopeptidase cleavage of the N-terminal Lys residue. In a parallel fashion, the inactive decapeptide AngI is generated by the action of renin on the plasma substrate angiotensinogen. By removal of the C-terminal His-Leu dipeptide, ACE generates the active peptide AngII. These two systems have opposing effects. AngII is a potent vasoconstrictor that also causes aldosterone release and Na$^+$ retention via activation of the AT_1 receptor; bradykinin is a vasodilator that stimulates Na$^+$ excretion by activating the B_2 receptor. ACE generates active AngII and, at the same time, inactivates bradykinin and kallidin; thus, its effects are prohypertensive, and ACE inhibitors are effective antihypertensive agents. The B_2 receptor mediates most of bradykinin's effects under normal circumstances, whereas synthesis of the B_1 receptor is induced by inflammatory mediators in inflammatory conditions. Both B_1 and B_2 receptors couple through G_q to activate PLC and increase intracellular Ca^{2+}; the physiological response depends on receptor distribution on particular cell types and occupancy by agonist peptides. For instance, on endothelial cells, activation of B_2 receptors results in Ca^{2+}-calmodulin–dependent activation of eNOS and generation of NO, which causes cyclic GMP accumulation and relaxation in neighboring smooth muscle cells. However, in endothelial cells under inflammatory conditions, B_1 receptor stimulation results in prolonged NO production via G_i and an acute MAP kinase–dependent activation of iNOS. On smooth muscle cells, activation of kinin receptors coupling through G_q results in an increased $[Ca^{2+}]_i$ and contraction. B_1 and B_2 receptors also can couple through G_i to activate PLA_2, causing the release of arachidonic acid and the local generation of prostanoids and other metabolites such as EDHF. Kallikrein also plays a role in the intrinsic blood coagulation pathway (see Chapter 36). CPM, carboxypeptidase M; CPN, carboxypeptidase N; EDHF, endothelial-derived hyperpolarizing factor.

des-Arg products of these peptides are the natural ligands for the bradykinin B_1 receptor (BDKB1R).

Kallikreins

Bradykinin and kallidin are cleaved from HMW or LMW kininogens by plasma or tissue kallikrein, respectively (see Figure 43–4). Plasma kallikrein and tissue kallikrein are distinct enzymes that are activated by different mechanisms (Bhoola et al., 1992). Plasma prekallikrein is an inactive protein of about 88 kDa that complexes with its substrate, HMW kininogen. The ensuing proteolytic cascade is normally restrained by the protease inhibitors present in plasma, most importantly C1-INH (the inhibitor of the activated first component of complement) and α_2 macroglobulin. Under experimental conditions, the plasma kallikrein-kinin system (also called the plasma contact system) is activated by the binding of factor XII (*Hageman factor*) to negatively charged surfaces. Bound factor XII, a protease that is common to both the kinin and the intrinsic coagulation cascades (see Chapter 36), slowly undergoes autoactivation and, in turn, activates prekallikrein. Importantly, kallikrein rapidly further activates factor XII, thereby exerting a positive feedback on the system. *In vivo*, the order of this process can be reversed. The binding of the HMW kininogen–prekallikrein heterodimer to a multiprotein receptor complex on endothelial cells leads to activation of the prekallikrein–HMW kininogen complex by either heat shock protein 90 (Hsp90) or prolylcarboxypeptidase to generate kallikrein, which can then activate factor XII to start the positive-feedback loop and cleave HMW kininogen to generate bradykinin (Kaplan and Joseph, 2014). This process may contribute to the symptoms of hereditary angioedema in patients who are deficient in C1-INH.

Human tissue kallikreins compose a 15-member gene family with high sequence identity that are clustered at chromosome 19q13.4 (Prassas et al., 2015). However, the classical “tissue kallikrein,” hK1, is the only family member to readily generate kallidin from LMW kininogen. Tissue kallikrein is synthesized as a 29-kDa preproprotein in the epithelial cells or secretory cells in several tissues, including salivary glands, pancreas, prostate, and renal distal nephron (Bhoola et al., 1992). Tissue kallikrein also is expressed in human neutrophils; it acts locally near its sites of origin. The synthesis of tissue preprokallikrein is controlled by a number of factors, including aldosterone in the kidney and salivary gland and androgens in certain other glands. Preprokallikrein is cleaved to the zymogen prokallikrein when secreted. The activation of prokallikrein to active tissue kallikrein requires proteolytic cleavage to remove a seven–amino acid propeptide, which can be accomplished *in vitro* by plasma kallikrein and by some serine and metalloproteases. However, the activating enzyme(s) *in vivo* is unknown.

Kininogens

The two substrates for the kallikreins, HMW kininogen (120 kDa) and LMW kininogen (66 kDa), are derived from a single gene by alternative splicing. The first 401 amino acids are identical (through the bradykinin sequence and 12 additional residues) and then the sequences diverge, with HMW kininogen containing a 56-kDa C-terminal light chain and LMW kininogen a 4-kDa light chain (Bhoola et al., 1992). HMW kininogen is cleaved by both plasma and tissue kallikrein to yield bradykinin and kallidin, respectively, whereas LMW kininogen is cleaved only by tissue kallikrein to produce kallidin. In addition to being substrates for kallikreins, kininogens are also cysteine protease inhibitors.

Metabolism of Kinins

The decapeptide kallidin is about as active as the nonapeptide bradykinin, even without conversion to bradykinin, which occurs when the N-terminal lysine residue is removed by an aminopeptidase (see Figure 43–4). The $t_{1/2}$ of kinins in plasma is only about 15 sec; 80% to 90% of the kinins may be destroyed in a single passage through the pulmonary vascular bed by enzymes present on the large endothelial surface area of the lung (Erdös and Skidgel, 1997). Plasma concentrations of bradykinin are difficult to measure because inadequate inhibition of kininogenases or kininases in the blood can lead to artifactual formation or degradation of bradykinin during blood collection. When care is taken to inhibit these processes, the reported physiological concentrations of bradykinin in blood are in the picomolar range.

The principal catabolizing enzyme in the lung and other vascular beds is kininase II, or ACE (angiotensin I–converting enzyme), a membrane-anchored peptidase on the surface of endothelial cells (see Chapter 30). Removal of the C-terminal dipeptide by ACE or neutral endopeptidase 24.11 (neprilysin) inactivates kinins (Figure 43–5) (Erdös and Skidgel, 1997). A slower-acting plasma enzyme, carboxypeptidase N (lysine carboxypeptidase, kininase I), releases the C-terminal arginine residue, producing [desArg^9]-bradykinin or [des-Arg^{10}]-kallidin (see Table 43–3 and Figures 43–4 and 43–5) (Skidgel and Erdös, 2007), both of which no longer activate B_2 receptors but are B_1 receptor agonists. Carboxypeptidase N is expressed in the liver and constitutively secreted into the blood. A rare familial carboxypeptidase N deficiency was associated with angioedema or urticaria, possibly due to increased bradykinin (Skidgel and Erdös, 2007). Carboxypeptidase M, which cleaves the C-terminal Arg of bradykinin about 3-fold faster than carboxypeptidase N, is a widely distributed plasma membrane–bound enzyme that is also found on lung microvascular endothelial cells (Zhang et al., 2013a). Finally, aminopeptidase P is a membrane enzyme on epithelial and endothelial cells that can cleave the N-terminal arginine of bradykinin, rendering it inactive and susceptible to further cleavage by dipeptidyl peptidase IV (Erdös and Skidgel, 1997) (see Figure 43–5).

Kinin Receptors and Their Signaling Pathways

The B_1 and B_2 kinin receptors are GPCRs whose signaling mediates most of the biological effects of the kallikrein-kinin system (Leeb-Lundberg et al., 2005). The B_2 receptor is constitutively expressed in most normal tissues, where it selectively binds intact bradykinin and kallidin (see Table 43–3 and Figure 43–4). The B_2 receptor mediates the effects of bradykinin and kallidin under normal circumstances, whereas synthesis of the B_1 receptor is induced by inflammatory conditions. The B_1 receptor is activated by the C-terminal des-Arg metabolites of bradykinin and kallidin produced by the actions of carboxypeptidases N and M. While [des-Arg^{10}]-kallidin is a potent B_1 receptor agonist, [desArg^9]-bradykinin is a relatively weak B_1 receptor agonist in humans as opposed to rodents where it is a potent agonist. Interestingly, carboxypeptidase M and the B_1 receptor interact on the cell surface to form an efficient signaling complex that enhances B_1 receptor agonist affinity and can lead to allosteric activation of B_1 receptor signaling by substrate binding to carboxypeptidase M (Zhang et al., 2013a, 2013b). B_1 receptors are normally absent or expressed at low levels in most tissues. B_1 receptor expression is upregulated by tissue injury and inflammation and by cytokines, endotoxins, and growth factors. Carboxypeptidase M expression also is increased by cytokines, to such a degree that B_1 receptor effects may predominate over B_2 effects (Zhang et al., 2013a).

Both B_1 and B_2 receptors couple through G_q to activate PLC and increase intracellular Ca^{2+}; the physiological response depends on receptor distribution on particular cell types, the cell environment, and mediators generated (Leeb-Lundberg et al., 2005). For example, on normal endothelial cells, activation of B_2 receptors results in G_q and Ca^{2+}-calmodulin–dependent activation of eNOS and short-term generation of NO, which causes cyclic GMP accumulation and relaxation in neighboring smooth muscle cells. However, direct activation of B_1 or B_2 receptors on smooth muscle cells leads to coupling through G_q and increased $[Ca^{2+}]_i$, resulting in contraction.

Inflammatory conditions alter receptor signaling in endothelial cells, such that B_2 receptor stimulation leads to prolonged eNOS-derived NO that depends on G_i-mediated activation of MEK1/2 and JNK1/2, whereas B_1 receptor activation couples through G_i and MAP kinase activation to cause ERK1/2-mediated phosphorylation and activation of iNOS (inducible nitric oxide synthase), which generates prolonged, high-output NO (Kuhr et al., 2010; Lowry et al., 2013). Both B_1 and B_2 receptor stimulation activate the proinflammatory transcription factor NF-κB (nuclear factor-κB) coupled through $G\alpha_q$ and βγ subunits and also activate the MAP kinase pathway (Leeb-Lundberg et al., 2005). B_1 and B_2 receptors also can couple through G_i to activate PLA_2, causing the release of arachidonic acid and the local generation of metabolites such as prostaglandins and vasodilator epoxyeicosatrienoic acids (EETs) (Campbell and Falck, 2007).

The B_1 and B_2 receptors differ in their time courses of downregulation after agonist stimulation; the B_2 receptor response is rapidly desensitized, whereas the B_1 response is not (Leeb-Lundberg et al., 2005). This likely is

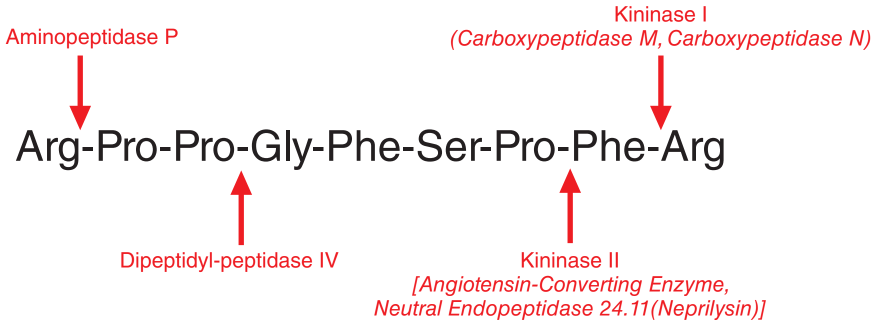

Aminopeptidase P
Kininase I
(Carboxypeptidase M, Carboxypeptidase N)
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
Dipeptidyl-peptidase IV
Kininase II
[Angiotensin-Converting Enzyme,
Neutral Endopeptidase 24.11(Neprilysin)]

Figure 43–5 *Schematic diagram of the degradation of bradykinin.* Arrows denote the primary cleavage sites in bradykinin. Bradykinin and kallidin are inactivated *in vivo* primarily by kininase II (ACE). Neutral endopeptidase 24.11 (neprilysin) cleaves bradykinin and kallidin at the same Pro-Phe bond as ACE and also is classified as a kininase II–type enzyme. In addition, aminopeptidase P can inactivate bradykinin by hydrolyzing the N-terminal Arg1-Pro2 bond, leaving bradykinin susceptible to further degradation by dipeptidyl peptidase IV. Bradykinin and kallidin are converted to their respective des-Arg^9 or des-Arg^{10} metabolites by kininase I–type carboxypeptidases M and N. Unlike the parent peptides, these kinin metabolites are potent ligands for B_1 kinin receptors but not B_2 kinin receptors.

due to modification at a Ser/Thr-rich cluster present in the C-terminal tail of the B_2 receptor that is not conserved in the B_1 receptor sequence. However, the B_1 receptor can heterodimerize with the B_2 receptor and in this form can be cross-desensitized by activation of the B_2 receptor with agonist (Zhang et al., 2015). Another difference between B_1 and B_2 receptors is that the B_1 receptor shows high constitutive activity when expressed.

Physiological and Pharmacological Effects of Kallikrein-Kinin Pathways

Kinins play important physiological and pathophysiological roles. Our understanding of the precise roles of kinins has been hampered by the difficulty in measuring activation of the pathways due to the short half-lives of kinins as well as the difficulties in preventing *ex vivo* activation of the pathways.

Physiological Effects of Kinins

Cardiovascular

Infusion of bradykinin causes vasodilation and lowers blood pressure. Bradykinin causes vasodilation by activating its B_2 receptor on endothelial cells, resulting in the generation of NO, prostacyclin, and a hyperpolarizing EET that is a CYP-derived metabolite of arachidonate (Campbell and Falck, 2007). Animals deficient in tissue kallikrein display arterial function abnormalities with impaired flow-dependent vasodilation and endothelial dysfunction. The endogenous kallikrein-kinin system plays a minor role in the regulation of normal blood pressure, but it may be important in hypertensive states. Urinary kallikrein concentrations are decreased in individuals with high blood pressure.

The kallikrein-kinin system is cardioprotective. Many of the beneficial effects of ACE inhibitors on cardiac function are attributable to enhancement of bradykinin effects, such as their antiproliferative activity or ability to increase tissue glucose uptake (Heitsch, 2003; Madeddu et al., 2007). Bradykinin contributes to the beneficial effect of preconditioning to protect the heart against ischemia and reperfusion injury. Bradykinin also stimulates tissue plasminogen activator release from the vascular endothelium and may contribute to the endogenous defense against some cardiovascular events, such as myocardial infarction and stroke (Heitsch, 2003; Madeddu et al., 2007).

As a substrate of the endopeptidase neprilysin, bradykinin is theorized to be involved in the cardioprotective mechanism of *sacubitril/valsartan*, a novel angiotensin receptor–neprilysin inhibitor (ARNI) combination drug that is used to decrease mortality in heart failure. The cardioprotective, natriuretic, and blood pressure–lowering effects of this drug are under investigation.

Kidney

Renal kinins act in a paracrine manner to regulate urine volume and composition. Kallikrein is synthesized and secreted by the connecting cells of the distal nephron. Tissue kininogen and kinin receptors are present in the cells of the collecting duct. Like other vasodilators, kinins increase renal blood flow. Bradykinin also causes natriuresis by inhibiting Na^+ reabsorption at the cortical collecting duct. Treatment with mineralocorticoids, ACE inhibitors, and inhibitors of neutral endopeptidase (neprilysin) increases renal kallikrein. Kinins have been shown to protect against diabetic nephropathy, thereby protecting against renal insufficiency.

Pathological Effects of Kinins

Angioedema

Plasma kinins increase permeability in the microcirculation, acting on the small venules to cause disruption of the interendothelial junctions. This, together with an increased hydrostatic pressure gradient, causes edema. The major mechanism of angioedema in patients with C1 inhibitor deficiency (hereditary angioedema due to C1 inhibitor deficiency and acquired C1 inhibitor deficiency) is increased generation of bradykinin due to dysregulation of the plasma kallikrein-kinin system (Busse and Christiansen, 2020). There is also evidence that some forms of hereditary angioedema with normal C1 inhibitor are due to bradykinin. Finally, angioedema associated with use of ACE inhibitors may be caused by the prolonged half-life of bradykinin when ACE is inhibited.

Respiratory Disease

Kinins have been implicated in allergic airway disorders such as asthma and rhinitis (Abraham et al., 2006). Inhalation of kinins causes bronchospasm mimicking an asthma attack in asthmatic patients but not in normal individuals. This bradykinin-induced bronchoconstriction is blocked by anticholinergic agents but not by antihistamines or cyclooxygenase inhibitors. Similarly, nasal challenge with bradykinin is followed by sneezing and glandular secretions in patients with allergic rhinitis, but not in normal individuals or those with nonallergic, noninfectious perennial rhinitis.

Anaphylaxis

Release of oversulfated proteoglycans from mast cells can activate the plasma kallikrein-kinin system in patients undergoing an anaphylactic reaction. In murine models of anaphylaxis, inhibition of bradykinin generation prevents complications such as hypothermia and death.

Pain

The kinins are powerful algesic agents that cause an intense burning pain when applied to the exposed base of a blister. Bradykinin excites primary sensory neurons and provokes the release of neuropeptides such as substance P, neurokinin A, and calcitonin gene–related peptide. Although there is overlap, B_2 receptors generally mediate acute bradykinin algesia, whereas the pain of chronic inflammation appears to involve increased numbers and activation of B_1 receptors.

Inflammation

Kinins participate in a variety of inflammatory conditions (Bhoola et al., 1992; Leeb-Lundberg et al., 2005). The B_1 receptors on inflammatory cells (e.g., macrophages) can elicit production of the inflammatory mediators IL-1 (interleukin-1) and TNFα (tissue necrosis factor α). Kinin levels are increased in a number of chronic inflammatory diseases and may be significant in gout, disseminated intravascular coagulation, inflammatory bowel disease, rheumatoid arthritis, and asthma. In addition, kinins and their receptors are associated with a variety of neuroinflammatory disorders, including neuropathic pain in diabetes, autoimmune encephalomyelitis, and Alzheimer's disease. Kinins may contribute to the skeletal changes seen in chronic inflammatory states; kinins stimulate bone resorption through B_1 and possibly B_2 receptors, perhaps by osteoblast-mediated osteoclast activation (see Chapter 52).

Diabetic Macular Edema

Diabetic macular edema is characterized in part by enhanced microvascular permeability with edema. Increased kallikrein activity has been found in the eyes of diabetic rats showing evidence of increased angiogenesis. Patients with diabetic retinopathy and diabetic macular degeneration have also been shown to have significantly increased plasma kallikrein activity in vitreous fluid. Early clinical studies are looking at the beneficial effect of kallikrein inhibitors for the treatment of diabetic macular edema.

Other Effects

Kinins promote dilation of the fetal pulmonary artery, closure of the ductus arteriosus, and constriction of the umbilical vessels, all of which occur in the transition from fetal to neonatal circulation. Kinins also affect the CNS, disrupting the blood-brain barrier and allowing increased CNS penetration. Kinins and kinin receptor signaling have been associated with neuroinflammatory disorders, such as neuropathic pain in diabetes, autoimmune encephalomyelitis, and Alzheimer's disease.

Drugs Acting on the Kallikrein-Kinin System

An imbalance between plasma kallikrein activity and its naturally-occuring inhibitors can contribute to disease, such as hereditary angioedema. Several classes of drugs can usefully modulate the activity of the kallikrein-kinin system, including inhibitors of plasma kallikrein, C1

TABLE 43–4 ■ PREPARATIONS AND DOSAGE OF KALLIKREIN-KININ ACTING DRUGS

DRUG	INDICATION	ADMINISTRATION^a	DOSE (adult)
Plasma Kallikrein Inhibitors			
Recombinant plasma kallikrein inhibitor			
Ecallantide	On-demand	SC	30 mg
Monoclonal antibody to plasma kallikrein			
Lanadelumab	Prophylactic	SC	300 mg q2-4 weeks
Oral plasma kallikrein inhibitor			
Berotralstat	Prophylactic	O	150 mg qDay
C1 Inhibitors			
Plasma-derived concentrates			
Berinert	On-demand	IV	20 IU/kg
Cinryze	Prophylactic	IV	1,000 IU q3-4 days
Haegarda	Prophylactic	SC	60 IU/kg q3-4 days
Recombinant C1 inhibitor			
Ruconest	On-demand	IV	50 IU/kg
Bradykinin B2 receptor antagonist			
Icatibant	On-demand	SC	30 mg

^{a}O, oral; SC, subcutaneous; IV, intravenous.

esterase inhibitors, and bradykinin receptor antagonists. Table 43–4 and the text below describe the currently available agents.

Kallikrein Inhibitors

Ecallantide

Ecallantide is a synthetic plasma kallikrein inhibitor approved for the treatment of acute attacks of hereditary angioedema in patients 12 years and older. It is administered by a healthcare professional (with appropriate medical support to manage possible anaphylaxis) subcutaneously at a total dose of 30 mg, divided into three 10-mg injections of 1 mL each. An additional dose of 30 mg may be administered within a 24-h period if the attack persists. The most common side effects (~3%–8% of patients) include headache, nausea, diarrhea, fever, injection site reactions, and nasopharyngitis. Safety has not been tested in pregnant or nursing women. Anaphylaxis has been reported in about 4% of treated patients, occurring within 1 h after dosing. Approximately 20% of patients treated with *ecallantide* develop antibodies to the drug and may be at a higher risk of hypersensitivity reactions on subsequent exposure. *Ecallantide* has been investigated as a potential treatment of angioedema caused by ACE inhibitors (Zuraw et al., 2013); however, studies conflict in demonstrating benefit (Bernstein et al., 2015; Lewis et al., 2015).

C1 Inhibitor

Concentrates of *C1 inhibitor* have been approved for the on-demand and prophylactic treatment of hereditary angioedema. C1 inhibitor acts on both of the proteases in the plasma kallikrein-kinin system (plasma kallikrein and active factor [F] XII), decreasing the production of bradykinin. *Cinryze* and *Berinert* are plasma-derived C1 inhibitor concentrates that are administered by intravenous infusion. *Berinert* is approved in the U.S. for on-demand treatment of hereditary angioedema attacks in adults and children. It is administered at a dose of 20 IU/kg by slow injection. *Cinryze* is approved for prophylactic use with a starting dose of 1000 IU twice weekly. *Ruconest* is a recombinant human C1 inhibitor that is approved for on-demand use at a dose of 50 IU/kg by slow intravenous injection in adults and adolescents. *Haegarda* is a more concentrated form of *Berinert* that is administered for long-term prophylaxis by subcutaneous injection at a dose of 60 IU/kg twice weekly in adults and children. All of the C1 inhibitor preparations are safe and generally well tolerated. At very high doses, C1 inhibitor may be thrombogenic, but this has not been an issue at normal doses. Extremely rare instances of allergic reactions to C1 inhibitor have been reported.

Lanadelumab

Lanadelumab is a fully human IgG1/kappa light chain monoclonal antibody produced in Chinese hamster ovary cells. *Lanadelumab* rapidly inhibits active plasma kallikrein, preventing HMW kininogen cleavage and generation of bradykinin. It is approved for the long-term prophylaxis of hereditary angioedema in patients 12 years and older. The starting dose of *lanadelumab* is 300 mg twice monthly, which can be reduced to 300 mg monthly if the patient does well after 6 months. *Lanadelumab* is well tolerated, with rare hypersensitivity reactions described.

Berotralstat

Berotralstat is an orally available small-molecule plasma kallikrein inhibitor. *Berotralstat* inhibits plasma kallikrein, preventing HMW kininogen cleavage and generation of bradykinin. It is approved for the long-term prophylaxis of hereditary angioedema in patients 12 years and older. The starting dose of *berotralstat* is 150 mg daily with food. The most common adverse reactions to *berotralstat* have been abdominal pain, vomiting, diarrhea, back pain, and gastroesophageal reflux disease, which are typically transient. In patients with chronic administration of agents that inhibit P-glycoprotein or BCRP (breast cancer resistance protein) (i.e., *cyclosporine*), the recommended dosage of *berotralstat* is one 110-mg capsule orally daily with food. *Berotralstat* can prolong the QT interval at doses above the recommended 150 mg daily.

Other Kallikrein Inhibitors

With the success of kallikrein inhibitors in treating hereditary angioedema due to C1 inhibitor deficiency, a number of other kallikrein inhibition strategies are being pursued in ongoing clinical trials for hereditary angioedema. These include other orally available small-molecule inhibitors, an antisense oligonucleotide directed at plasma kallikrein, and CRISPR-Cas9–mediated knockdown of plasma kallikrein. The efficacy of kallikrein inhibitors for diabetic macular edema is also being studied in clinical trials.

Aprotinin is a natural proteinase inhibitor that inhibits mediators of the inflammatory response, fibrinolysis, and thrombin generation, including kallikrein and plasmin. *Aprotinin* was employed clinically to reduce blood loss in patients undergoing coronary artery bypass surgery, but unfavorable survival statistics in retrospective and prospective studies resulted in its discontinuation.

Kinin Receptor Antagonists

The utility of specific kinin receptor antagonists currently is being investigated in diverse areas such as pain, inflammation, chronic inflammatory diseases, and cardiovascular diseases (Campos et al., 2006). The beneficial effects of ACE inhibitor therapy rest in part on enhancing bradykinin activity (e.g., on the heart, kidney, blood pressure; see Chapter 30); this has led to the suggestion that kinin agonists could be therapeutically beneficial (Heitsch, 2003; Rhaleb et al., 2011).

Icatibant

The selective B_2 receptor antagonist *icatibant* has been approved in the E.U. and U.S. for treatment of acute episodes of swelling in patients more than 18 years of age with hereditary angioedema. It is administered by a healthcare professional, or self-administered by the patient after training, at a dose of 30 mg in 3 mL of solution by subcutaneous injection in the abdomen. Additional doses may be administered at intervals of at least 6 h if the response is inadequate or symptoms recur, not to exceed three doses in any 24-h period. A common side effect experienced by most patients is a local reaction at the injection site (e.g., redness, bruising, swelling, burning, itching). A small percentage of patients have experienced fever, elevated transaminase, dizziness, nausea, headache, or rash.

Safety has not been tested in pregnant or nursing women. *Icatibant* was investigated in randomized, placebo-controlled trials for treatment of ACE inhibitor–associated angioedema but was shown to lack clinical utility (Sinert et al., 2017; Straka et al., 2017).

Other Kinin Receptor Antagonists

Orally available B_2 and B_1 bradykinin receptor antagonists have been developed. An oral potent B_2 receptor is currently in early-phase clinical trials for hereditary angioedema due to C1 inhibitor deficiency. Despite several phase I studies being started, no B_1 receptor antagonists are currently undergoing clinical testing.

FXII Inhibitors

Garadacimab is a fully human monoclonal IgG4 antibody directed against the active site of FXIIa, the activated protease form of coagulation factor XII. A phase II clinical trial using three doses of subcutaneous *garadacimab* given every 4 weeks demonstrated significant efficacy in patients with hereditary angioedema due to C1 inhibitor deficiency. A pivotal phase III study is currently in progress.

FXIIa inhibitors are also being considered for additional indications, including prevention of clotting/thrombosis, sepsis, multiple sclerosis, and Alzheimer's disease. Orally available FXIIa inhibitors are in development.

ACE Inhibitors

The ACE inhibitors, widely used in the treatment of hypertension, congestive heart failure, and diabetic nephropathy, block the conversion of angiotensin (Ang) I to AngII and also block the degradation of bradykinin by ACE (see Figure 43–4 and Chapter 30). Numerous studies demonstrated that bradykinin contributes to many of the protective effects of ACE inhibitors (Heitsch, 2003; Madeddu et al., 2007). The search is on to find a suitable stable B_2 agonist for clinical evaluation that provides cardiovascular benefit without proinflammatory effects.

A rare side effect of ACE inhibitors is angioedema, which is likely due to the inhibition of kinin metabolism by ACE (Zuraw et al., 2013). A common side effect of ACE inhibitors that may be related to enhanced kinin levels is a chronic, nonproductive cough that dissipates when the drug is stopped. Bradykinin may also contribute to the therapeutic effects of the AT_1 receptor antagonists. During AT_1 receptor blockade, AngII signaling through the unopposed AT_2 subtype receptor is enhanced, causing an increase in bradykinin concentrations, which has beneficial effects on cardiovascular and renal function (Padia and Carey, 2013). However, the increase in bradykinin levels is likely more modest than that achieved by ACE inhibitors, as reflected by the lower incidence of angioedema in patients taking AT_1 receptor antagonists (Zuraw et al., 2013).

Acknowledgment: *Randal A. Skidgel contributed to this chapter in the previous edition of this book. We have retained some of his text in the current edition.*

Drug Facts for Your Personal Formulary: *H_1 Antagonists*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
First-Generation Antihistamines: H_1 receptor inverse agonists • Most have central and anticholinergic effects • Use with caution in children and in adults >65 years of age		
Doxepin	• Tricyclic antidepressant • Insomnia • Pruritis (topical cream) • Pruritis (atopic dermatitis, eczema, lichen simplex) (cream)	• Causes significant sedation/drowsiness • Anticholinergic effects • Increased risk of suicidal thoughts (children, adolescents, and young adults)
Carbinoxamine Clemastine Diphenhydramine Dimenhydrinate	• Symptoms of allergic response • Mild urticaria • Insomnia (diphenhydramine) • Motion sickness (dimenhydrinate, diphenhydramine)	• Pronounced tendency to cause sedation • Significant anticholinergic effects • GI side effects are low • Carbinoxamine and diphenhydramine: adjunct to epinephrine for anaphylaxis
Pyrilamine (only available as an ingredient in over-the-counter combination preparations)	• Symptoms of allergic response	• Anticholinergic effects • Central effects < other first-generation drugs • GI side effects are quite common
Chlorpheniramine Dexchlorpheniramine Brompheniramine Dexbrompheniramine (component of cold medicine)	• Allergic conjunctivitis • Allergic rhinitis • Anaphylaxis (adjunct), histamine-mediated angioedema, dermatographism, pruritus, sneezing, urticaria (brompheniramine) • Symptoms of allergic response	• Less drowsiness than other first-generation drugs; CNS stimulation side effects more common
Hydroxyzine	• Pruritis • Sedation • Antianxiety • Atopic dermatitis • Antiemetic • Urticaria	• CNS depressant action may contribute to antipruritic effects
Cyclizine (discontinued in the U.S.) Meclizine (not for use in children)	• Motion sickness • Nausea/vomiting • Vertigo	• Antinausea properties due to prominent anticholinergic effects • Less likely to cause drowsiness than other first-generation drugs • Meclizine, most used, long effect (≥8 h)
Promethazine	• Antiemetic • Motion sickness • Pruritus • Sedation • Symptoms of allergic response (off-label use)	• Risk of fatal respiratory depression in children, especially <2 years • May lower seizure threshold • Has local anesthetic activity • Most potent antihistamine antiemetic

Drug Facts for Your Personal Formulary: H_1 *Antagonists (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Cyproheptadine	• Allergic conjunctivitis • Allergic rhinitis • Anaphylaxis • Histamine-mediated angioedema • Pruritus, allergy • Vasomotor rhinitis • Urticaria • Dermatographism	• May increase appetite, cause weight gain • Has significant anticholinergic activity • Also blocks serotonin effects by antagonizing the $5HT_{2A}$ receptor
Second-Generation Antihistamines: H_1 receptor inverse agonists • Lack significant central and anticholinergic effects		
Olopatadine (nasal and ophthalmic only)	• Allergic conjunctivitis • Allergic rhinitis • Ocular pruritus • Rhinorrhea • Sneezing	• Approved for once-daily dosing • Eye drops may cause headaches in some • Nasal spray can cause epistaxis and nasal ulceration or septal perforation • Some increase in risk of somnolence with nasal spray • Nasal spray minor side effects include bitter taste and headache
Acrivastine (only marketed in combination with pseudoephedrine)	• Allergic rhinitis • Nasal congestion • Allergic symptoms	• ~40% metabolized by CYPs, reducing potential for drug interactions • Somewhat higher risk of mild sedation than other second-generation drugs
Cetirizine Levocetirizine	• Allergic rhinitis • Atopic dermatitis (cetirizine) • Urticaria (chronic idiopathic)	• Somewhat higher risk of mild sedation than other second-generation drugs; more potent levocetirizine can be used at lower dose with less risk of sedation • Only ~30% (cetirizine) or ~1% (levocetirizine) metabolized by CYPs, reducing potential for drug interactions
Loratadine Desloratadine	• Allergic rhinitis • Chronic idiopathic urticaria • Exercise-induced bronchospasm prophylaxis (loratadine) • Pruritus (desloratadine)	• Desloratadine is the active metabolite of loratadine • 24-h duration of activity so only once-a-day dosing is required
Fexofenadine	• Allergic rhinitis • Chronic idiopathic urticaria	• Is the active metabolite of terfenadine (withdrawn from the market due to risk of torsades de pointes) • Only ~8% metabolized by CYPs, reducing potential for drug interactions
Alcaftadine (ophthalmic only)	• Allergic conjunctivitis • Ocular pruritus	• In addition to mast cell–stabilizing and anti-inflammatory properties, its H_4 antagonist activity may give superior relief from ocular itching • Approved for once-daily dosing • Most common adverse reactions (<4%) are eye irritation, redness, and pruritis
Bepotastine (ophthalmic only)	• Allergic conjunctivitis • Ocular pruritus	• Has mast cell–stabilizing and anti-inflammatory properties • Most common (~25%) adverse reaction is mild taste • Other minor (2%–5%) reactions are eye irritation, headache, and nasopharyngitis
Ketotifen (ophthalmic only)	• Allergic conjunctivitis • Ocular pruritus	• Has mast cell–stabilizing and anti-inflammatory properties • Most common (~10%–25%) adverse reactions are red eyes and mild headache or rhinitis
Azelastine (nasal and ophthalmic only)	• Allergic conjunctivitis • Allergic rhinitis (alone and combined with fluticasone) • Ocular pruritus • Vasomotor rhinitis	• Has mast cell–stabilizing and anti-inflammatory properties • Eye drops may cause transient eye burning/stinging • Some increase in risk of somnolence with nasal spray • Minor side effects with eye drops and nasal spray include bitter taste and headache
Emedastine (ophthalmic only)	• Allergic conjunctivitis • Ocular pruritus	• Lacks mast cell–stabilizing and anti-inflammatory properties • Common side effect: headache (~11%) • Minor reactions (<5%): abnormal dreams, bad taste, eye irritation
Epinastine (ophthalmic only)	• Allergic conjunctivitis • Ocular pruritus	• In addition to mast cell–stabilizing and anti-inflammatory properties, its H_2 antagonist activity may reduce eyelid edema • Common side effect (~10%): symptoms of upper respiratory infection • Minor ocular reactions: burning sensation, folliculosis, hyperemia, and pruritis

Drug Facts for Your Personal Formulary: H_1 *Antagonists* (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Drugs Acting on the Kallikrein-Kinin System		
Recombinant Kallikrein Inhibitor Ecallantide	• Acute attacks of hereditary angioedema	• Approved for patients ≥12 years old • Patients may develop antibodies to the drug • Risk of hypersensitivity on subsequent exposure • Must be administered by healthcare professional
C1 Inhibitors Various plasma-derived concentrates	• On-demand and prophylactic treatment of hereditary angioedema	• Approved for adult and pediatric patients • Inhibit plasma kallikrein and active FXII
Antibody to plasma kallikrein Lanadelumab	• Prophylactic treatment of hereditary angioedema	• Approved for patients ≥12 years old • Fully human monoclonal antibody to plasma kallikrein
Kinin receptor antagonists Icatibant	• Acute attacks of hereditary angioedema	• Approved for patients ≥18 years old • B_2 kinin receptor antagonist • Commonly produces local reaction at injection site
Small molecule plasma inhibitor Berotralstat	• Prophylactic treatment of hereditary angioedema	• Approved for patients ≥12 years old • May interact with P-gp or BCRP inhibitors

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Chapter 44

Pulmonary Pharmacology

Peter J. Barnes

MECHANISMS OF ASTHMA

MECHANISMS OF COPD

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Pulmonary pharmacology concerns understanding how drugs act on the lung and the pharmacological therapy of pulmonary diseases. Much of pulmonary pharmacology is concerned with the effects of drugs on the airways and the therapy of airway obstruction, particularly asthma and chronic obstructive pulmonary disease (COPD), which are among the most common diseases of the airways, although there are marked differences in inflammatory mechanisms and response to therapy between these diseases. This chapter discusses the pharmacotherapy of obstructive airways disease, particularly therapy with bronchodilators, which act mainly by reversing airway smooth muscle contraction, and anti-inflammatory drugs, which suppress the inflammatory response in the airways. This chapter focuses on the pulmonary pharmacology of β_2 adrenergic agonists and corticosteroids; the basic pharmacology of these classes of agents is presented elsewhere (see Chapters 14 and 50).

This chapter also discusses other drugs used to treat obstructive airway diseases, such as mucolytics and respiratory stimulants, and covers the drug therapy of cough, the most common respiratory symptom. Drugs used in the treatment of pulmonary hypertension (see Chapter 35) or lung infections, including tuberculosis (see Chapter 65), are covered elsewhere.

Mechanisms of Asthma

Asthma is a chronic inflammatory disease of the airways that is characterized by activation of *mast cells*, infiltration of *eosinophils*, *T helper 2 (T_H2) lymphocytes, and innate type 2 lymphocytes (ILC2)* (Figure 44–1) (Papi et al., 2018). Mast cell activation by allergens and physical stimuli releases *bronchoconstrictor mediators*, such as *histamine, leukotriene (LT) D_4*, and *prostaglandin (PG) D_2*, which cause airway smooth muscle contraction, vasodilation, microvascular leakage, and plasma exudation.

Many of the symptoms of asthma are due to airway smooth muscle contraction, and therefore, bronchodilators are important as symptom relievers. Whether airway smooth muscle is intrinsically abnormal in asthma is not clear, but increased contractility of airway smooth muscle may contribute to airway hyperresponsiveness, the physiological hallmark of asthma.

Chronic inflammation in allergic asthma may initially be driven by allergen exposure, but it appears to become autonomous so that asthma is essentially incurable. The inflammation may be orchestrated by dendritic cells that regulate T_H2 cells that drive eosinophilic inflammation and by IgE formation by B lymphocytes. Nonallergic asthma is also eosinophilic, orchestrated by T_H2 and ILC2 cells.

Abbreviations

ACh: acetylcholine
AUC: area under the curve
BDP: beclomethasone dipropionate
cAMP: 3′-5′ cyclic adenosine monophosphate, cyclic AMP
CCR: C-C chemokine receptor
COMT: catechol-*O*-methyl transferase
COPD: chronic obstructive pulmonary disease
CRTh2: chemokine receptor homologous molecule expressed on Th2 lymphocytes
cys-LT: cysteinyl-leukotriene
DPI: dry powder inhaler
F_{eNO}: fractional exhaled nitric oxide
FEV_1: forced expiratory volume in 1 sec
GI: gastrointestinal
GPCR: G protein-coupled receptor
GR: glucocorticoid receptor
HDAC: histone deacetylase
HFA: hydrofluoroalkane
HPA: hypothalamic-pituitary-adrenal
ICS: inhaled corticosteroid
Ig: immunoglobulin
IL: interleukin
ILC2: innate type 2 lymphocyte
IP_3: inositol 1,4,5-trisphosphate
LABA: long-acting β_2 agonist
LAMA: long-acting muscarinic antagonist
LTRA: leukotriene cys-LT_1-receptor antagonist
5-LO: 5′-lipoxygenase
LT: leukotriene
MAO: monoamine oxidase
MAP: mitogen-activated protein
MDI: metered-dose inhaler
MMAD: mass median aerodynamic diameter
MMP: matrix metalloproteinase
NF-κB: nuclear factor kappa B
PDE: cyclic nucleotide phosphodiesterase
PG: prostaglandin
PKA: protein kinase A
pMDI: pressurized metered-dose inhaler
SABA: short-acting β_2 agonists
SAMA: short-acting muscarinic antagonist
TAS2R: taste 2 receptor
Tc1 cell: cytotoxic T lymphocyte
T_H17: T helper 17 cell
T_H2: T helper 2 lymphocyte
TNF: tumor necrosis factor
TRP: transient receptor potential
TSLP: thymic stromal lymphopoietin
VIP: vasoactive intestinal polypeptide

Airway epithelium plays an important role through the release of multiple inflammatory mediators and through the release of growth factors in an attempt to repair the damage caused by inflammation. The inflammatory process in asthma is mediated through the release of more than 100 inflammatory mediators, including lipid mediators, cytokines, chemokines, and growth factors.

Chronic inflammation may lead to structural changes (remodeling) in the airways, including an increase in the number (hyperplasia) and size (hypertrophy) of airway smooth muscle cells, blood vessels (angiogenesis), and mucus-secreting cells (goblet cell hyperplasia). A characteristic histological feature of asthma is collagen deposition (fibrosis) below the basement membrane of the airway epithelium (termed subepithelial cell fibrosis) (see Figure 44–1). This appears to be the result of eosinophilic inflammation and is found even at the onset of asthmatic symptoms. The complex inflammation of asthma is suppressed by corticosteroids in most patients, but even if asthma is well controlled, the inflammation and symptoms return if corticosteroids are discontinued. Asthma usually starts in early childhood, then may disappear during adolescence and reappear in adulthood. It is characterized by variable airflow obstruction and typically shows a good therapeutic response to bronchodilators and corticosteroids. Asthma severity usually does not change, so that patients with mild asthma rarely progress to severe asthma and patients with severe asthma usually have this from the onset, although some patients, particularly with late-onset asthma, show a progressive loss of lung function like patients with COPD. Patients with severe asthma may have a pattern of inflammation more similar to COPD and are characterized by reduced responsiveness to corticosteroids (Ray et al., 2016).

Mechanisms of COPD

COPD involves inflammation of the respiratory tract with a pattern that differs from that of asthma. In COPD, there is a *predominance of neutrophils, macrophages, cytotoxic T lymphocytes (Tc1 cells), and T helper 17 (T_H17) cells*. The inflammation *predominantly affects small airways*, resulting in progressive small-airway narrowing and fibrosis (chronic obstructive bronchiolitis) and inflammation in the lung parenchyma, resulting in alveolar wall destruction (emphysema) (Figure 44–2) (Agustí and Hogg, 2019; Barnes et al., 2015). These pathological changes result in airway closure on expiration, leading to air trapping and hyperinflation, particularly on exercise (dynamic hyperinflation). This accounts for shortness of breath on exertion and exercise limitation that are characteristic symptoms of COPD.

Bronchodilators reduce air trapping by dilating peripheral airways and are the mainstay of treatment in COPD. In contrast to asthma, the airflow obstruction of COPD tends to be progressive. The inflammation in the peripheral lung of patients with COPD is also mediated by multiple inflammatory mediators and cytokines, but the pattern of mediators differs from that of asthma. In contrast to asthma, the inflammation in patients with COPD is largely corticosteroid resistant, and there are currently no safe and effective anti-inflammatory treatments. Many patients with COPD have comorbidities, including ischemic heart disease, hypertension, congestive heart failure, diabetes, osteoporosis, skeletal muscle wasting, depression, chronic renal disease, and anemia (Divo and Celli, 2020). These diseases may occur together as part of multimorbidity, as diseases of accelerated aging with common pathogenetic mechanisms (Barnes et al., 2019).

Routes of Drug Delivery to the Lungs

Drugs may be delivered to the lungs by oral or parenteral routes and also by inhalation. The choice depends on the drug and on the respiratory disease.

Inhaled Route

Inhalation (Figure 44–3) is the preferred mode of delivery of many drugs with a direct effect on airways, particularly for asthma and COPD (Lavorini et al., 2019). It is the only way to deliver some drugs, such as cromolyn sodium and anticholinergic drugs, and is the preferred route of delivery for β_2 agonists and corticosteroids to reduce systemic side effects. Antibiotics may be delivered by inhalation in patients with chronic respiratory sepsis (e.g., in cystic fibrosis). The major advantage of inhalation is the delivery of drug to the airways in doses that are effective with a much lower risk of systemic side effects. This is particularly important with the use of inhaled corticosteroids (ICS), which largely avoids systemic side effects. In addition, inhaled bronchodilators have a more rapid onset of action than when taken orally.

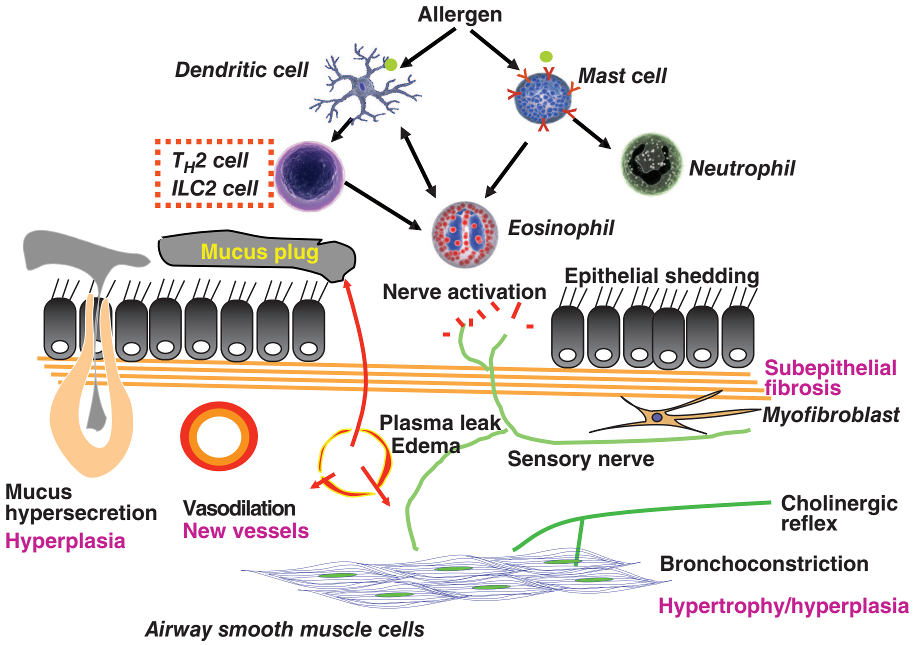
Allergen
Dendritic cell
Mast cell
Neutrophil
T_H2 cell
ILC2 cell
Eosinophil
Mucus plug
Nerve activation
Epithelial shedding
Subepithelial fibrosis
Myofibroblast
Plasma leak
Edema
Sensory nerve
Mucus hypersecretion
Hyperplasia
Vasodilation
New vessels
Cholinergic reflex
Bronchoconstriction
Hypertrophy/hyperplasia
Airway smooth muscle cells

Figure 44–1 *Cellular mechanisms of asthma.* Myriad inflammatory cells are recruited and activated in the airways, where they release multiple inflammatory mediators, which can also arise from structural cells. These mediators lead to bronchoconstriction, plasma exudation and edema, vasodilation, mucus hypersecretion, and activation of sensory nerves. Chronic inflammation leads to structural changes, including subepithelial fibrosis (basement membrane thickening), airway smooth muscle hypertrophy and hyperplasia, angiogenesis, and hyperplasia of mucus-secreting cells.

Particle Size

The size of particles for inhalation is of critical importance in determining the site of deposition in the respiratory tract. The optimum size for particles to settle in the airways is 2- to 5-μm MMAD (mass median aerodynamic diameter). Larger particles settle out in the upper airways, whereas smaller particles remain suspended and are therefore exhaled. There is increasing interest in delivering drugs to small airways, particularly in COPD and severe asthma (Usmani and Barnes, 2012). This involves delivering drug particles of about 1-μm MMAD, which is now possible using drugs formulated in hydrofluoroalkane (HFA) propellant.

Pharmacokinetics

Of the total drug delivered, only 10% to 20% enters the lower airways with a conventional pressurized metered-dose inhaler (pMDI). Drugs are absorbed from the airway lumen and have direct effects on target cells of the airway. Drugs may also be absorbed into the bronchial circulation and

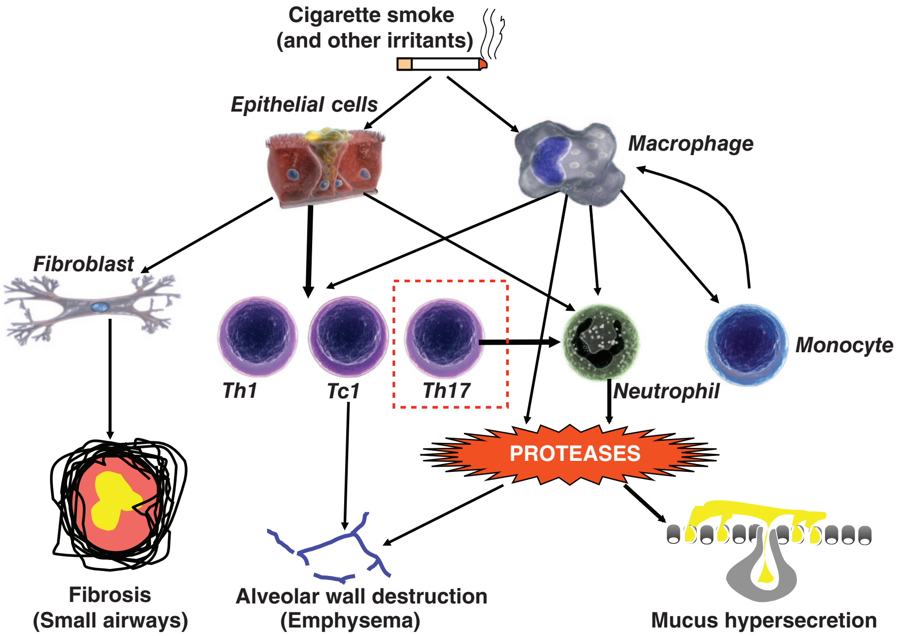
Cigarette smoke
(and other irritants)
Epithelial cells
Macrophage
Fibroblast
Th1
Tc1
Th17
Neutrophil
Monocyte
PROTEASES
Fibrosis
(Small airways)
Alveolar wall destruction
(Emphysema)
Mucus hypersecretion

Figure 44–2 *Cellular mechanisms in COPD.* Cigarette smoke and other irritants activate epithelial cells and macrophages in the lung to release mediators that attract circulating inflammatory cells, including monocytes (which differentiate to macrophages within the lung), neutrophils, and T lymphocytes (T_H1, Tc1, and T_H17 cells). Fibrogenic factors released from epithelial cells and macrophages lead to fibrosis of small airways. Release of proteases results in alveolar wall destruction (emphysema) and mucus hypersecretion (chronic bronchitis).

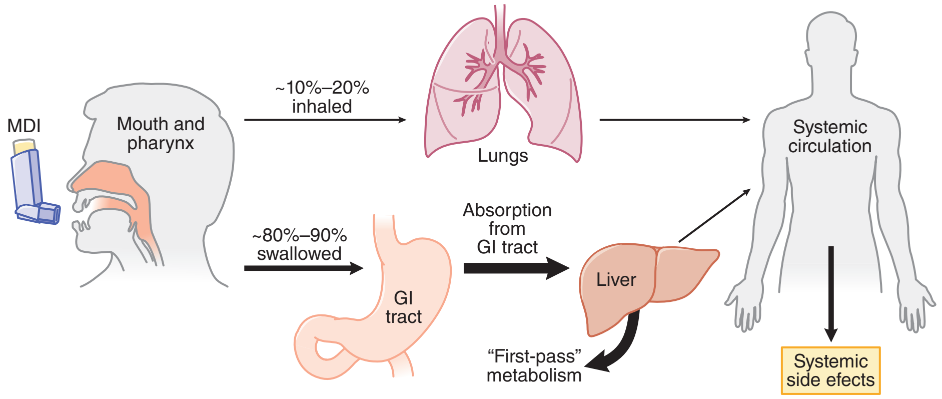

Figure 44–3 *Schematic representation of the deposition of inhaled drugs (e.g., corticosteroids, β_2 agonists).* Inhalation therapy deposits drugs directly, but not exclusively, in the lungs. Distribution between lungs and oropharynx depends mostly on the particle size and the efficiency of the delivery method. Most material will be swallowed and absorbed, entering systemic circulation after undergoing the first-pass effect in the liver. Some drug will also be absorbed into the systemic circulation from the lungs. Use of a large-volume spacer will reduce the amount of drug deposited on the oropharynx, thereby reducing the amount swallowed and absorbed from the GI tract and thus limiting systemic effects.

then distributed to more peripheral airways. Drugs with higher molecular weights tend to be retained to a greater extent in the airways. Nevertheless, several drugs have greater therapeutic efficacy when given by the inhaled route. The ICS *ciclesonide* is a prodrug activated by esterases in the respiratory tract to the active principle *des-ciclesonide*. More extensive pulmonary distribution of a drug with a smaller MMAD increases alveolar deposition and thus is likely to increase absorption from the lungs into the general circulation, resulting in more systemic side effects. Thus, although HFA pMDIs deliver more ICS to smaller airways, there is also increased systemic absorption, so that the therapeutic ratio may not be changed.

Delivery Devices

Pressurized Metered-Dose Inhalers. Drugs are propelled from a canister in the pMDI with the aid of a propellant, previously with a chlorofluorocarbon (Freon) but now replaced by an HFA that is more ozone friendly, that is, does not contribute to ozone depletion in Earth's upper atmosphere. These devices are convenient, portable, and typically deliver 50 to 200 doses of drug.

Spacer Chambers. Large-volume spacer devices between the pMDI and the patient reduce the velocity of particles entering the upper airways and the size of the particles by allowing evaporation of liquid propellant. This reduces the amount of drug that impinges on the oropharynx and increases the proportion of drug inhaled into the lower airways. Application of spacer chambers is useful in the reduction of the oropharyngeal deposition of ICS and the consequent reduction in the local side effects of these drugs. Spacer devices are also useful in delivering inhaled drugs to small children who are not able to use a pMDI. Children as young as 3 years of age can use a spacer device fitted with a face mask.

Dry Powder Inhalers. Drugs may also be delivered as a micronized dry powder, using devices that deliver a fine powder dispersed by air turbulence on inhalation, which requires a minimum inspiratory flow. Children younger than 7 years of age find it difficult to use a dry powder inhaler (DPI). DPIs have been developed for systemic delivery of peptides and proteins, such as insulin, but have proved to be problematic because of consistency of dosing.

Nebulizers. Two types of nebulizer are available. *Jet nebulizers* are driven by a stream of gas (air or oxygen), whereas *ultrasonic nebulizers* use a rapidly vibrating piezoelectric crystal and thus do not require a source of compressed gas. The nebulized drug may be inspired during tidal breathing, and it is possible to deliver much higher doses of drug compared with a pMDI. Nebulizers are therefore useful in treating acute exacerbations of asthma and COPD, for delivering drugs when airway obstruction is extreme, for delivering inhaled drugs to infants and small children who cannot use the other inhalation devices, and for giving drugs such as antibiotics when relatively high doses must be delivered.

Oral Route

Drugs for treatment of pulmonary diseases may also be given orally. The oral dose is much higher than the inhaled dose required to achieve the same effect (typically by a ratio of about 20:1), so that systemic side effects are more common. *When there is a choice of inhaled or oral route for a drug (e.g., β_2 agonist or corticosteroid), the inhaled route is always preferable*, and the oral route should be reserved for the few patients unable to use inhalers (e.g., small children, patients with physical problems such as severe arthritis of the hands). *Theophylline* is ineffective by the inhaled route and therefore must be given systemically. Corticosteroids may have to be given orally for parenchymal lung diseases (e.g., in interstitial lung diseases).

Parenteral Route

The intravenous route should be reserved for delivery of drugs in the severely ill patient who is unable to absorb drugs from the gastrointestinal (GI) tract. Side effects are generally frequent due to the high plasma concentrations. Biologics are usually given by subcutaneous injection.

Bronchodilators

Bronchodilator drugs relax constricted airway smooth muscle *in vitro* and cause immediate reversal of airway obstruction in asthma *in vivo* (Cazzola et al., 2012). They also prevent bronchoconstriction (and thereby provide bronchoprotection). Three main classes of bronchodilator are in current clinical use:

- β_2 Adrenergic agonists (sympathomimetics)
- *Theophylline* (a methylxanthine)
- Anticholinergic agents (muscarinic receptor antagonists)

Bronchodilators may directly relax airway smooth muscle or may cause bronchodilation indirectly by blocking the effects of bronchoconstrictor mediators or neurotransmitters. For example, *anti-LTs* (LT receptor antagonists and 5′-lipoxygenase inhibitors) have a small bronchodilator effect in some asthmatic patients and appear to prevent bronchoconstriction. *Corticosteroids*, although they gradually improve airway obstruction, have no direct effect on contraction of airway smooth muscle and therefore are not considered to be bronchodilators.

β_2 Adrenergic Agonists

Inhaled β_2 agonists are the bronchodilator treatment of choice in asthma because they are the most effective bronchodilators and have minimal side effects when used correctly. Systemic, short-acting, and nonselective β agonists, such as *isoproterenol* (*isoprenaline*) or *metaproterenol*, should only be used as a last resort.

Chemistry

The development of β_2 agonists is based on substitutions in the catecholamine structure of norepinephrine and epinephrine (see Chapters 10 and 14). The catechol ring consists of hydroxyl groups in the 3 and 4 positions of the benzene ring. Norepinephrine differs from epinephrine only in the terminal amine group; in general, further modification at this site confers β receptor selectivity. Many inhaled β_2-selective agonists are approved, and although there may be differences in potency, there are no clinically significant differences in selectivity. Inhaled β_2-selective drugs in current clinical use have a similar duration of action (3–6 h).

Administration of long-acting β_2 agonists (LABAs) by inhalation provides a much longer duration of effect (Cazzola et al., 2019b). *Salmeterol* and *formoterol* provide bronchodilation and bronchoprotection for more than 12 h and are usually given twice daily. *Formoterol* has a bulky substitution in the aliphatic chain and has moderate lipophilicity, which appears to keep the drug in the membrane close to the receptor, so it behaves as a slow-release drug; however, when diluted in plasma, *formoterol* loses this property, thereby avoiding long-term side effects, and can be used as a reliever. *Salmeterol* has a long aliphatic chain that may aid in anchoring the drug within the receptor binding cleft ("exosite") and that may thus contribute to *salmeterol*'s long duration. Once-daily β_2 agonists (sometimes called ultra-LABAs), such as *indacaterol*, *vilanterol*, and *olodaterol*, have a duration of action that exceeds 24 h; these agents are suitable for once-daily dosing.

Mode of Action

Occupation of β_2 receptors by agonists results in the activation of the G_s–adenylyl cyclase–3′-5′ cyclic adenosine monophosphate (cAMP)–protein kinase A (PKA) pathway, resulting in phosphorylative events leading to bronchial smooth muscle relaxation (Figure 44–4). β_2 Agonists act as functional antagonists of constriction, relaxing airway smooth muscle irrespective of the constrictor stimuli. β_2 Receptors are localized to several different airway cells in addition to smooth muscle, where they may have additional effects. β_2 Agonists also may cause bronchodilation *indirectly* by inhibiting the release of bronchoconstrictor mediators from inflammatory cells and of bronchoconstrictor neurotransmitters from airway nerves. These mechanisms include the following:

- Prevention of mediator release from isolated human lung mast cells (via β_2 receptors)
- Prevention of microvascular leakage and thus the development of bronchial mucosal edema after exposure to mediators (e.g., histamine, LTD_4, and PGD_2)
- Increase in *mucus secretion* from submucosal glands and *ion transport* across airway epithelium (may enhance mucociliary clearance, reversing defective clearance found in asthma and COPD)
- *Reduction in neurotransmission* in human airway *cholinergic nerves* by an action at presynaptic β_2 receptors to inhibit acetylcholine (ACh) release

Although these additional effects of β_2 agonists may be relevant to the prophylactic use of these drugs against various challenges, their rapid bronchodilator action is probably attributable to their direct effect on smooth muscle of all airways.

Anti-inflammatory Effects

Whether β_2 agonists have anti-inflammatory effects in asthma is controversial. The inhibitory effects of β_2 agonists on mast cell mediator release and microvascular leakage are clearly anti-inflammatory, suggesting that β_2 agonists may modify *acute* inflammation. However, β_2 agonists do not appear to have a significant inhibitory effect on the *chronic* inflammation of asthmatic airways, which is suppressed by corticosteroids. This has now been confirmed by several biopsy and bronchoalveolar lavage studies in patients with asthma who are taking regular β_2 agonists (including LABAs), that demonstrated no significant reduction in the number or activation in inflammatory cells in the airways, in contrast to resolution of the inflammation that occurs with ICS. This may be related to the fact that effects of β_2 agonists on macrophages, eosinophils, and lymphocytes are rapidly desensitized.

Clinical Use

Short-Acting β_2 Agonists. Inhaled short-acting β_2 agonists (SABAs) are the most widely used and effective bronchodilators in the treatment of asthma. When inhaled from pMDIs or DPIs, they are convenient, easy to use, rapid in onset, and without significant systemic side effects. These agents are effective in protecting against various asthma triggers, such as exercise, cold air, and allergens. SABAs are the bronchodilators of choice in treating acute severe asthma. The nebulized route of administration is easier and safer than intravenous administration and just as effective. Inhalation is preferable to oral administration because systemic side effects are less. *SABAs, such as albuterol (salbutamol), should be used "as required" by symptoms and not on a regular basis in the treatment of mild asthma; increased use indicates the need for more anti-inflammatory therapy.*

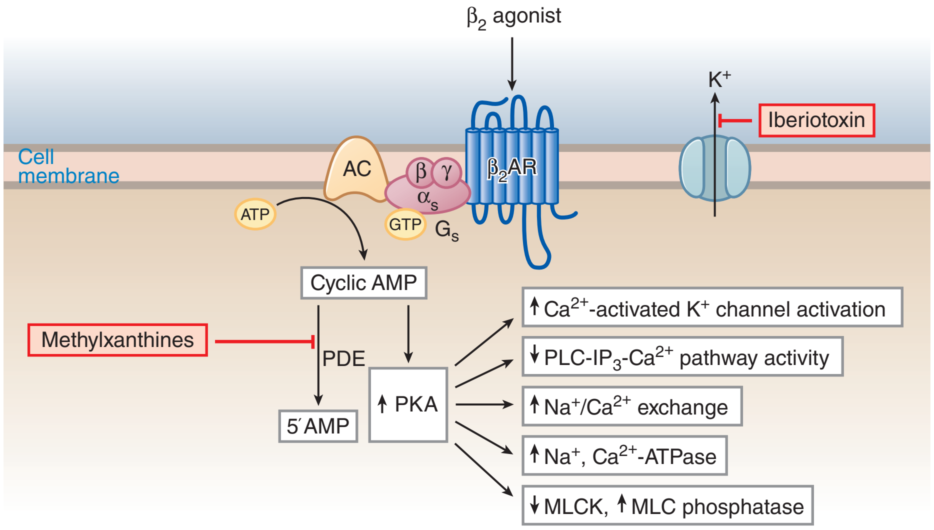

Figure 44–4 *Molecular actions of β_2 agonists to induce relaxation of airway smooth muscle cells.* Activation of β_2 receptors (β_2AR) results in activation of adenylyl cyclase (AC) via G_s, leading to an increase in intracellular cAMP and activation of PKA. PKA phosphorylates a variety of target substrates, resulting in opening of Ca^{2+}-activated K^+ channels (K_{Ca}), thereby facilitating hyperpolarization, decreased phosphoinositide (PI) hydrolysis, increased Na^+/Ca^{2+} exchange, increased Na^+, Ca^{2+}-ATPase activity, and decreased myosin light chain kinase (MLCK) activity and increased myosin light chain (MLC) phosphatase. β_2 Receptors may also couple to K_{Ca} via G_s. IP_3, inositol 1,4,5-trisphosphate; PDE, cyclic nucleotide phosphodiesterase; PLC, phospholipase C.

Oral β_2 agonists are occasionally indicated as an additional bronchodilator. Slow-release preparations (e.g., slow-release *albuterol* and *bambuterol* [not available in the U.S.]) may be indicated in nocturnal asthma; however, these agents have an increased risk of side effects. Several SABAs are available; they are resistant to uptake and enzymatic degradation by COMT (catechol-*O*-methyl transferase) and monoamine oxide (MAO); all are usable by inhalation and orally, have a similar duration of action (~3–4 h; less in severe asthma), and similar side effects. Differences in β_2 receptor selectivity have been claimed but are not clinically important. Drugs in clinical use include *albuterol, levalbuterol, metaproterenol, terbutaline,* and *pirbuterol*, as well as several drugs not available in the U.S. (*fenoterol, tulobuterol,* and *rimiterol*).

Long-Acting Inhaled β_2 Agonists. The LABAs *salmeterol and formoterol* (and *arformoterol,* the active enantiomer of *formoterol*) have proved to be a significant advance in asthma and COPD therapy. These drugs have a bronchodilator action of more than 12 h and protect against bronchoconstriction for a similar period (Cazzola et al., 2019b). They improve asthma control (when given twice daily) compared with regular treatment with SABAs (four to six times daily). Once-daily LABAs, such as *indacaterol, vilanterol*, and *olodaterol*, with a duration of over 24 h, are more effective in patients with COPD than twice-daily LABAs and more frequent SABAs (Cazzola et al., 2019b).

Tolerance to the bronchodilator effect of *formoterol* and the bronchoprotective effects of *formoterol* and *salmeterol* has been demonstrated but is of doubtful clinical significance. Although both *formoterol* and *salmeterol* have a similar duration of effect in clinical studies, there are differences. *Formoterol* has a more rapid onset of action and is an almost-full agonist, whereas *salmeterol* is a partial agonist with a slower onset of action. These differences might confer a theoretical advantage for *formoterol* in more severe asthma, whereas it may also make it more likely to induce tolerance. However, no significant clinical differences between *salmeterol* and *formoterol* have been found in the treatment of patients with severe asthma. More importantly, *formoterol* does not have long-lasting side effects, unlike *salmeterol* and other ultra-LABAs and is the only LABA that can be used as rescue therapy.

In COPD, LABAs are effective bronchodilators that may be used alone or in combination with LAMAs or ICSs. LABAs improve symptoms and exercise tolerance by reducing both air trapping and exacerbations. *In patients with asthma, LABAs should never be used alone because they do not treat the underlying chronic inflammation, and this may increase the risk of life-threatening and fatal asthma exacerbations; rather, LABAs should always be used in combination with an ICS in a fixed-dose combination inhaler.* LABAs are an effective add-on therapy to ICSs and are more effective than increasing the dose of an ICS when asthma is not controlled at low doses.

Combination Inhalers. Combination inhalers that contain a LABA and a corticosteroid (e.g., *fluticasone propionate/salmeterol, budesonide/formoterol, fluticasone furoate/vilanterol*) are now widely used in the treatment of asthma and COPD. In asthma, combining a LABA with a corticosteroid offers complementary synergistic actions (Barnes, 2002). The combination inhaler is more convenient for patients, simplifies therapy, and improves adherence with the ICS. Also, delivering the two drugs in the same inhaler ensures they are delivered simultaneously to the same cells in the airways, allowing the beneficial molecular interactions between LABAs and corticosteroids to occur. Combination inhalers are now the preferred therapy for patients with persistent asthma. These combination inhalers are also more effective in patients with COPD than a LABA and an ICS alone, but the mechanisms accounting for this beneficial interaction are less well understood than in patients with asthma.

Stereoselective β_2 Agonists. *Albuterol* is a racemic mixture of active *R*- and inactive *S*-isomers. Although *R-albuterol* (*levalbuterol*) was more potent than racemic *R/S-albuterol* in some studies, careful dose responses showed no advantage in terms of efficacy and no evidence that the *S-albuterol* is detrimental in asthmatic patients. Because *levalbuterol* is usually more expensive than normally used racemic *albuterol*, this therapy has no clear clinical advantage. Stereoselective *formoterol* (*R,R-formoterol*, *arformoterol*) has now been developed as a nebulized solution but also appears to offer no clinical advantage over racemic *formoterol* in patients with COPD (Loh et al., 2015).

β_2 Receptor Polymorphisms. Several single-nucleotide polymorphisms and haplotypes of the human *ADRβ2*, which affect the structure of β_2 receptors, have been described. The common variants are Gly^{16}Arg and Gln^{27}Glu, which have *in vitro* effects on receptor desensitization, but clinical studies have shown inconsistent effects on the bronchodilator responses to SABAs and LABAs (Hikino et al., 2019). Some studies have shown that patients with the common homozygous Arg^{16}Arg variant have more frequent adverse effects and a poorer response to SABAs than heterozygotes or Gly^{16}Gly homozygotes, but overall, these differences are small, and there appears to be no clinical value in measuring *ADRβ2* genotype. Bleecker and colleagues (2007) found no differences in responses to LABAs between these genotypes. A recent study suggested that the Gly^{16} to Arg substitution may be clinically relevant in treating childhood asthma (Turner et al., 2016).

Side Effects. Unwanted effects are dose-related and due to stimulation of extrapulmonary β receptors (Table 44–1 and Chapter 14). Side effects are not common with inhaled therapy but quite common with oral or intravenous administration.

- *Muscle tremor* due to stimulation of β_2 receptors in skeletal muscle is the most common side effect. It may be more troublesome with elderly patients and thus is frequently encountered in patients with COPD.
- *Tachycardia* and *palpitations* are due to reflex cardiac stimulation secondary to peripheral vasodilation, from direct stimulation of atrial β_2 receptors (human heart has a relatively high proportion of β_2 receptors; see Chapter 14), and possibly also from stimulation of myocardial β_1 receptors as the doses of β_2 agonist are increased.
- *Hypokalemia* is a potentially serious side effect. This is due to β_2 receptor stimulation of potassium entry into skeletal muscle, which may be secondary to a rise in insulin secretion. Hypokalemia might be serious in the presence of hypoxia, as in acute asthma, when there may be a predisposition to cardiac arrhythmias (Chapter 34). In practice, however, significant arrhythmias after nebulized β_2 agonists are rarely observed in acute asthma or patients with COPD.
- *Ventilation-perfusion* (*V/Q*) *mismatch* due to pulmonary vasodilation in blood vessels previously constricted by hypoxia results in the shunting of blood to poorly ventilated areas and a fall in arterial oxygen tension. Although in practice the effect of β_2 agonists on Pao$_2$ is usually very small (<5 mmHg fall), occasionally in severe COPD it can be large, although it may be prevented by giving additional inspired oxygen.
- *Metabolic* effects (increase in free fatty acid, insulin, glucose, pyruvate, and lactate) are usually seen only after large systemic doses.

Tolerance. Continuous treatment with an agonist often leads to tolerance, which may be due to downregulation of the receptor (see Chapter 14). Tolerance of nonairway β_2 receptor–mediated responses, such as tremor and cardiovascular and metabolic responses, is readily induced in normal and asthmatic subjects. In asthmatic patients, tolerance to the bronchodilator effects of β_2 agonists has not usually been found. However, tolerance develops to the bronchoprotective effects of β_2 agonists, and this is more marked with indirect bronchoconstrictors that activate mast cells (e.g., adenosine, allergen, and exercise) than with direct bronchoconstrictors,

TABLE 44–1 ■ SIDE EFFECTS OF β_2 AGONISTS

- Muscle tremor (direct effect on skeletal muscle β_2 receptors)
- Tachycardia (direct effect on atrial β_2 receptors, reflex effect from increased peripheral vasodilation via β_2 receptors)
- Hypokalemia (direct β_2 effect on skeletal muscle uptake of K$^+$)
- Restlessness
- Hypoxemia (↑ ventilation/perfusion mismatch due to reversal of hypoxic pulmonary vasoconstriction)
- Metabolic effects (↑ FFA, glucose, lactate, pyruvate, insulin)

such as histamine and methacholine. The reason for the relative resistance of airway smooth muscle β_2 responses to desensitization remains uncertain but may reflect the large receptor reserve: More than 90% of β_2 receptors may be lost without any reduction in the relaxation response. The high level of *ADRβ2 expression* in airway smooth muscle compared with peripheral lung may also contribute to the resistance to tolerance and reflect a high rate of β receptor synthesis. In addition, the expression of GRK2, which phosphorylates and inactivates occupied β_2 receptors, is very low in airway smooth muscle. By contrast, there is no receptor reserve in inflammatory cells, GRK2 expression is high, and tolerance to β_2 agonists rapidly develops at these sites.

Experimental studies have shown that corticosteroids prevent the development of tolerance in airway smooth muscle and prevent and reverse the fall in pulmonary β receptor density. However, ICSs fail to prevent the tolerance to the bronchoprotective effect of inhaled β_2 agonists, possibly because they do not reach airway smooth muscle in a high enough concentration.

Long-Term Safety. Because of a possible relationship between adrenergic drug therapy and the rise in asthma deaths in several countries during the early 1960s, doubts were cast on the long-term safety of β agonists. An epidemiological study examining the links between drugs prescribed for asthma and death or near death from asthma attacks found a marked increase in the risk of death with high doses of all inhaled β_2 agonists. The risk was greater with *fenoterol*, but when the dose is adjusted to the equivalent dose for *albuterol*, there is no significant difference in the risk for these two drugs.

The link between high β_2 agonist usage and increased asthma mortality does not prove a causal association: patients with more severe and poorly controlled asthma, who are more likely to have an increased risk of fatal attacks, are more likely to be using higher doses of β_2 agonist inhalers and less likely to be using effective anti-inflammatory treatment. Indeed, in the patients who used regular inhaled steroids, there was a significant reduction in risk of death. Studies in the literature support the following:

SABAs should be used only on demand for symptom control, and if they are required frequently (more than three times weekly), an ICS is needed.

The safety of LABAs in asthma remains controversial. LABA treatment has been associated with increased near-fatal exacerbations and death, but concomitant treatment with an ICS appears to obviate this risk, so it is *recommended that LABAs should be used only when ICSs are also prescribed (preferably in the form of a combination inhaler so that the LABAs can never be taken without the ICSs)* (Busse et al., 2018). All LABAs approved in the U.S. carry a black-box warning cautioning against overuse. There are fewer safety concerns with LABA use in COPD. No major adverse effects and no evidence of cardiovascular problems were reported in several large and prolonged studies (Kew et al., 2013).

Future Developments

The β agonists will continue to be the bronchodilators of choice for asthma because they are effective in all patients and have few or no side effects when used in low doses. When used as required for symptom control, inhaled β_2 agonists appear safe. Use of large doses of inhaled β_2 agonists indicates poor asthma control; such patients should be assessed and appropriate controller medication used. LABAs are a useful option for long-term control in asthma and COPD. In patients with asthma, LABAs should probably only be used in a fixed combination with an ICS to prevent the potential danger associated with LABAs alone. There is little advantage to be gained by improving β_2 receptor selectivity because most of the side effects of these agents are due to β_2 receptor stimulation (muscle tremor, tachycardia, hypokalemia). Once-daily inhaled β_2 agonists are useful in patients with COPD and may have additive effects with long-acting muscarinic antagonists (LAMAs). *Formoterol* combined with an ICS is now recommended as the rescue bronchodilator of choice; this combination is more effective and avoids the potential dangers of overuse of short-acting β_2 agonists (Bateman et al., 2018; O'Byrne et al., 2018).

Muscarinic Cholinergic Antagonists

The basic pharmacology of the antimuscarinic agents is presented in Chapter 11.

HISTORY

Datura stramonium (jimson weed) and related species of the nightshade family contain a mixture of muscarinic antagonists (*atropine, hyoscyamine, scopolamine*) and were smoked for relief of asthma two centuries ago. Subsequently, the purified plant alkaloid *atropine* was introduced for treating asthma. Due to the significant side effects of *atropine*, particularly drying of secretions, less-soluble quaternary compounds, such as *atropine methylnitrate* and *ipratropium bromide*, have been developed. These compounds are topically active and are not significantly absorbed from the respiratory or GI tracts.

Mode of Action

As competitive antagonists of endogenous ACh at muscarinic receptors, these agents inhibit the direct constrictor effect on bronchial smooth muscle mediated via the M_3-G_q-PLC-IP_3-Ca^{2+} pathway (see Chapters 3 and 11). The efficacy stems from the role played by the parasympathetic nervous system in regulating bronchomotor tone. The effects of ACh on the respiratory system include bronchoconstriction and tracheobronchial mucus secretion. Thus, antimuscarinic drugs antagonize these effects of ACh, resulting in bronchodilation and reduced mucus secretion. ACh may also result in structural changes such as airway fibrosis and an increase in neutrophilic inflammation (Kistemaker and Gosens, 2015).

Acetylcholine may be released from airway cells other than neurons, including epithelial cells (Kummer and Krasteva-Christ, 2014). The synthesis of ACh in epithelial cells is increased by inflammatory stimuli (such as tumor necrosis factor [TNF] α), which increase the expression of choline acetyltransferase, which could contribute to cholinergic effects in airway diseases. Muscarinic receptors are expressed in airway smooth muscle of small airways that do not appear to be significantly innervated by cholinergic nerves; these receptors may be a mechanism of cholinergic narrowing in peripheral airways that could be relevant in COPD, responding to locally synthesized, nonneuronal ACh.

Myriad mechanical, chemical, and immunological stimuli elicit reflex bronchoconstriction via vagal pathways, and cholinergic pathways may play an important role in regulating acute bronchomotor responses in animals. Anticholinergic drugs will inhibit only ACh-mediated bronchoconstriction and will have no blocking effect on the *direct* effects of inflammatory mediators, such as histamine and LTs. Furthermore, cholinergic antagonists probably have little or no effect on mast cells, microvascular leak, or the chronic inflammatory response.

Clinical Use

In asthmatic patients, anticholinergic drugs are less effective as bronchodilators than β_2 agonists and offer less-efficient protection against bronchial challenges. LAMAs are currently used as an additional bronchodilator in asthmatic patients not controlled on maximal doses of LABA. Nebulized anticholinergic drugs are effective in acute severe asthma but less effective than β_2 agonists. In the acute and chronic treatment of asthma, anticholinergic drugs may have an additive effect with β_2 agonists and should therefore be considered when control of asthma is not adequate with nebulized β_2 agonists. A muscarinic antagonist should be considered when there are problems with *theophylline* or when inhaled β_2 agonists cause a troublesome tremor in elderly patients.

In COPD, anticholinergic drugs may be as effective as or even superior to β_2 agonists. Their relatively greater effect in COPD than in asthma may be explained by an inhibitory effect on vagal tone, which, although not necessarily increased in COPD, may be the only reversible element of airway obstruction and one that is exaggerated by geometric factors in the narrowed airways of patients with COPD (Figure 44–5). Anticholinergic drugs reduce air trapping and improve exercise tolerance in patients with COPD.

Therapeutic Choices

The short-acting muscarinic antagonist (SAMA) *ipratropium bromide* is available as a pMDI and nebulized preparation. The onset of

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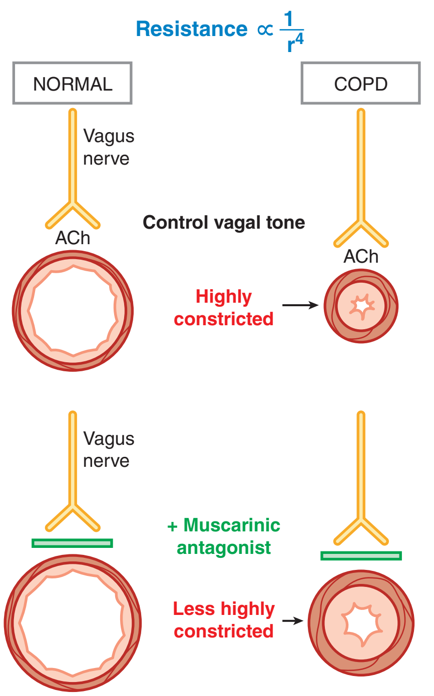


Figure 44–5 *Anticholinergic drugs inhibit vagally mediated airway tone, thereby producing bronchodilation.* This effect is small in normal airways but is greater in airways of patients with COPD, which are structurally narrowed and have higher resistance to airflow because airway resistance is inversely related to the fourth power of the radius r.

bronchodilation is relatively slow and is usually maximal 30 to 60 min after inhalation but may persist for 6 to 8 h. It is usually given by MDI three or four times daily on a regular basis, rather than intermittently for symptom relief, in view of its slow onset of action, but has now been replaced by LAMAs, such as *tiotropium bromide*.

Long-Acting Muscarinic Antagonists

Several LAMAs have now been developed for the treatment of COPD and, more recently, severe asthma. *Tiotropium bromide* is a long-acting anticholinergic drug that is suitable for once-daily dosing as a DPI or via a soft mist inhaler device and is more effective than *ipratropium* four times daily according to several studies; it also significantly reduces exacerbations. *Tiotropium* binds to all muscarinic receptor subtypes but dissociates slowly from M_3 and M_1 receptors, giving it a degree of kinetic receptor selectivity for these receptors compared with M_2 receptors, from which it dissociates more rapidly. Thus, compared with *ipratropium*, *tiotropium* is less likely to antagonize M_2-mediated inhibition of ACh release (the resulting increase in ACh could counteract the blockade of M_3 receptor–mediated bronchoconstriction) (see Chapter 11). Over a 4-year period, *tiotropium* improved lung function and health status and reduced exacerbations and all-cause mortality (Tashkin et al., 2008). Although there was no effect on disease progression overall, there was a small effect in the patients with moderate COPD (Global Initiative for Chronic Obstructive Lung Disease [GOLD] stage 2; GOLD, 2021) (Decramer et al., 2009).

Glycopyrrolate bromide and *umeclidinium bromide* are also once-daily LAMAs with very similar clinical effects to *tiotropium*, whereas *aclidinium bromide* is given twice daily (Cazzola et al., 2013). LAMAs are now the bronchodilators of choice for patients with COPD in preference to LABAs alone (Cho et al., 2018). LAMAs are also effective as additional bronchodilators in patient with asthma not adequately controlled with maximal ICS/LABA therapy, although not all patients respond and there is little effect on symptoms and only a small additional protection against exacerbations (Kerstjens et al., 2015).

Combination Inhalers

There are additive bronchodilator effects between anticholinergics and β_2 agonists in patients with COPD, which has led to the development of fixed-dose combinations. SABA/SAMA combinations, such as *albuterol/ipratropium*, are popular as a reliever. Several studies have demonstrated additive effects of these two drugs, thus providing an advantage over increasing the dose of β_2 agonist in patients who experience side effects.

LABA/LAMA dual combination inhalers have also been developed, including *indacaterol/glycopyrrolate, vilanterol/umeclidinium,* and *olodaterol/tiotropium* (all once daily), and *formoterol/glycopyrrolate* and *formoterol/aclidinium* (twice daily), which all show beneficial effects on lung function compared with either LABA or LAMA alone, although they may not be clearly beneficial in terms of reducing exacerbations (Mammen et al., 2020).

Adverse Effects

Inhaled anticholinergic drugs are generally well tolerated. On stopping inhaled anticholinergics, a small rebound increase in airway responsiveness has been described. Systemic side effects after SAMA or LAMA are uncommon during normal clinical use because there is little systemic absorption. Because cholinergic agonists can stimulate *mucus secretion*, there has been concern that anticholinergics may reduce secretion and lead to more viscous mucus. However, *ipratropium bromide* and *tiotropium bromide*, even in high doses, have no detectable effect on mucociliary clearance in either normal subjects or in patients with airway disease. A significant unwanted effect is the unpleasant *bitter taste* of inhaled *ipratropium*, which may contribute to poor compliance. Nebulized *ipratropium bromide* may precipitate *glaucoma* in elderly patients due to a direct effect of the nebulized drug on the eye. This may be prevented by nebulization with a mouthpiece rather than a face mask.

Reports of *paradoxical bronchoconstriction* with *ipratropium bromide*, particularly when given by nebulizer, were largely explained as effects of the hypotonic nebulizer solution and by antibacterial additives, such as benzalkonium chloride and EDTA. This problem has not been described with *tiotropium bromide* or other LAMAs. Occasionally, bronchoconstriction may occur with *ipratropium bromide* given by metered-dose inhaler (MDI). It is possible that this is due to blockade of prejunctional M_2 receptors on airway cholinergic nerves that normally inhibit ACh release.

LAMAs cause dryness of the mouth in 10% to 15% of patients, but this usually disappears during continued therapy. Urinary retention is occasionally seen in elderly patients.

Future Developments

The LABA/LAMA fixed-combination inhalers are likely to become the bronchodilators of choice in patients with COPD, and LAMAs are added to ICS/LABA combinations in severe asthma.

Triple inhalers that have the ICS/LABA/LAMA combination are now available and include *fluticasone furoate/vilanterol/umeclidinium* once daily and *budesonide/formoterol/glycopyrrolate* and *beclomethasone propionate/formoterol/glycopyrrolate* given twice daily (Ritondo et al., 2021). Triple inhalers are more effective than LABA/LAMA or ICS/LABA combinations in reducing exacerbations in COPD patients and may reduce cardiovascular mortality (Lipson et al., 2018; Rabe et al., 2020). Triple inhalers may also be the treatment of choice for patients with asthma-COPD overlap (Park et al., 2021).

Dual-action drugs that are both muscarinic antagonists and β_2 agonists (known as MABAs) are also in clinical development, but it has proved difficult to balance the β agonist and anticholinergic activities (Hughes et al., 2012).

Phosphodiesterase Inhibitors

The phosphodiesterase (PDE) inhibitors relax smooth muscle and inhibit inflammatory cells through an increase in cellular cAMP and its sequelae. PDE4 is the predominant PDE isoform in inflammatory cells, including mast cells, eosinophils, neutrophils, T lymphocytes, macrophages, and structural cells such as sensory nerves and epithelial cells, suggesting that PDE4 inhibitors could be useful as an anti-inflammatory treatment in both asthma and COPD (Spina and Page, 2017). In animal models of asthma, PDE4 inhibitors reduce eosinophil infiltration and responses to allergen; in COPD, they are effective against smoke-induced inflammation and emphysema.

Of the four subfamilies of PDE4, PDE4D is the major form whose inhibition is associated with vomiting, a limiting side effect. Inhibition of PDE4B is important for anti-inflammatory effects.

To overcome the problems of dose-limiting side effects, compounds selective for PDE4B have been developed but have not shown any clinical advantage (Goto et al., 2014). Several inhaled PDE4 inhibitors have been developed that avoid side effects seen with oral administration, but most lack efficacy (Phillips, 2020). One potent inhaled PDE4 inhibitor, CHF6001, reduces the late response to allergen in patients with mild asthma but provides no significant improvement in COPD patients (Singh et al., 2020). A dual PDE3/4 inhibitor provides bronchodilation in patients with COPD when given by nebulization but its anti-inflammatory effects are uncertain (Franciosi et al., 2013).

Roflumilast

Roflumilast is an oral nonselective PDE4 inhibitor that is converted to its more active and long-acting metabolite, roflumilast *N*-oxide.

ADME. The drug is well absorbed after oral administration (bioavailability ~80%). It is extensively metabolized to its *N*-oxide active metabolite by CYPs 3A4 and 1A2. Thus, agents that inhibit these CYPs (e.g., *erythromycin*, *ketoconazole*, *fluvoxamine*, *enoxacin*, *cimetidine*) will increase the area under the curve (AUC) for *roflumilast*. Conversely, *rifampicin*, an inducer of CY3A4, will decrease *roflumilast*'s C_{max} and AUC; thus, a strong CYP inducer could reduce the effectiveness of *roflumilast*. The drug and its active metabolite are largely excreted as conjugates in the urine.

Therapeutic Use. *Roflumilast* is currently used as an additional therapy in patients with severe COPD with chronic bronchitis who continue to have exacerbations despite treatment with triple inhaled therapy (ICS + LABA + LAMA). *Roflumilast* is approved for patients with COPD with severe disease (force expiratory volume in 1 sec [FEV_1] <50% predicted, frequent exacerbations, and chronic bronchitis). Given once daily by mouth (500-mg tablet), it reduces exacerbations by approximately 20% but has little effect on symptoms and lung function (Calverley et al., 2009); it is effective on top of long-acting bronchodilators and ICSs (Martinez et al., 2016).

In patients with mild asthma, *roflumilast* provides improvement in FEV_1 similar to an ICS; its has similar positive effects when added to *montelukast* in moderate to severe asthma (Bateman et al., 2016). Thus, it may be suitable for treating patients with predominantly neutrophilic inflammation. It should not be used for acute bronchospasm.

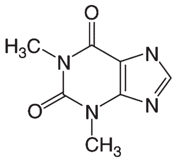
Untoward Effects; Drug Interactions. The relatively weak efficacy of *roflumilast* is due to dose limitations as a result of side effects, particularly diarrhea, headaches, and nausea, which are also due to PDE4 inhibition. *Roflumilast* is contraindicated in severe liver impairment (Child-Pugh B or C). There are insufficient data on the safety of *roflumilast* during pregnancy; the drug should be used with caution and only if the benefits outweigh the risk to the fetus.

Ensifentrine

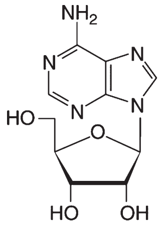
Ensifentrine (RPL554) is an inhaled PDE3/4 inhibitor with 3.5 orders of magnitude selectivity for PDE3 over PDE4 (Cazzola et al., 2019a). Inhaled *ensifentrine* induces bronchodilatation in asthmatic and COPD patients, which is likely to be due to PDE3 inhibition. Anti-inflammatory effects have not been convincingly demonstrated, likely explained by its low PDE4 inhibitory activity. While *ensifentrine* produces an additional bronchodilator response in COPD when added to an inhaled LAMA, it has not been demonstrated to have further bronchodilatation in COPD patients on a LABA-LAMA combination inhaler, so its clinical value is questionable. *Ensifentrine* can reduce symptoms in COPD patients (Watz et al., 2020). Although most of the clinical studies have been performed using a nebulized formulation, dry powder and pMDI formulations have now been developed. The drug is in phase III studies in COPD patients.

Methylxanthines

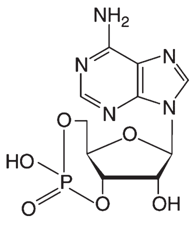
Methylxanthines, such as *theophylline*, which are related to caffeine, have been used in the treatment of asthma since 1930, and *theophylline* is still widely used in developing countries because it is inexpensive. *Theophylline* became more useful with the introduction of reliable slow-release preparations. However, inhaled β_2 agonists are far more effective as bronchodilators, and ICSs have a greater anti-inflammatory effect. In patients with severe asthma and COPD, it still remains a useful drug as an add-on therapy (Barnes, 2013b).



THEOPHYLLINE



ADENOSINE



CYCLIC AMP

Chemistry

Theophylline is a methylxanthine similar in structure to the common dietary xanthines caffeine and theobromine. Several substituted derivatives have been synthesized, but only two appear to have any advantage over *theophylline*: *Enprofylline* is a more potent bronchodilator and may have fewer toxic effects because it does not antagonize adenosine receptors; *doxofylline*, a methylxanthine available in some countries, inhibits PDEs similarly to *theophylline* but is less active as an adenosine antagonist and has a more favorable side effect profile (Cazzola and Matera, 2020). Many salts of *theophylline* have also been marketed; the most common is *aminophylline*. Other salts do not have any advantage. *Theophylline* remains the major methylxanthine in clinical use.

Mechanism of Action

In addition to its bronchodilator action, *theophylline* has many nonbronchodilator effects that may be relevant to its effects in asthma and COPD (Figure 44–6). Several molecular mechanisms of action have been proposed:

- *Inhibition of PDEs*. *Theophylline* is a nonselective PDE inhibitor, but the degree of inhibition is relatively modest at concentrations of *theophylline* that are within the therapeutic range. PDE inhibition and the concomitant elevation of cellular cAMP and cyclic GMP likely account for the bronchodilator action of *theophylline*. Several isoenzyme families of PDE have now been recognized, and those important in smooth muscle relaxation include PDE3, PDE4, and PDE5, but inhibition of PDE3 is likely to be a main mechanism of bronchodilation,
- *Adenosine receptor antagonism*. *Theophylline* antagonizes adenosine receptors at therapeutic concentrations. Adenosine causes bronchoconstriction in airways from asthmatic patients by releasing histamine and LTs. Antagonism of adenosine A_1 receptors may be responsible for serious side effects, including cardiac arrhythmias and seizures.
- *Interleukin (IL)-10 release*. IL-10 has a broad spectrum of anti-inflammatory effects, and its secretion is reduced in asthma and COPD. IL-10 release is increased by *theophylline*, and this effect may be mediated via PDE inhibition, although this has not been seen at the low doses that are effective in asthma.
- *Effects on gene transcription*. *Theophylline* prevents the translocation of the proinflammatory transcription factor nuclear factor-κB (NF-κB) into the nucleus, potentially reducing the expression of inflammatory genes in asthma and COPD (Ichiyama et al., 2001). However, these effects are seen at high concentrations and may be mediated by inhibition of PDE.
- *Effects on apoptosis*. Prolonged survival of granulocytes due to a reduction in apoptosis may be important in perpetuating chronic inflammation in asthma (eosinophils) and COPD (neutrophils). *Theophylline* promotes apoptosis in eosinophils and neutrophils *in vitro*, an effect mediated by antagonism of adenosine A_{2A} receptors

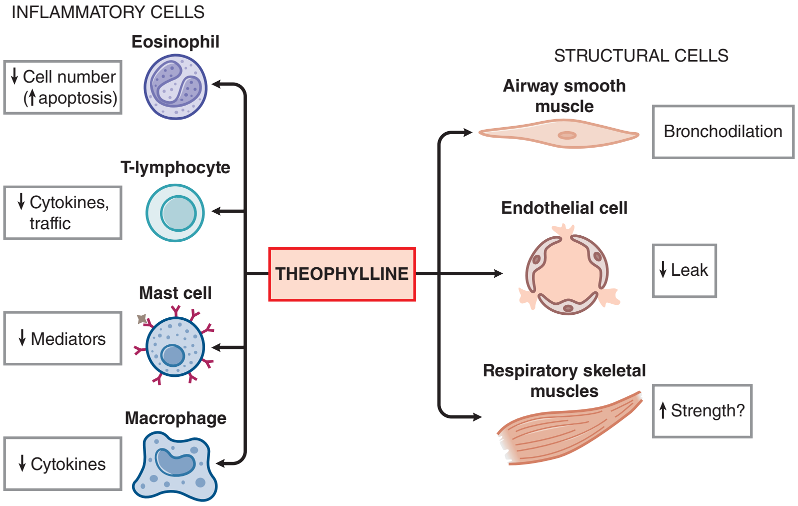


Figure 44–6 *Theophylline affects multiple cell types in the airway.*

(Yasui et al., 2000). *Theophylline* also induces apoptosis in T lymphocytes via PDE inhibition.

- *Histone deacetylase (HDAC) activation.* Recruitment of HDAC2 by glucocorticoid receptors (GRs) switches off activated inflammatory genes. Therapeutic concentrations of *theophylline* activate HDAC, thereby enhancing the anti-inflammatory effects of corticosteroids (Cosio et al., 2004). This mechanism is independent of PDE inhibition or adenosine antagonism and is mediated by inhibition of phosphoinositide-3-kinase-δ, which is activated by oxidative stress (To et al., 2010). Low concentrations of *theophylline* (1–5 mg/L) increase HDAC activity assessed in bronchial biopsies of asthmatic patients and correlate with the reduction in eosinophil numbers in the biopsy.

Nonbronchodilator Effects

Theophylline has clinical benefit in asthma and COPD at plasma concentrations less than 10 mg/L, sufficiently low that such effects are unlikely to be fully explained by its PDE3 inhibition. *Theophylline* has anti-inflammatory effects in asthma; chronic oral treatment with *theophylline* inhibits the late response to inhaled allergen, reduces infiltration of eosinophils and $CD4^+$ lymphocytes into the airways after allergen challenge, and reduces eosinophils in bronchial walls and sputum (Lim et al., 2001). In patients with COPD, *theophylline* reduces the total number and proportion of neutrophils in induced sputum, the concentration of CXCL8, and neutrophil chemotactic responses (Culpitt et al., 2002). *Theophylline* withdrawal in patients with COPD results in worsening of disease. In vitro, *theophylline* increases responsiveness to corticosteroids and reverses steroid resistance in cells from COPD patients (Ito et al., 2002) via activastion of HDAC2 (Ito et al., 2002).

Pharmacokinetics and Metabolism

Theophylline has antiasthmic effects other than bronchodilation below 10 mg/L, so the therapeutic range is now considered as 5 to 15 mg/L (28–83 μM). The dose of *theophylline* required to give these therapeutic concentrations varies among subjects, largely because of differences in plasma clearance of the drug. *Theophylline* is rapidly and completely absorbed, but there are large interindividual variations in clearance due to differences in hepatic metabolism. *Theophylline* is metabolized in the liver, mainly by CYP1A2; many factors influence hepatic metabolism and clearance of *theophylline* (Table 44–2).

Because of these variations in clearance, individualization of *theophylline* dosage is required, and plasma concentrations should be measured 4 h after the last dose with slow-release preparations when steady state has been achieved. There is no significant circadian variation in *theophylline* metabolism, although there may be delayed absorption at night related to supine posture.

Preparations and Routes of Administration

Intravenous aminophylline, an ethylene diamine ester of *theophylline* that is water soluble, has been used for many years in the treatment of acute severe asthma. The recommended dose is 6 mg/kg given intravenously over 20 to 30 min, followed by a maintenance dose of 0.5 mg/kg per hour. If the patient is already taking *theophylline* or there are any factors that decrease clearance, these doses should be halved, and the plasma level checked more frequently. Nebulized β_2 agonists are now preferred over intravenous *aminophylline* for acute exacerbations of asthma and COPD.

Oral immediate-release *theophylline* tablets or elixirs, which are rapidly absorbed, give wide fluctuations in plasma levels and are not recommended. Several sustained-release preparations are now available that are absorbed at a constant rate and provide steady plasma concentrations over a 12- to 24-h period. Both slow-release *aminophylline* and *theophylline* are available and are equally effective (although the ethylene diamine component of *aminophylline* has been implicated in allergic reactions). For continuous treatment, twice-daily therapy (~8 mg/kg twice daily) is needed. For nocturnal asthma, a single dose of slow-release *theophylline* at night is often effective. Once optimal doses have been determined, routine monitoring of plasma concentrations is usually not necessary unless a change in clearance is suspected or evidence of toxicity emerges.

TABLE 44–2 ■ FACTORS AFFECTING CLEARANCE OF THEOPHYLLINE

Increased clearance

- Enzyme induction (mainly CYP1A2) by coadministered drugs (e.g., rifampicin, barbiturates, ethanol)
- Smoking (tobacco, marijuana) via CYP1A2 induction
- High-protein, low-carbohydrate diet
- Barbecued meat
- Childhood

Decreased clearance

- CYP inhibition (cimetidine, erythromycin, ciprofloxacin, allopurinol, fluvoxamine, zileuton, zafirlukast)
- Congestive heart failure
- Liver disease
- Pneumonia
- Viral infection and vaccination
- High-carbohydrate diet
- Old age

Clinical Use

In patients with acute asthma, intravenous *aminophylline* is less effective than nebulized β_2 agonists and should therefore be reserved for those patients who fail to respond to, or are intolerant of, β agonists. *Theophylline* should not be added routinely to nebulized β_2 agonists because it does not increase the bronchodilator response and may increase their side effects. *Theophylline* has been used as a controller in the management of mild persistent asthma, although it is usually found to be less effective than low doses of ICSs. Addition of low-dose *theophylline* to an ICS in patients who are not adequately controlled provides better symptom control and lung function than doubling the dose of ICS (Lim et al., 2000). LABAs are more effective as an add-on therapy, but *theophylline* is considerably less expensive and may be the only affordable add-on treatment when the costs of medication are limiting.

Theophylline is still used as a bronchodilator in COPD, but inhaled anticholinergics and β_2 agonists are preferred. *Theophylline* tends to be added to these inhaled bronchodilators in patients with more severe disease and can give additional clinical improvement when added to a LABA (Ram et al., 2005). However, low-dose *theophylline* does not reduce exacerbations when added to ICS in patients with severe COPD (Devereux et al., 2018) or reduce exacerbations with or without a low dose of oral corticosteroids (Jenkins et al., 2020b).

Side Effects

Unwanted effects of *theophylline* are usually related to plasma concentration and tend to occur at C_p greater than 15 mg/L. The most common side effects are headache, nausea, and vomiting (due to inhibition of PDE4), abdominal discomfort, and restlessness (Table 44–3). There may also be increased acid secretion (PDE inhibition) and diuresis (antagonism of adenosine A_1 receptors). *Theophylline* may lead to behavioral disturbance and learning difficulties in schoolchildren. At high concentrations, cardiac arrhythmias may occur due to inhibition of cardiac PDE3 and antagonism of cardiac A_1 receptors. At very high concentrations, seizures may occur due to central A_1 receptor antagonism. Use of low doses of *theophylline*, targeting plasma concentrations of 5 to 10 mg/L, largely avoids side effects and drug interactions.

Summary and Future Developments

Theophylline use has been declining, partly because of the problems with side effects, but mainly because more effective therapy with β_2 agonists and ICSs has been introduced. Oral *theophylline* remains a useful add-on treatment in some patients with difficult asthma and appears to have effects beyond those provided by steroids. Rapid-release *theophylline* preparations are the only affordable antiasthma medication in some developing countries. There is increasing evidence that *theophylline* has some antiasthmic effect at doses that are lower than those needed for bronchodilation, and plasma levels of 5 to 15 mg/L are recommended.

Novel Classes of Bronchodilator

Currently, the most effective bronchodilators are LABAs for asthma and a LAMA for COPD. Inventing new classes of bronchodilator has been difficult; several agents have had problems with vasodilator side effects because they relax vascular smooth muscle to a greater extent than airway smooth muscle.

TABLE 44–3 ■ SIDE EFFECTS OF THEOPHYLLINE AND MECHANISMS

SIDE EFFECT	PROPOSED MECHANISM
Nausea and vomiting	PDE4 inhibition
Headaches	PDE4 inhibition
Gastric discomfort	PDE4 inhibition
Diuresis	A_1 receptor antagonism
Behavioral disturbance (?)	?
Cardiac arrhythmias	PDE3 inhibition, A_1 receptor antagonism
Epileptic seizures	A_1 receptor antagonism

Magnesium Sulfate

Magnesium sulfate ($MgSO_4$) is useful as an additional bronchodilator in children and adults with acute severe asthma. Intravenous or nebulized $MgSO_4$ benefits adults and children with severe exacerbations (FEV_1 <30% of predicted value), giving improvement in lung function when added to nebulized β_2 agonist and a reduction in hospital admissions (Knightly et al., 2017). The treatment is cheap and well tolerated, although the clinical benefit appears small. Side effects include flushing and nausea but are usually minor. $MgSO_4$ appears to act as a bronchodilator and may reduce cytosolic Ca^{2+} concentrations in airway smooth muscle cells. The concentration of *magnesium* is lower in serum and erythrocytes of asthmatic patients than in normal controls and correlates with airway hyperresponsiveness, although the improvement in acute severe asthma after *magnesium* does not correlate with plasma concentrations. The effects of intravenous $MgSO_4$ in COPD are minimal, and there are too few studies to make any firm recommendation (Vafadar Moradi et al., 2021).

Potassium Channel Openers

The K^+ channel openers such as *cromakalim* or *levcromakalim* (the *levo*-isomer of *cromakalim*) open ATP-dependent K^+ channels in smooth muscle, leading to membrane hyperpolarization and relaxation of airway smooth muscle (Pelaia et al., 2002). Clinical studies in asthma have been disappointing, with no bronchodilation or protection against bronchoconstrictor challenges. These agents are dose-limited by cardiovascular side effects so their development has been stopped. Ca^{2+}-activated large conductance K^+ channels (maxi-K channels) are also opened by β_2 agonists; so maxi-K activators are being considered as bronchodilators.

Vasoactive Intestinal Polypeptide Analogues

Vasoactive intestinal polypeptide (VIP) is a 28-amino acid peptide. It binds to two G protein-coupled receptors (GPCRs), $VPAC_1$ and $VPAC_2$, both of which couple primarily to G_s to stimulate the adenylyl cyclase–cAMP–PKA pathway leading to relaxation of smooth muscle. VIP is a potent dilator of human airway smooth muscle *in vitro* but is not effective in patients because it is rapidly metabolized (plasma $t_{1/2}$ ~2 min); in addition, VIP causes vasodilator side effects. More stable analogues of VIP, such as Ro 25-1533, which selectively stimulates VIP receptors in airway smooth muscle (via $VPAC_2$), have been synthesized. Inhaled Ro 25-1533 has a rapid bronchodilator effect in asthmatic patients, but it is not as prolonged as *formoterol* (Linden et al., 2003).

Bitter Taste Receptor Agonists

Bitter taste receptors (TAS2R) are GPCRs that are expressed in airway smooth muscle and mediate bronchodilation in response to agonists, such as *quinine* and *chloroquine*, even after β_2 receptor desensitization (Nayak et al., 2019). However, current agonists are weak, so more potent drugs are needed.

Other Inhibitors of Smooth Muscle Contraction

Agents that inhibit the contractile machinery of airway smooth muscle, including rho kinase inhibitors, inhibitors of myosin light chain kinase, and allosteric modulators of smooth muscle myosin (Sirigu et al., 2016), are also in development. Because such agents would also cause vasodilation, it would be necessary to administer them by inhalation.

Corticosteroids

The introduction of *ICSs*, as a way of reducing the requirement and side effects of oral glucocorticosteroids, has revolutionized the treatment of chronic asthma (Barnes, 2017). Because asthma is a chronic inflammatory disease, ICSs are considered first-line therapy in all but patients with the mildest disease. In marked contrast, ICSs are much less effective in COPD and should be used only in patients with severe disease who have frequent exacerbations and increased blood eosinophils. Oral corticosteroids remain the mainstay of treatment of several other pulmonary diseases, such as sarcoidosis and pulmonary eosinophilic syndromes. The general pharmacology of corticosteroids is presented in Chapter 50.

Mechanism of Action

Corticosteroids enter target cells and bind to GRs in the cytoplasm (see Figure 50–7. There is only one type of GR that binds corticosteroids and no evidence for the existence of subtypes that might mediate different aspects of corticosteroid action (Barnes, 2011). The steroid-GR complex moves into the nucleus, where it binds to specific sequences on the upstream regulatory elements of certain target genes, resulting in increased (or, rarely, decreased) transcription of the gene, with subsequent increased (or decreased) synthesis of the gene products.

The GRs may also interact with protein transcription factors and coactivator molecules in the nucleus and thereby influence the synthesis of certain proteins independently of any direct interaction with DNA. The repression of transcription factors, such as AP-1 and NF-κB, is likely to account for many of the anti-inflammatory effects of steroids in asthma. Corticosteroids reverse the activating effect of these proinflammatory transcription factors on histone acetylation by recruiting HDAC2 to inflammatory genes that have been activated through acetylation of associated histones (Figure 44–7). GRs are acetylated when corticosteroids are bound and bind to DNA in this acetylated state as dimers, whereas the acetylated GR has to be deacetylated by HDAC2 to interact with inflammatory genes and NF-κB (Ito et al., 2006).

There may be additional mechanisms that are also important in the anti-inflammatory actions of corticosteroids. Corticosteroids have potent inhibitory effects on MAP kinase signaling pathways through the induction of MAP kinase phosphatase 1, which may inhibit the expression of multiple inflammatory genes.

Anti-inflammatory Effects in Asthma

Corticosteroids have widespread effects on gene transcription, increasing the transcription of several anti-inflammatory genes and suppressing transcription of many inflammatory genes. Steroids have inhibitory effects on many inflammatory and structural cells that are activated in asthma and prevent the recruitment of inflammatory cells into the airways (Figure 44–8). In patients with mild asthma, the inflammation may be completely resolved after inhaled steroids.

Steroids potently inhibit the formation of multiple inflammatory cytokines, particularly cytokines released from T_H2 cells. Corticosteroids also decrease eosinophil survival by inducing apoptosis. Corticosteroids inhibit the expression of multiple inflammatory genes in airway epithelial cells, probably the most important action of ICSs in suppressing asthmatic inflammation. Corticosteroids also prevent and reverse the increase in vascular permeability due to inflammatory mediators and may therefore lead to resolution of airway edema. Steroids have a direct inhibitory effect on mucus glycoprotein secretion from airway submucosal glands, as well as indirect inhibitory effects by downregulation of inflammatory stimuli that stimulate mucus secretion.

Corticosteroids have no direct effect on contractile responses of airway smooth muscle; improvement in lung function after ICSs is presumably due to an effect on the chronic airway inflammation, edema, and airway hyperresponsiveness. A single dose of an ICS has no effect on the early response to allergen (reflecting the ICS's lack of effect on mast cell mediator release) but inhibits the late response (which may be due to an effect on macrophages, eosinophils, and airway wall edema) and also inhibits the increase in airway hyperresponsiveness.

The ICSs have rapid anti-inflammatory effects, reducing airway hyperresponsiveness and inflammatory mediator concentrations in sputum within a few hours (Erin et al., 2008). However, it may take several weeks or months to achieve maximal effects on airway hyperresponsiveness,

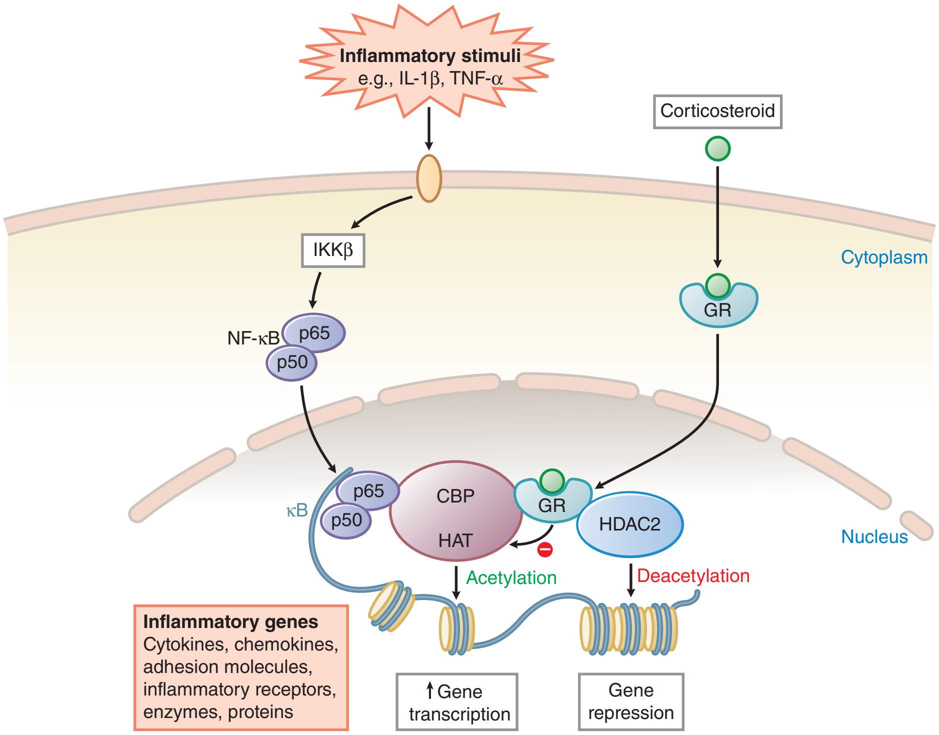


Figure 44–7 *Mechanism of anti-inflammatory action of corticosteroids in asthma.* Inflammatory stimuli (IL-1β, TNF-α, etc.) activate IKKβ, leading to activation of the transcription factor NF-κB. A dimer of p50 and p65 NF-κB proteins translocates to the nucleus and binds to specific κB recognition sites and to coactivators, such as the CREB-binding protein (CBP), which have intrinsic histone acetyltransferase (HAT) activity. This results in acetylation of core histones and consequent increased expression of genes encoding multiple inflammatory proteins. Cytosolic GRs bind corticosteroids; the receptor-ligand complexes translocate to the nucleus and bind to coactivators to inhibit HAT activity in two ways: directly and, more importantly, by recruiting HDAC2, which reverses histone acetylation, leading to the suppression of activated inflammatory genes.

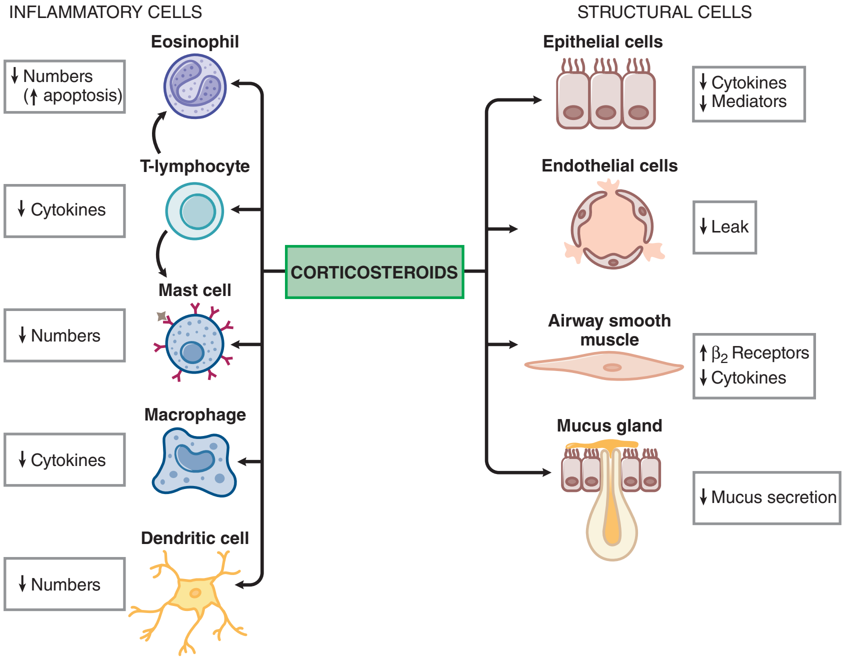


Figure 44–8 *Effect of corticosteroids on inflammatory and structural cells in the airways.*

presumably reflecting the slow healing of the damaged inflamed airway. It is important to recognize that corticosteroids *suppress* inflammation in the airways but do not cure the underlying disease. When steroids are withdrawn, there is a recurrence of the same degree of airway hyperresponsiveness, although in patients with mild asthma, it may take several months to return.

Interaction with β_2 Adrenergic Receptors

Steroids potentiate the effects of β agonists on bronchial smooth muscle and prevent and reverse β receptor desensitization in airways *in vitro* and *in vivo* (Newton and Giembycz, 2016). At a molecular level, corticosteroids increase the transcription of the β_2 receptor gene in human lung *in vitro* and in the respiratory mucosa *in vivo* and also increase the stability of its messenger RNA. They also prevent or reverse uncoupling of β_2 receptors to G_s. In experimental systems, corticosteroids prevent downregulation of β_2 receptors.

β_2 Agonists also enhance the action of GRs, resulting in increased nuclear translocation of liganded GR receptors and enhancing the binding of GRs to DNA. This effect has been demonstrated in sputum macrophages of asthmatic patients after an ICS and inhaled LABA (Barnes, 2017). This suggests that β_2 agonists and corticosteroids enhance each other's beneficial effects in asthma therapy.

Pharmacokinetics

The pharmacokinetics of oral corticosteroids are described in Chapter 50. The pharmacokinetics of ICSs are important in relation to systemic effects (Barnes, 2017). The fraction of steroid that is inhaled into the lungs acts locally on the airway mucosa but may be absorbed from the airway and alveolar surface. Thus, a portion of an inhaled dose reaches the systemic circulation. Furthermore, the fraction of inhaled steroid that is deposited in the oropharynx is swallowed and absorbed from the gut. The absorbed fraction may be metabolized in the liver (first-pass metabolism) before reaching the systemic circulation (see Figure 44–3). The use of a spacer chamber reduces oropharyngeal deposition and therefore reduces systemic absorption of ICSs, although this effect is minimal in corticosteroids with a high first-pass metabolism. Mouth rinsing and discarding the rinse have a similar effect, and this procedure should be used with high-dose dry powder steroid inhalers when spacer chambers cannot be used.

Beclomethasone dipropionate (BDP) and *ciclesonide* are prodrugs that release the active corticosteroid after the ester group is cleaved by esterases in the lung. *Ciclesonide* is available as an MDI for asthma and as a nasal spray for allergic rhinitis. *Budesonide, fluticasone propionate*, and *mometasone furoate* have a greater first-pass metabolism than BDP and are therefore less likely to produce systemic effects at high inhaled doses.

Routes of Administration and Dosing

Inhaled Corticosteroids in Asthma

Inhaled corticosteroids are recommended as first-line therapy for patients with persistent asthma. They should be started in any patient who needs to use a β_2 agonist inhaler for symptom control more than twice weekly. They are effective in mild, moderate, and severe asthma and in children as well as adults (Barnes, 2017).

Most of the benefit may be obtained from doses of less than 400 μg BDP or equivalent. However, some patients (with relative corticosteroid resistance) may benefit from higher doses (up to 2000 μg/day). For most patients, ICSs should be used twice daily, a regimen that improves adherence once control of asthma has been achieved (which may require four-time daily dosing initially or a course of oral steroids if symptoms are severe). Administration once daily of some steroids (e.g., *budesonide*, *mometasone*, and *ciclesonide* in mild asthma and *fluticasone furoate* in all patients) is effective when doses of 400 μg or less are needed. If a dose greater than 800 μg daily via pMDI is used, a spacer device should be employed to reduce the risk of oropharyngeal side effects. ICSs may be used in children in the same way as in adults; at doses of 400 μg/day or less, there is no evidence of significant growth suppression (Agertoft and Pedersen, 2000; Pedersen, 2001). The dose of ICS should be the minimal dose that controls asthma; once control is achieved, the dose should be slowly reduced. Nebulized corticosteroids (e.g., *budesonide*) are useful in the treatment of small children who are not able to use other inhaler devices.

ICSs may also be used in combination with a rapidly acting β_2 agonist such as *formoterol* or *albuterol* (anti-inflammatory reliever) as a reliever in patients with mild asthma. The combination gives better control of asthma and exacerbations with lower overall doses of ICS than with regular low-dose ICS (Bateman et al., 2018; O'Byrne et al., 2018). The ICS-*formoterol* inhaler as twice-daily maintenance (single inhaler maintenance and reliever therapy [SMART]) is not yet approved in the U.S. (Global Initiative for Asthma, 2021; Jenkins et al., 2020a).

Inhaled Corticosteroids in COPD

ICSs are far less effective in the treatment of COPD than asthma (Ernst et al., 2015), and their major effect is reducing exacerbations in selected patients who have frequent exacerbations (≥2 severe exacerbations/year) and have increased blood eosinophils (>300 cells/μL). Corticosteroids do not appear to have any significant anti-inflammatory effect in COPD; there appears to be an active resistance mechanism, which may be explained by impaired activity of HDAC2 as a result of oxidative stress (Barnes, 2013a). ICSs have no effect on the progression of COPD, even when given to patients with presymptomatic disease; in addition, ICSs have no effect on mortality (Vestbo et al., 2016). Many patients are treated unnecessarily with high doses of ICS, and because these doses may cause long-term systemic side effects, ICSs can be safely withdrawn in the absence of frequent exacerbations with a high blood eosinophil count (Calverley et al., 2017). For COPD patients who benefit from ICS, it is convenient to administer the agent via a triple fixed-dose inhaler in combination with a LABA and a LAMA (Ritondo et al., 2021).

Patients with cystic fibrosis and bronchiectasis, which involve chronic neutrophilic inflammation of the airways, are also resistant to high doses of ICS.

Systemic Steroids

Intravenous steroids are indicated in acute asthma if lung function is less than 30% predicted and in patients who show no significant improvement with nebulized β_2 agonist. *Hydrocortisone* is the steroid of choice because it has the most rapid onset (5–6 h after administration), compared with 8 h with *prednisolone*. It is common to give *hydrocortisone* 4 mg/kg initially, followed by a maintenance dose of 3 mg/kg every 6 h. *Methylprednisolone* is also available for intravenous use. Intravenous therapy is usually given until a satisfactory response is obtained, and then oral *prednisolone* may be substituted. *Oral prednisone* or *prednisolone* (40–60 mg) has a similar effect to intravenous *hydrocortisone* and is easier to administer. A high dose of *inhaled fluticasone propionate* (2000 μg daily) is as effective as a course of oral *prednisolone* in controlling acute exacerbations of asthma in a family practice setting and in children in an emergency department setting, although this route of delivery is more expensive (Manjra et al., 2000).

Prednisone and *prednisolone* are the most commonly used oral steroids. Maximal beneficial effect is usually achieved with 30 to 40 mg *prednisone* daily, although a few patients may need 60 to 80 mg daily to achieve control of symptoms. The usual maintenance dose is about 10 to 15 mg/day. Short courses of oral steroids (30–40 mg *prednisolone* daily for 1–2 weeks) are indicated for exacerbations of asthma; the dose may be tapered over 1 week after the exacerbation is resolved (the taper is not strictly necessary after a short course of therapy, but patients find it reassuring). Oral steroids are usually given as a single dose in the morning because this coincides with the normal diurnal increase in plasma cortisol and produces less adrenal suppression than if given in divided doses or at night. Oral corticosteroids are used to treat acute exacerbations of COPD, but the effect is small (Walters et al., 2018).

Adverse Effects

Corticosteroids inhibit corticotropin and cortisol secretion by a negative-feedback effect on the pituitary gland (see Chapter 46). Hypothalamic-pituitary-adrenal (HPA) axis suppression depends on dose and usually only occurs with doses of *prednisone* greater than 7.5 to 10 mg/day. Significant suppression after short courses of corticosteroid therapy is not usually a problem, but prolonged suppression may occur after several months or years. *Steroid doses after prolonged oral therapy must be reduced slowly*. Symptoms of "steroid withdrawal syndrome" include lassitude, musculoskeletal pains, and, occasionally, fever. HPA suppression with inhaled steroids is usually seen only when the daily inhaled dose exceeds 2000 μg BDP or its equivalent daily.

Side effects of long-term oral corticosteroid therapy include fluid retention, increased appetite, weight gain, osteoporosis, capillary fragility, hypertension, peptic ulceration, diabetes, cataracts, and psychosis. Their frequency tends to increase with age. Very occasionally, adverse reactions (such as anaphylaxis) to intravenous *hydrocortisone* have been described, particularly in *aspirin*-sensitive asthmatic patients.

TABLE 44–4 ■ SIDE EFFECTS OF INHALED CORTICOSTEROIDS

Local side effects
Dysphonia
Oropharyngeal candidiasis
Cough
Systemic side effects
Adrenal suppression and insufficiency
Growth suppression
Bruising
Osteoporosis
Cataracts
Glaucoma
Metabolic abnormalities (glucose, insulin, triglycerides)
Psychiatric disturbances (euphoria, depression)
Pneumonia

The incidence of systemic side effects after ICSs is an important consideration, particularly in children (Table 44–4). Initial studies suggested that adrenal suppression occurred only with inhaled doses greater than 1500 to 2000 μg/day (Patel et al., 2020). More sensitive measurements of systemic effects include indices of bone metabolism, such as serum osteocalcin and urinary pyridinium cross-links, and in children, knemometry, which may be increased with inhaled doses as low as 400 μg/day BDP in some patients (Agertoft and Pedersen, 2000). The clinical relevance of these measurements is not yet clear. Nevertheless, it is important to reduce the likelihood of systemic effects by using the lowest dose of ICS needed to control the asthma and by use of a large-volume spacer to reduce oropharyngeal deposition.

Several systemic effects of inhaled steroids have been described and include dermal thinning and skin capillary fragility (relatively common in elderly patients after high-dose inhaled steroids). Other side effects, such as cataract formation and osteoporosis, are reported but often in patients who are also receiving courses of oral steroids (Patel et al., 2020). There is some evidence that use of high-dose ICSs is associated with cataract and glaucoma, but it is difficult to dissociate the effects of ICS from the effects of courses of oral steroids that these patients usually require. There has been particular concern about the use of inhaled steroids in children because of growth suppression (Agertoft and Pedersen, 2000).

The ICSs may have *local side effects* due to the deposition of inhaled steroid in the oropharynx. The most common problem is hoarseness and weakness of the voice (dysphonia) due to atrophy of the vocal cords following laryngeal deposition of steroid; it may occur in up to 40% of patients and is noticed particularly by patients who need to use their voices during their work (lecturers, teachers, and singers). Throat irritation and coughing after inhalation are common with MDIs and appear to be due to additives because these problems are not usually seen if the patient switches to a DPI. There is no evidence for atrophy of the lining of the airway. Oropharyngeal candidiasis occurs in about 5% of patients. There is no evidence for increased lung infections, including tuberculosis, in patients with asthma. High doses of ICSs increase the risk of pneumonia in patients with COPD; the risk appears to be higher with *fluticasone propionate* than *budesonide* (Lodise et al., 2020). In COPD patients, ICSs may also cause osteoporosis, fractures, and an increase in diabetes, conditions for which these patients already have an increased risk (Suissa et al., 2010).

Corticosteroid MDIs with HFA propellants produce smaller aerosol particles and may have a more peripheral deposition, making them useful in treating patients with more severe asthma.

Therapeutic Choices

Numerous ICSs are now available, including BDP, *triamcinolone*, *flunisolide*, *budesonide*, *hemihydrate*, *fluticasone propionate*, *mometasone furoate*, *ciclesonide*, and *fluticasone furoate*. All are equally effective as antiasthma drugs, but there are differences in their pharmacokinetics: *Budesonide*, *fluticasone*, *mometasone*, and *ciclesonide* have a lower oral bioavailability than BDP because they are subject to greater first-pass hepatic metabolism; this results in reduced systemic absorption from the fraction of the inhaled drug that is swallowed (Derendorf et al., 2006) and thus reduced adverse effects. At high doses (>1000 μg), *budesonide* and *fluticasone propionate* have fewer systemic effects than BDP and *triamcinolone* (not marketed in the U.S.), and they are preferred in patients who need high doses of ICSs and in children. *Ciclesonide* is a prodrug that is converted to the active metabolite by esterases in the lung, giving it low oral bioavailability and a high therapeutic index (Mukker et al., 2016). *Fluticasone furoate* has the longest duration of action, is suitable for once-daily dosing, and is usually combined with the ultra-LABA *vilanterol* (Syed, 2015).

When doses of ICS exceed 800 μg BDP or equivalent daily, a large-volume spacer is recommended to reduce oropharyngeal deposition and systemic absorption in the case of BDP. All currently available ICSs are absorbed from the lung into the systemic circulation, so that some systemic absorption is inevitable. However, the amount of drug absorbed does not appear to have clinical effects in doses of less than 800 μg BDP equivalent. Although there are potency differences among corticosteroids, there are relatively few comparative studies, partly because dose comparison of corticosteroids is difficult due to their long time course of action and the relative flatness of their dose-response curves.

Future Developments

Early treatment with ICSs in both adults and children may give a greater improvement in lung function than if treatment is delayed, likely reflecting the fact that corticosteroids are able to modify the underlying inflammatory process and prevent structural changes (fibrosis, smooth muscle hyperplasia, etc.). ICSs are currently recommended for patients with persistent asthmatic symptoms (e.g., need for an inhaled β_2 agonist more than twice a week). A major change in the use of ICS is in combination with a rapid-acting β_2 agonist as a safer and more effective rescue therapy. The combination is now the preferred treatment for all asthmatic patients.

Developing new corticosteroids with fewer systemic effects is desirable. It has been possible to develop corticosteroids that dissociate the DNA-binding effect of corticosteroids (which mediates most of the adverse effects) from the inhibitory effect on transcription factors such as NF-κB (which mediates much of the anti-inflammatory effect). Such "dissociated steroids" or selective GR agonists should, theoretically, retain anti-inflammatory activity but have a reduced risk of adverse effects; achieving this separation of desired and adverse effects is proving difficult *in vivo* (Zhang et al., 2020). Several nonsteroidal selective GR agonists are now in development for asthma (Rogliani et al., 2020).

Corticosteroid resistance is a major barrier to effective therapy in patients with severe asthma, in asthmatic patients who smoke, and in patients with COPD and cystic fibrosis (Barnes, 2013a). "Steroid-resistant" asthma is thought to be due to reduced anti-inflammatory actions of corticosteroids. In patients with COPD and some patients with severe asthma, there is a reduction in HDAC2 expression that reduces corticosteroid responsiveness; this is potentially reversible by theophylline.

Mediator Antagonists

Over 100 different inflammatory mediators have been implicated in both asthma and COPD, suggesting that inhibition of synthesis or antagonists of receptors for these mediators may be beneficial (Barnes, 2004). However, specific inhibitors have been largely disappointing in both diseases, indicating the redundancy of inflammatory mediators. The variability in patient response to antileukotriene agents in asthma suggests that there may be biomarkers that select good responders, for example, those patients with increased leukotriene production, such as increased urinary LTE_4.

Antihistamines

Histamine mimics many of the features of asthma and is released from mast cells in acute asthmatic responses, suggesting that antihistamines may be useful in asthma therapy. There is, however, little evidence that histamine H_1 receptor antagonists provide any useful clinical benefit. Newer antihistamines, such as *cetirizine* and *azelastine*, have some beneficial effects, but this may be unrelated to H_1 receptor antagonism. Antihistamines are not recommended in the routine management of asthma.

Antileukotrienes

There is considerable evidence that cys-LTs (cysteinyl-leukotrienes) are produced in asthma and that they have potent effects on airway function, inducing bronchoconstriction, airway hyperresponsiveness, plasma exudation, mucus secretion, and eosinophilic inflammation (Figure 44–9; also see Chapter 41). These findings led to the development of 5′-lipoxygenase (5-LO) enzyme inhibitors (of which *zileuton* is the only drug marketed) and leukotriene cys-LT_1-receptor antagonists (LTRAs), including *montelukast*, *zafirlukast*, and *pranlukast* (not available in the U.S.) (Yokomizo et al., 2018).

Clinical Studies

Leukotriene cys-LT_1-receptor antagonists are widely used in the management of asthma; they can be administered orally and are relatively well tolerated. They effectively inhibit the effects of inhaled cys-LTs, but they are less effective than ICS in controlling asthma and preventing exacerbations, reflecting the fact that many other mediators are likely to contribute to clinical asthma. Even in *aspirin*-sensitive asthma, in which increased production of cys-LTs is thought to play an important role, LTRAs are less effective than ICS. When added to ICS, they provide little further control of asthma and are less effective than LABAs as add-on treatments for asthma (Chauhan et al., 2017). In patients with severe asthma who are not controlled on high doses of ICS and LABA, LTRAs do not provide any additional benefit (Robinson et al., 2001).

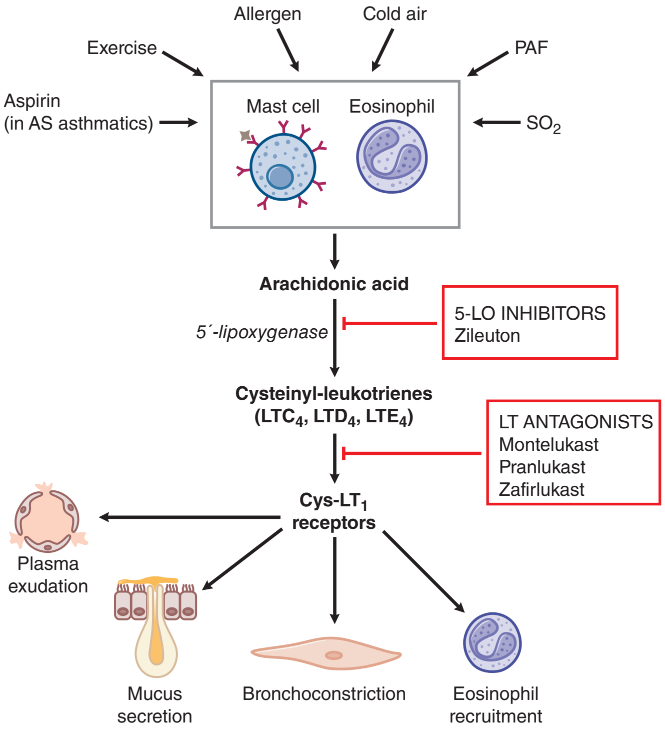


Figure 44–9 *Effects of cysteinyl-LTs on the airways and their inhibition by anti-LTs.* AS, *aspirin* sensitive; PAF, platelet-activating factor.

The 5-LO inhibitor *zileuton* appears to be more effective than LTRAs in asthma control and treating *aspirin*-sensitive asthma, which may be explained by inhibition of other mediators, such as LTB_4, which is chemoattractant to neutrophils, and 5-oxo-eicosatetraenoic acid (5-Oxo-ETE), which is chemotactic for eosinophils. However, *zileuton* is not available in most countries, and there have been concerns about frequent side effects, particularly hepatic dysfunction, and the need for frequent dosing (four times daily).

In COPD, cys-LTs are not elevated in exhaled breath condensate, and LTRAs have no role in the therapy of COPD. By contrast, LTB_4 is elevated in COPD, suggesting that 5-LO inhibitors that reduce LTB_4 synthesis could have some potential benefit by reducing neutrophil inflammation. However, a pilot study failed to indicate any clear benefit of a 5-LO inhibitor in COPD patients (Gompertz and Stockley, 2002).

Adverse Effects

Leukotriene cys-LT_1-receptor antagonists are usually well tolerated but are associated with headaches and eosinophilic granulosis with polyangiitis. *Montelukast* has been associated with an increase in serious neuropsychiatric events in children and adults, including suicidal thoughts, and now has a black-box warning by the FDA (Glockler-Lauf et al., 2019).

Immunomodulatory Therapies

Immunosuppressive Therapy

Immunosuppressive therapy (e.g., *methotrexate, cyclosporine A, gold, intravenous immunoglobulin*) has been considered in asthma when other treatments have been unsuccessful or to reduce the dose of maintenance oral steroids required. However, immunosuppressive treatments are less effective and have a greater propensity for side effects than oral corticosteroids and therefore cannot be routinely recommended.

Biologic Therapies

Several antibodies that block cytokines or antibodies are now available for the treatment of severe asthma, although not yet recommended for COPD (Barnes, 2018).

Anti-IgE

Increased immunoglobulin E (IgE) is a fundamental feature of allergic asthma. *Omalizumab* is a humanized monoclonal antibody that blocks the binding of IgE to high-affinity IgE receptors (FcεR1) on mast cells and thus prevents their activation by allergens (Figure 44–10). It blocks binding on IgE to low-affinity IgE receptors (FcεRII, CD23) on other inflammatory cells, including T and B lymphocytes, macrophages, and possibly eosinophils, to inhibit chronic inflammation. *Omalizumab* also reduces levels of circulating IgE. In addition, *omalizumab* enhances the secretion of type 1 interferons after exposure to rhinovirus and reduces FcεRI expression on dendritic cells, resulting in enhanced protection against viral infection (Gill et al., 2018). This may account for the effect of *omalizumab* to reduce virally induced exacerbations (see below).

Clinical Use. *Omalizumab* is used for the treatment of patients with severe allergic asthma. The antibody is administered subcutaneously every 2 to 4 weeks, and the dose is determined by the titer of circulating total IgE. *Omalizumab* reduces the requirement for oral corticosteroids and ICSs and markedly reduces asthma exacerbations. Not all patients respond, and there are no clear clinical predictors of clinical response, necessitating a trial of therapy (usually over 4 months); high blood eosinophils and fractional exhaled nitric oxide (F_{eNO}) may be associated with a better response (Casale et al., 2019). *Omalizumab* has greater than 50% excellent clinical effectiveness in adults and children with severe asthma (Humbert et al., 2018). *Omalizumab* also has some efficacy in nonatopic asthma with normal circulating IgE levels, a result presumed to reflect reduced local IgE responses (Pillai et al., 2016). Because of its high cost, this treatment is generally used only in patients with very severe allergic asthma who are poorly controlled even on oral corticosteroids and in patients with very severe concomitant allergic rhinitis. It may also be of value in protecting against anaphylaxis during specific immunotherapy. *Omalizumab* is effective in preventing asthma exacerbations if administered prior to the exacerbation season (Teach et al., 2015). The major side effect of *omalizumab* is an anaphylactic response, which is uncommon (<0.1%).

Anti–IL-5

Interleukin-5 plays a pivotal role in eosinophilic inflammation and is also involved in eosinophil survival and priming through activation of the specific receptor, IL-5Rα, which is mainly expressed on eosinophils.

Anti–IL-5 and anti–IL-5 receptor (IL-5Rα) blocking antibodies inhibit eosinophilic inflammation in asthmatic patients (Figure 44–11). Three antibodies are approved for use in severe eosinophilic (type 2 immunity) asthma. The anti–IL-5 antibodies *mepolizumab* (given subcutaneously every 4 weeks) and *reslizumab* (given IV every 4 weeks) block circulating IL-5. *Benralizumab* (given subcutaneously every 8 weeks) blocks IL-5Rα (Menzella et al., 2020). *Benralizumab*, by binding to eosinophil IL-5Rα,

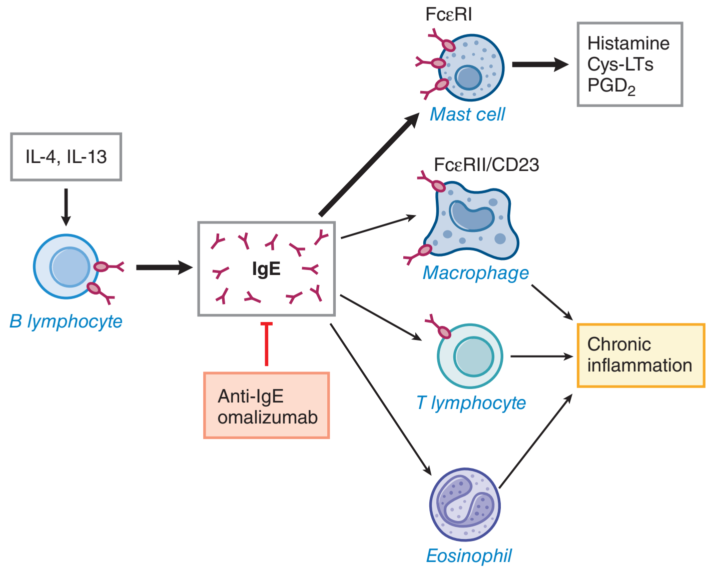


Figure 44–10 *Immunoglobulin E plays a central role in allergic diseases.* Blocking IgE using an antibody, such as *omalizumab*, is a rational therapeutic approach. IgE may activate high-affinity receptors (FcεRI) on mast cells as well as low-affinity receptors (FcεRII, CD23) on other inflammatory cells. *Omalizumab* prevents these interactions and the resulting inflammation.

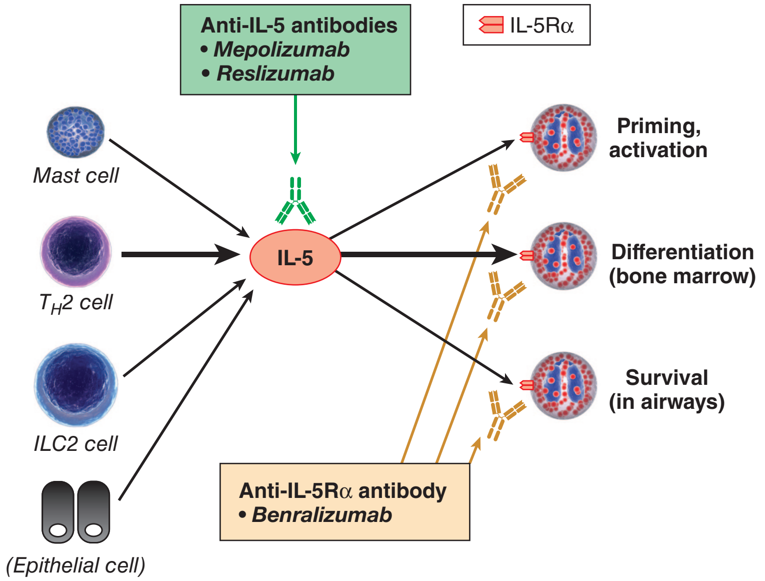


Figure 44–11 *Anti–IL-5 therapies.* IL-5, released predominantly from T_H2 and ILC2 lymphocytes, stimulates eosinophilic inflammation. IL-5 action can be inhibited by the antibodies *mepolizumab* and *reslizumab*. The IL-5 receptor (IL-5Rα) can be blocked and rendered unresponsive to IL-5 by the antibody *benralizumab*.

causes apoptosis and a more rapid resolution of eosinophilic inflammation in the airways. These therapies all reduce exacerbations by around 50%, with small improvements in symptoms, quality of life, and lung function in severe asthma patients (blood eosinophil count of >300 cells/μL). They also reduce the maintenance dose of oral corticosteroids by at least 50%, permitting many steroid-dependent asthmatics to come off steroid treatments completely. Anti–IL-5 treatments have not been effective in reducing exacerbations in COPD patients, even when there is an increase in blood eosinophils (Criner et al., 2019; Pavord et al., 2017).

Anti–IL-5 therapy is well tolerated, with only occasional anaphylaxis. There is no evidence for any increase in worm or parasitic infections, although these treatments are not yet used much in countries where these infectious diseases are prevalent.

Anti–IL-4/13

Interleukin-4 helps to direct IgE synthesis and eosinophilic inflammation; blocking IL-4 has been ineffective in clinical studies. Similarly, blocking IL-13 has also proved to be ineffective in clinical studies of severe eosinophilic asthma. However, blocking IL-4Rα, the common receptor for these cytokines, with *dupilumab* appears to be very effective in these patients. *Dupilumab* (given subcutaneously every 2 weeks) reduces exacerbations, improves symptoms and lung function in patients with moderate to severe asthma (Castro et al., 2018), and reduces the requirement for oral corticosteroids in steroid-dependent patients (Rabe et al., 2018). *Dupilumab* reduces F_{eNO}, which is regulated by IL-4α stimulation of inducible nitric oxide synthase, but has no effect on blood eosinophils. The drug is well tolerated. An important clinical advantage is that *dupilumab* is effective in treating atopic dermatitis and rhinosinusitis, which are common comorbidities in severe asthma (Muñoz-Bellido et al., 2022).

Anti-TNF

Tumor necrosis factor-α (TNFα) plays an amplifying role in inflammation. TNFα is increased in sputum, bronchoalveolar lavage, and plasma of patients with severe asthma and COPD. These findings suggest that anti-TNF therapies, which are clinically effective in other chronic inflammatory diseases (e.g., rheumatoid arthritis and inflammatory bowel disease), would be useful therapies in severe asthma and COPD. A large, controlled trial of an anti-TNF blocking antibody (*golimumab*) showed no effect on exacerbations or asthma control in patients with severe asthma. Side effects included increased incidence of pneumonia (Wenzel et al., 2009). Similarly, the anti-TNF blocking antibody *infliximab* provided no clinical benefits in COPD patients, but side effects were common, including pneumonia and increased incidence of lung cancer (Rennard et al., 2007).

Specific Immunotherapy: Desensitization to Allergens

Theoretically, specific immunotherapy against common allergens should be effective in preventing asthma. Although this treatment is effective in allergic rhinitis due to single allergens such as grass pollen, there is little evidence that desensitizing injections to common allergens are effective in controlling chronic asthma (Rolland et al., 2009).

Sublingual immunotherapy is safer, and several studies have shown its clinical benefit in asthma, although it is uncertain whether this treatment reduces exacerbations and improves the quality of life (Calderón and Bacharier, 2021; Fortescue et al., 2020).

Specific immunotherapy induces the secretion of the anti-inflammatory cytokine IL-10 from regulatory helper T lymphocytes, and this blocks costimulatory signal transduction in T cells (via CD28) so that they are unable to react to allergens presented by antigen-presenting cells (see Figures 38–3 and 38–4). Applying an understanding of the cellular processes involved might lead to safer and more effective approaches in the future. More specific immunotherapies may be developed with recombinant allergens, cloned allergen epitopes, T-cell peptide fragments of allergens, CpG oligonucleotides, and vaccines of conjugates of allergen and TLR-9 (toll-like receptor-9) to stimulate T_H1 immunity and suppress T_H2 immunity (Shamji and Durham, 2017).

Cromones

Cromolyn sodium (*disodium cromoglycate*) is a derivative of *khellin*, an Egyptian herbal remedy, and was found to protect against allergen challenge without any bronchodilator effect. A structurally related drug, *nedocromil sodium*, which has a similar pharmacological profile to *cromolyn*, was subsequently developed. Although *cromolyn* was popular in the past because of its good safety profile, its use has sharply declined with the more widespread use of the more effective ICSs, particularly in children. Cromones are no longer recommended for asthma therapy. Interest continues in these compound as downregulators of mast cell activity (Paivandy and Pejler, 2021).

New Drugs in Development for Airway Disease

Several new classes of drug are in development for asthma and COPD, but clinical development has been slow, and many treatments have either proved to be ineffective or are limited by toxicology and side effect profiles (Gross and Barnes, 2017).

Novel Mediator Antagonists

Many mediators have been implicated in asthma and COPD, many of which have redundant functions, so it is not surprising that inhibiting single mediators, such as bradykinin and platelet-activating factor, has been ineffective in asthma. However, blocking a mediator that is upstream in an inflammatory cascade might be more effective, particularly with cytokines that play a pivotal role in a cytokine network (Barnes, 2018).

DP_2 Antagonists

Prostaglandin D_2 is predominantly released from mast cells in asthmatic patients and mediates bronchoconstriction via thromboxane receptors and vasodilatation via DP_1 receptors (DPr_1). PGD_2 is a chemoattractant for T_H2 lymphocytes, ILC2, and eosinophils via the DP_2 receptor (DPr_2, previously known as CRTh2). Several small-molecule DPr_2 antagonists have been developed for the treatment of asthma. After promising initial results, one such agent, *fevipiprant*, failed to meet the primary endpoint of reduced exacerbations in patients with moderate to severe eosinophilic asthma, and development has now stopped (Brightling et al., 2021). Other DP_2 antagonists are also in clinical development, but this class of drug appears to be far less effective than anti–IL-5 antibodies in eosinophilic asthma.

Antioxidants

Oxidative stress is important in severe asthma and COPD and may contribute to corticosteroid resistance. Existing antioxidants include vitamins C and E and *N*-acetyl-cysteine. These drugs have weak effects, but more potent antioxidants are in development, including activators of the transcription factor Nrf2. Although several new antioxidants are in development for the treatment of airway disease, none is currently approved (Barnes, 2020).

Cytokine Modifiers

In addition to blocking antibodies against T2 cytokines mentioned above, antibodies against other critical cytokines have been tested in asthma and COPD.

Interleukin-1, released from the NLRP3 inflammasome, is thought to play an important role in asthma associated with obesity (Wood et al., 2019). In COPD, anti–IL-1 antibodies, such as *canakinumab*, have proved to be disappointing. NLRP3 inflammasome inhibitors are in development.

Interleukin-17 is important in orchestrating chronic neutrophilic inflammation and is secreted mainly from T_H17 and ILC3 cells. It may play a role in severe neutrophilic asthma. An IL-17 receptor antibody, *brodalumab*, was ineffective in severe asthma (Busse et al., 2013). An anti–IL-17 antibody was also reported to be ineffective in COPD patients (Eich et al., 2017).

Thymic stromal lymphopoietin (TSLP) is an upstream cytokine or alarmin that shows increased expression in airway epithelial cells in asthmatic patients, particularly in severe disease. TSLP activates T2 immunity in the airway, with the recruitment of T_H2 and ILC2 cells to drive eosinophilic inflammation (Porsbjerg et al., 2020). *Tezepelumab* is an antibody that blocks TSLP and inhibits both IL-5 (with reduced blood eosinophils) and IL-4/13 (with reduced F_{eNO}). In severe eosinophilic asthma, *tezepelumab* induces a 70% reduction in exacerbations as well as improvement in symptoms and lung function. *Tezepelumab* also has effects in non-type 2 asthma, reflecting its effects on other types of immunity. Tezepelumab-ekko is FDA-approved as add-on maintenence treatment of severe asthma in patients 12 years and older.

Interleukin-33 is another alarmin that regulates T2 immunity with a profile similar to that of TSLP. Antibodies that block IL-33 and its receptor ST2 are currently in clinical trials for asthma and COPD (Porsbjerg et al., 2020).

Chemokine Receptor Antagonists

Many chemokines are involved in asthma and COPD and play a key role in recruitment into the lungs of inflammatory cells, such as eosinophils, neutrophils, macrophages, and lymphocytes. Chemokine receptors are attractive targets because they are GPCRs with defined binding pockets (Taylor et al., 2019); small-molecule inhibitors are now in development. Antagonists of the various C-C motif chemokine receptors (CCRs) could block recruitment of eosinophils, neutrophils, and monocytes in the airways of patients with asthma; to date, the compounds tested have not proven clinically useful. A promising CCR4 antagonist attenuated allergic lung inflammation in an animal model of asthma (Zhang et al., 2017).

Protease Inhibitors

Several proteolytic enzymes are involved in the chronic inflammation of airway diseases. Mast cell tryptase has several effects on airways, including increasing responsiveness of airway smooth muscle to constrictors, increasing plasma exudation, potentiating eosinophil recruitment, and stimulating fibroblast proliferation. Tryptase inhibitors have so far proved to be disappointing in clinical studies.

In COPD, proteases are involved in the degradation of connective tissue, particularly the breakdown of elastin fibers in the development of emphysema. Matrix metalloproteinase 9 (MMP9) appears to be the predominant elastolytic enzyme in emphysema, and several selective MMP9 inhibitors are now in development.

New Anti-inflammatory Drugs

Mitogen-Activated Protein Kinase Inhibitors

Mitogen-activated protein (MAP) kinase pathways are involved in chronic inflammation in asthma and COPD (Barnes, 2016). *Losmapimod* and related compounds block the p38 MAP kinase pathway. These drugs inhibit the synthesis of many inflammatory cytokines, chemokines, and inflammatory enzymes. To date, the clinical use of inhibitors of p38 MAP kinase for COPD and asthma has been disappointing or dose-limited by side effects.

Mucoregulators

Mucus hypersecretion occurs in chronic bronchitis, COPD, cystic fibrosis, and asthma (Fahy and Dickey, 2010). In chronic bronchitis, mucus hypersecretion is related to chronic irritation by cigarette smoke and may involve neural mechanisms and the activation of neutrophils to release enzymes such as neutrophil elastase and proteinase 3 that have powerful stimulatory effects on mucus secretion. Mast cell–derived chymase is also a potent mucus secretagogue. This suggests that several classes of drugs may be developed to control mucus hypersecretion. Mucus secretion is regulated by epidermal growth factor receptors (EGFRs), but a nebulized EGFR inhibitor has been ineffective in COPD (Woodruff et al., 2010).

Systemic anticholinergic drugs appear to reduce mucociliary clearance, but this is not observed with either *ipratropium bromide* or *tiotropium bromide*, presumably reflecting their poor absorption from the respiratory tract. β_2 Agonists increase mucus production and mucociliary clearance and increase ciliary beat frequency *in vitro*. Because inflammation leads to mucus hypersecretion, anti-inflammatory treatments should reduce mucus hypersecretion; ICSs are very effective in reducing increased mucus production in asthma.

Mucolytics

Several agents reduce the viscosity of sputum *in vitro*. One group consists of derivatives of cysteine that reduce the disulfide bridges that bind glycoproteins to other proteins, such as albumin and secretory IgA. These drugs also act as antioxidants and may therefore reduce airway inflammation. Only *N-acetylcysteine* is available in the U.S.; *carbocysteine, methylcysteine, erdosteine*, and *bromhexine* are available elsewhere. Orally administered, these agents are relatively well tolerated, but clinical studies in chronic bronchitis, asthma, and bronchiectasis have been disappointing. *N-Acetylcysteine* is not currently recommended for COPD management.

DNAse (*dornase alfa*) reduces mucus viscosity in sputum of patients with cystic fibrosis and is indicated if there is significant symptomatic and lung function improvement after a trial of therapy (Yang and Montgomery, 2018). There is no evidence that *dornase alfa* is effective in COPD or asthma, however.

Osmotic agents, such as nebulized hypertonic saline and *mannitol* given by DPI, give marginal clinical benefit in cystic fibrosis but have demonstrated efficacy in chronic bronchitis and COPD (Nevitt et al., 2020).

Expectorants

Expectorants are oral drugs that are supposed to enhance the clearance of mucus. Although expectorants were once commonly prescribed, there is little or no objective evidence for their efficacy (Albrecht et al., 2017). Lacking evidence for their efficacy, the FDA has removed most expectorants from the market in a review of over-the-counter drugs. With the exception of *guaifenesin*, no agents are approved as expectorants in the U.S. For patients who find it difficult to clear mucus, adequate hydration and inhalation of steam may be of some benefit.

Antitussives

Although cough is a common symptom of airway disease, its mechanisms are poorly understood, and current treatment is unsatisfactory (Song and Chung, 2020). Viral infections of the upper respiratory tract are the most common cause of cough; postviral cough is usually self-limiting and commonly patient medicated. Their wide use notwithstanding, over-the-counter cough medications are largely ineffective. Because cough is a defensive reflex, its suppression may be inappropriate in bacterial lung infection. Before treatment with antitussives, it is important to identify underlying causal mechanisms that may require therapy.

Whenever possible, treat the underlying cause, not the cough. Asthma commonly presents as cough, and the cough will usually respond to ICSs. A syndrome characterized by cough in association with sputum eosinophilia but no airway hyperresponsiveness, termed *eosinophilic bronchitis*, also responds to ICSs (Diver et al., 2019). Nonasthmatic cough does not respond to ICSs but sometimes responds to anticholinergic therapy. The cough associated with postnasal drip of sinusitis responds to antibiotics (if warranted), nasal decongestants, and intranasal steroids. The cough associated with angiotensin-converting enzyme inhibitors (in ~15% of patients treated) responds to lowering the dose or withdrawal of the drug and substitution of an AT_1 receptor antagonist (see Chapter 30).

Gastroesophageal reflux is a common cause of cough through a reflex mechanism and occasionally as a result of acid aspiration into the lungs. This cough may respond to suppression of gastric acid with an H_2 receptor antagonist or a proton pump inhibitor (see Chapter 53). Several treatments that have been assessed in the treatment of refractory cough (Song and Chung, 2020).

Opiates

Opiates have a central mechanism of action on μ opioid receptors in the medullary cough center. There is some evidence that they may have additional peripheral action on cough receptors in the proximal airways. *Codeine* and *pholcodine* (not available in the U.S.) are commonly used, but there is little evidence that they are clinically effective, particularly on postviral cough; in addition, they are associated with sedation and constipation. *Morphine* and *methadone* are effective but indicated only for intractable cough associated with bronchial carcinoma.

Dextromethorphan

Dextromethorphan is a centrally active *N*-methyl-ᴅ-aspartate receptor antagonist. It may also antagonize opioid receptors. Despite the fact that it is in numerous over-the-counter cough suppressants and used commonly to treat cough, it is poorly effective. In children with acute nocturnal cough, it is not significantly different from placebo in reducing cough (Dicpinigaitis et al., 2014). It can cause hallucinations at higher doses and has significant abuse potential.

Local Anesthetics

Benzonatate, a local anesthetic, acts peripherally by anesthetizing the stretch receptors located in the respiratory passages, lungs, and pleura. By dampening the activity of these receptors, *benzonatate* may reduce the cough reflex. The recommended dose is 100 mg, three times per day, and up to 600 mg/day, if needed. Severe allergic reactions have been reported in patients allergic to *para-aminobenzoic acid*, a metabolite of *benzonatate*.

Neuromodulators

Gabapentin and *pregabalin* are γ-aminobutyric acid analogues that inhibit neurotransmission and have been used in neuropathic pain syndromes. They have been shown to benefit chronic idiopathic cough (Gibson and Vertigan, 2015). Side effects of somnolence and dizziness are common at higher doses, so it is usual to initiate therapy at lower doses.

Other Drugs

Several other drugs reportedly have small benefits in protecting against cough challenges or in reducing cough in pulmonary diseases. These drugs include *moguisteine* (not available in the U.S.), which acts peripherally and appears to open ATP-sensitive K^+ channels. *Theobromine*, a naturally occurring methylxanthine, reduces cough induced by tussive agents. Although the expectorant *guaifenesin* is not typically known as a cough suppressant, it is significantly better than placebo in reducing acute viral cough and inhibits cough-reflex sensitivity in patients with upper respiratory tract infections (Dicpinigaitis et al., 2014).

Novel Antitussives

There is a need to develop new, more effective therapies for cough, particularly drugs that act peripherally to avoid sedation. There are close analogies between chronic cough and sensory hyperesthesia, so new therapies with novel antitussives are likely to arise from pain research.

Transient Receptor Potential Antagonists

Several types of transient receptor potential (TRP) ion channels have been described on airway sensory nerves and may be activated by various mediators and physical factors, resulting in cough. TRPV1 (previously called the *vanilloid receptor*) is activated by capsaicin, H^+, and bradykinin, all of which are potent tussive agents. *TRPV1 inhibitors* block cough induced by capsaicin and bradykinin and are effective in some models of cough. In a clinical study of an oral TRPV1 inhibitor, there was protection against capsaicin-induced cough but no clinical improvement in chronic idiopathic cough after long-term treatment (Belvisi et al., 2017). Side effects of these drugs are loss of temperature regulation and hyperthermia, which has prevented clinical development.

Transient receptor potential A1 is emerging as a more promising novel target for antitussives. This channel is activated by oxidative stress and many irritants and may be sensitized by inflammatory cytokines. Several selective TRPA1 antagonists are now in development. TRPV4 may also activate cough and may be activated by ATP (Bonvini et al., 2015).

ATP Receptor Antagonists

Adenosine triphosphate is a potent tussive agent and stimulates cough in patients with asthma and COPD via activation of $P2X_3$ receptors on afferent nerves (see Table 16–7). A *$P2X_3$ antagonist* (*gefapixant*) is effective in reducing chronic idiopathic cough, although abnormal taste (dysgeusia) is a frequent side effect (Dicpinigaitis et al., 2020). *Gefapixant* is named after the late Geof Burnstock, a pioneer of purinergic signaling.

Drugs for Dyspnea and Ventilatory Control

Drugs for Dyspnea

Bronchodilators should reduce breathlessness in patients with airway obstruction. Chronic oxygen use may have a beneficial effect, but in a few patients, dyspnea may be extreme. Drugs that reduce breathlessness may also depress ventilation in parallel and may be dangerous in severe asthma and COPD. Some patients show a beneficial response to *dihydrocodeine* and *diazepam*; however, these drugs must be used with caution because of the risk of ventilatory depression (Currow et al., 2014). *Slow-release morphine tablets* may also be helpful in patients with COPD with extreme dyspnea. Nebulized *morphine* may reduce breathlessness in COPD and act on pulmonary opioid receptors. Nebulized *furosemide* has some efficacy in treating dyspnea a variety of causes, but the evidence is not yet sufficiently convincing to recommend this as routine therapy (Barbetta et al., 2017).

Ventilatory Stimulants

Selective respiratory stimulants are indicated if ventilation is impaired as a result of overdose with sedatives, in postanesthetic respiratory depression, and in idiopathic hypoventilation. Respiratory stimulants are rarely indicated in COPD because respiratory drive is already maximal, and further stimulation of ventilation may be counterproductive because of the increase in energy expenditure caused by the drugs.

Doxapram

At low doses (0.5 mg/kg IV), *doxapram* stimulates carotid chemoreceptors; at higher doses, it stimulates medullary respiratory centers. Its effect is transient; thus, intravenous infusion (0.3–3 mg/kg per min) is needed for sustained effect. Unwanted effects include nausea, sweating, anxiety, and hallucinations, and at higher doses, increased pulmonary and systemic pressure. Both the kidney and the liver participate in the clearance of *doxapram*; thus, use caution if hepatic or renal function is impaired. In COPD, the infusion of *doxapram* is restricted to 2 h. The use of *doxapram* to treat ventilatory failure in COPD has now largely been replaced by noninvasive ventilation.

Almitrine

Almitrine bismesylate is a piperazine derivative that appears to selectively stimulate peripheral chemoreceptors and is without central actions. *Almitrine* stimulates ventilation only when there is hypoxia. Long-term use of *almitrine* is associated with peripheral neuropathy, limiting its availability in most countries, including the U.S.

Acetazolamide

The carbonic anhydrase inhibitor *acetazolamide* (see Chapter 29) induces metabolic acidosis and thereby stimulates ventilation; it is not widely used because the metabolic imbalance it produces may be detrimental in the face of respiratory acidosis. It has a small beneficial effect in respiratory failure in patients with COPD. The drug has proved useful in prevention of high-altitude (mountain) sickness (Faisy et al., 2016).

Naloxone

Naloxone is a competitive opioid antagonist that is indicated only if ventilatory depression is due to overdose of opioids.

Flumazenil

Flumazenil, a benzodiazepine antagonist, can reverse respiratory depression due to benzodiazepine overdose (Chapter 22).

Drug Facts for Your Personal Formulary: *Asthma and COPD Therapeutics*

Drug	Therapeutic Uses	Clinical Tips
Short-Acting β_2 Agonists: Inhaled bronchodilators for symptom relief and acute bronchodilation		
Albuterol (salbutamol)	• Asthma, COPD, and exercise-induced bronchospasm • Inhaled: 180 μg (2 puffs) every 4–6 h as needed • Nebulized: 2.5 mg via oral inhalation every 6–8 h as needed over 5–15 min • Oral: 2–4 mg by mouth every 6–8 h	• Also available nebulized and inhaled as levalbuterol (active isomer, so half the dose) • May need to be nebulized with oxygen in severe exacerbation • Adverse effects: tachycardia, palpitations, muscle tremors, and hyperkalemia
Levalbuterol (L-albuterol)	• Bronchodilator • Inhaled (MDI nebulizer)	• Half of doses of racemic albuterol • No advantage over racemic albuterol • Adverse effects: tachycardia, palpitations, muscle tremors, and hyperkalemia
Pirbuterol	• 400 μg (2 puffs) every 4–6 h as needed • Inhaled (MDI nebulizer)	• Similar to albuterol • Adverse effects: tachycardia, palpitations, muscle tremors, and hyperkalemia
Long-Acting β_2 Agonists: Add-on therapy to ICSs in asthma; can be used alone in COPD		
Formoterol	• Asthma as add-on to ICS • Maintenance and treatment of severe COPD • Inhaled: 12 μg (contents of 1 capsule) every 12 h • Nebulized 20 μg in 2 mL, twice per day	• Used as maintenance, usually in a combination with an ICS • Can also be used as a reliever of bronchospasm • Adverse effects: tachycardia, palpitations, muscle tremors, and hyperkalemia
Arformoterol Salmeterol Indacaterol Olodaterol	• Arformoterol for severe COPD • Maintenance treatment for COPD • Arformoterol, inhaled (nebulized), 15 μg in 2 mL twice daily • Salmeterol, inhaled 50 μg twice daily • Indacaterol, inhaled (DPI) 75 μg once daily • Olodaterol, inhaled 2.5 μg once daily	• Cannot be used as a reliever, only for maintenance treatment for COPD • Adverse effects: tachycardia, palpitations, muscle tremors, and hyperkalemia
Anticholinergics: Muscarinic receptor antagonists inhaled as bronchodilators		
Ipratropium bromide Albuterol/ipratropium combination	• Inhaled, 2 puffs (17 μg/puff) 3–4 times/day • Combination albuterol 103 μg/ipratropium 18 μg/puff; 2 puffs 4 times daily	• Largely replaced by LAMAs • Avoid spraying in eyes • Adverse effects include dry mouth, tachycardia, urinary retention, glaucoma • Combination with albuterol may be used as a reliever
Tiotropium bromide	• 2.5 μg via oral inhalation (2 puffs of 1.25 μg/actuation) once daily	• Caution in patients with urinary retention or glaucoma history
Umeclidinium bromide	• Inhaled (DPI) 62.5 μg (1 puff) once daily	
Aclidinium bromide	• Inhaled (DPI) 400 μg (1 puff) twice daily	
Glycopyrrolate	• Inhaled (DPI) 1 capsule (15.6 μg) inhaled twice daily	

Drug Facts for Your Personal Formulary: *Asthma and COPD Therapeutics (continued)*

Drug	Therapeutic Uses	Clinical Tips
LAMA-LABA Combination Inhalers: Maintenance treatment for COPD		
Glycopyrrolate/ indacaterol	• Inhaled (DPI) 1 inhalation (glycopyrrolate 15.6 μg/ indacaterol 27.5 μg) twice daily	• Side effects of anticholinergics and β_2 agonists as above • Maintenance treatment for COPD
Umeclidinium/ vilanterol	• Inhaled (DPI) 1 inhalation (umeclidinium 62.5 μg/25 μg vilanterol) once daily	
Tiotropium/olodaterol	• Inhaled (mist inhaler), 2 inhalations (containing 2.5 μg tiotropium/2.5 μg of olodaterol per inhalation) once daily	
Inhaled Corticosteroids: Maintenance treatment for asthma		
Beclomethasone dipropionate (BDP)	• Inhaled (MDI, DPI); 88 μg (1 spray = 44 μg) twice daily • Not to exceed 440 μg twice daily	• More systemic effects than other ICSs: orally bioavailable BDP is converted to an active metabolite, beclomethasone monopropionate, following absorption • Local effects: hoarse voice, candidiasis • Systemic effects: growth suppression, bruising, adrenal suppression
Fluticasone propionate	• Inhaled (MDI, DPI); 50, 100, 250 μg 2 puffs, twice daily • Do not exceed 1000 μg daily	• Fewer systemic effects than BDP • Local effects: hoarse voice, candidiasis
Budesonide	• Inhaled via jet nebulizer either once daily or divided into 2 doses (maximum daily dose 0.5 mg/day)	• Fewer systemic effects than BDP • Used in children less than 8 who cannot use DPI • Local effects: hoarse voice, candidiasis
Ciclesonide	• Inhaled (MDI) 80 μg twice daily	• Least-systemic effects of all ICSs; may be effective once daily • Local effects: hoarse voice, candidiasis
ICS/LABA Combination Inhalers: Maintenance treatment in asthma and COPD		
Fluticasone propionate/ salmeterol	• Inhaled (DPI) • Starting dosage based on asthma severity	• Use lowest dose that maintains asthma control • Use only in severe COPD or asthma-COPD overlap • Adverse effects as for ICSs and LABAs
Budesonide/formoterol	• Inhaled (MDI) (80 μg budesonide and 4.5 μg formoterol per inhalation) twice daily	
Fluticasone furoate/ vilanterol	• Inhaled (DPI) 1 inhalation (fluticasone furoate 100 μg/ vilanterol 25 μg) once daily	
Systemic Corticosteroids: Short course or oral maintenance for asthma (and COPD)		
Prednisone Prednisolone	• Oral: 40–80 mg once daily or divided dose for 3–10 days for acute exacerbation • Minimal dose for maintenance	• Prednisone converted to prednisolone in the liver • Bruising, weight gain, edema, osteoporosis, diabetes, cataracts, adrenal suppression (see Chapter 46)
Hydrocortisone succinate	• IM/IV: 100–500 mg every 12 h for acute severe asthma	• Only if patient not able to take oral steroids
Methylprednisolone	• IV: 100–1000 mg for acute severe asthma	• Rarely indicated because of steroid side effects
Antileukotrienes (Leukotriene Modifiers) for Asthma Maintenance		
Montelukast Zafirlukast Zileuton	• Oral: montelukast (10 mg once/day); zafirlukast (20 mg twice/day); zileuton (600 mg four times/day or 1200 mg twice/day)	• Less effective than ICS in asthma • Headache, Churg-Strauss syndrome • Zileuton may cause hepatic dysfunction (do not use if alanine aminotransferase increased)
Methylxanthines: Add-on maintenance treatment of severe asthma and COPD		
Theophylline (oral) Aminophylline (IV)	• Aminophylline (IV) is indicated for severe exacerbation that does not respond to nebulized β agonists; shorter action than theophylline	• Interaction with drugs that affect CYP1A2; see Table 44–2 • Nausea, headaches, diuresis, arrhythmias, seizures
Phosphodiesterase Inhibitor		
Roflumilast	• COPD • Mild asthma with mainly neutrophilic inflammation	• PDE4 inhibitor • Do not use for bronchospasm • Contraindicated in severe liver disease • Subject to interaction with inhibitors and inducers of CYP4A3

Drug Facts for Your Personal Formulary: *Asthma and COPD Therapeutics (continued)*

Drug	Therapeutic Uses	Clinical Tips
Immunomodulators (consult FDA package insert prior to use)		
Omalizumab	• Severe allergic asthma • Non-atopic asthma	• Anti-IgE • Prevents IgE activation of mast cells
Reslizumab, Mepolizumab	• ↓ severe eosinic inflammation in asthma	• mAbs; bind circulating IL-5 • Can ↓ oral corticosteroid need
Benralizumab (antibody to IL-5 receptor)	• ↓ severe eosinic inflammation in asthma	• Antibody blocks IL-5 receptor • Causes apoptosis of eosinophils
Dupilumab (antibody to IL-4/13 receptor)	• Moderate-to-severe asthma to improve lung function and reduce exacerbations	• IL-4Rα blocker • Improves co-morbidities of asthma (atopic dermatitis, rhinosinusitis)
Tezepelumab-ekko (human mAb to TSLP)	• Add-on therapy for patients 12 years and older with severe asthma; 210 mg subcutaneously every 4 weeks	• Treat any helminth infections prior to use • As treatment progresses, decrease steroids *gradually*, if appropriate • Avoid concomitatnt use of live attenuated vaccines

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Chapter 45

Hematopoietic Agents: Growth Factors, Minerals, and Vitamins

Michael Choi and Thomas J. Kipps

HEMATOPOIESIS

GROWTH FACTOR PHYSIOLOGY

ERYTHROPOIESIS-STIMULATING AGENTS

- Erythropoietin
- Sequestration of Transforming Growth Factor β Superfamily Ligands

MYELOID GROWTH FACTORS

- Granulocyte-Macrophage Colony-Stimulating Factor
- Granulocyte Colony-Stimulating Factor

THROMBOPOIETIC GROWTH FACTORS

- Interleukin-11
- Thrombopoietin

IRON DEFICIENCY AND OTHER HYPOCHROMIC ANEMIAS

- The Bioavailability of Iron
- Metabolism of Iron
- Iron Requirements; Availability of Dietary Iron
- Treatment of Iron Deficiency
- Copper, Pyridoxine, and Riboflavin

VITAMIN B_{12}, FOLIC ACID, AND THE TREATMENT OF MEGALOBLASTIC ANEMIAS

- Cellular Roles of Vitamin B_{12} and Folic Acid
- Vitamin B_{12} and Human Health

FOLIC ACID AND HUMAN HEALTH

Hematopoiesis

The finite life span of most mature blood cells requires their continuous replacement, a process termed *hematopoiesis*. New cell production must respond to basal needs and states of increased demand. Erythrocyte production can increase more than 20-fold in response to anemia or hypoxemia, leukocyte production increases dramatically in response to systemic infections, and platelet production can increase 10- to 20-fold when platelet consumption results in thrombocytopenia.

The regulation of blood cell production is complex. Hematopoietic stem cells are rare marrow cells that manifest self-renewal and lineage commitment, resulting in cells destined to differentiate into the 10 or more distinct blood cell lineages. For the most part, this process occurs in the marrow cavities of the skull, vertebral bodies, pelvis, and proximal long bones; it involves interactions among hematopoietic stem and progenitor cells and the cells and complex macromolecules of the marrow stroma and is influenced by a number of soluble and membrane-bound hematopoietic growth factors. Several hormones and cytokines have been identified and cloned that affect hematopoiesis, permitting their production in quantities sufficient for research and, in some cases, therapeutic use. Clinical applications range from the treatment of primary hematological diseases (e.g., aplastic anemia, congenital neutropenia) to use as adjuncts in the treatment of severe infections and in the management of patients with kidney failure or those undergoing cancer chemotherapy or marrow transplantation.

Hematopoiesis also requires an adequate supply of minerals (e.g., iron, cobalt, and copper) and vitamins (e.g., folic acid, vitamin B_{12}, pyridoxine, ascorbic acid, and riboflavin); deficiencies generally result in characteristic anemias or, less frequently, a general failure of hematopoiesis (Rojas-Hernandez and Oo, 2018). Therapeutic correction of a specific deficiency state depends on the accurate diagnosis of the basis for the anemia and on knowledge about the correct dose, formulation, and route of administration of the deficient mineral(s) or vitamin(s).

Growth Factor Physiology

Steady-state hematopoiesis encompasses the tightly regulated production of more than 400 billion blood cells each day. The hematopoietic organ also is unique in adult physiology in that several mature cell types are derived from a much smaller number of multipotent progenitors, which develop from a more limited number of pluripotent hematopoietic stem cells. Such cells are capable of maintaining their own number and differentiating under the influence of cellular and humoral factors to produce the large and diverse number of mature blood cells.

Our understanding of stem cell differentiation owes much to the *in vitro* culture of marrow cells. Using the results from clonal cultures in semisolid medium, stem cell differentiation can be described as a series of developmental steps that produce mixed blood cell lineage colonies, which give rise to large, immature and small, mature single-lineage burst-forming units (BFUs) and colony-forming units (CFUs), respectively, for each of the major blood cell types. These early progenitors (BFUs and CFUs) are capable of further proliferation and differentiation, increasing their number by some 30-fold. It is at this most mature stage of development that the lineage-committed growth factors (colony-stimulating factors [CSFs] for monocytes [M-CSFs] and granulocytes [G-CSFs], erythropoietin, and thrombopoietin) exert their primary proliferative and differentiative effects. Overall, proliferation and maturation of the CFU for each cell line can amplify the resulting mature cell product by another 30-fold or more, generating more than 1000 mature cells from each committed stem cell.

Hematopoietic and lymphopoietic growth factors are glycoproteins produced by a number of marrow cells and peripheral tissues. They are active at very low concentrations and typically affect more than one committed cell lineage. Most interact synergistically with other factors and stimulate production of additional growth factors, a process termed *networking*. Growth factors generally exert actions at several points in the processes of cell proliferation and differentiation and in mature cell function. However, the network of growth factors that contributes to any given cell lineage depends absolutely on a nonredundant, lineage-specific

Abbreviations

BFU: burst-forming units
CFU: colony-forming units
CFU-E: CFU erythrocyte
CFU-GM: CFU granulocyte and macrophage
CFU-Meg: CFU megakaryocyte
CH$_3$H$_4$PteGlu$_1$: methyltetrahydrofolate
CKD: chronic kidney disease
CoA: coenzyme A
CSF: colony-stimulating factor
dTMP: deoxythymidine monophosphate
dUMP: deoxyuridine monophosphate
ESA: erythropoiesis-stimulating agent
FIGLU: formiminoglutamic acid
G-CSF: granulocyte colony-stimulating factor
GI: gastrointestinal
GM-CSF: granulocyte-macrophage colony-stimulating factor
HFE: high Fe, hereditary hemochromatosis protein, hemeostatic iron regulator
HIF: hypoxia-inducible factor
HIV: human immunodeficiency virus
IL: interleukin
IRE: iron-regulating element
IRP: iron-regulating protein
ITP: immune thrombocytopenia
M-CSF: monocyte-/macrophage-stimulating factor
MDS: myelodysplastic syndromes
PBSC: peripheral blood stem cell
PteGlu: pteroylglutamic acid, folic acid
rHuMGDF: recombinant human megakaryocyte growth and development factor
SAM: *S*-adenosylmethionine
TPO: thrombopoietin
TRA: thrombopoietin receptor agonist
TGFβ: transforming growth factor β
VHL: von Hippel-Lindau

factor, such that absence of factors that stimulate developmentally early progenitors is compensated for by redundant cytokines, but loss of the lineage-specific factor leads to a specific cytopenia.

Some of the overlapping and nonredundant effects of the more important hematopoietic growth factors are illustrated in Figure 45–1 and Table 45–1.

Erythropoiesis-Stimulating Agents

Erythropoiesis-stimulating agent (ESA) is the term given to a pharmacological substance that stimulates red blood cell production.

Erythropoietin

Erythropoietin is the most important regulator of the proliferation of committed erythroid progenitors (CFU-E, colony forming units, erythrocyte) and their immediate progeny. In its absence, severe anemia is invariably present, commonly seen in patients with renal failure. Erythropoiesis is controlled by a feedback system in which a sensor in the kidney detects changes in oxygen delivery to modulate the erythropoietin secretion. The sensor mechanism is now understood at the molecular level (Haase, 2010).

Hypoxia-inducible factor (HIF), a heterodimeric (HIF-1α and HIF-1β) transcription factor, enhances expression of multiple hypoxia-inducible genes, such as vascular endothelial growth factor and erythropoietin. HIF-1α is labile due to its prolyl hydroxylation and subsequent polyubiquitination and degradation, aided by the von Hippel-Lindau (VHL) protein. During states of hypoxia, the prolyl hydroxylase is inactive, allowing the accumulation of HIF-1α and activating erythropoietin expression, which in turn stimulates rapid expansion of erythroid progenitors. Specific alteration of VHL leads to an oxygen-sensing defect, characterized by constitutively elevated levels of HIF-1α and erythropoietin, with resultant polycythemia (Gordeuk et al., 2004). A second isoform of HIF, HIF-2α, is

HISTORICAL PERSPECTIVE

Modern concepts of hematopoietic cell growth and differentiation derive from experiments done in the 1950s. Till and McCulloch demonstrated that individual hematopoietic cells could form macroscopic hematopoietic colonies in the spleens of irradiated mice, thereby establishing the concept of discrete hematopoietic stem cells (i.e., the presence of a multilineage clonal splenic colony appearing 11 days after transplantation implied that a single cell lodged and expanded into several cell lineages). This concept now has been expanded to include normal human marrow cells. Moreover, such cells now can be prospectively identified.

The basis for identifying soluble growth factors was provided by Sachs and independently by Metcalf, who developed clonal, *in vitro* assays for hematopoietic progenitor cells. Such hematopoietic colonies first developed only in the presence of conditioned culture medium from leukocytes or tumor cell lines. Individual growth factors then were isolated based on their activities in clonal *in vitro* assays, assays that were instrumental in purifying a hierarchy of progenitor cells committed to individual and combinations of mature blood cells (Kondo et al., 2003).

In 1906, Paul Carnot postulated the existence of a circulating growth factor that controls red blood cell development. He observed an increase in the red cell count in rabbits injected with serum obtained from anemic animals and postulated the existence of a factor that he called hemopoietin. Only in the 1950s did Reissmann, Erslev, and Jacobsen and coworkers define the origin and actions of the hormone, now called erythropoietin. Subsequently, extensive studies of erythropoietin were carried out in patients with anemia and polycythemia, leading to the purification of erythropoietin from urine and the subsequent cloning of the erythropoietin gene. The high-level expression of erythropoietin in cell lines has allowed for its purification and use in humans with anemia.

Similarly, the existence of specific leukocyte growth factors was suggested by the capacity of different conditioned culture media to induce the *in vitro* growth of colonies containing different combinations of granulocytes and monocytes. An activity that stimulated the production of both granulocytes and monocytes was purified from murine lung-conditioned medium, leading to cloning of granulocyte-macrophage colony-stimulating factor (GM-CSF), first from mice (Gough et al., 1984) and subsequently from humans (Wong et al., 1985). Finding an activity that stimulated the exclusive production of neutrophils permitted the cloning of granulocyte colony-stimulating factor (G-CSF) (Welte et al., 1985). Subsequently, a megakaryocyte colony-stimulating factor termed thrombopoietin was purified and cloned (Kaushansky, 1998).

Growth factors that support lymphocyte growth were identified using assays that measured the capacity of the cytokine to promote lymphocyte proliferation *in vitro*. This permitted the identification of the growth-promoting properties of IL-7, IL-4, or IL-15 for all lymphocytes, B cells, or NK (natural killer) cells, respectively (Goodwin et al., 1989; Grabstein et al., 1994). Recombinant expression of these complementary DNAs permitted production of sufficient quantities of biologically active growth factors for clinical investigations, allowing for the demonstration of the potential clinical utility of such factors.

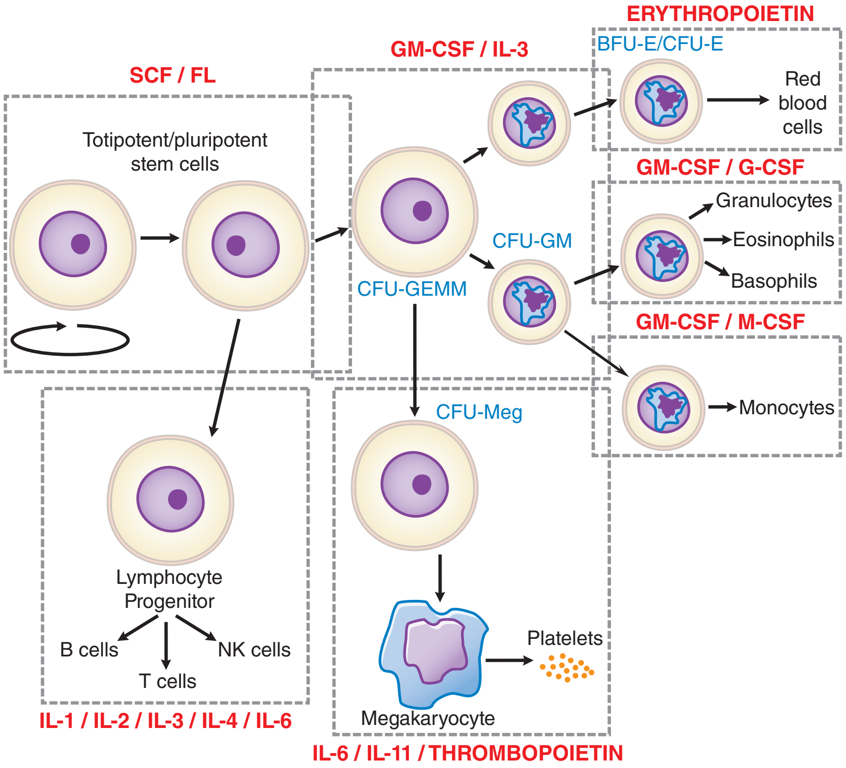


Figure 45–1 *Sites of action of hematopoietic growth factors in the differentiation and maturation of marrow cell lines.* A self-sustaining pool of marrow stem cells differentiates under the influence of specific hematopoietic growth factors to form a variety of hematopoietic and lymphopoietic cells. SCF, FL, IL-3, and GM-CSF, together with cell-cell interactions in the marrow, stimulate stem cells to form a series of BFUs and CFUs: CFU-GEMM, CFU-GM, CFU-Meg, BFU-E, and CFU-E. After considerable proliferation, further differentiation is stimulated by synergistic interactions with growth factors for each of the major cell lines—G-CSF, M-CSF, thrombopoietin, and erythropoietin. Each of these factors also influences the proliferation, maturation, and in some cases the function of the derivative cell line (Table 45–1). BFU-E, burst forming unit, erythrocyte; CFU-GEMM, colony-forming unit, granulocyte, erythrocyte, monocyte, and megakaryocyte; FL, FLT3 (FMS tyr kinase 3) ligand; SCF, stem cell factor.

an important regulator of the expression of genes that contribute to iron absorption (Mastrogiannaki et al., 2013); a genetic gain-of-function mutation in HIF-2α also induces erythrocytosis in patients (Percy et al., 2008).

Erythropoietin is expressed primarily in peritubular interstitial cells of the kidney. Erythropoietin contains 193 amino acids, of which the first 27 are cleaved during secretion. The final hormone is heavily glycosylated and has a molecular mass of about 30 kDa. After secretion, erythropoietin binds to a receptor on the surface of committed erythroid progenitors in the marrow and is internalized. With anemia or hypoxemia, synthesis rapidly increases by 100-fold or more, serum erythropoietin levels rise, and marrow progenitor cell survival, proliferation, and maturation are dramatically stimulated. This finely tuned feedback loop can be disrupted by kidney disease, marrow damage, or a deficiency in iron or an essential vitamin. With an infection or an inflammatory state, erythropoietin secretion, iron delivery, and progenitor proliferation all are suppressed by inflammatory cytokines, but this accounts for only part of the resultant anemia; interference with iron metabolism also is a result of inflammatory mediator actions on the hepatic protein hepcidin (Drakesmith and Prentice, 2012). Loss of hepcidin-producing liver mass or genetic or acquired conditions that repress hepcidin production by the liver may lead to iron overload (Pietrangelo, 2016).

Preparations

Preparations of recombinant human erythropoietin include *epoetin alfa, epoetin beta, epoetin omega*, and *epoetin zeta*, which differ almost exclusively in carbohydrate modifications due to manufacturing differences and are supplied in single-use vials or syringes containing 500 to 40,000 units for intravenous or subcutaneous administration. When injected intravenously, epoetin alfas are cleared from plasma with a $t_{1/2}$ of 4 to 8 h. However, the effect on marrow progenitors lasts much longer, and once-weekly dosing can be sufficient to achieve an adequate response. An engineered epoetin alfa, *darbepoetin*, which displays a longer circulatory half-life, is also available for use in patients with indications similar to those for other epoetins. Based on phage display technology, small peptide agonists of the erythropoietin receptor were identified and developed into clinical agents by coupling to polyethylene glycol. One such erythropoiesis-stimulating peptide, *peginesatide*, was approved for the treatment of anemia due to chronic kidney disease (CKD); postmarketing reports of serious hypersensitivity reactions and anaphylaxis necessitated its removal from the market.

Recombinant human erythropoietin (*epoetin alfa*) is nearly identical to the endogenous hormone. The carbohydrate modification pattern of *epoetin alfa* differs slightly from the native protein, but this difference apparently does not alter kinetics, potency, or immunoreactivity of the drug. Modern assays can detect these differences and thereby identify athletes who use the recombinant product for "blood doping."

Therapeutic Uses, Monitoring, and Adverse Effects

Recombinant erythropoietin therapy, in conjunction with adequate iron intake, can be highly effective in a number of anemias, especially those associated with poor erythropoietic response. *Epoetin alfa* is effective in the treatment of anemias associated with surgery, AIDS, cancer chemotherapy, prematurity, and certain chronic inflammatory conditions. *Darbepoetin alfa* also has been approved for use in patients with anemia associated with CKD. A Cochrane analysis could not demonstrate the superiority of one form of ESA over any another.

During erythropoietin therapy, absolute or functional iron deficiency may develop. Functional iron deficiency (i.e., normal ferritin levels but low transferrin saturation) presumably results from the inability to mobilize iron stores rapidly enough to support the increased erythropoiesis. Supplemental iron therapy is recommended for all patients whose serum ferritin is less than 100 μg/L or whose serum transferrin saturation

TABLE 45–1 ■ HEMATOPOIETIC GROWTH FACTORS

Erythropoietin (EPO)

- Stimulates proliferation and maturation of committed erythroid progenitors to increase red cell production

Stem cell factor (SCF, c-kit ligand, Steel factor) and FLT 3 ligand (FL)

- Act synergistically with a wide range of other colony-stimulating factors and interleukins to stimulate pluripotent and committed stem cells
- FL also stimulates both dendritic and natural killer (NK) cells (antitumor response)
- SCF also stimulates mast cells and melanocytes

Interleukins

IL-1, IL-3, IL-5, IL-6, IL-9, and IL-11

- Act synergistically with each other and SCF, GM-CSF, G-CSF, and EPO to stimulate BFU erythrocytes (BFU-E), CFU-GEMM, CFU-GM, CFU-E, and CFU-Meg growth
- Numerous immunological roles, including stimulation of B-cell and T-cell growth

IL-5

- Controls eosinophil survival and differentiation

IL-6; IL-6 and IL-11

- IL-6 stimulates human myeloma cells to proliferate
- IL-6 and IL-11 stimulate BFU megakaryocytes to increase platelet production

IL-1, IL-2, IL-4, IL-7, and IL-12

- Stimulate growth and function of T cells, B cells, NK cells, and monocytes
- Co-stimulate B, T, and lymphokine-activated killer (LAK) cells

IL-8 and IL-10

- Numerous immunological activities involving B- and T-cell functions
- IL-8 acts as a chemotactic factor for basophils and neutrophils

Granulocyte-macrophage colony-stimulating factor (GM-CSF)

- Acts synergistically with SCF, IL-1, IL-3, and IL-6 to stimulate CFU-GM and CFU-Meg to increase neutrophil and monocyte production
- With EPO may promote BFU-E formation
- Enhances migration, phagocytosis, superoxide production, and antibody-dependent cell-mediated toxicity of neutrophils, monocytes, and eosinophils
- Prevents alveolar proteinosis

Granulocyte colony-stimulating factor (G-CSF)

- Stimulates CFU granulocytes to increase neutrophil production
- Enhances phagocytic and cytotoxic activities of neutrophils

Monocyte/macrophage colony-stimulating factor (M-CSF, CSF-1)

- Stimulates CFU macrophages (CFU-Ms) to increase monocyte precursors
- Activates and enhances function of monocyte/macrophages

Macrophage colony-stimulating factor (M-CSF)

- Stimulates CFU-M to increase monocyte/macrophage precursors
- Acts in concert with tissues and other growth factors to determine the proliferation, differentiation, and survival of a range of cells of the mononuclear phagocyte system

Thrombopoietin (TPO, *Mpl* ligand)

- Stimulates the self-renewal and expansion of hematopoietic stem cells
- Stimulates stem cell differentiation into megakaryocyte progenitors
- Selectively stimulates megakaryocytopoiesis to increase platelet production
- Acts synergistically with other growth factors, especially IL-6 and IL-11

is below 20%. During initial therapy and after any dosage adjustment, the hematocrit is determined once a week (patients infected with HIV [human immunodeficiency virus] and those with cancer) or twice a week (patients with renal failure) until it has stabilized in the target range and the maintenance dose has been established; the hematocrit then is monitored at regular intervals. If the hematocrit increases by more than 4 points in any 2-week period, the dose should be decreased. Due to the time required for erythropoiesis and the erythrocyte half-life, hematocrit changes lag behind dosage adjustments by 2 to 6 weeks. The dose of *darbepoetin* should be decreased if the hemoglobin increase exceeds 1 g/dL in any 2-week period because of the association of excessive rate of rise of hemoglobin with adverse cardiovascular events.

The use of ESAs is associated with an increased risk of thrombosis. During hemodialysis, patients receiving *epoetin alfa* or *darbepoetin* may require increased anticoagulation. The risk of thrombotic events is higher in adults with ischemic heart disease or congestive heart failure receiving *epoetin alfa* therapy with the goal of reaching a normal hematocrit (42%) than in those with a lower target hematocrit of 30% (Bennett et al., 2008). ESA use is associated with increased rates of cancer recurrence and decreased on-study survival in patients in whom the drugs are administered for cancer-induced or for chemotherapy-induced anemia (Bohlius et al., 2009). Due to those concerns, the FDA issued "black-box" warnings regarding increased risk of death, serious adverse cardiovascular reactions, and stroke when ESAs were administered to target a final hemoglobin concentrations near normal physiological levels. In June 2011, the FDA specified that ESAs should not be used to increase hemoglobin to concentrations above 11 g/dL.

The most common side effect of *epoetin alfa* therapy is aggravation of hypertension, which occurs in 20% to 30% of patients and most often is associated with a rapid rise in hematocrit levels. Hypertensive encephalopathy and seizures have occurred in patients with chronic renal failure treated with *epoetin alfa*. For this reason, ESAs should not be used in patients with pre-existing uncontrolled hypertension. Patients may require initiation of, or increases in, antihypertensive therapy. Headache, tachycardia, edema, shortness of breath, nausea, vomiting, diarrhea, injection site stinging, and flu-like symptoms (e.g., arthralgias and myalgias) also have been reported in conjunction with *epoetin alfa* therapy.

Anemia of Chronic Renal Failure

Patients with anemia secondary to CKD are ideal candidates for *epoetin alfa* therapy as the disease represents a true hormone deficiency state. ESA use for patients with CKD can reduce the need for red cell transfusions. Dosing is individualized to the lowest dose necessary to reduce the need for transfusions. The response in predialysis, peritoneal dialysis, and hemodialysis patients depends on the severity of the renal failure, the erythropoietin dose and route of administration, and iron availability (Besarab et al., 1999; Kaufman et al., 1998). The subcutaneous route of administration is preferred over the intravenous route because absorption is slower and the amount of drug required is reduced by 20% to 40%, although the intravenous route is typically used for patients who already are on hemodialysis.

Patients with a hemoglobin of less than 10 g/dL are started on doses of 50 to 100 units/kg of *epoetin alfa*, given subcutaneously, three times a week. Patients who are not on dialysis can receive 10,000 to 20,000 units every other week. The dose of *epoetin alfa* should be adjusted to obtain a gradual rise in the hematocrit over a 2- to 4-month period to a final hemoglobin of less than 11 g/dL. Treatment to hemoglobin levels greater than 11 g/dL is not recommended. The final maintenance dose of *epoetin alfa* can vary from 10 units/kg to more than 300 units/kg, with an average dose of 75 units/kg, three times a week. Children less than 5 years of age generally require a higher dose. Resistance to therapy is common in patients who develop an inflammatory illness or become iron deficient, so close monitoring of general health, iron status, or other causes of anemia is essential. Less-common causes of resistance include occult blood loss, folic acid deficiency, carnitine deficiency, inadequate dialysis, aluminum toxicity, or osteitis fibrosa cystica secondary to hyperparathyroidism. ESA therapy is typically discontinued if a patient does respond over a 12-week escalation period, as further increases in dose may increase the risks without improving the benefit of therapy.

Darbepoetin alfa is approved for use in patients who are anemic secondary to CKD. The recommended starting dose is 0.45 μg/kg administered intravenously or subcutaneously once weekly or 0.75 μg/kg administered every 2 weeks, with dose adjustments depending on the response. As with *epoetin alfa*, side effects tend to occur when patients experience a rapid rise in hemoglobin concentration; a rise of less than 1 g/dL every 2 weeks generally is considered safe.

Anemia in Patients With AIDS

Epoetin alfa therapy has been approved for the treatment of HIV-infected patients with anemia due to *zidovudine* therapy (Fischl et al., 1990). Excellent responses to doses of 100 to 300 units/kg, given subcutaneously three times a week, generally are seen in patients with *zidovudine*-induced anemia. However, a more recent analysis of erythropoietin therapy in patients with HIV infection failed to support its routine use (Martí-Carvajal et al., 2011). The reason for the difference between 1990 and 2011 may reflect the more effective therapy for HIV in the highly active antiretroviral therapy era, such that the origin of anemia in HIV-infected individuals today is different from what it was at the onset of the AIDS epidemic. Accordingly, ESAs are not commonly used in HIV-infected individuals, except for those who have therapy indications similar to those without HIV infection, such as anemia associated with renal failure.

Anemia Associated With Hematological Malignancy

Guidelines support the use of recombinant erythropoietin in patients with low-grade myelodysplastic syndrome. In this setting, neutropenia often dictates the use of G-CSF, which frequently augments the erythroid response to erythropoietin. In responding patients, the response duration is usually 2 to 3 years. A baseline serum erythropoietin level may help to predict the response; most patients with blood levels greater than 500 IU/L are unlikely to respond to any dose of the drug. Most patients treated with *epoetin alfa* experience an improvement in their anemia and their sense of well-being (Littlewood et al., 2001).

Anemia in Patients With Cancer Undergoing Chemotherapy

Epoetin alfa therapy, 150 units/kg three times a week or 450 to 600 units/kg once a week, can reduce the transfusion requirement in patients with cancer undergoing chemotherapy as well as lead to reduced anemia-related symptoms. Previous therapeutic guidelines (Rizzo et al., 2002) recommended the use of *epoetin alfa* in patients with chemotherapy-associated anemia when hemoglobin levels fall below 10 g/dL, basing the decision to treat less-severe anemia (hemoglobin 10–12 g/dL) on clinical circumstances. Following these recommendations, case reports suggested a direct effect of both *epoetin alfa* and *darbepoetin alfa* in stimulation of tumor cells. A meta-analysis of a large number of patients and clinical trials estimated the risk of death at about 10% higher than for cancer patients who were not treated with these agents (Bohlius et al., 2009). Based on these results, new guidelines were issued (Rizzo et al., 2010) and subsequently updated (Bohlius et al., 2019). ESAs may be offered to patients with symptomatic chemotherapy-associated anemia with hemoglobin values of less than 10 g/dL who cannot tolerate or refuse red blood cell transfusion and whose cancer treatment is palliative. Again, the goal of ESA treatment should not be to normalize the hemoglobin values, but to mitigate symptoms of anemia that typically associate with hemoglobin values of less than or equal to 10 g/dL. Treatment with iron may be necessary to improve the response to ESAs for patients with low serum ferritin.

Use in Perioperative Patients

Epoetin alfa has been used perioperatively to treat anemia (hematocrit 30%–36%) and reduce the need for allogeneic erythrocyte transfusion in nonanemic patients during and following surgery in patients with moderate or large anticipated blood loss. Patients undergoing elective orthopedic and cardiac procedures have been treated with 150 to 300 units/kg of *epoetin alfa* once daily for the 10 days preceding surgery, on the day of surgery, and for 4 days after surgery. As an alternative, 600 units/kg can be given on days 21, 14, and 7 before surgery, with an additional dose on the day of surgery. ESAs are used in this manner to correct anemia in patients who cannot receive transfusions. Most Jehovah's Witnesses who refuse red blood cell transfusions find it acceptable to receive recombinant *epoetin alfa*.

Other Uses

Epoetin alfa has been evaluated for the treatment of the anemia of prematurity, in which impaired erythropoietin production is central to the pathogenesis. However, *epoetin alfa* appears to have limited efficacy in decreasing the number of blood donors to which the infant is exposed; a more effective approach is to limit the amount of blood drawn from the infant and to use satellite packs that divide one unit of blood into smaller aliquots that can be given at successive intervals. This approach mitigates the risk of therapy. In this regard, it should be noted that administration of *epoetin alfa* to premature infants was associated with an increased incidence of retinopathy of prematurity in one retrospective study (Ohlsson and Aher, 2020).

Competitive athletes have used *epoetin alfa* to increase their hemoglobin levels ("blood doping") in an attempt to improve their athletic performance. Unfortunately, this misuse of the drug has been implicated in the deaths of several athletes and is strongly discouraged.

Sequestration of Transforming Growth Factor β Superfamily Ligands

Transforming growth factor β (TGFβ) superfamily ligands are soluble growth factors that regulate hematopoiesis. Canonical signaling by TGFβ family ligand-receptor complexes is transduced by SMAD proteins, which inhibit hematopoietic stem cell proliferation. Under physiological conditions, erythroid progenitor cells produce ligands such as activins and growth differentiation factors (GDFs), which inhibit terminal erythroid differentiation by induction of apoptosis and cell-cycle arrest in erythroblasts, in a tightly regulated feedback loop. However, in disorders of ineffective erythropoiesis, such as thalassemia and myelodysplastic syndrome, there is a constitutive increase of SMAD2 and SMAD3 signaling, which inhibits red cell maturation (Figure 45–2).

Luspatercept is a recombinant fusion protein that consists of a human IgG1 Fc domain linked with a modified extracellular domain of the activin receptor 2B (ActRIIB), which binds and traps select TGFβ family ligands. *Luspatercept* binds GDF8, GDF11, and activin B; has lower affinity for activin A; and does not bind TGFβ1, TGFβ2, or TGFβ3. This pattern of ligand trapping decreases SMAD2 and SMAD3 signaling, enabling erythroblast differentiation and erythroid maturation, with fewer nonhematological effects (Kubasch et al., 2021).

Therapeutic Uses, Monitoring, and Adverse Effects

Beta Thalassemia. *Luspatercept* is indicated for adult patients with beta thalassemia who require regular red cell transfusions (Capellini et al., 2020; Piga et al., 2019). Approximately 20% of patients had at least a 33% reduction in transfusion burden during weeks 13 to 24 of treatment (the primary endpoint of the phase III clinical trial), compared to 4.5% of patients randomized to placebo. Eleven percent of patients achieved transfusion independence or at least an 8-week interval. Of patients initially randomized to *luspatercept*, approximately 70% were still receiving treatment and experiencing a reduction in transfusion burden at the end of a 2-year period (prior to planned unmasking and crossover).

Thromboembolic events occurred in 3.6% of patients treated with *luspatercept* (compared to 0.9% of patients in the placebo group). All events were in patients who had undergone splenectomy and had other risk factors for thromboembolic disease (e.g., prior venous thrombosis). It is recommended to consider thromboprophylaxis in patients at increased risk and to monitor all patients for signs and symptoms of thromboembolic disease. Other adverse events include transient bone pain, arthralgias, dizziness, hypertension, and hyperuricemia.

The starting dose of *luspatercept* is 1 mg/kg once every 3 weeks by subcutaneous injection by a healthcare professional. It is recommended to delay dosing for hemoglobin levels of 11.5 g/dL or greater (if not influenced

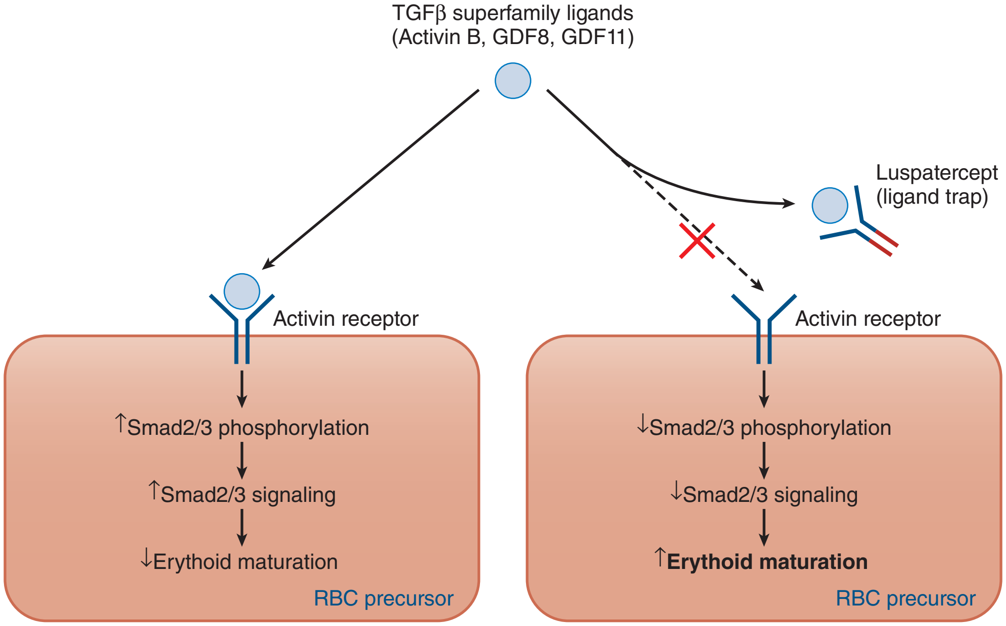


Figure 45–2 *Mechanism of action of luspatercept.* Erythroid development is regulated by opposing inhibitory and stimulatory influences of growth factors, transduced by various members of the SMAD family. Several ligands of the TGFβ superfamily can bind to and activate activin receptors, resulting in the phosphorylation of SMAD2/3. Phospho-SMAD2/3 is an inhibitory signal for erythroid maturation. In myelodysplastic syndromes, there is an imbalance of the usual regulation: The SMAD2/3 signaling pathway in erythroid cells is overactive, impairing erythroid maturation and resulting in anemia (**left panel**). *Luspatercept* is a fusion protein consisting of a modified activin receptor and the Fc domain of human IgG1. *Luspatercept* sequesters activin B, GDF8, and GDF11 (members of the TGFβ superfamily), preventing their interaction with the activin receptor, thereby reducing SMAD2/3 signaling and dis-inhibiting and promoting erythroid maturation (**right panel**). GDF, growth differentiation factor; RBC, red blood cell.

by recent transfusion). Patients who do not achieve a reduction in red blood cell transfusion burden after at least 6 weeks may increase the dose to 1.25 mg/kg, which is the maximum dose. Patients should discontinue use of *luspatercept* if they do not have a decrease in transfusion burden after 9 weeks of treatment at this maximum dose or if there is unacceptable toxicity.

Myelodysplastic Syndromes. The FDA has approved use of *luspatercept* for the treatment of adult patients with myelodysplastic syndromes (MDS), specifically those with very low- to intermediate-risk MDS with ring sideroblasts or myelodysplastic/myeloproliferative neoplasms with ring sideroblasts and thrombocytosis, with anemia despite ESA and requiring 2 or more red blood units over an 8-week period. In a phase III trial, the primary response rate (defined as transfusion independence for 8 weeks or longer) was 38%. Approximately 70% of patients had an increase in hemoglobin of at least 1.5 g/dL (Fenaux et al., 2020).

The most common side effects of *luspatercept* reported by patients with MDS include mild fatigue, diarrhea, asthenia, nausea, dizziness, and back pain. Thromboembolism has not been associated with *luspatercept* in patients with MDS. To date, the use of *luspatercept* has not been associated with an increased risk of progression to acute myeloid leukemia. However, long-term follow-up is ongoing, as patients within this subgroup of MDS typically have only a low risk of progression to acute myeloid leukemia.

The recommended starting dose for patients with MDS is 1 mg/kg once every 3 weeks by subcutaneous injection. The dose may be increased up to 1.75 mg/kg if patients are not red blood cell transfusion–free. Specific dose modification for response is provided in the prescribing information.

Myeloid Growth Factors

The myeloid growth factors are glycoproteins that stimulate the proliferation and differentiation of one or more myeloid cell types. Recombinant forms of several growth factors have been produced, including GM-CSF, G-CSF, interleukin-3 (IL-3), M-CSF or CSF-1, and stem cell factor (see Table 45–1), although only G-CSF and GM-CSF have found meaningful clinical applications.

Myeloid growth factors are produced naturally by a number of different cells, including fibroblasts, endothelial cells, macrophages, and T cells (Figure 45–3). These factors are active at extremely low concentrations and act via membrane receptors of the cytokine receptor superfamily to activate the Jak/STAT signal transduction pathway. GM-CSF can stimulate proliferation, differentiation, and function of a number of the myeloid cell lineages (see Figure 45–1). It acts synergistically with other growth factors, including erythropoietin, at the level of the BFU. GM-CSF stimulates CFU-GM (CFU granulocyte and macrophage), CFU-M (CFU macrophage), CFU-E, and CFU-Meg (CFU megakaryocyte) to increase cell production. GM-CSF also enhances the migration, phagocytosis,

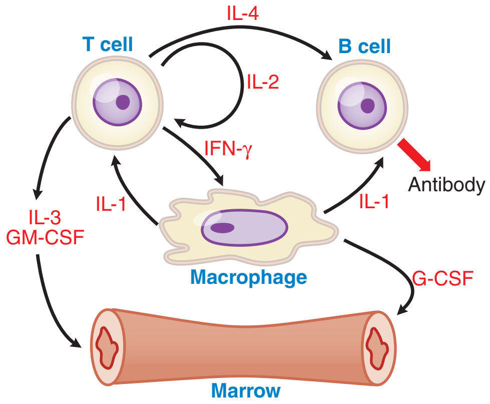


Figure 45–3 *Cytokine-cell interactions.* Macrophages, T cells, B cells, and marrow stem cells interact via several cytokines (IL-1, IL-2, IL-3, IL-4, interferon [IFN] γ, GM-CSF, and G-CSF) in response to a bacterial or a foreign antigen challenge. See Table 45–1 for the functional activities of these various cytokines.

superoxide production, and antibody-dependent cell-mediated toxicity of neutrophils, monocytes, and eosinophils (Weisbart et al., 1987).

The activity of G-CSF is restricted to neutrophils and their progenitors, stimulating their proliferation, differentiation, and function. It acts primarily on CFU-G (CFU granulocyte), although it also can play a synergistic role with IL-3 and GM-CSF in stimulating other cell lines. G-CSF enhances phagocytic and cytotoxic activities of neutrophils. G-CSF reduces inflammation by inhibiting IL-1, tumor necrosis factor, and interferon γ. G-CSF also mobilizes primitive hematopoietic cells, including hematopoietic stem cells, from the marrow into the peripheral blood (Sheridan et al., 1992). This observation has virtually transformed the practice of stem cell transplantation, such that more than 90% of all such procedures today use G-CSF–mobilized peripheral blood cells as the donor stem cell product.

Granulocyte-Macrophage Colony-Stimulating Factor

Recombinant human GM-CSF (*sargramostim*) is a glycoprotein with 127 amino acids. The primary therapeutic effect of *sargramostim* is the stimulation of myelopoiesis.

The initial clinical application of *sargramostim* was in patients undergoing autologous marrow transplantation. By shortening the duration of neutropenia, transplant morbidity was significantly reduced without a change in long-term survival or risk of inducing an early relapse of the malignant process (Brandt et al., 1988).

The role of GM-CSF therapy in allogeneic transplantation is less clear. Its effect on neutrophil recovery is less pronounced in patients receiving prophylactic treatment of graft-versus-host disease. However, it may improve survival in transplant patients who exhibit early graft failure (Nemunaitis et al., 1990).

It also has been used to mobilize CD34-positive progenitor cells for peripheral blood stem cell (PBSC) collection for transplantation after myeloablative chemotherapy (Haas et al., 1990). *Sargramostim* has been used to shorten the period of neutropenia and reduce morbidity in patients receiving intensive cancer chemotherapy (Gerhartz et al., 1993). It also stimulates myelopoiesis in some patients with cyclic neutropenia, myelodysplasia, aplastic anemia, or AIDS-associated neutropenia.

Sargramostim is administered by subcutaneous injection or slow intravenous infusion at doses of 125 to 500 μg/m^2 per day. Plasma levels of GM-CSF rise rapidly after subcutaneous injection and then decline with a $t_{1/2}$ of 2 to 3 h. When given intravenously, infusions should be maintained over 3 to 6 h. With the initiation of therapy, there is a transient decrease in the absolute leukocyte count secondary to cell margination and pulmonary vascular sequestration. This is followed by a dose-dependent, biphasic increase in leukocyte counts over the next 7 to 10 days. Once the drug is discontinued, the leukocyte count returns to baseline within 2 to 10 days. When GM-CSF is given in lower doses, the response is primarily neutrophilic, whereas monocytosis and eosinophilia are observed at larger doses. After hematopoietic stem cell transplantation or intensive chemotherapy, *sargramostim* is given daily during the period of maximum neutropenia until a sustained rise in the granulocyte count is observed. Frequent blood counts are essential to avoid an excessive rise in the granulocyte count. Higher doses are associated with more pronounced side effects, including bone pain, malaise, flu-like symptoms, fever, diarrhea, dyspnea, and rash. An acute reaction to the first dose, characterized by flushing, hypotension, nausea, vomiting, and dyspnea, with a fall in arterial oxygen saturation due to granulocyte sequestration in the pulmonary circulation, occurs in sensitive patients. With prolonged administration, a few patients may develop a capillary leak syndrome, with peripheral edema and pleural and pericardial effusions. Other serious side effects include transient supraventricular arrhythmia, dyspnea, and elevation of serum creatinine, bilirubin, and hepatic enzymes.

Granulocyte Colony-Stimulating Factor

Recombinant human G-CSF, *filgrastim*, is a glycoprotein with 175 amino acids. The principal action of *filgrastim* is the stimulation of CFU-G to increase neutrophil production (see Figure 45–1). Several forms of G-CSF are now available, including two longer-acting pegylated forms (*pegfilgrastim* and *lipegfilgrastim*), *tbo-filgrastim,* and biosimilar *filgrastim-sndz*. The biosimilar approval pathway had not been established at the time of *tbo-filgrastim*'s Biologics License Application, but its pharmacokinetic parameters, safety, and efficacy do not differ substantially from those of *filgrastim*.

Filgrastim is effective in the treatment of severe neutropenia after autologous hematopoietic stem cell transplantation and high-dose cancer chemotherapy (Lieschke and Burgess, 1992). Like GM-CSF, *filgrastim* shortens the period of severe neutropenia and reduces morbidity secondary to bacterial and fungal infections (Hammond et al., 1989). G-CSF also is effective in the treatment of severe congenital neutropenias. *Filgrastim* therapy can improve neutrophil counts in some patients with myelodysplasia or marrow damage (moderately severe aplastic anemia or tumor infiltration of the marrow). The neutropenia of patients with AIDS receiving *zidovudine* also can be partially or completely reversed.

Filgrastim is routinely used in patients undergoing PBSC collection for stem cell transplantation. It promotes the release of CD34$^+$ progenitor cells from the marrow, reducing the number of collections necessary for transplant. G-CSF–induced mobilization of stem cells into the circulation also has the potential to enhance repair of other damaged organs in which PBSCs might play a role. PBSC grafts have a higher cell dose and somewhat more committed progenitor cells than steady-state marrow grafts, resulting in faster engraftment and faster immunological reconstitution.

Filgrastim is administered by subcutaneous injection or intravenous infusion (over at least 30 min) at doses of 1 to 20 μg/kg per day. The usual starting dose in a patient receiving myelosuppressive chemotherapy is 5 μg/kg per day, rounded to the nearest vial size, for example 300 or 480 μg daily. The distribution and clearance rate from plasma ($t_{1/2}$ of 3.5 h) are similar for both routes of administration. As with GM-CSF therapy, G-CSF administered after hematopoietic stem cell transplantation or intensive cancer chemotherapy will increase granulocyte production and shorten the period of severe neutropenia. Frequent blood cell counts should be obtained to determine the effectiveness of the treatment and guide dosage adjustment. In patients who received intensive myelosuppressive cancer chemotherapy, daily administration of G-CSF for 14 to 21 days or more may be necessary to correct the neutropenia.

A prolonged half-life allows for single dose administration of *pegfilgrastim*, rather than daily administration. The recommended dose for *pegfilgrastim* is fixed at 6 mg for patients weighing more than 20 kg, administered subcutaneously once per chemotherapy cycle. Due to potential sensitivity of dividing myeloid cells to cytotoxic chemotherapy, *pegfilgrastim* is typically administered 24 h after chemotherapy and at least 14 days prior to the next planned chemotherapy dose (Lyman et al., 2017).

Adverse Reactions

Adverse reactions to *filgrastim* include mild-to-moderate bone pain in patients receiving high doses over a protracted period, local skin reactions following subcutaneous injection, and rare cutaneous necrotizing vasculitis. Patients with a history of hypersensitivity to proteins produced by *Escherichia coli* should not receive the drug; the same holds for patients with sickle cell anemia, as it has been known to precipitate severe crises and even death. Mild-to-moderate splenomegaly has been observed in patients on long-term therapy.

In 2004 and 2006, two papers were published suggesting that previously healthy stem cell donors receiving human G-CSF for mobilization displayed marrow cell changes concerning for the development of future malignancy. Previous studies have shown an increase in myeloid leukemia in patients with breast cancer receiving G-CSF for neutropenia. However, careful follow-up has failed to reveal any meaningful increase in myeloid leukemia in normal stem cell donors administered G-CSF.

Selecting From Available Preparations

There are limited comparative data from randomized controlled trial and insufficient data to recommend one CSF over others for primary prevention of febrile neutropenia. In practice, most institutions use G-CSF rather than GM-CSF. Guidelines from the American Society of Clinical Oncology support the use of all G-CSF preparations, including biosimilars (Smith et al., 2015).

Thrombopoietic Growth Factors

Interleukin-11

Interleukin-11 (IL-11) is a cytokine that stimulates hematopoiesis, intestinal epithelial cell growth, and osteoclastogenesis and inhibits adipogenesis. IL-11 also enhances megakaryocyte maturation *in vitro*. Recombinant human IL-11, *oprelvekin* ($t_{1/2}$ ~7 h), leads to a thrombopoietic response in 5 to 9 days when administered daily to normal subjects.

The drug is administered to patients at 25 to 50 µg/kg per day subcutaneously. *Oprelvekin* is approved for use in patients undergoing chemotherapy for nonmyeloid malignancies with severe thrombocytopenia (platelet count <20 × 10^9/L), and it is administered until the platelet count returns to more than 100 × 10^9/L. The major complications of therapy are fluid retention and associated cardiac symptoms, such as tachycardia, palpitation, edema, and shortness of breath; this is a significant concern in elderly patients and often requires concomitant therapy with diuretics. Also reported are blurred vision, injection site rash or erythema, and paresthesias.

Thrombopoietin

Thrombopoietin (TPO), a glycoprotein produced by the liver, marrow stromal cells, and other organs, is the primary regulator of platelet production. Two forms of recombinant TPO have been tested for clinical use. One is a truncated version of the native protein, termed recombinant human megakaryocyte growth and development factor (rHuMGDF), that is covalently modified with polyethylene glycol to increase the circulatory $t_{1/2}$. The second is the full-length polypeptide termed recombinant human thrombopoietin (rHuTPO). While use in thrombocytopenic clinical trial subjects was found to be safe, the use of rHuMGDF in a clinical trial of normal platelet donors, designed to boost the quantity of donated platelets, led to donor thrombocytopenia in several subjects due to the immunogenicity of this agent (Li et al., 2001). This experience led to both agents being abandoned for clinical use and to the development of small molecular mimics of recombinant TPO, termed thrombopoietin receptor agonists (TPO-RA or TRA), also called TPO mimetics.

Thrombopoietin Receptor Agonists

Thrombopoietin receptor agonists (TRAs) stimulate production of megakaryocytes and platelets in the marrow by activating the TPO receptor. TRAs are FDA-approved for use in patients with immune thrombocytopenia (ITP), and several are approved for patients with liver disease scheduled to undergo surgery. *Romiplostim* contains four copies of a small peptide that binds with high affinity to the TPO receptor, grafted onto an immunoglobulin scaffold. *Romiplostim* is safe and efficacious in patients with ITP (Kuter et al., 2008). The drug is administered weekly by subcutaneous injection, starting with a dose of 1 µg/kg, titrated to a maximum of 10 µg/kg, until the platelet count increases above 50 × 10^9/L.

The other approved agents are considered nonpeptide TRAs, derived from small-molecule screens for chemicals that bind to and activate the TPO receptor. *Eltrombopag* is a small organic TRA that is administered orally; the recommended starting dose is 50 mg/day, titrated to 75 mg depending on platelet response. A lower dose (25 mg/day) is used in patients of Asian descent or with impaired liver function. *Avatrombopag* is another oral nonpeptide that is more potent than *eltrombopag* and has less stringent dietary restrictions or need for liver function test monitoring than *eltrombopag*. *Avatrombopag* is approved for treatment of patients with chronic ITP who have insufficient response to prior treatment (Bussel et al., 2014), as well as for adults with chronic liver disease and thrombocytopenia scheduled for surgery. For the latter, *avatrombopag* is started 10 to 13 days prior to the scheduled procedure and dosed for 5 consecutive days, such that the surgery is 5 to 8 days after the last dose (Terrault et al., 2014). *Lusutrombopag*, another oral nonpeptide TRA, is also approved to increase platelet counts in patients with thrombocytopenia in the setting of liver disease who are scheduled for elective surgery (Peck-Radosavljevic et al., 2019).

Clinical uses of TRAs include treatment of patients with chronic ITP, chronic hepatitis C–associated thrombocytopenia, severe aplastic anemia, and chronic liver disease–associated thrombocytopenia (preprocedure as outlined above). TRAs are under investigation for use in raising the platelet counts of patients with MDS, congenital thrombocytopenia due to muscle heavy-chain myosin-9 (MYH9) mutation, HIV-associated thrombocytopenia, radiation injury, or chemotherapy-induced thrombocytopenia.

Risks of TRAs include thrombosis. *Eltrombopag* was associated with risk of portal vein thrombosis in patients with advanced liver disease, although many cases had platelet counts greater than 200,000/µL and there could have been other causes of thrombosis. The risk of thrombosis was not noted in trials of preprocedural *avatrombopag* or *lusutrombopag*, perhaps due to the limited duration of the trials. The risk of thrombosis was not increased in patients with chronic ITP treated with TRAs compared to placebo. Patients on TRAs have been noted to develop marrow fibrosis that generally is reversible and not associated with development of other cytopenias (Janssens et al., 2016). Given the short life span of platelets, especially in patients with ITP, platelet counts may drop precipitously after abrupt discontinuation of TRA, even below the prior baseline. This rebound thrombocytopenia was seen in the clinical trials, which mandated complete treatment hold for platelet counts above 400,000/µL. While the product labeling adopted this dosing scheme, patients should be closely monitored for large fluctuations in platelet counts.

Iron Deficiency and Other Hypochromic Anemias

The Bioavailability of Iron

Iron exists in the environment largely as ferric oxide, ferric hydroxide, and polymers. In this state, its biological availability is limited unless solubilized by acid or chelating agents. For example, bacteria and some plants produce high-affinity chelating agents that extract iron from the surrounding

HISTORICAL PERSPECTIVE

The modern understanding of iron metabolism began in 1937 with the work of McCance and Widdowson on iron absorption and excretion and Heilmeyer and Plotner's measurement of iron in plasma (Beutler, 2002). In 1947, Laurell described a plasma iron transport protein that he called *transferrin* (Laurell, 1951). Around the same time, Hahn and coworkers used radioisotopes to measure iron absorption and define the role of the intestinal mucosa to regulate this function (Hahn, 1948). In the next decade, Huff and associates initiated isotopic studies of internal iron metabolism. The subsequent development of practical clinical measurements of serum iron, transferrin saturation, plasma ferritin, and red cell protoporphyrin permitted the definition and detection of the body's iron store status and iron-deficient erythropoiesis. In 1994, Feder and colleagues identified the *HFE* (High Fe, Hereditary Hemochromatosis Protein, or Homeostatic Iron Regulator) gene, which is mutated in type 1 hemochromatosis (Feder et al., 1996). Subsequently, Ganz and colleagues discovered a peptide produced by the liver, which was termed *hepcidin* (Park et al., 2001), now known to be the master regulator of iron homeostasis and to play a role in anemia of chronic disease (Ganz and Nemeth, 2011).

TABLE 45–2 ■ THE BODY CONTENT OF IRON

	mg of Fe/kg of body weight	
	MALE	**FEMALE**
Essential iron		
Hemoglobin	31	28
Myoglobin and enzymes	6	5
Storage iron	13	4
Total	50	37

environment. Most mammals have little difficulty in acquiring iron; this is explained by ample iron intake and perhaps also by a greater efficiency in absorbing iron. Humans, however, appear to be an exception. Although total dietary intake of elemental iron in humans usually exceeds requirements, the bioavailability of the iron in the diet is limited.

Iron deficiency is the most common nutritional cause of anemia in humans. It can result from inadequate iron intake, malabsorption, blood loss, or an increased requirement, as with pregnancy. When severe, it results in a characteristic microcytic, hypochromic anemia. In addition to its role in hemoglobin, iron is an essential component of myoglobin, heme enzymes (e.g., cytochromes, catalase, and peroxidase), and the metalloflavoprotein enzymes (e.g., xanthine oxidase and α-glycerophosphate oxidase). Iron deficiency can affect metabolism in muscle independently of the effect of anemia on O_2 delivery. This may reflect a reduction in the activity of iron-dependent mitochondrial enzymes. Iron deficiency also has been associated with behavioral and learning problems in children, abnormalities in catecholamine metabolism, and possibly impaired heat production.

Metabolism of Iron

The body store of iron is divided between essential iron-containing compounds and excess iron, which is held in storage (Table 45–2). *Hemoglobin* dominates the essential fraction. Each hemoglobin molecule contains four atoms of iron, amounting to 1.1 mg (20 μmol) of iron/mL of red blood cells. Other forms of essential iron include *myoglobin* and a variety of heme and nonheme iron-dependent enzymes. *Ferritin* is a protein-iron storage complex that exists as individual molecules or as aggregates. *Apoferritin* (molecular weight ~450 kDa) is composed of 24 polypeptide subunits that form an outer shell around a storage cavity for polynuclear hydrous ferric oxide phosphate. More than 30% of the weight of ferritin may be iron (4000 atoms of iron per ferritin molecule). Ferritin aggregates, referred to as *hemosiderin* and visible by light microscopy, constitute about one-third of normal stores. The two predominant sites of iron storage are the reticuloendothelial system and the hepatocytes.

Internal exchange of iron is accomplished by the plasma protein *transferrin*, a 76-kDa glycoprotein that has two binding sites for ferric iron. Iron is delivered from transferrin to intracellular sites by means of specific transferrin receptors in the plasma membrane. The iron-transferrin complex binds to the receptor, and the ternary complex is internalized through clathrin-coated pits by receptor-mediated endocytosis. A proton-pumping ATPase lowers the pH of the intracellular vesicular compartment (the endosomes) to about 5.5. Iron subsequently dissociates, and the receptor returns the apotransferrin to the cell surface, where it is released into the extracellular environment. Cells regulate their expression of transferrin receptors and intracellular ferritin in response to the iron supply (De Domenico et al., 2008). The synthesis of apoferritin and transferrin receptors is regulated posttranscriptionally by two iron-regulating proteins, IRP1 and IRP2. These IRPs are cytosolic RNA-binding proteins that bind to iron-regulating elements (IREs) present in the 5′ or 3′ untranslated regions of mRNA encoding apoferritin or the transferrin receptors, respectively. Binding of these IRPs to the 5′ IRE of apoferritin mRNA represses translation, whereas binding to the 3′ IRE of mRNA encoding the transferrin receptors enhances transcript stability, thereby increasing protein production.

The flow of iron through the plasma amounts to a total of 30 to 40 mg/day in the adult (~0.46 mg/kg of body weight). The major internal circulation of iron involves the erythron and reticuloendothelial cells (Figure 45–4). About 80% of the iron in plasma goes to the erythroid marrow to be packaged into new erythrocytes; these normally circulate for about 120 days before being catabolized by the reticuloendothelial system. At that time, a portion of the iron is immediately returned to the plasma bound to transferrin, while another portion is incorporated into the ferritin stores of reticuloendothelial cells and returned to the circulation more gradually. With abnormalities in erythrocyte

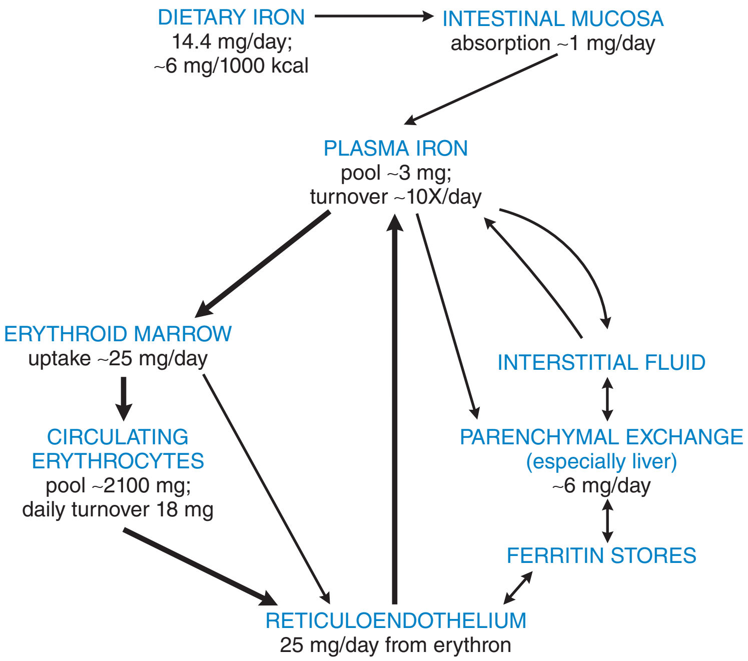


Figure 45–4 *Iron metabolism in humans (excretion omitted).*

TABLE 45–3 ■ IRON REQUIREMENTS FOR PREGNANCY

	AVERAGE IRON AMOUNT (mg)
Expansion of maternal red cell mass	450
Fetal iron requirements and loading	270
Placental iron requirement and storage	90
Blood loss	
Basal maternal iron loss	230
Blood loss at delivery	150
OVERALL IRON REQUIREMENT FOR PREGNANCY	**1190**

Estimated iron amounts for pregnancy, based on a 120-lb woman.
Source: Iron requirements and iron balance during pregnancy have recently been reviewed (Fisher AL, Nemeth E. Iron homeostasis during pregnancy. *Am J Clin Nutr*, **2017**, *106*(suppl 6):1567S–1574S; Georgieff MK. Iron deficiency in pregnancy. *Am J Obstet Gynecol*, **2020**, *223*:516–524).

maturation, the predominant portion of iron assimilated by the erythroid marrow may be rapidly localized in the reticuloendothelial cells as defective red cell precursors are broken down; this is termed *ineffective erythropoiesis*. The rate of iron turnover in plasma may be reduced by half or more with red cell aplasia, with all the iron directed to the hepatocytes for storage.

The human body conserves its iron stores to a remarkable degree. Only 10% of the total is lost per year by normal men (i.e., ~1 mg/day). Two-thirds of this iron is excreted from the gastrointestinal (GI) tract as extravasated red cells, iron in bile, and iron in exfoliated mucosal cells. The other third is accounted for by small amounts of iron in desquamated skin and in the urine. Additional losses of iron occur in women due to menstruation. Although the average loss in menstruating women is about 0.5 mg per day, 10% of menstruating women lose more than 2 mg per day. Pregnancy and lactation impose an even greater requirement for iron (Table 45–3). Other causes of iron loss include blood donation, the use of anti-inflammatory drugs that cause bleeding from the gastric mucosa, and GI disease with associated bleeding.

The limited physiological losses of iron point to the primary importance of absorption in determining the body's iron content (Garrick and Garrick, 2009). After acidification and partial digestion of food in the stomach, iron is presented to the intestinal mucosa as either inorganic iron or heme iron. A ferrireductase, duodenal cytochrome B, located on the luminal surface of absorptive cells of the duodenum and upper small intestine, reduces the iron to the ferrous state, which is the substrate for divalent metal (ion) transporter 1 (DMT1, SLC11A2). DMT1 transports the iron to the basolateral membrane, where it is taken up by another transporter, *ferroportin* (Fpn; SLC40A1), and subsequently reoxidized to Fe^{3+}, primarily by hephaestin (Hp; *HEPH*), a transmembrane copper-dependent ferroxidase. Apotransferrin binds the resultant oxidized Fe^{3+}. The hepatic protein, hepcidin, binds to ferroportin, inducing its internalization and degradation, thus limiting the amount of iron released into the blood (Camaschella, 2013). Conditions that enhance the levels of hepcidin, such as inflammation, can result in decreased gut iron absorption, reduced serum iron, and inadequate iron available for developing red blood cells. Conversely, when hepcidin levels are low, such as in hemochromatosis, iron overload occurs due to excessive ferroportin-mediated iron influx.

Genetic polymorphism and consequent dysfunction in hepcidin or in proteins regulating its expression can result in inadequate levels of hepcidin and cause hereditary hemochromatosis (Pietrangelo, 2016). This can be due to polymorphism in *HFE*, resulting in a Cys→Tyr change at position 282 (C282Y) in the HFE protein, or pathogenic mutations in hepcidin (*HAMP*), ferroportin (*FPN*), hemojuvelin (*HJV*), or transferrin receptor 2 (*TfR2*). The phenotype may vary, ranging from severe, as in *HJV*– or *HAMP*–juvenile-onset hemochromatosis, to relatively milder forms of adult-onset hemochromatosis, resulting from defects in *FPN* or *TfR2*. Acquired hemochromatosis can result from excessive amounts of parenteral iron, such as may occur in multiple transfusions for hereditary anemia or acquired aplastic anemia, from loss of hepcidin-producing liver mass, or with disease factors such as hepatitis C or chronic alcoholism that impair the production of hepcidin.

Iron Requirements; Availability of Dietary Iron

Adult men must absorb only 13 μg of iron/kg of body weight/d (~1 mg/day), whereas menstruating women require about 21 μg/kg (~1.4 mg) per day. In the last two trimesters of pregnancy, requirements increase to about 80 μg/kg (5–6 mg) per day; infants have similar requirements due to their rapid growth (Table 45–4).

The difference between dietary supply and requirements is reflected in the size of iron stores, which are low or absent when iron balance is precarious and high when iron balance is favorable. In infants after the third month of life and in pregnant women after the first trimester, stores of iron are negligible. Menstruating women have approximately one-third the stored iron found in adult men (see Table 45–2).

Although the iron content of the diet obviously is important, of greater nutritional significance is the bioavailability of iron in food. Heme iron, which constitutes only 6% of dietary iron, is far more available and is absorbed independent of the diet composition; it therefore represents 30% of iron absorbed (Conrad and Umbreit, 2000). The nonheme fraction represents the larger amount of dietary iron ingested by the economically underprivileged. In a vegetarian diet, nonheme iron is absorbed poorly because of the inhibitory action of a variety of dietary components, particularly phosphates. Ascorbic acid and meat facilitate the

TABLE 45–4 ■ DAILY IRON ABSORPTION REQUIREMENT

SUBJECT	IRON REQUIREMENT (μg/kg)	AVAILABLE IRON IN POOR DIET–GOOD DIET (μg/kg)	SAFETY FACTOR: AVAILABLE/REQUIREMENT
Infant	67	33–66	0.5–1
Child	22	48–96	2–4
Adolescent (male)	21	30–60	1.5–3
Adolescent (female)	20	30–60	1.5–3
Adult (male)	13	26–52	2–4
Adult (female)	21	18–36	1–2
Mid-to-late pregnancy	80	18–36	0.22–0.45

The numbers in columns 2 and 3 refer to iron absorption via the GI tract in micrograms per kilogram body weight. As noted in Figure 45–4, of 14.4 mg of dietary iron presented to the GI tract each day, only about 1 mg is absorbed. See text concerning factors influencing iron absorption and differential absorption of heme versus nonheme iron.

absorption of nonheme iron. In developed countries, the normal adult diet contains about 6 mg of iron per 1000 calories, providing an average daily intake for adult men of between 12 and 20 mg and for adult women of between 8 and 15 mg. Foods high in iron (>5 mg/100 g) include organ meats such as liver and heart, brewer's yeast, wheat germ, egg yolks, oysters, and certain dried beans and fruits; foods low in iron (<1 mg/100 g) include milk and milk products and most nongreen vegetables. Iron also may be added from cooking in iron pots. In assessing dietary iron intake, it is important to consider not only the amount of iron ingested but also its bioavailability.

Iron Deficiency

The prevalence of iron deficiency anemia in the U.S. is on the order of 1% to 4% and depends on the economic status of the population (McLean et al., 2009). In developing countries, up to 20% to 40% of infants and pregnant women may be affected. Better iron balance has resulted from the practice of fortifying flour, the use of iron-fortified formulas for infants, and the prescription of medicinal iron supplements during pregnancy.

Iron deficiency anemia results from dietary intake of iron that is inadequate to meet normal requirements (nutritional iron deficiency), blood loss, or interference with iron absorption (Camaschella, 2015). More severe iron deficiency is usually the result of blood loss, either from the GI tract or, in women, from the uterus. Finally, treatment of patients with *erythropoietin* can result in a functional iron deficiency. Iron deficiency in infants and young children can lead to behavioral disturbances and can impair development, which may not be fully reversible. Iron deficiency in children also can lead to an increased risk of lead toxicity secondary to pica and an increased absorption of heavy metals. Premature and low-birthweight infants are at greatest risk for developing iron deficiency, especially if they are not breast fed or do not receive iron-fortified formula (Finch, 2015). After the age of 2 to 3 years, the requirement for iron declines until adolescence, when rapid growth combined with irregular dietary habits again increase the risk of iron deficiency. Adolescent girls are at greatest risk; the dietary iron intake of most girls ages 11 to 18 is insufficient to meet their requirements.

Treatment of Iron Deficiency

General Therapeutic Principles

The response of iron deficiency anemia to iron therapy is influenced by several factors, including the severity of anemia, the ability of the patient to tolerate and absorb oral iron, and the presence of other complicating illnesses, such as those resulting in chronic blood loss or anatomic or physiological defects that interfere with oral iron absorption. Therapeutic effectiveness is best measured by the resulting increase in the rate of production of red cells. The magnitude of the marrow response to iron therapy is proportional to the severity of the anemia (level of erythropoietin stimulation) and the amount of iron delivered to marrow precursors.

Clinically, the effectiveness of iron therapy is best evaluated by tracking the reticulocyte response and the rise in the hemoglobin or the hematocrit. An increase in the reticulocyte count is not observed for at least 4 to 7 days after beginning therapy. A measurable increase in the hemoglobin level takes even longer. A decision regarding the effectiveness of treatment should not be made for 4 weeks after the start of treatment. An increase of 2 g/dL or more in the concentration of hemoglobin by that time should be considered a positive response, assuming that no other change in the patient's clinical status can account for the improvement and that the patient has not been transfused.

If the response to iron treatment is inadequate, the diagnosis must be reconsidered. A full laboratory evaluation should be conducted, and poor compliance by the patient or the presence of a concurrent inflammatory disease should be explored. A source of continued bleeding obviously should be sought. If no other explanation can be found, an evaluation of the patient's ability to absorb oral iron should be considered. There is no justification for merely continuing oral iron therapy beyond 3 to 4 weeks if a favorable response has not occurred.

For patients treated with oral iron, once a response is demonstrated, therapy may be extended beyond normalization of the hemoglobin if it is desirable to replenish iron stores. This may require a considerable period of oral therapy because the rate of absorption of iron by the intestine will decrease markedly as iron stores are reconstituted. The prophylactic use of oral iron should be reserved for patients at high risk, including pregnant women, women with excessive menstrual blood loss, and infants. Iron supplements also may be of value for rapidly growing infants who are consuming substandard diets and for adults with a recognized cause of chronic blood loss. Except for infants, in whom the use of supplemented formulas is routine, the use of over-the-counter mixtures of vitamins and minerals to prevent iron deficiency should be discouraged.

Parenteral iron treatment may be necessary for patients with excessive chronic blood loss that exceeds the capacity for oral iron absorption or patients with anatomic or physiological conditions that preclude sufficient absorption. Several formulations allow for rapid repletion of iron stores with much lower risk of anaphylaxis than previous high-molecular-weight iron dextran products.

Therapy With Oral Iron

Orally administered ferrous sulfate is often the treatment of choice for iron deficiency due to cost-effectiveness, especially in resource-poor settings. The patient's ability to tolerate and absorb medicinal iron is a key factor in determining the rate of response to oral iron therapy. The small intestine regulates absorption and, with increasing doses of oral iron, limits the entry of iron into the bloodstream. This provides a natural ceiling on how much iron can be supplied by oral therapy. In the patient with moderately severe iron deficiency anemia, tolerable doses of oral iron will deliver, at most, 40 to 60 mg of iron per day to the erythroid marrow. This is an amount sufficient for production rates of two to three times normal.

Ferrous salts are absorbed about three times as well as ferric salts. Variations in the particular ferrous salt have relatively little effect on bioavailability; the sulfate, fumarate, succinate, gluconate, aspartate, other ferrous salts, and polysaccharide-ferrihydrite complex are absorbed to approximately the same extent. The effective dose of all these preparations is based on iron content.

Other iron compounds have utility in fortification of foods. Reduced iron (metallic iron, elemental iron) is as effective as ferrous sulfate, provided that the material employed has a small particle size. Large-particle ferrum reductum and iron phosphate salts have much lower bioavailability. Ferric edetate has been shown to have good bioavailability and to have advantages for maintenance of the normal appearance and taste of food.

The amount of iron in iron tablets is important. It also is essential that the coating of the tablet dissolves rapidly in the stomach. Delayed-release preparations are available, but absorption from such preparations varies. Ascorbic acid (≥200 mg) increases the absorption of medicinal iron by at least 30%. However, the increased uptake is associated with an increase in the incidence of side effects. Preparations that contain other compounds with therapeutic action, such as vitamin B_{12}, folate, or cobalt, are not recommended because the patient's response to the combination cannot easily be interpreted.

Untoward Effects of Oral Preparations of Iron. Side effects of oral iron preparations include heartburn, nausea, upper gastric discomfort, and diarrhea or constipation. A good policy is to initiate therapy at a small dosage and then gradually to increase the dosage to that desired. Only individuals with underlying disorders that augment the absorption of iron run the hazard of developing iron overload (hemochromatosis).

Dosing. Traditionally, the treatment of adults with iron deficiency has been 150 to 200 mg of elemental iron in up to three divided dose (e.g., ferrous sulfate 325 mg three times daily, equaling 195 mg of elemental iron per day), of which 25 mg is absorbed and utilized. However, this convention has been challenged by data demonstrating that dosing more than once a day may be counterproductive by decreasing iron absorption (due to increased hepcidin levels) and increasing the risk of side

TABLE 45–5 ■ AVERAGE RESPONSE TO ORAL IRON

TOTAL DOSE OF IRON (mg/day)	ESTIMATED ABSORPTION		INCREASE IN BLOOD HEMOGLOBIN (g/L/day)
	%	mg	
35	40	14	0.7
105	24	25	1.4
195	18	35	1.9
390	12	45	2.2

effects (Moretti et al., 2015). Therefore, once-daily administration of ferrous sulfate 325 mg on an empty stomach is a typical dosage that maximizes absorption while maintaining high tolerance (Düzen Oflas et al., 2020).

Children weighing 15 to 30 kg can take half the average adult dose; small children and infants can tolerate relatively large doses of iron (e.g., 5 mg/kg). When the object is the prevention of iron deficiency in pregnant women, for example, doses of 15 to 30 mg of iron per day are adequate. Bioavailability of iron is reduced with food and by concurrent antacids. For a rapid response or to counteract continued bleeding, as much as 120 mg of iron may be administered four times a day.

The duration of treatment is governed by the rate of recovery of hemoglobin (Table 45–5) and the desire to create iron stores. The former depends on the severity of the anemia. With a daily rate of repair of 2 g of hemoglobin per liter of whole blood, the red cell mass usually is reconstituted within 1 to 2 months. Thus, an individual with a hemoglobin of 50 g/L may achieve a normal complement of 150 g/L in about 50 days, whereas an individual with a hemoglobin of 100 g/L may take only half that time. The creation of stores of iron requires many months of oral iron administration. The rate of absorption decreases rapidly after recovery from anemia, and after 3 to 4 months of treatment, stores may increase at a rate of not much more than 100 mg/month. Much of the strategy of continued therapy depends on the estimated future iron balance. Patients with an inadequate diet may require continued therapy with low doses of iron. If the bleeding has stopped, no further therapy is required after the hemoglobin has returned to normal. With continued bleeding, long-term, high-dose therapy clearly is indicated.

Iron Poisoning. Large amounts of ferrous salts are toxic, but fatalities are rare in adults. Most deaths occur in children, particularly between the ages of 12 and 24 months. As little as 1 to 2 g of iron may cause death, but 2 to 10 g usually are ingested in fatal cases. All iron preparations should be kept in childproof bottles. Signs and symptoms of severe poisoning may occur within 30 min after ingestion or may be delayed for several hours. They include abdominal pain, diarrhea, or vomiting of brown or bloody stomach contents containing pills. Of particular concern are pallor or cyanosis, lassitude, drowsiness, hyperventilation due to acidosis, and cardiovascular collapse. If death does not occur within 6 h, there may be a transient period of apparent recovery, followed by death in 12 to 24 h. The corrosive injury to the stomach may result in pyloric stenosis or gastric scarring.

In the evaluation of a child thought to have ingested iron, a color test for iron in the gastric contents and determination of the concentration of iron in plasma can be performed. If the latter is less than 63 μmol (3.5 mg/L), the child is not in immediate danger. However, vomiting should be induced when there is iron in the stomach, and an X-ray should be taken to evaluate the number of pills remaining in the small bowel (iron tablets are radiopaque). When the plasma concentration of iron is greater than the total iron-binding capacity (63 μmol; 3.5 mg/L), *deferoxamine* should be administered (see Chapter 76). The speed of diagnosis and therapy is important. With early treatment, the mortality from iron poisoning can be reduced from 45% to about 1%. *Deferiprone* and *deferasirox* are oral iron chelators that are FDA approved for treatment of patients with thalassemia who have iron overload.

Therapy With Parenteral Iron

Parenteral iron (IV iron) administration may be an effective alternative to oral iron. Previously, parenteral iron formulations carried a higher risk of anaphylactic and anaphylactoid reactions. However, this concern has been addressed by evidence of safety and efficacy of newer IV iron formulations. Thus, IV iron may be considered sooner in the treatment paradigm of iron-deficient patients. Common indications are iron malabsorption (e.g., sprue, short-bowel syndrome), oral iron intolerance, as a routine supplement to total parenteral nutrition, and in patients who are receiving erythropoietin. Parenteral iron can be given to iron-deficient patients to create iron stores within one or two sessions, something that would take months to achieve by the oral route.

Several iron formulations are available in the U.S. (Larson and Coyne, 2014). These include *ferumoxytol*, *iron dextran*, *sodium ferric gluconate*, *iron sucrose*, *ferric carboxymaltose*, and *iron isomaltoside* (Table 45–6).

Ferumoxytol. *Ferumoxytol* is a semisynthetic carbohydrate-coated superparamagnetic iron oxide nanoparticle approved for treatment of iron deficiency anemia in patients with CKD; the *ferumoxytol* must be administered as a 1.02-g infusion over a relatively short infusion time of 15 min (Auerbach et al., 2013). Indications for *ferric gluconate* and *iron sucrose* are limited to patients with CKD and documented iron deficiency, although broader applications are being advocated (Larson and Coyne, 2014).

Iron Dextran. *Iron dextran* injection is a colloidal solution of *ferric oxyhydroxide* complexed with polymerized dextran (molecular weight ∼180 kDa) that contains 50 mg/mL of elemental iron. The use of low-molecular-weight *iron dextran* has reduced the incidence of toxicity relative to that observed with high-molecular-weight preparations. *Iron dextran* can be administered by intravenous (preferred) or intramuscular injection. Injection of a therapeutic dose should be initiated only after a test dose of 0.5 mL (25 mg of iron). Given intravenously in a dose less than 500 mg, the *iron dextran* complex is cleared with a plasma $t_{1/2}$ of 6 h. When 1 g or more is administered intravenously as total-dose therapy, reticuloendothelial cell clearance is constant at 10 to 20 mg/h.

Intramuscular injection of *iron dextran* should be initiated only after a test dose of 0.5 mL (25 mg of iron). If no adverse reactions are observed, the injections can proceed. The daily dose ordinarily should not exceed 0.5 mL (25 mg of iron) for infants weighing less than 4.5 kg,

TABLE 45–6 ■ INTRAVENOUS IRON FORMULATIONS

DRUG	DOSING
Ferumoxytol	Single dose of 1020 mg; or 2 doses of 510 mg, given 3–8 days apart
Iron dextran, low molecular weight	Single dose of 1000 mg, or multiple doses of 100 mg
Ferric gluconate	Multiple doses of 125–250 mg
Iron sucrose	Multiple doses of 100–300 mg
Ferric carboxymaltose	If weight ≥50 mg: 2 doses of 750 mg, given 7 or more days apart. If weight <50 kg: 2 doses of 15 mg/kg, given 7 or more days apart
Iron isomaltoside (ferric derisomaltose)	If weight ≥50 mg: single dose of 1000 mg If weight <50 kg: single dose of 20 mg/kg Or up to 3 doses of 500 mg given over 7 days

Product labeling is available on the FDA website: https://www.accessdata.fda.gov/scripts/cder/daf/.

1 mL (50 mg of iron) for children weighing less than 9 kg, and 2 mL (100 mg of iron) for other patients. However, local reactions and the concern about malignant change at the site of injection make intramuscular administration inappropriate except when the intravenous route is inaccessible. The patient should be observed for signs of immediate anaphylaxis and for an hour after injection for any signs of vascular instability or hypersensitivity, including respiratory distress, hypotension, tachycardia, or back or chest pain. Delayed hypersensitivity reactions also are observed, especially in patients with rheumatoid arthritis or a history of allergies. Fever, malaise, lymphadenopathy, arthralgias, and urticaria can develop days or weeks following injection and last for prolonged periods of time. Use *iron dextran* with extreme caution in patients with rheumatoid arthritis or other connective tissue diseases and during the acute phase of an inflammatory illness. Once hypersensitivity is documented, *iron dextran* therapy must be abandoned.

With multiple total-dose infusions such as those sometimes used in the treatment of chronic GI blood loss, accumulations of slowly metabolized *iron dextran* stores in reticuloendothelial cells can be impressive. The plasma ferritin level also can rise to levels associated with iron overload. It seems prudent, however, to withhold the drug whenever the plasma ferritin rises above 800 μg/L.

Sodium Ferric Gluconate. *Sodium ferric gluconate* is an intravenous iron preparation with a molecular size of about 295 kDa and an osmolality of 990 mOsm/L. Administration of *ferric gluconate* at doses ranging from 62.5 to 125 mg during hemodialysis is associated with transferrin saturation exceeding 100%. Unlike *iron dextran*, which requires processing by macrophages that may require several weeks, about 80% of *sodium ferric gluconate* is delivered to transferrin within 24 h. *Sodium ferric gluconate* also has a lower risk of inducing serious anaphylactic reactions than *iron dextran* (Sengolge et al., 2005).

Iron Sucrose. *Iron sucrose* is a complex of polynuclear iron (III)–hydroxide in sucrose (Beguin and Jaspers, 2014). Following intravenous injection, the complex is taken up by the reticuloendothelial system, where it dissociates into iron and sucrose. *Iron sucrose* is generally administered in daily amounts of 100 to 200 mg within a 14-day period to a total cumulative dose of 1000 mg. Like *sodium ferric gluconate*, *iron sucrose* appears to be better tolerated and to cause fewer adverse events than *iron dextran* (Hayat, 2008). This agent is FDA-approved for the treatment of iron deficiency in patients with CKD. Chronic use has the potential to cause renal tubulointerstitial damage (Agarwal, 2006).

Ferric Carboxymaltose. *Ferric carboxymaltose* is an iron complex consisting of a ferric hydroxide core and a carbohydrate shell (Keating, 2015). With this preparation, a replenishment dose of up to 1000 mg of iron can be administered in 15 min. Intravenous administration results in transient elevations in serum iron, serum ferritin, and transferrin saturation, with subsequent correction in hemoglobin levels and replenishment of depleted iron stores. *Ferric carboxymaltose* is rapidly cleared from the circulation, becoming distributed (~80%) in the marrow, as well as the liver and spleen. Common reported drug-related adverse effects include headache, dizziness, nausea, abdominal pain, constipation, diarrhea, rash, and injection site reactions. However, the incidence of drug-related adverse events appears similar to that of patients treated with oral *ferrous sulfate*. *Ferric carboxymaltose* is FDA-approved for therapy of iron deficiency anemia.

Iron Isomaltoside. *Iron isomaltoside* (also called ferric derisomaltose) consists of iron strongly bound to a chemically modified isomalto-oligosaccharides (Auerbach et al., 2019). Unlike the branched dextran polysaccharides in *iron dextran*, the isomaltoside carbohydrate moiety is linear and unbranched, which is theorized to lower its potential immunogenicity. The strength of iron binding enables a controlled slow release of bioavailable iron, mitigating the risk of toxicity from labile free iron. *Iron isomaltoside* was introduced in Europe in 2010. The FDA approved its use in the U.S. in 2020 as an infusion of up to 1 g (or 20 mg/kg if weight is less than 50 kg) for adult patients with an intolerance to oral iron or an unsatisfactory response to oral iron therapy or for patients with CKD not requiring hemodialysis. Conveniently, *iron isomaltoside* can provide full correction of an iron deficit with a single infusion.

Copper, Pyridoxine, and Riboflavin

Copper

Copper has redox properties similar to those of iron, which simultaneously are essential and potentially toxic to the cell. Cells have virtually no free copper. Instead, copper is stored by metallothioneins and distributed by specialized chaperones to sites that make use of its redox properties. Transfer of copper to nascent cuproenzymes is performed by individual or collective activities of P-type ATPases, ATP7A and ATP7B, which are expressed in all tissues (Nevitt et al., 2012). In mammals, the liver is the organ most responsible for the storage, distribution, and excretion of copper. Mutations in ATP7A or ATP7B that interfere with this function have been found responsible for Wilson disease or Menkes syndrome (steely hair syndrome) (de Bie et al., 2007), respectively, which can result in life-threatening hepatic failure.

Copper deficiency is extremely rare; the amount present in food is more than adequate to provide the needed body complement of slightly more than 100 mg. Even in clinical states associated with hypocupremia (sprue, celiac disease, and nephrotic syndrome), effects of copper deficiency usually are not demonstrable. Anemia due to copper deficiency has been described in individuals who have undergone intestinal bypass surgery, in those who are receiving parenteral nutrition, in malnourished infants, and in patients ingesting excessive amounts of zinc (Willis et al., 2005). Copper deficiency interferes with the absorption of iron and its release from reticuloendothelial cells. In humans, the prominent findings have been leukopenia, particularly granulocytopenia, and anemia. Concentrations of iron in plasma are variable, and the anemia is not always microcytic. When a low plasma copper concentration is determined in the presence of leukopenia and anemia, a therapeutic trial with copper is appropriate. Daily doses up to 0.1 mg/kg of *cupric sulfate* have been given by mouth, or 1 to 2 mg per day may be added to the solution of nutrients for parenteral administration.

Pyridoxine

Patients with either hereditary or acquired sideroblastic anemia characteristically have impaired hemoglobin synthesis and accumulate iron in the perinuclear mitochondria of erythroid precursor cells, so-called ringed sideroblasts. Hereditary sideroblastic anemia is an X-linked recessive trait with variable penetrance and expression that results from mutations in the erythrocyte form of δ-aminolevulinate synthase.

Oral therapy with *pyridoxine* is of proven benefit in correcting the sideroblastic anemias associated with the antituberculosis drugs *isoniazid* and *pyrazinamide*, which act as vitamin B_6 antagonists. A daily dose of 50 mg of *pyridoxine* completely corrects the defect without interfering with treatment, and routine supplementation of *pyridoxine* often is recommended (see Chapter 65). In contrast, if pyridoxine is given to counteract the sideroblastic abnormality associated with administration of *levodopa*, the effectiveness of *levodopa* in controlling Parkinson's disease is decreased. *Pyridoxine* therapy does not correct the sideroblastic abnormalities produced by *chloramphenicol* or lead. Patients with idiopathic acquired sideroblastic anemia generally fail to respond to oral *pyridoxine*, and those individuals who appear to have a *pyridoxine*-responsive anemia require prolonged therapy with large doses of the vitamin, 50 to 500 mg/day. The occasional patient who is refractory to oral *pyridoxine* may respond to parenteral administration of *pyridoxal phosphate*. However, oral *pyridoxine* in doses of 200 to 300 mg/day produces intracellular concentrations of *pyridoxal phosphate* equal to or greater than those generated by therapy with the phosphorylated vitamin.

Riboflavin

The spontaneous appearance in humans of red cell aplasia due to riboflavin deficiency undoubtedly is rare, if it occurs at all. Riboflavin deficiency has been described in combination with infection and protein deficiency, both of which are capable of producing hypoproliferative anemia. However, it seems reasonable to include riboflavin in the nutritional management of patients with gross, generalized malnutrition.

Vitamin B_{12}, Folic Acid, and the Treatment of Megaloblastic Anemias

Vitamin B_{12} and folic acid are dietary essentials. A deficiency of either vitamin impairs DNA synthesis in any cell in which chromosomal replication and division are taking place. Because tissues with the greatest rate of cell turnover show the most dramatic changes, the hematopoietic system is especially sensitive to deficiencies of these vitamins.

Cellular Roles of Vitamin B_{12} and Folic Acid

The major roles of vitamin B_{12} and folic acid in intracellular metabolism are summarized in Figure 45–5. Intracellular vitamin B_{12} is maintained as two active coenzymes: methylcobalamin and deoxyadenosylcobalamin.

Methylcobalamin (CH_3B_{12}) supports the *methionine synthetase* reaction, which is essential for normal metabolism of folate (Weissbach, 2008). Methyl groups contributed by methyltetrahydrofolate (CH_3H_4PteGlu$_1$) are used to form methylcobalamin, which then acts as a methyl group donor for the conversion of homocysteine to methionine. This folate-cobalamin interaction is pivotal for normal synthesis of purines and pyrimidines and, therefore, of DNA. The methionine synthetase reaction is largely responsible for the control of the recycling of folate cofactors; the maintenance of intracellular concentrations of folylpolyglutamates; and, through the synthesis of methionine and its product *S*-adenosylmethionine (SAM), the maintenance of a number of methylation reactions.

Deoxyadenosylcobalamin (deoxyadenosyl B_{12}) is a cofactor for the *mitochondrial mutase* enzyme that catalyzes the isomerization of l-methylmalonyl coenzyme A (CoA) to succinyl CoA, an important reaction in carbohydrate and lipid metabolism. This reaction has no direct relationship to the metabolic pathways that involve folate.

Because methyltetrahydrofolate is the principal folate congener supplied to cells, the transfer of the methyl group to cobalamin is essential for the adequate supply of tetrahydrofolate (H_4PteGlu$_1$). Tetrahydrofolate is a precursor for the formation of intracellular folylpolyglutamates; it also acts as the acceptor of a one-carbon unit in the conversion of serine to glycine, with the resultant formation of 5,10-methylenetetrahydrofolate (5,10-CH_2H_4PteGlu). The last derivative donates the methylene group to deoxythymidine monophosphate (dUMP) for the synthesis of deoxyuridine monophosphate (dTMP)—an extremely important reaction in DNA synthesis. In the process, the 5,10-CH_2H_4PteGlu is converted to dihydrofolate (H_2PteGlu). The cycle then is completed by the reduction of the H_2PteGlu to H_4PteGlu by dihydrofolate reductase, the step that is blocked by folate antagonists such as methotrexate (see Chapter 70). As shown in Figure 45–5, other pathways also lead to the synthesis of 5,10-methylenetetrahydrofolate. These pathways are important in the metabolism of formiminoglutamic acid (FIGLU) and purines and pyrimidines.

Deficiency of either vitamin B_{12} or folate decreases the synthesis of methionine and SAM and consequently interferes with protein biosynthesis, a number of methylation reactions, and the synthesis of polyamines. In addition, the cell responds to the deficiency by redirecting folate metabolic pathways to supply increasing amounts of methyltetrahydrofolate; this tends to preserve essential methylation reactions at the expense of nucleic acid synthesis. With vitamin B_{12} deficiency, methylenetetrahydrofolate reductase activity increases, directing available intracellular folates into the methyltetrahydrofolate pool (not shown in Figure 45–5). The methyltetrahydrofolate then is trapped by the lack of sufficient vitamin B_{12} to accept and transfer methyl groups, and subsequent steps in folate metabolism that require tetrahydrofolate are deprived of substrate. This process provides a common basis for the development of megaloblastic anemia with deficiency of either vitamin B_{12} or folic acid.

The mechanisms responsible for the neurological lesions of vitamin B_{12} deficiency are less well understood (Solomon, 2007). Damage to the myelin sheath is the most obvious lesion in this neuropathy. This observation led to the early suggestion that the deoxyadenosyl B_{12}–dependent methylmalonyl CoA mutase reaction, a step in propionate metabolism, is related to the abnormality. However, other evidence suggests that the deficiency of methionine synthetase and the block of the conversion of methionine to SAM are more likely to be responsible.

HISTORY

The discovery of vitamin B_{12} and folic acid is a dramatic story that began almost 200 years ago and includes two Nobel Prize–winning discoveries. Beginning in 1824, Combe and Addison wrote a series of case reports describing what must have been megaloblastic anemias (still known as Addisonian pernicious anemia). Austin Flint in 1860 first described a severe gastric atrophy and called attention to its possible relationship to the anemia. After Whipple's observation in 1925 that liver is a source of a potent hematopoietic substance for iron-deficient dogs, Minot and Murphy carried out Nobel Prize–winning experiments that demonstrated the effectiveness of the feeding of liver to reverse pernicious anemia. Soon thereafter, Castle defined the need for both intrinsic factor, a substance secreted by the parietal cells of the gastric mucosa, and extrinsic factor, the vitamin-like material provided by crude liver extracts. Nearly 20 years passed before Rickes and coworkers and Smith and Parker isolated and crystallized vitamin B_{12}; Dorothy Crowfoot Hodgkin received the 1964 Nobel Prize in Chemistry "for her determinations by X-ray techniques of the structures of important biochemical substances," including the crystal structure of vitamin B_{12}.

As attempts were being made to purify extrinsic factor, Wills and her associates described a macrocytic anemia in women in India that responded to a factor present in crude liver extracts but not in the purified fractions known to be effective in pernicious anemia. This factor, first called Wills' factor and later vitamin M, is now known to be folic acid. The term *folic acid* was coined by Mitchell and coworkers in 1941, after its isolation from leafy vegetables. From more recent work, we know that neither vitamin B_{12} nor folic acid as purified from foodstuffs is the active coenzyme in humans. During extraction, active labile forms are converted to stable congeners of vitamin B_{12} and folic acid, cyanocobalamin and PteGlu (pteroylglutamic acid), respectively. These congeners must be modified *in vivo* to be effective. Despite our knowledge of the intracellular metabolic pathways in which these vitamins function as required cofactors, many questions remain, such as: What is the relationship of vitamin B_{12} deficiency to the neurological abnormalities that occur in megaloblastic anemia?

Vitamin B_{12} and Human Health

Humans depend on exogenous sources of vitamin B_{12} (see structure in Figure 45–6). In nature, the primary sources are certain microorganisms that grow in soil or the intestinal lumen of animals that synthesize the vitamin. The daily nutritional requirement of 3 to 5 μg must generally be obtained from animal by-products in the diet. However, some vitamin B_{12} is available from legumes, which are contaminated with bacteria that can synthesize vitamin B_{12}, and vegetarians often fortify their diets with a wide range of vitamins and minerals; thus, strict vegetarians rarely develop vitamin B_{12} deficiency. The terms *vitamin B_{12}* and *cyanocobalamin* are used interchangeably as generic terms for all of the cobamides active in humans. Preparations of vitamin B_{12} for therapeutic use contain either cyanocobalamin or hydroxocobalamin because only these derivatives remain active after storage.

Metabolic Functions

The active coenzymes methylcobalamin and 5-deoxyadenosylcobalamin are essential for cell growth and replication. Methylcobalamin is required for the conversion of homocysteine to methionine and its derivative SAM. In addition, when concentrations of vitamin B_{12} are inadequate, folate becomes "trapped" as methyltetrahydrofolate to cause a functional deficiency of other required intracellular forms of folic acid. The hematological abnormalities in vitamin B_{12}–deficient patients result from this

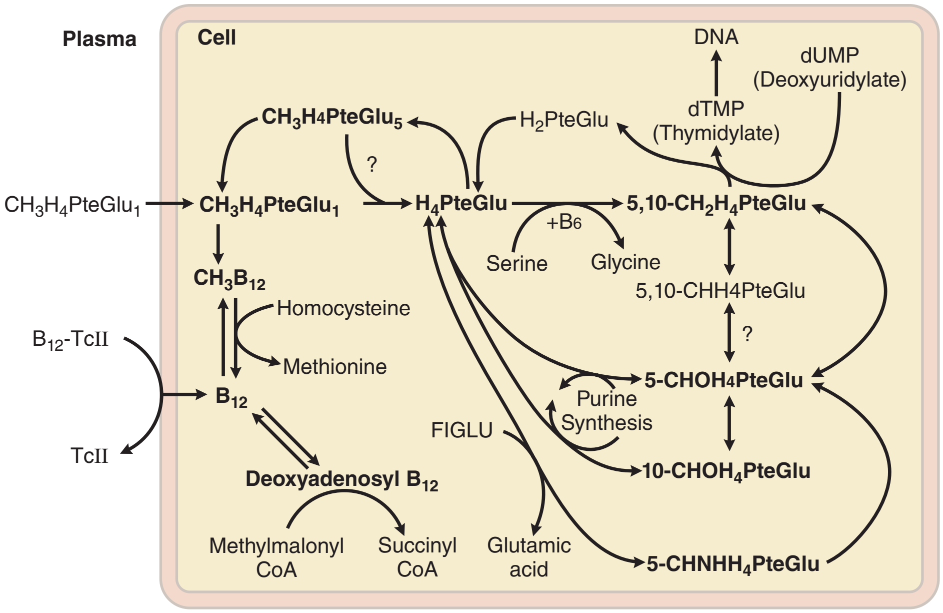

Plasma
Cell
DNA
dUMP
(Deoxyuridylate)
$CH_3H_4PteGlu_5$
$H_2PteGlu$
dTMP
(Thymidylate)
?
$CH_3H_4PteGlu_1$
$CH_3H_4PteGlu_1$
$H_4PteGlu$
$5,10\text{-}CH_2H_4PteGlu$
+B6
Serine
Glycine
CH_3B_{12}
5,10-CHH4PteGlu
Homocysteine
?
B_{12}-TcII
Methionine
5-CHOH4PteGlu
B_{12}
Purine
Synthesis
FIGLU
TcII
10-$CHOH_4$PteGlu
Deoxyadenosyl B_{12}
Methylmalonyl
CoA
Succinyl
CoA
Glutamic
acid
5-$CHNHH_4$PteGlu

Figure 45–5 *Interrelationships and metabolic roles of vitamin B_{12} and folic acid.* See text for explanation. See Figure 45–6 for structures of vitamin B_{12} congeners and Figure 45–8 for structures of the various folate congeners. FIGLU, formiminoglutamic acid, which arises from the catabolism of histidine; TcII, transcobalamin II.

process. Deoxyadenosylcobalamin (deoxyadenosyl B_{12}) is required for the rearrangement of methylmalonyl CoA to succinyl CoA.

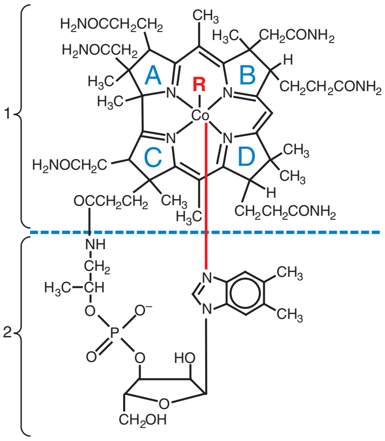
ADME and Daily Requirements

In the presence of gastric acid and pancreatic proteases, dietary vitamin B_{12} is released from food and salivary-binding protein and bound to gastric intrinsic factor. When the vitamin B_{12}–intrinsic factor complex reaches the ileum, it interacts with a receptor on the mucosal cell surface and is actively transported into circulation. Vitamin B_{12} deficiency in adults is rarely the result of a deficient diet per se; rather, it usually reflects a defect in one or another aspect of this complex sequence of absorption (Figure 45–7). Antibodies to parietal cells or intrinsic factor complex also can play a prominent role in producing a deficiency. Several intestinal conditions can interfere with absorption, including pancreatic disorders (loss of pancreatic protease secretion), bacterial overgrowth, intestinal parasites, sprue, and localized damage to ileal mucosal cells by disease or as a result of surgery.

Absorbed vitamin B_{12} binds to transcobalamin II, a plasma β globulin, for transport to tissues. The supply of vitamin B_{12} available for tissues is directly related to the size of the hepatic storage pool and the amount of vitamin B_{12} bound to transcobalamin II (see Figure 45–7). Vitamin B_{12} bound to transcobalamin II is rapidly cleared from plasma and preferentially distributed to hepatic parenchymal cells. As much as 90% of the body's stores of vitamin B_{12}, from 1 to 10 mg, is in the liver. Vitamin B_{12} is stored as the active coenzyme with a turnover rate of 0.5 to 8 µg per day. The recommended daily intake of the vitamin in adults is 2.4 µg. Approximately 3 µg of cobalamins are secreted into bile each day, 50% to 60% of which is not destined for reabsorption. Interference with reabsorption by intestinal disease can progressively deplete hepatic stores of the vitamin.

Vitamin B_{12} Deficiency

The plasma concentration of vitamin B_{12} is the best routine measure of B_{12} deficiency and normally ranges from 150 to 660 pM (~200 to 900 pg/mL). Deficiency should be suspected whenever the concentration falls below 150 pM. The correlation is excellent except when the plasma concentrations of transcobalamin I and III are increased, as occurs with hepatic disease or a myeloproliferative disorder. Inasmuch as the vitamin B_{12} bound to these transport proteins is relatively unavailable to cells,


1
2
A
B
C
D
R
Co
$H_2NOCCH_2CH_2$
H_2NOCCH_2
CH_3
H_3C
CH_2CONH_2
H
$CH_2CH_2CONH_2$
H_3C
H_3C
N
N
N
N
CH_3
CH_3
H_2NOCH_2C
H
$OCCH_2CH_2$
CH_3
H_3C
$CH_2CH_2CONH_2$
NH
CH_2
H_3C-CH
O
O^-
P
O
O
HO
O
CH_2OH
N
N
CH_3
CH_3

Vitamin B_{12} Congeners

Permissive Name	*R Group*
Cyanocobalamin (Vitamin B_{12})	–CN
Hydroxocobalamin	–OH
Methylcobalamin	$-CH_3$
5'-Deoxyadenosylcobalamin	–5'-Deoxyadenosyl

Figure 45–6 *The structures and nomenclature of vitamin B_{12} congeners.* The vitamin B_{12} molecule has three major portions: (1) a planar group porphyrin-like ring structure with four reduced pyrrole rings (A–D) linked to a central cobalt atom and extensively substituted with methyl, acetamide, and propionamide residues; (2) a 5,6-dimethylbenzimidazolyl nucleotide, which links almost at right angles to the planar nucleus with bonds to the cobalt atom and to the propionate side chain of the C pyrrole ring; and (3) a variable R group—the most important of which are found in the stable compounds cyanocobalamin and hydroxocobalamin and the active coenzymes methylcobalamin and 5-deoxyadenosylcobalamin.

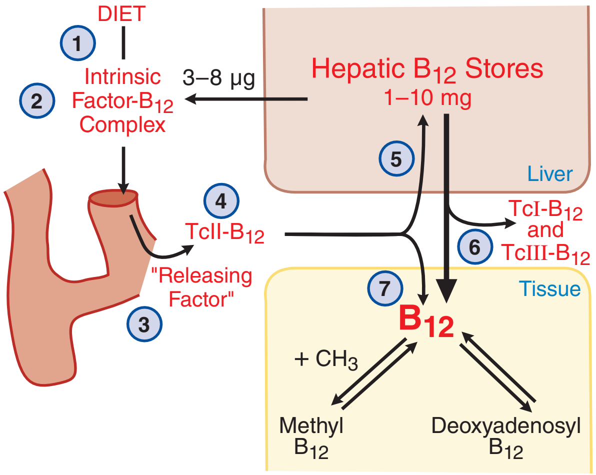


Figure 45–7 *Absorption and distribution of vitamin B_{12}.* Deficiency of vitamin B_{12} can result from a congenital or acquired defect in (1) inadequate dietary supply; (2) inadequate secretion of intrinsic factor (classical pernicious anemia); (3) ileal disease; (4) congenital absence of TcII; or (5) rapid depletion of hepatic stores by interference with reabsorption of vitamin B_{12} excreted in bile. The utility of measurements of the concentration of vitamin B_{12} in plasma to estimate supply available to tissues can be compromised by liver disease and (6) the appearance of abnormal amounts of TcI and TcIII in plasma. The formation of methylcobalamin requires (7) normal transport into cells and an adequate supply of folic acid as $CH_3H_4PteGlu_1$. TcI, TcII, transcobalamins I and II.

tissues can become deficient when the concentration of vitamin B_{12} in plasma is normal or even high. In subjects with congenital absence of transcobalamin II, megaloblastic anemia occurs despite relatively normal plasma concentrations of vitamin B_{12}; the anemia will respond to parenteral doses of vitamin B_{12} that exceed the renal clearance.

Vitamin B_{12} deficiency is recognized clinically by its impact on the hematopoietic and nervous systems. The sensitivity of the hematopoietic system relates to its high rate of cell turnover. Other tissues with high rates of cell turnover (e.g., mucosa and cervical epithelium) also have high requirements for the vitamin. As a result of an inadequate supply of vitamin B_{12}, DNA replication becomes highly abnormal. Once a hematopoietic stem cell is committed to enter a programmed series of cell divisions, the defect in chromosomal replication results in an inability of maturing cells to complete nuclear divisions while cytoplasmic maturation continues at a relatively normal rate. This results in the production of morphologically abnormal cells and death of cells during maturation, a phenomenon referred to as *ineffective hematopoiesis*. Severe deficiency affects all cell lines; pronounced pancytopenia results.

The diagnosis of a vitamin B_{12} deficiency usually can be made using measurements of the serum vitamin B_{12} or serum methylmalonate (which is somewhat more sensitive and useful in identifying metabolic deficiency in patients with normal serum vitamin B_{12} levels). In managing a patient with severe megaloblastic anemia, a therapeutic trial using very small doses of the vitamin can be used to confirm the diagnosis. Serial measurements of the reticulocyte count, serum iron, and hematocrit are performed to define the characteristic recovery of normal red cell production. The Schilling test can be used to measure the absorption of the vitamin and delineate the mechanism of the disease. By performing the Schilling test with and without added intrinsic factor, it is possible to discriminate between intrinsic factor deficiency by itself and primary ileal cell disease. *Vitamin B_{12} deficiency can irreversibly damage the nervous system*. Because the neurological damage can be dissociated from the changes in the hematopoietic system, vitamin B_{12} deficiency must be considered in elderly patients with dementia or psychiatric disorders, even if they are not anemic (Spence, 2016).

Vitamin B_{12} Therapy

Vitamin B_{12} has an undeserved reputation as a health tonic and has been used for a number of disease states. Numerous multivitamin preparations are marketed either as nutritional supplements or for the treatment of anemia; many are supplemented with intrinsic factor. Although the combination of oral vitamin B_{12} and intrinsic factor would appear to be ideal for patients with an intrinsic factor deficiency, such preparations are not reliable.

Vitamin B_{12} is available for injection or oral administration; combinations with other vitamins and minerals also can be given orally or parenterally. The choice of a preparation always depends on the cause of the deficiency. Oral administration cannot be relied on for effective therapy in the patient with a marked deficiency of vitamin B_{12} and abnormal hematopoiesis or neurological deficits. The treatment of choice for vitamin B_{12} deficiency is cyanocobalamin administered by intramuscular or subcutaneous injection, never intravenously. Cyanocobalamin is administered in doses of 1 to 1000 μg. Tissue uptake, storage, and utilization depend on the availability of transcobalamin II. Doses greater than 100 μg are cleared rapidly from plasma into the urine, and administration of larger amounts of vitamin B_{12} will not result in greater retention of the vitamin. Administration of 1000 μg is of value in the performance of the Schilling test. After isotopically labeled vitamin B_{12} is administered orally, the compound that is absorbed can be quantitatively recovered in the urine if 1000 μg of *cyanocobalamin* is administered intramuscularly. This unlabeled material saturates the transport system and tissue binding sites, so more than 90% of the labeled and unlabeled vitamin is excreted during the next 24 h.

Effective use of the vitamin B_{12} depends on accurate diagnosis and an understanding of the following general principles of therapy:

- Vitamin B_{12} should be given prophylactically only when there is a reasonable probability that a deficiency exists or will exist (i.e., dietary deficiency in the strict vegetarian, the predictable malabsorption of vitamin B_{12} in patients who have had a gastrectomy, and certain diseases of the small intestine) (Del Villar Madrigal et al., 2015). When GI function is normal, an oral prophylactic supplement of vitamins and minerals, including vitamin B_{12}, may be indicated. Otherwise, the patient should receive monthly injections of cyanocobalamin.
- The relative ease of treatment with vitamin B_{12} should not prevent a full investigation of the etiology of the deficiency. The initial diagnosis usually is suggested by macrocytic anemia or an unexplained neuropsychiatric disorder.
- Therapy always should be as specific as possible. Although a large number of multivitamin preparations are available, the use of shotgun vitamin therapy in the treatment of vitamin B_{12} deficiency can be dangerous: Sufficient folic acid may be given to result in a hematological recovery that can mask continued vitamin B_{12} deficiency and permit neurological damage to develop or progress.
- Although a classical therapeutic trial with small amounts of vitamin B_{12} can help confirm the diagnosis, acutely ill elderly patients may not be able to tolerate the delay in the correction of a severe anemia. Such patients require supplemental blood transfusions and immediate therapy with folic acid and vitamin B_{12} to guarantee rapid recovery.
- Long-term therapy with vitamin B_{12} must be evaluated at intervals of 6 to 12 months in patients who are otherwise well. If there is an additional illness or a condition that may increase the requirement for the vitamin (e.g., pregnancy), reassessment should be performed more frequently.

Treatment of the Acutely Ill Patient. The therapeutic approach depends on the severity of the illness. In uncomplicated pernicious anemia, in which the abnormality is restricted to a mild or moderate anemia without leukopenia, thrombocytopenia, or neurological signs or symptoms, the administration of vitamin B_{12} alone will suffice. Moreover, therapy may be delayed until other causes of megaloblastic anemia have been excluded and sufficient studies of GI function have been performed to reveal the underlying cause of the disease. In this situation, a therapeutic trial with small amounts of parenteral vitamin B_{12} (1–10 μg/day) can confirm the presence of an uncomplicated vitamin B_{12} deficiency.

In contrast, patients with neurological changes or severe leukopenia or thrombocytopenia associated with infection or bleeding require emergency treatment. Effective therapy must not wait for detailed diagnostic tests. Once the megaloblastic erythropoiesis has been confirmed and sufficient blood collected for later measurements of vitamin B_{12} and folic acid, the patient should receive intramuscular injections of 100 μg of cyanocobalamin and 1 to 5 mg of folic acid. For the next 1 to 2 weeks, the patient should receive daily intramuscular injections of 100 μg of cyanocobalamin, together with a daily oral supplement of 1 to 2 mg of folic acid. Because an effective increase in red cell mass will not occur for 10 to 20 days, the patient with a markedly depressed hematocrit and tissue hypoxia also should receive a transfusion of 2 to 3 units of packed red blood cells. If congestive heart failure is present, diuretics can be administered to prevent volume overload.

The first objective hematological change is the disappearance of the megaloblastic morphology of the marrow. As the ineffective erythropoiesis is corrected, the concentration of iron in plasma falls dramatically as the metal is used in the formation of hemoglobin, usually within the first 48 h. Full correction of precursor maturation in marrow with production of an increased number of reticulocytes begins about the second or third day and peaks 3 to 5 days later. Patients with complicating iron deficiency, an infection or other inflammatory state, or renal disease may be unable to correct their anemia. Therefore, it is important to monitor the reticulocyte index over the first several weeks. If it does not continue at elevated levels while the hematocrit is below 35%, plasma concentrations of iron and folic acid should again be determined and the patient reevaluated for an illness that could inhibit the response of the marrow. The degree and rate of improvement of neurological signs and symptoms depend on the severity and the duration of the abnormalities. Those that have been present for only a few months usually disappear relatively rapidly. When a defect has been present for many months or years, full return to normal function may never occur.

Long-Term Therapy With Vitamin B_{12}. Once begun, vitamin B_{12} therapy must be maintained for life. This fact must be impressed on the patient and family, and a system must be established to guarantee continued monthly injections of cyanocobalamin.

Intramuscular injection of 100 μg of cyanocobalamin every 4 weeks is usually sufficient. Patients with severe neurological symptoms and signs may be treated with larger doses of vitamin B_{12} in the period immediately after the diagnosis. Doses of 100 μg per day or several times per week may be given for several months with the hope of encouraging faster and more complete recovery. It is important to monitor vitamin B_{12} concentrations in plasma and to obtain peripheral blood counts at intervals of 3 to 6 months to confirm the adequacy of therapy. Because refractoriness to therapy can develop at any time, evaluation must continue throughout the patient's life. Intranasal preparations are available for maintenance following normalization of vitamin B_{12}–deficient patients without nervous system involvement.

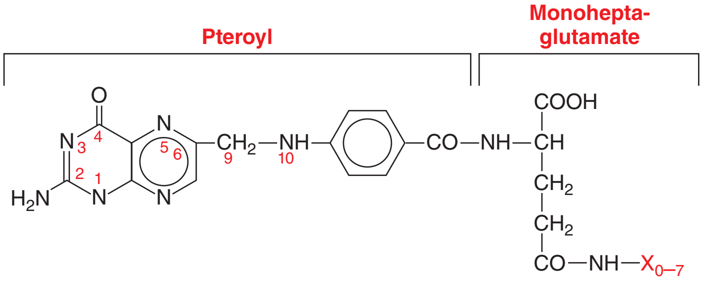
Folic Acid and Human Health

Biochemical Roles of Folate

Pteroylglutamic acid (Figure 45–8) is the common pharmaceutical form of folic acid. It is not the principal folate congener in food or the active coenzyme for intracellular metabolism. After absorption, PteGlu is rapidly reduced at the 5, 6, 7, and 8 positions to *tetrahydrofolic acid* (H_4PteGlu), which then acts as an acceptor of a number of one-carbon units. These are attached at either the 5 or the 10 position of the pteridine ring or may bridge these atoms to form a new five-membered ring. The most important forms of the coenzyme are synthesized by the reactions depicted in Figure 45–5, and each plays a specific role in intracellular metabolism:

- *Conversion of homocysteine to methionine*. This reaction requires CH_3H_4PteGlu as a methyl donor and uses vitamin B_{12} as a cofactor.
- *Conversion of serine to glycine*. This reaction requires tetrahydrofolate as an acceptor of a methylene group from serine and uses pyridoxal phosphate as a cofactor. It results in the formation of 5,10-CH_2H_4PteGlu, an essential coenzyme for the synthesis of dTMP.
- *Synthesis of thymidylate*. 5,10-CH_2H_4PteGlu donates a methylene group and reducing equivalents to dUMP for the synthesis of dTMP—a rate-limiting step in DNA synthesis.
- *Histidine metabolism*. H_4PteGlu also acts as an acceptor of a formimino group in the conversion of FIGLU to glutamic acid.
- *Synthesis of purines*. Two steps in the synthesis of purine nucleotides require the participation of 10-$CHOH_4$PteGlu as a formyl donor in reactions catalyzed by ribotide transformylases: the formylation of glycinamide ribonucleotide and the formylation of 5-aminoimidazole-4-carboxamide ribonucleotide. By these reactions, carbon atoms at positions 8 and 2, respectively, are incorporated into the growing purine ring.
- *Utilization or generation of formate*. This reversible reaction uses H_4PteGlu and 10-$CHOH_4$PteGlu.

Daily Requirements. Many food sources are rich in folates, especially fresh green vegetables, liver, yeast, and some fruits. However, lengthy cooking can destroy up to 90% of the folate content of such food. Generally, a standard U.S. diet provides 50 to 500 μg of absorbable folate per



Position	Radical	Congener	
N^5	$—CH_3$	CH_3H_4PteGlu	Methyltetrahydrofolate
N^5	—CHO	5-$CHOH_4$PteGlu	Folinic acid (citrovorum factor)
N^{10}	—CHO	10-$CHOH_4$PteGlu	10-Formyltetrahydrofolate
$N^{5,10}$	=CH—	5,10-CHH_4PteGlu	5,10-Methenyltetrahydrofolate
$N^{5,10}$	$—CH_2—$	5,10-CH_2H_4PteGlu	5,10-Methylenetetrahydrofolate
N^5	—CHNH	$CHNHH_4$PteGlu	Formiminotetrahydrofolate
N^{10}	$—CH_2OH$	CH_2OHH_4PteGlu	Hydroxymethyltetrahydrofolate

Figure 45–8 *The structures and nomenclature of PteGlu (folic acid) and its congeners.* X represents additional residues of glutamate; polyglutamates are the storage and active forms of the vitamin. The number of residues of glutamate is variable.

day, although individuals with high intakes of fresh vegetables and meats will ingest as much as 2 mg/day. In the normal adult, the recommended daily intake is 400 μg; pregnant or lactating women and patients with high rates of cell turnover (such as patients with a hemolytic anemia) may require 500 to 600 μg or more per day. For the prevention of neural tube defects, a daily intake of at least 400 μg of folate in food or in supplements beginning a month before pregnancy and continued for at least the first trimester is recommended. Folate supplementation also is being considered in patients with elevated levels of plasma homocysteine.

ADME. As with vitamin B_{12}, the diagnosis and management of deficiencies of folic acid depend on an understanding of the transport pathways and intracellular metabolism of the vitamin (Figure 45–9). Folates present in food are largely in the form of reduced polyglutamates, and absorption requires transport and the action of a *pteroylglutamyl carboxypeptidase* associated with mucosal cell membranes. The mucosae of the duodenum and upper part of the jejunum are rich in dihydrofolate reductase and can methylate most of or all the reduced folate that is absorbed. Because most absorption occurs in the proximal portion of the small intestine, it is not unusual for folate deficiency to occur when the jejunum is diseased. Both nontropical and tropical sprues are common causes of folate deficiency and megaloblastic anemia.

Once absorbed, folate is transported rapidly to tissues as $CH_3H_4PteGlu$. Although certain plasma proteins do bind folate derivatives, they have a greater affinity for nonmethylated analogues. The role of such binding proteins in folate homeostasis is not well understood. An increase in binding capacity is detectable in folate deficiency and in certain disease states, such as uremia, cancer, and alcoholism. A constant supply of $CH_3H_4PteGlu$ is maintained by food and by an enterohepatic cycle of the vitamin. The liver actively reduces and methylates PteGlu (and H_2 or $H_4PteGlu$) and then transports the $CH_3H_4PteGlu$ into bile for reabsorption by the gut and subsequent delivery to tissues. This pathway may provide 200 μg or more of folate each day for recirculation to tissues. The importance of the enterohepatic cycle was suggested by animal studies that showed a rapid reduction of the plasma folate concentration after either drainage of bile or ingestion of alcohol, which apparently blocks the release of $CH_3H_4PteGlu$ from hepatic parenchymal cells.

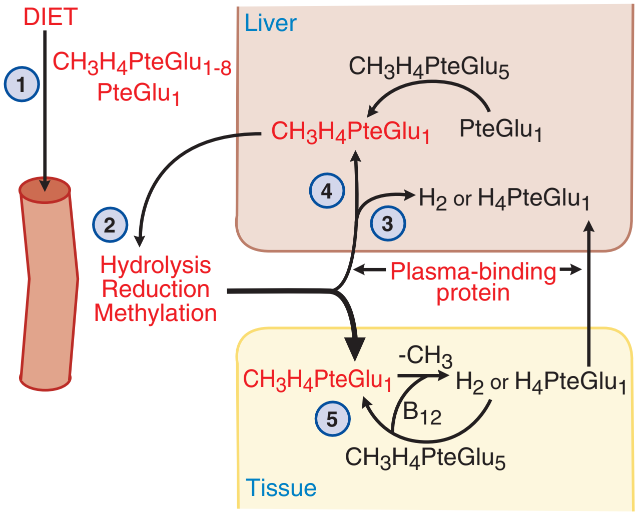


Figure 45–9 *Absorption and distribution of folate derivatives.* Dietary sources of folate polyglutamates are hydrolyzed to the monoglutamate, reduced, and methylated to $CH_3H_4PteGlu_1$ during GI transport. Folate deficiency commonly results from (1) inadequate dietary supply and (2) small intestinal disease. In patients with uremia, alcoholism, or hepatic disease, there may be defects in (3) the concentration of folate-binding proteins in plasma and (4) the flow of $CH_3H_4PteGlu_1$ into bile for reabsorption and transport to tissue (the folate enterohepatic cycle). Finally, vitamin B_{12} deficiency will (5) "trap" folate as $CH_3H_4PteGlu$, thereby reducing the availability of $H_4PteGlu_1$ for its essential roles in purine and pyrimidine synthesis.

Folate Deficiency. Folate deficiency is a common complication of diseases of the small intestine that interfere with the absorption of folate from food and the recirculation of folate through the enterohepatic cycle. The prevalence of folate deficiency in persons over age 65 is relatively high due to reduced dietary intake or intestinal malabsorption (Araujo et al., 2015). In acute or chronic alcoholism, daily intake of folate in food may be severely restricted, and the enterohepatic cycle of the vitamin may be impaired by toxic effects of alcohol on hepatic parenchymal cells; this is the most common cause of folate-deficient megaloblastic erythropoiesis and the most amenable to therapy, via reinstitution of a normal diet. Disease states characterized by a high rate of cell turnover, such as hemolytic anemias, also may be complicated by folate deficiency. In addition, drugs that inhibit dihydrofolate reductase (e.g., *methotrexate* and *trimethoprim*) or that interfere with the absorption and storage of folate in tissues (e.g., certain anticonvulsants and oral contraceptives) can lower the concentration of folate in plasma and may cause a megaloblastic anemia (Hesdorffer and Longo, 2015).

Folate deficiency is recognized by its impact on the hematopoietic system. As with vitamin B_{12}, this fact reflects the increased requirement associated with high rates of cell turnover. The megaloblastic anemia that results from folate deficiency cannot be distinguished from that caused by vitamin B_{12} deficiency. In contrast to vitamin B_{12} deficiency, folate deficiency is rarely, if ever, associated with neurological abnormalities. After deprivation of folate, megaloblastic anemia develops much more rapidly than it does following interruption of vitamin B_{12} absorption (e.g., gastric surgery). This observation reflects the fact that body stores of folate are limited. Although the rate of induction of megaloblastic erythropoiesis may vary, a folate-deficiency state may appear in 1 to 4 weeks, depending on the individual's dietary habits and stores of the vitamin.

Folate deficiency is implicated in the incidence of neural tube defects (Wallingford et al., 2013). An inadequate intake of folate also can result in elevations in plasma homocysteine. Because even moderate hyperhomocysteinemia is considered an independent risk factor for coronary artery and peripheral vascular disease and for venous thrombosis, the role of folate as a methyl donor in the homocysteine-to-methionine conversion is receiving increased attention (Stanger and Wonisch, 2012).

General Principles of Therapy. The therapeutic use of folic acid is limited to the prevention and treatment of deficiencies of the vitamin. As with vitamin B_{12} therapy, effective use of the vitamin depends on accurate diagnosis and an understanding of the mechanisms that are operative in a specific disease state. The following general principles of therapy should be respected:

- Dietary supplementation is necessary when there is a requirement that may not be met by a "normal" diet. The daily ingestion of a multivitamin preparation containing 400 to 500 μg of folic acid has become standard practice before and during pregnancy to reduce the incidence of neural tube defects and for as long as a woman is breastfeeding. In women with a history of a pregnancy complicated by a neural tube defect, an even larger dose of 4 mg/day has been recommended. Patients on total parenteral nutrition should receive folic acid supplements as part of their fluid regimen because liver folate stores are limited. Adult patients with a disease state characterized by high cell turnover (e.g., hemolytic anemia) generally require 1 mg of folic acid given once or twice a day. The 1-mg dose also has been used in the treatment of patients with elevated levels of homocysteine.
- Any patient with folate deficiency and a megaloblastic anemia should be evaluated carefully to determine the underlying cause of the deficiency state. This should include evaluation of the effects of medications, the amount of alcohol intake, the patient's history of travel, and the function of the GI tract.
- Therapy always should be as specific as possible. Multivitamin preparations should be avoided unless there is good reason to suspect deficiency of several vitamins.
- The potential danger of mistreating a patient who has vitamin B_{12} deficiency with folic acid must be kept in mind. The administration of large doses of folic acid can result in an apparent improvement of the megaloblastic anemia because PteGlu is converted by dihydrofolate

reductase to H_4PteGlu; this circumvents the methylfolate "trap." However, folate therapy does not prevent or alleviate the neurological defects of vitamin B_{12} deficiency, and these may progress and become irreversible.

Therapeutic Use of Folate. Folic acid is marketed as oral tablets containing PteGlu or l-methylfolate, as an aqueous solution for injection (5 mg/mL), and in combination with other vitamins and minerals. Folinic acid (leucovorin calcium, citrovorum factor) is the 5-formyl derivative of tetrahydrofolic acid. The principal therapeutic uses of folinic acid are to circumvent the inhibition of dihydrofolate reductase as a part of high-dose *methotrexate* therapy and to potentiate *fluorouracil* in the treatment of colorectal cancer (see Chapter 70). It also has been used as an antidote to counteract the toxicity of folate antagonists such as *pyrimethamine* or *trimethoprim*. Folinic acid provides no advantage over folic acid, is more expensive, and therefore is not recommended. A single exception is the megaloblastic anemia associated with congenital dihydrofolate reductase deficiency.

Evaluation of the serum folate level can help exclude folate deficiency, but only in patients whose serum folate levels exceed 5.0 ng/mL; 2% to 5% of healthy adults can have serum folate levels that are below this level. Red cell folate levels (reference range >140 ng/mL) reflect chronic folate levels, are less affected by acute ingestion of folate than are serum levels, but are more time consuming and costly to measure. Serum folate concentrations more frequently show a higher correlation with serum homocysteine, which is a sensitive marker of deficiency (Farrell et al., 2013). In any case, a patient with a low serum or red cell folate level should have follow-up tests that include serum homocysteine (reference range, 5–16 mM), which is elevated in B_{12} and folate deficiency, and serum methylmalonic acid (reference range, 70–270 mM), which is elevated only in B_{12} deficiency.

Untoward Effects. There have been rare reports of reactions to parenteral injections of folic acid and *leucovorin*. Oral folic acid usually is not toxic. Folic acid in large amounts may counteract the antiepileptic effect of *phenobarbital*, *phenytoin*, and *primidone* and increase the frequency of seizures in susceptible children. The FDA recommends that oral tablets of folic acid be limited to strengths of 1 mg or less.

Acknowledgment: *Kenneth Kaushansky contributed to this chapter in the previous edition of this book. We have retained some of his text in the current edition.*

Drug Facts for Your Personal Formulary: *Hematopoietic Agents: Growth Factors, Minerals, and Vitamins*

Drug	Therapeutic Use (Principle Labeled Indications)	Clinical Pharmacology and Tips
Erythropoietin-Stimulating Agents (ESAs): Stimulate red blood cell production; bind to erythropoietin (EPO) receptor on erythroid progenitor cells		
Epoetin alfa	• Anemia due to CKD • Anemia due to chemotherapy in patients with cancer	• Use the lowest dose sufficient to reduce the need for transfusions • ↑ risk of death, cardiovascular event, or stroke if administered to a target hemoblobin >11 g/dL • Not indicated in patients receiving chemotherapy when expected outcome is curative or when the anemia can be managed by transfusion • Darbepoetin alfa may be dosed less frequently, but the two ESAs have similar efficacy
Darbepoetin alfa	• Anemia due to CKD • Anemia due to chemotherapy in patients with cancer	
Granulocyte Colony-Stimulating Factors (GCSFs): Stimulate production, maturation, and activation of neutrophils		
Filgrastim	• Chemotherapy-induced myelosuppression in nonmyeloid malignancies • Acute myeloid leukemia following induction or consolidation chemotherapy • After bone marrow transplantation • Severe chronic neutropenia	• Indicated for prophylactic use to ↓ risk of neutropenic fever when risk is anticipated to be ~20% or greater • Not intended as routine treatment of established neutropenic fever
Pegfilgrastim	• Prevention of chemotherapy-induced neutropenia	• Compared to filgrastim: prolonged duration of effect and reduced renal clearance • Typically administered 24 h after chemotherapy and at least 14 days prior to the next chemotherapy dose
Thrombopoietin Receptor Agonists (TRAs): Stimulate production of megakaryocytes and platelets in the marrow		
Romiplostim	• Immune thrombocytopenia (ITP) with insufficient response to corticosteroids, immune globulin, or splenectomy	• Use only when degree of thrombocytopenia and clinical condition ↑ risk of bleeding; not intended to completely normalize platelet count
Eltrombopag	• Severe aplastic anemia (first-line in combination with immune suppression) • Chronic hepatitis C virus (HCV)–associated thrombocytopenia • ITP with insufficient response to corticosteroids, immune globulin, or splenectomy	• As above, not intended to completely normalize platelet count • In patients with chronic HCV, with interferon and ribavirin may ↑ risk of hepatic decompensation
Avatrombopag	• Chronic ITP with insufficient response to a previous treatment • Chronic liver disease–associated thrombocytopenia who are scheduled to undergo a procedure	• In chronic ITP, use lowest dose necessary to achieve platelet counts >50,000/μL • For preprocedural use: 5-day course beginning avatrombopag 10 days prior to scheduled procedure; conduct procedure 5–8 days after the last dose
Lusutrombopag	• Chronic liver disease–associated thrombocytopenia with a scheduled procedure	• 3 mg once daily for 7 days, beginning 8–14 days prior to scheduled procedure; conduct procedure 2–8 days after the last lusutrombopag dose

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Drug Facts for Your Personal Formulary: *Hematopoietic Agents: Growth Factors, Minerals, and Vitamins* (*continued*)

Drug	Therapeutic Use (Principle Labeled Indications)	Clinical Pharmacology and Tips
TFGβ Inhibitor: Stimulates red blood cell productions by promoting erythroid maturation in conditions with ineffective erythropoiesis		
Luspatercept	• Anemia due to beta thalassemia • Anemia due to myelodysplastic syndrome (MDS) with ring sideroblasts (RS) or MDS/myeloproliferative neoplasm with RS and thrombocytosis	• Monitor blood pressure • Monitor for signs/symptoms of thromboembolism
Oral Iron: Corrects iron deficiency and builds iron stores necessary for hemoglobin synthesis		
Ferrous sulfate and other iron salts	• Iron deficiency anemia	• 65 mg of elemental iron (e.g., ferrous sulfate 325-mg tablet) every other day, maximizing relative absorption • Daily dosing ⇒ ↓ absorption, but may be reasonable to improve adherence • Ferrous salts have equivalent efficacy • Enteric-coated and slow-/sustained-release forms not preferred (poor absorption)
Parenteral Iron: Corrects iron deficiency in clinical situations when there is a need for rapid repletion, or poor absorption or lack of response to oral iron		
Iron dextran	• Iron deficiency in patients with intolerance to oral iron or unsatisfactory response to oral iron	• Administer 25-mg test dose first • 1 g can be given as a single dose • Administered as a slow infusion
Sodium ferric gluconate	• Iron deficiency in patients with CKD undergoing hemodialysis in conjunction with supplemental EPO therapy	• Multiple doses of 125–250 mg • 125-mg dose is administered over 1 h • Avoided in pregnancy due to benzyl alcohol preservative (potential fetal risk)
Iron sucrose	• Iron deficiency in patients with CKD	• Multiple doses of 100–300 mg • Administered as a slow IV injection or infusion • Less preferred in pregnancy due to the need for multiple infusions rather than single replacement dose
Ferumoxytol	• Iron deficiency anemia in adults with an intolerance or unsatisfactory response to oral iron or who have CKD	• Single dose of 1020 mg or two doses of 510 mg, given 3–8 days apart • Administered over at least 15 min (30 min for 1020-mg dose) • Monitor for signs of hypersensitivity for ≥30 min after infusion
Ferric carboxymaltose	• Iron deficiency anemia in patients with intolerance to oral iron or unsatisfactory response to oral iron or who have non–dialysis-dependent CKD	• Two doses of 750 mg (or 15 mg/kg if weight <50 kg), given 7 or more days apart • Administer as a slow IV push over 15 min
Ferric derisomaltose	• Iron deficiency anemia in patients with intolerance to oral iron or unsatisfactory response to oral iron or who have non–dialysis-dependent CKD	• Single dose of 100 mg (20 mg/kg if weight <50 kg) • Administer doses up to 1 g over at least 20 min • Administer doses >1 g over at least 30 min • 500 mg can be administered as slow IV bolus injection (250 mg/min) • Use in pregnancy not prospectively studied, but retrospective review notes safety/efficacy profile similar to that typically observed with other formulations
Agents for Megaloblastic Anemias: Folic acid and vitamin B_{12} are necessary for purine and pyrimidine synthesis		
Cyanocobalamin (vitamin B_{12})	• Treatment of pernicious anemia or vitamin B_{12} deficiency due to dietary deficiencies or malabsorption • Prevention of vitamin B_{12} deficiency in patients with increased requirements	• Oral: 1000–2000 μg daily • Intramuscular (IM) or deep subcutaneous (SQ): 1000 μg • Initial therapy for severe/symptomatic disease is IM or deep SQ 1000 μg daily three times per week for 1 week, then once weekly for 4–8 weeks; maintenance doses: 1000 μg IM/SQ monthly, or 1000–2000 μg orally daily when symptoms have resolved • For mild/asymptomatic deficiency: 1000 μg weekly for 4–8 weeks, then monthly maintenance; or 1000–2000 μg orally daily in patients without impaired GI absorption • Continue indefinitely unless a reversible cause of deficiency has been addressed
Folic acid	• Treatment of megaloblastic and macrocytic anemias due to folate deficiency	• 1–5 mg orally once daily
Leucovorin calcium (folinic acid)	• Rescue agent after high-dose methotrexate (MTX) • Treatment of advanced colorectal cancer • Treatment of megaloblastic anemias due to folic acid deficiency when oral therapy is not feasible	• 5-Formyl derivative of tetrahydrofolic acid • Colon cancer: potentiates the effect of 5-fluorouracil by binding and stabilizing 5-dUMP and thymidylate synthetase • Starting 24 h after MTX treatment to replenish active folate stores

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Endocrine Pharmacology

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Chapter 46

Introduction to Endocrinology: The Hypothalamic-Pituitary Axis

Dequina A. Nicholas and Mark A. Lawson

ENDOCRINOLOGY AND HORMONES: GENERAL CONCEPTS

THE HYPOTHALAMIC-PITUITARY-ENDOCRINE AXIS

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- Physiology of Oxytocin
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Endocrinology and Hormones: General Concepts

Endocrinology analyzes the biosynthesis of hormones, their sites of production, and the sites and mechanisms of their action and interaction. The term *hormone* is of Greek origin and classically refers to a chemical messenger that circulates in body fluids and produces specific effects on cells distant from the hormone's point of origin. The major functions of hormones include the regulation of energy storage, production, and utilization; the adaptation to new environments or conditions of stress; the facilitation of growth and development; and the maturation and function of the reproductive system. Although hormones were originally defined as products of ductless glands, we now appreciate that many tissues and cell types not classically considered as "endocrine" (e.g., the heart, kidneys, GI tract, adipocytes, stem cells, and neurons) synthesize and secrete hormones that play key physiological roles. The current understanding of hormones emphasizes their cellular origin and action. Broadly, the field of endocrinology also includes the consideration of substances that act by means of autocrine and paracrine mechanisms, the influence of neurons—particularly those in the hypothalamus—that regulate endocrine function through synaptic or peptide hormone action, and the reciprocal interactions of cytokines and other components of the immune system with the endocrine system.

Conceptually, hormones may be divided into two classes based on mechanism of action:

- Hormones that act predominantly via *nuclear receptors* to modulate transcription in target cells (e.g., steroid hormones, retinoids, thyroid hormone, and vitamin D)
- Hormones that typically act via *membrane receptors* to exert rapid effects on signal transduction pathways (e.g., peptide and amino acid hormones)

Notably, the steroid hormones operate through both mechanisms, and their effect on cells is determined by the receptor complement in an individual cell. The receptors for both classes of hormones provide tractable targets for a diverse group of compounds that are among the most widely used drugs in clinical medicine.

The Hypothalamic-Pituitary-Endocrine Axis

Many of the classic endocrine hormones (e.g., cortisol, thyroid hormone, sex steroids, GH) are regulated by complex reciprocal interactions among the hypothalamus, anterior pituitary, and target organs or tissues (Table 46–1). The basic organization of the hypothalamic-pituitary-endocrine axis is summarized in Figure 46–1.

Discrete sets of hypothalamic neurons produce different releasing and inhibiting hormones, which are axonally transported to the median eminence. On stimulation, either synaptic or by other intrahypothalamic hormones, these neurons secrete their respective hypothalamic hormones into the hypothalamic-adenohypophyseal portal veins, which connect to the *anterior pituitary* gland. The *hypothalamic hormones* bind to membrane receptors on specific subsets of pituitary cells and regulate the secretion of the corresponding *pituitary hormones*. The pituitary hormones circulate to the target tissues where they activate cognate receptors to exert cell-specific effects or stimulate the synthesis and secretion of the target endocrine hormones. These interactions follow *feed-forward regulation* in which the originating hypothalamic signal is amplified by the anterior pituitary, then elicits a regulated response from target tissues and stimulates the production of hormones by the endocrine targets. In contrast, the *posterior pituitary* contains the endings of nerve axons arising from the hypothalamus, forming the neurohypophysis, which has direct

Abbreviations

AC: adenylyl cyclase
ACTH: corticotropin, formerly adrenocorticotrophic hormone
ADH: antidiuretic hormone
CG: chorionic gonadotropin
COX: cyclooxygenase
CRH: corticotropin-releasing hormone
DA: dopamine
ELISA: enzyme-linked immunosorbent assay
FP: prostaglandin F receptor
FSH: follicle-stimulating hormone, follitropin
GH: growth hormone
GHR: GH receptor
GHRH: growth hormone–releasing hormone
GI: gastrointestinal
GnRH: gonadotropin-releasing hormone
GPCR: G protein-coupled receptor
hCG: human chorionic gonadotropin
5HT: 5-hydroxytryptamin serotonin
IGF-1: insulin-like growth factor 1
IGFBP: IGF-binding protein
IRS: insulin receptor substrate
LH: luteinizing hormone; lutropin
MRI: magnetic resonance imaging
α-MSH: α-melanocyte–stimulating hormone
NO: nitric oxide
NPY: neuropeptide Y
OXTR: oxytocin receptor
POMC: pro-opiomelanocortin
PRL: prolactin
SC: subcutaneous
SHC: Src homology-containing protein
SHP2: Src-homology-2-domain-containing protein tyrosine phosphatase 2
SST: somatostatin
SSTR: SST receptor
TRH: thyrotropin-releasing hormone
TSH: thyroid-stimulating hormone, thyrotropin
VIP: vasoactive intestinal peptide

access to the circulation and does not rely on intermediate action of the pituitary (see Figure 46–1).

Typically, feed-forward signals are controlled by *negative feedback*, which permits precise regulation of hormone levels and reestablishment of homeostasis after activation of a secretory event (Figure 46–2). The endocrine target hormone circulates to both the hypothalamus and pituitary, where it acts via specific receptors to inhibit the production and secretion of both its hypothalamic-releasing hormone and the regulatory pituitary hormone. In addition, other brain regions have inputs to the hypothalamic hormone–producing neurons, and hormones produced independently by other tissues target both the hypothalamus and anterior pituitary, further integrating the regulation of hormone levels in response to diverse stimuli.

TABLE 46–1 ■ HORMONES THAT INTEGRATE THE HYPOTHALAMIC-PITUITARY-ENDOCRINE AXIS

HYPOTHALAMIC HORMONE	EFFECT ON PITUITARY TROPHIC (SIGNAL) HORMONE	TARGET HORMONE(S)
Growth hormone–releasing hormone	↑↑ Growth hormone	IGF-1
Somatostatin	↓ Growth hormone ↓ Thyroid-stimulating hormone	
Dopamine	↓ Prolactin	—
Corticotropin-releasing hormone	↑ Corticotropin	Cortisol
Thyrotropin-releasing hormone	↑ Thyroid-stimulating hormone ↑ Prolactin	Thyroid hormone
Gonadotropin-releasing hormone	↑ Follicle-stimulating hormone ↑ Luteinizing hormone	Estrogen (f) Progesterone/estrogen (f) Testosterone (m)

f, female; m, male; ↑, increased production; ↓, decreased production.

Pituitary Hormones and Their Hypothalamic-Releasing Factors

The anterior pituitary hormones can be classified into three different groups based on their structural features (Table 46–2):

- Pro-opiomelanocortin (POMC)-derived hormones include *corticotropin* (ACTH) and *α-melanocyte–stimulating hormone* (α-MSH). These are derived from POMC by proteolytic processing (see Figures 23–3 and 50–1).
- Somatotropic hormones include *growth hormone* (GH) and prolactin (PRL). In humans, the somatotropic family also includes placental lactogen.

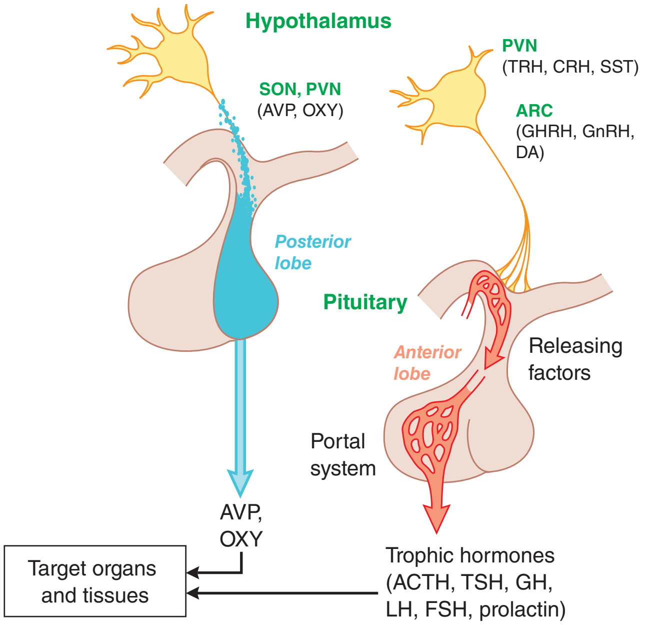

Figure 46–1 *Organization of the anterior and posterior pituitary gland.* Hypothalamic neurons in the supraoptic (SON) and paraventricular (PVN) nuclei synthesize arginine vasopressin (AVP) or oxytocin (OXY). Most of their axons project directly to the posterior pituitary, from which AVP and OXY are secreted into the systemic circulation to regulate their target tissues. Neurons that regulate the anterior lobe cluster in the mediobasal hypothalamus, including the PVN and the arcuate (ARC) nuclei. They secrete hypothalamic releasing hormones, which reach the anterior pituitary via the hypothalamic-adenohypophyseal portal system and stimulate distinct populations of pituitary cells. These cells, in turn, secrete the trophic (signal) hormones, which regulate endocrine organs and other tissues. ARC, arcuate; AVP, arginine vasopressin; OXY, oxytocin; PVN, paraventricular nuclei; SON, supraoptic nuclei; see Abbreviations list for other abbreviations.

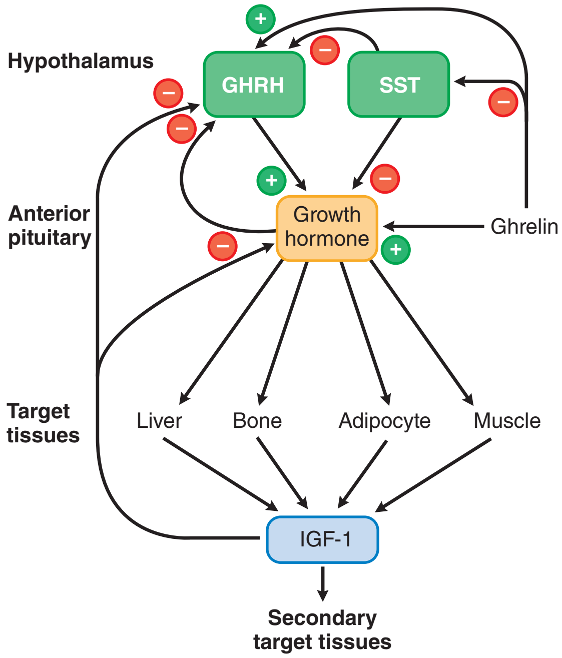

Figure 46–2 *Growth hormone secretion and actions.* Two hypothalamic factors, GHRH and SST, stimulate or inhibit the release of GH from the pituitary, respectively. Insulin-like growth factor-1 (IGF-1), a product of GH action on peripheral tissues, causes negative-feedback inhibition of GH release by acting at the hypothalamus and the pituitary. The actions of GH can be direct or indirect (mediated by IGF-1). See text for discussion of the other agents that modulate GH secretion and of the effects of locally produced IGF-1. Inhibition, –; stimulation, +.

- The glycoprotein hormones include *thyroid-stimulating hormone* (TSH, also called thyrotropin), *luteinizing hormone* (LH, also called lutropin), and *follicle-stimulating hormone* (FSH, also called follitropin). In humans, the glycoprotein hormone family also includes placental *human chorionic gonadotropin* (hCG).

The synthesis and release of *anterior pituitary hormones* are influenced by the CNS. Their secretion is positively regulated by a group of peptides referred to as *hypothalamic-releasing hormones* (see Figure 46–1). These include *corticotropin-releasing hormone* (CRH), *growth hormone–releasing hormone* (GHRH), *gonadotropin-releasing hormone* (GnRH), and *thyrotropin-releasing hormone* (TRH). *Somatostatin* (SST), another hypothalamic peptide, negatively regulates secretion of pituitary GH and TSH. The neurotransmitter *dopamine* (DA) inhibits the secretion of PRL by lactotropes.

Posterior pituitary hormones, which are synthesized by hypothalamic neurons and secreted from the neurohypophysis, include *oxytocin* and *arginine vasopressin* (also called antidiuretic hormone [ADH]). Arginine vasopressin plays an important role in water homeostasis (see Chapter 29); oxytocin plays important roles in labor and parturition and in milk letdown as discussed in the sections that follow. In contrast to other anterior pituitary hormone regulatory models, oxytocin is not regulated by feedback control. Rather, only the feed-forward component of the axis exists, and secretion is reduced by cessation of the stimulatory input.

Growth Hormone and Prolactin

Growth hormone and PRL are structurally related members of the somatotropic hormone family and share many biological features. The somatotropes and lactotropes, the pituitary cells that produce and secrete GH and PRL, respectively, are subject to strong inhibitory input from hypothalamic neurons. For PRL, dopaminergic input is the dominant negative regulator of secretion. GH and PRL act via membrane receptors that belong to the class 1 cytokine receptor family and modulate target cell function via very similar signal transduction pathways (see Chapter 3).

Structures of Growth Hormone and Prolactin

Table 46–2 presents some features of the somatotropic family of hormones. GH is secreted by somatotropes as a heterogeneous mixture of peptides. The principal form is a single unglycosylated polypeptide chain of 22 kDa that has two disulfide bonds. Alternative splicing produces a smaller form (~20 kDa) with equal bioactivity that makes up 5% to 10% of circulating GH. Recombinant human GH consists entirely of the 22 kDa form, which allows detection of GH abuse. In the circulation, a 55 kDa protein, which is derived from the extracellular domain of the proteolytically cleaved GHRH receptor, binds approximately 45% of the 22 kDa and 25% of the 20 kDa forms. A second protein unrelated to the GHR also binds approximately 5% to 10% of circulating GH with lower affinity. Bound GH is cleared more slowly and has a biological $t_{1/2}$ about 10 times that of unbound GH, suggesting that the bound hormone may provide a GH reservoir that dampens acute fluctuations in GH levels associated with its pulsatile secretion.

Human PRL is synthesized by lactotropes. A portion of the secreted hormone is glycosylated at a single Asn residue. In the circulation, multimeric forms of PRL occur, as do degradation products of 16 kDa and 18 kDa. As with GH, the biological significance of these polymeric and degraded forms is not known.

TABLE 46–2 ■ PROPERTIES OF THE PROTEIN HORMONES OF THE HUMAN ADENOHYPOPHYSIS AND PLACENTA

CLASS AND HORMONE	MASS (daltons)	PEPTIDE CHAINS	AMINO ACID RESIDUES AND COMMENTS	
POMC-derived hormones[a]				These peptides are derived by proteolytic processing of the common precursor, POMC.
Corticotropin	4500	1	39	
α-Melanocyte–stimulating hormone	1650		13	
Somatotropic family of hormones				
Growth hormone	22,000	1	191	Receptors for these hormones belong to the cytokine superfamily.
Prolactin	23,000		199	
Placental lactogen	22,125		190	
Glycoprotein hormones				
Luteinizing hormone	29,400	2	β-121	These are heterodimeric glycoproteins with a common α subunit of 92 amino acids and unique β subunits that determine biological specificity and $t_{1/2}$.
Follicle-stimulating hormone	32,600		β-111	
Human chorionic gonadotropin	38,600		β-145	
Thyroid-stimulating hormone	28,000		β-118	

[a] See Figure 23–3 and 50–1 and associated text for further discussion of POMC-derived peptides, including ACTH and α-melanocyte–stimulating hormone.

Human placental lactogen, structurally similar to GH and PRL, is synthesized in pregnant females, with maximal levels near term. Human placental lactogen alters the mother's metabolism mainly by reducing maternal insulin sensitivity to favor fetal nutrition (Cattini et al., 2020).

Regulation of Growth Hormone Secretion

Daily GH secretion varies throughout life. GH secretion is high in children, peaks during puberty, and then decreases with age in adulthood. GH is secreted in discrete but irregular pulses, and the amplitude of secretory pulses is greatest at night. GH secretion is stimulated by GHRH and ghrelin and is subject to feedback inhibition by GH itself, SST, and *insulin-like growth factor 1* (IGF-1; see Figure 46–2).

Growth Hormone–Releasing Hormone

GHRH, a peptide with 44 amino acids produced by hypothalamic neurons, stimulates GH secretion (see Figure 46–2) by binding to a specific GPCR on somatotropes in the anterior pituitary. The stimulated GHRH receptor couples to G_s to raise intracellular levels of cAMP and Ca^{2+}, thereby stimulating GH synthesis and secretion. Loss-of-function mutations of the GHRH receptor cause a rare form of short stature in humans.

Ghrelin

Ghrelin, a 28-amino-acid peptide, stimulates GH secretion through actions on a GPCR called the GH secretagogue receptor. Ghrelin is synthesized predominantly in endocrine cells in the fundus of the stomach but also is produced at lower levels at several other sites, including the pituitary and hypothalamus. Hypothalamic ghrelin is thought to be a stimulus for GH release through actions on pituitary somatotropes and hypothalamic GHRH-secreting neurons.

Both fasting and hypoglycemia increase circulating stomach-derived ghrelin levels, and this, in turn, stimulates appetite and increases food intake, apparently by central actions on NPY and agouti-related peptide neurons in the hypothalamus. The physiologic role of stomach-derived ghrelin in GH secretion is unclear due to a lack of phenotype in ghrelin and GH secretagogue receptor knockout models and conflicting results of clinical studies attempting to correlate circulating levels of ghrelin with GH secretion (Nass et al., 2011).

Other Stimuli

Several neurotransmitters, drugs, metabolites, and other stimuli modulate the release of GHRH or SST and thereby affect GH secretion. DA, 5HT, and α_2 adrenergic receptor agonists stimulate GH release, as do hypoglycemia, exercise, stress, emotional excitement, sex steroids, and ingestion of protein-rich meals. In contrast, β adrenergic receptor agonists, free fatty acids, glucose, IGF-1, and GH itself inhibit release.

Feedback Control of GH Secretion

Growth hormone secretion is regulated by negative-feedback loops. The negative-feedback action of GH is mediated through GH itself, in part by SST, which is synthesized in widely distributed neurons (Ergun-Longmire and Wajnrajch, 2013) and via its major peripheral effector *IGF-1* (see Figure 46–2).

Insulin-like Growth Factor 1. The inhibitory effect of IGF-1 on GH secretion is predominantly through direct effects on the anterior pituitary gland but also at the hypothalamus via stimulation of SST secretion. After its synthesis and release, IGF-1 interacts with receptors on the cell surface that mediate its biological activities. This receptor is present in essentially all tissues and binds IGF-1 and the related growth factor, IGF-2, with high affinity. The type 1 IGF receptor is closely related to the insulin receptor and consists of a heterotetramer with intrinsic tyrosine kinase activity. The signal transduction pathway for the insulin receptor is described in detail in Chapter 51.

Somatostatin. SST is synthesized as a 92-amino-acid precursor and processed by proteolytic cleavage to generate two peptides: SST-28 and SST-14 (Figure 46–3). SST exerts its effects by binding to and activating a family of five related GPCRs that signal through G_i to inhibit cAMP formation and to activate K^+ channels and protein phosphotyrosine phosphatases.

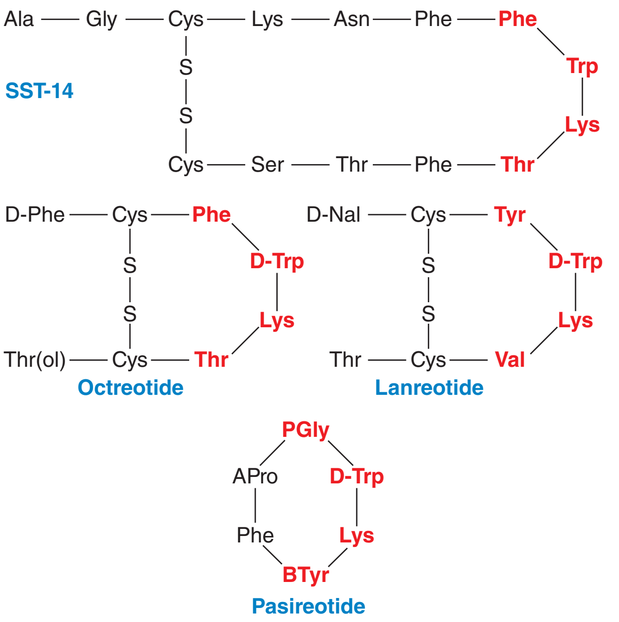

Figure 46–3 *Structures of SST-14 and selected synthetic analogues.* Residues that play key roles in binding to SST receptors are shown in red. *Octreotide*, *lanreotide*, and *pasireotide* are clinically available synthetic analogues of SST. APro, [(2-aminoethyl) aminocarboxyl oxy]-L-proline; D-Nal, 3-(2-napthyl)-D-alanyl; PGly, phenylglycine; BTyr, benzyltyrosine.

There are five SSTR subtypes. $SSTR_{1–4}$ bind the two forms of SST with approximately equal affinity. $SSTR_5$ has a 10- to 15-fold greater affinity for SST-28. $SSTR_2$ and $SSTR_5$ are the most important for regulation of GH secretion, and studies suggested that these two SSTRs form functional heterodimers with distinctive signaling behavior (Grant et al., 2008). SST exerts direct effects on somatotropes in the pituitary and indirect effects mediated via GHRH neurons in the arcuate nucleus.

Regulation of Prolactin Secretion

Prolactin is unique among the anterior pituitary hormones in that hypothalamic regulation of its secretion is predominantly inhibitory. The major regulator of PRL secretion is DA, which interacts with the D_2 receptor, a GPCR on lactotropes, to inhibit PRL secretion (Figure 46–4). TRH and hypothalamic VIP have PRL-releasing properties, but their physiologic significance is uncertain. Many of the physiological factors that influence GH secretion also affect PRL secretion. Thus, sleep, stress, hypoglycemia, exercise, and sex steroids increase the secretion of both hormones.

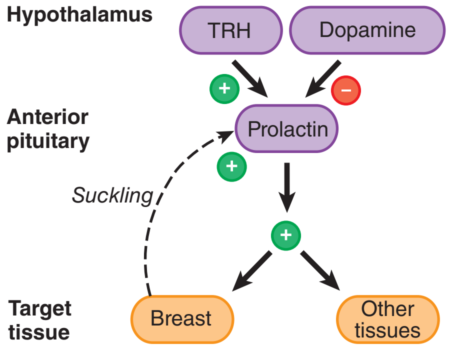

Figure 46–4 *Prolactin secretion and actions.* PRL is the only anterior pituitary hormone for which a unique stimulatory releasing factor has not been identified. TRH and VIP, however, can stimulate PRL release; DA inhibits it. Suckling induces PRL secretion, and PRL not only affects lactation and reproductive functions but also has effects on many other tissues. PRL is not under feedback control by peripheral hormones.

PRL acts predominantly in women, both during pregnancy and in the postpartum period while breastfeeding. During pregnancy, the maternal serum PRL level starts to increase at 8 weeks of gestation, peaks to 150 to 250 ng/mL at term, and declines thereafter to prepregnancy levels unless the mother breastfeeds the infant. Suckling or breast manipulation in nursing mothers transmits signals from the breast to the hypothalamus via the spinal cord and the median forebrain bundle, causing elevation of circulating PRL levels. PRL levels can rise 10-fold within 30 min of stimulation. This response is distinct from milk letdown, which is mediated by oxytocin release from the posterior pituitary gland. The suckling response becomes less pronounced after several months of breastfeeding, and PRL concentrations eventually decline to prepregnancy levels. PRL also is synthesized by decidual cells early in pregnancy (accounting for the high levels of PRL in amniotic fluid during the first trimester of human pregnancy).

Molecular and Cellular Basis of Growth Hormone and Prolactin Action

All effects of GH and PRL result from their interactions with specific membrane receptors on target tissues (Figure 46–5). Receptors for GH and PRL belong to the class 1 cytokine receptor family and thus have sequence homology with the receptors for leptin, erythropoietin, granulocyte-macrophage colony-stimulating factor, and several of the interleukins. Within this receptor family (except for GHR), a Trp-Ser-X-Trp-Ser motif is conserved in the extracellular ligand-binding domain. In addition to this domain, these receptors also have a single membrane-spanning region and an intracellular domain that mediates signal transduction in response to ligand-induced conformational changes.

Growth hormone receptor is activated by the binding of a single GH to two receptor monomers to form a GH-$[\text{GHR}]_2$ ternary complex. GH first binds one monomer of the GHR dimer at the high-affinity GH site 1, followed by a second, lower-affinity interaction of GH with the other GHR at GH site 2. The ligand-occupied GHR dimer lacks inherent tyrosine kinase activity but provides docking sites for two molecules of JAK2, a cytoplasmic tyrosine kinase of the Janus kinase family. Instead, GH binding induces a conformational change that juxtaposes the JAK2 molecules and leads to *trans*-phosphorylation and autoactivation of JAK2, with consequent tyrosine phosphorylation of docking sites on the cytoplasmic segments of the GHR and of proteins that mediate downstream signaling events (see Figure 46–5; Chia, 2014). These include STAT proteins, SHC (an adapter protein that regulates the Ras/MAPK signaling pathway), and IRS-1 and IRS-2 (insulin receptor substrates 1 and 2, proteins that activate the PI3K pathway). One critical target of STAT5 is the gene encoding IGF-1, a mediator of many of the effects of GH (see Figure 46–2). The fine control of GH action also involves feedback regulatory events that subsequently turn off the GH signal. As part of its action, GH induces the expression of a family of suppressor of cytokine signaling (SOC) proteins and a group of protein tyrosine phosphatases (including SHP2) that, by different mechanisms, disrupt the communication of the activated GHR with JAK2 (Flores-Morales et al., 2006).

The effects of PRL on target cells also result from interactions with a cytokine family receptor that is widely distributed and signals through many of the same pathways as the GHR (Bernard et al., 2015). Alternative splicing of the PRL receptor gene on chromosome 5 gives rise to multiple forms of the receptor that are identical in the extracellular domain but differ in their cytoplasmic domains. In addition, soluble forms that correspond to the extracellular domain of the receptor are found in circulation. Unlike human GH and placental lactogen, which also bind to the PRL receptor and thus are lactogenic, PRL binds specifically to the PRL receptor and has no somatotropic (GH-like) activity.

Physiology of Growth Hormone and Prolactin

The most striking physiological effect of GH is the stimulation of the longitudinal growth of bones. GH also increases bone mineral density after the epiphyses have closed, increases muscle mass, increases the glomerular filtration rate, and stimulates preadipocyte differentiation into adipocytes. GH has potent anti-insulin actions in both the liver and peripheral tissues (e.g., adipocytes and muscle) that decrease glucose utilization and increase lipolysis, but most of its anabolic and growth-promoting effects are mediated indirectly through the induction of IGF-1. IGF-1 interacts

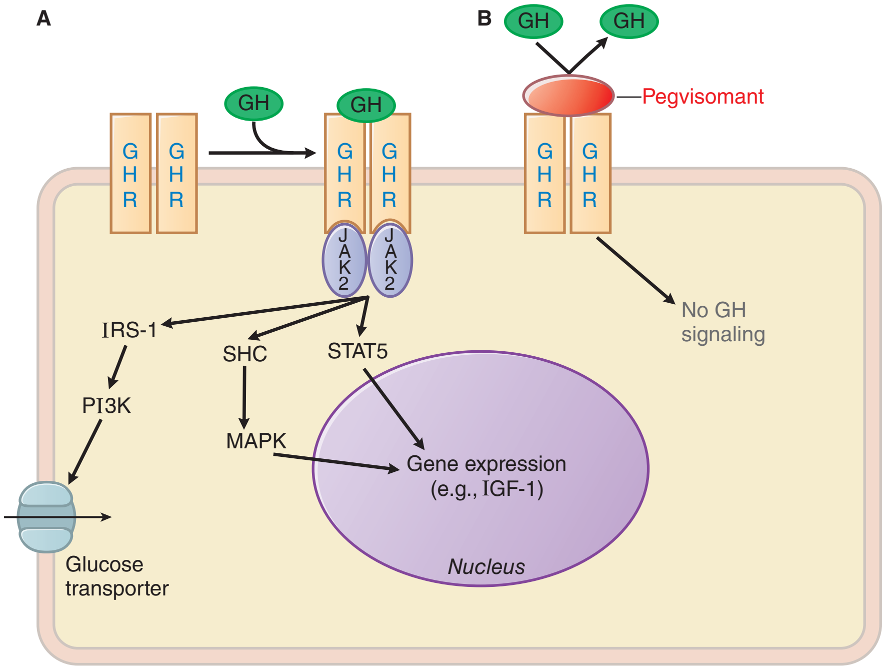

Figure 46–5 *Mechanisms of GH and PRL action and of GHR antagonism.* **A.** GH and two GHRs form a ternary complex that induces association and Tyr autophosphorylation of JAK2 and of docking sites on the cytoplasmic tail of GHRs. JAK2 phosphorylates cytoplasmic proteins that activate downstream signaling pathways, including STAT5 and mediators upstream of MAPK, which ultimately modulate gene expression. The structurally related PRL receptor also is a ligand-activated homodimer that recruits the JAK-STAT signaling pathway. GHR also activates IRS-1, which may mediate the increased expression of glucose transporters on the plasma membrane. **B.** *Pegvisomant*, a recombinant pegylated variant of human GH, is a high-affinity GH antagonist that interferes with GH binding.

with receptors on the cell surface that mediate its biological activities. Circulating IGF-1 is associated with a family of IGF-binding proteins (IGFBP) that serve as transport proteins and may mediate certain aspects of IGF-1 signaling. Most IGF-1 in circulation is bound to IGFBP-3 and another protein called the acid-labile subunit.

The essential role of IGF-1 in growth is evidenced by patients with loss-of-function mutations in both alleles of the *IGF1* gene. These patients have severe intrauterine and postnatal growth retardation that is unresponsive to GH but responsive to recombinant human IGF-1 (Walenkamp and Wit, 2008).

The PRL effects are limited primarily to the mammary gland, where PRL plays an important role in inducing growth and differentiation of the ductal and lobuloalveolar epithelia and is essential for lactation. Target genes by which PRL induces mammary development include those encoding milk proteins (e.g., caseins and whey acidic protein), genes important for intracellular structure (e.g., keratins), and genes important for cell-cell communication (e.g., amphiregulin). PRL receptors are present in many other sites, including the hypothalamus, liver, adrenal glands, testes, ovaries, prostate, and immune system, suggesting that PRL may play multiple roles outside the breast. The physiological effects of PRL at these sites remain poorly characterized.

Pathophysiology of Growth Hormone and Prolactin

Distinct endocrine disorders result from either excessive or deficient GH production. In contrast, PRL predominantly affects endocrine function when produced in excess.

Excess Production

Syndromes of excess secretion of GH and PRL typically are caused by somatotrope or lactotrope adenomas that oversecrete the respective hormones. These adenomas often retain some features of the normal regulation described previously, thus permitting pharmacological modulation of secretion—an important modality in therapy.

Clinical Manifestations of Excess Growth Hormone. GH excess causes distinct clinical syndromes depending on the age of the patient. If the epiphyses are unfused, GH excess causes increased longitudinal growth, resulting in *gigantism*. In adults, GH excess causes *acromegaly*. The symptoms and signs of acromegaly (e.g., arthropathy, carpal tunnel syndrome, generalized visceromegaly, macroglossia, hypertension, glucose intolerance, headache, lethargy, excess perspiration, and sleep apnea) progress slowly, and diagnosis is often delayed. Mortality is increased at least 2-fold relative to age-matched controls, predominantly due to increased death from cardiovascular disease. Treatments that normalize GH and IGF-1 levels reverse this increased risk of mortality and ameliorate most of the other symptoms and signs.

Clinical Manifestations of Excess Prolactin. *Hyperprolactinemia* is a relatively common endocrine abnormality that can result from hypothalamic or pituitary diseases that interfere with the delivery of inhibitory dopaminergic signals, from renal failure, from primary hypothyroidism associated with increased TRH levels, or from treatment with DA receptor antagonists. Most often, hyperprolactinemia is caused by PRL-secreting pituitary adenomas. Manifestations of PRL excess in women include galactorrhea, amenorrhea, and infertility. In men, hyperprolactinemia causes loss of libido, erectile dysfunction, and infertility.

Diagnosis of Growth Hormone and Prolactin Excess. Although acromegaly should be suspected in patients with the appropriate symptoms and signs, diagnostic confirmation requires the demonstration of increased circulating GH or IGF-1. The definitive diagnostic test for acromegaly is the oral glucose tolerance test. Whereas normal subjects suppress their GH level to less than 1 ng/mL in response to an oral glucose challenge, patients with acromegaly either fail to suppress or show a paradoxical increase in GH level.

In patients with hyperprolactinemia, the major question is whether conditions other than a PRL-producing adenoma are responsible for the elevated PRL level. Medications that inhibit DA signaling can cause moderate elevations in PRL (e.g., antipsychotics, *metoclopramide*), as can primary hypothyroidism, pituitary mass lesions that interfere with DA delivery to the lactotropes, and pregnancy. Thus, thyroid function and pregnancy tests are indicated, as is MRI to look for a pituitary adenoma or other defect that might elevate serum PRL.

Impaired Production

Clinical Manifestations of Growth Hormone Deficiency. Children with GH deficiency present with short stature, delayed bone age, and a low age-adjusted growth velocity. GH deficiency in adults is associated with decreased muscle mass and exercise capacity, decreased bone density, impaired psychosocial function, and increased mortality from cardiovascular causes. The diagnosis of GH deficiency should be considered in children with height more than 2 to 2.5 standard deviations below normal, delayed bone age, a decreased growth velocity, and a predicted adult height substantially below the mean parental height. In adults, overt GH deficiency usually results from pituitary lesions caused by a functioning or nonfunctioning pituitary adenoma, secondary to trauma, or related to surgery or radiotherapy for a pituitary or suprasellar mass (Ergun-Longmire and Wajnrajch, 2013). Almost all patients with multiple deficits in other pituitary hormones also have deficient GH secretion.

Clinical Manifestations of Prolactin Deficiency. PRL deficiency may result from conditions that damage the pituitary gland. Inasmuch as the sole clinical manifestation of PRL deficiency is failure of postpartum lactation, PRL is not given as part of endocrine replacement therapy.

Pharmacotherapy of Growth Hormone and Prolactin Disorders

Treatment of Growth Hormone Excess

The initial treatment modality in gigantism/acromegaly is selective removal of the adenoma by transsphenoidal surgery. Radiation and drugs that inhibit GH secretion or action are given if surgery does not result in cure (Katznelson et al., 2014). Pituitary irradiation may be associated with significant long-term complications, including visual deterioration and pituitary dysfunction. Thus, increased attention has been given to the pharmacological management of acromegaly.

Somatostatin Analogues

The development of synthetic analogues of SST has revolutionized the medical treatment of acromegaly. The goal of treatment is to decrease GH levels to less than 2.5 ng/mL after an oral glucose tolerance test and to bring IGF-1 levels to within the normal range for age and sex. The two SST analogues used widely are *octreotide* and *lanreotide*, synthetic derivatives that have longer half-lives than SST and bind preferentially to SST_2 and SST_5 receptors (see Figure 46–3).

Octreotide. *Octreotide* exerts pharmacological actions similar to those of SST. *Octreotide* (100 μg) administered subcutaneously three times daily is 100% bioactive. Peak effects are seen within 30 min, serum $t_{1/2}$ is about 90 min, and duration of action is about 12 h. An equally effective, long-acting, slow-release form, *octreotide LAR*, is administered intramuscularly in a dose of 10, 20, or 30 mg once every 4 weeks. In addition to its effect on GH secretion, *octreotide* can decrease tumor size, although tumor growth generally resumes after *octreotide* treatment is stopped.

Lanreotide. *Lanreotide* autogel is a long-acting octapeptide SST analogue that causes prolonged suppression of GH secretion when administered by deep subcutaneous injection of 60, 90, or 120 mg every 4 weeks. Its efficacy appears comparable to that of the long-acting formulation of *octreotide*.

Pasireotide. *Pasireotide* is a long-acting cyclohexapeptide SST analogue that is approved for the treatment of Cushing disease (excessive cortisol production triggered by increases in ACTH release due to a pituitary adenoma; see Chapter 50) in patients who are ineligible for pituitary surgery or in whom surgery has failed. *Pasireotide* binds to multiple SST receptors

(1, 2, 3, and 5) but has its highest affinity for the SST_5 receptor. In a head-to-head study, a greater percentage of subjects administered *pasireotide LAR* reached treatment goals compared to those given *octreotide LAR*. *Pasireotide LAR* also is approved for treatment of acromegaly.

Adverse Effects. Gastrointestinal side effects—including diarrhea, nausea, and abdominal pain—occur in up to 50% of patients receiving all three SST analogues. The incidence and severity of these side effects are similar for the three analogues. The symptoms usually diminish over time and do not require cessation of therapy. Approximately 25% of patients receiving these drugs develop multiple tiny gallstones, presumably due to decreased gallbladder contraction and bile secretion. Bradycardia and QT prolongation may occur in patients with underlying cardiac disease. Inhibitory effects on TSH secretion rarely lead to hypothyroidism, but thyroid function should be evaluated periodically. *Pasireotide* suppresses ACTH secretion in Cushing disease and may lead to a decrease in cortisol secretion and to hypocortisolism. All SST analogues decrease insulin secretion, but the simultaneous reduction in GH levels results in a reduction in insulin resistance. For *octreotide* and *lanreotide*, most patients will experience no change in glucose tolerance; however, depending on the relative effects on insulin secretion versus resistance, some patients may experience a worsening and others an improvement in glucose tolerance. *Pasireotide*, in addition, decreases the secretion of glucagon-like peptide 1 and glucose insulinotropic peptide, two incretins that facilitate insulin secretion and inhibit glucagon secretion. As a result, glucose tolerance usually worsens significantly and antihyperglycemic therapy is often needed.

Other Therapeutic Uses. SST blocks not only GH secretion but also the secretion of other hormones, growth factors, and cytokines. Thus, the slow-release formulations of SST analogues have been used to treat symptoms associated with metastatic carcinoid tumors (e.g., flushing and diarrhea) and adenomas secreting VIP (e.g., watery diarrhea). *Octreotide* and *lanreotide* can also be used to treat patients with thyrotrope adenomas that oversecrete TSH who have failed surgery. *Octreotide* is used for treatment of acute variceal bleeding and for perioperative prophylaxis in pancreatic surgery. Modified forms of *octreotide* labeled with indium or technetium have been used for diagnostic imaging of neuroendocrine tumors, such as pituitary adenomas and carcinoids; modified forms labeled with β emitters such as ^{90}Y have been used in selective destruction of SST_2 receptor–positive tumors.

Growth Hormone Receptor Antagonist

Pegvisomant. *Pegvisomant* is approved for the treatment of acromegaly. It is a GH analogue with amino acid substitutions that disrupt the interaction at GH site 2, effectively functioning as a GHR antagonist. *Pegvisomant* binds to the receptor and causes its internalization but cannot trigger the conformational change that stimulates JAK-STAT signaling or IGF-1 secretion (see Figure 46–5).

The drug is administered subcutaneously as a 40-mg loading dose, followed by administration of 10 mg/day. Based on serum IGF-1 levels, the dose is titrated at 4- to 6-week intervals to a maximum of 30 mg/day. *Pegvisomant* should not be used in patients with an unexplained elevation of hepatic transaminases, and liver function tests should be monitored in all patients. In addition, lipohypertrophy has occurred at injection sites, sometimes requiring cessation of therapy; this is believed to reflect the inhibition of direct actions of GH on adipocytes. Because of concerns that loss of negative feedback by GH and IGF-1 may increase the growth of GH-secreting adenomas, careful follow-up by pituitary MRI is strongly recommended.

Pegvisomant can also be given weekly, in addition to SST analogues, when IGF-1 levels are not fully controlled by the latter drugs (Lim and Fleseriu, 2017). *Pegvisomant* differs structurally from native GH and induces the formation of specific antibodies in about 15% of patients. Nevertheless, the development of tachyphylaxis due to these antibodies has not been reported.

Treatment of Prolactin Excess

The therapeutic options for patients with prolactinomas include transsphenoidal surgery, radiation, and treatment with DA receptor agonists that suppress PRL production via activation of D_2 receptors. Because of the very high efficacy of DA receptor agonists, they are generally regarded as the initial treatment of choice, with surgery and radiation reserved for patients who either do not respond or are intolerant of DA receptor agonists (Melmed et al., 2011).

Dopamine Receptor Agonists

Bromocriptine, *cabergoline*, and *quinagolide* effectively reduce PRL levels, thereby relieving the inhibitory effect of hyperprolactinemia on ovulation and permitting most patients with prolactinomas to become pregnant. *Quinagolide* should not be used when pregnancy is intended. These agents generally decrease both PRL secretion and the size of the adenoma. Over time, especially with *cabergoline*, the prolactinoma may decrease in size to the extent that the drug can be discontinued without recurrence of the hyperprolactinemia.

Bromocriptine. *Bromocriptine* is the DA receptor agonist against which newer agents are compared. *Bromocriptine* is a semisynthetic ergot alkaloid (see Chapter 15) that interacts with D_2 receptors to inhibit release of PRL; to a lesser extent, it also activates D_1 dopamine receptors. The oral dose of *bromocriptine* is well absorbed; however, only 7% of the dose reaches the systemic circulation because of extensive first-pass metabolism in the liver. *Bromocriptine* has a short elimination $t_{1/2}$ (between 2 and 8 h) and thus is usually administered in divided doses. To avoid the need for frequent dosing, a slow-release oral form is available outside the U.S. *Bromocriptine* may be administered vaginally (2.5 mg once daily), with fewer GI side effects.

Bromocriptine normalizes serum PRL levels in 70% to 80% and decreases tumor size in more than 50% of patients with prolactinomas. Hyperprolactinemia and tumor growth recur on cessation of therapy in most patients. At higher concentrations, *bromocriptine* is used in the management of Parkinson disease (see Chapter 21). *Bromocriptine mesylate* (1.6–4.8 mg/day) is approved as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus.

Adverse Effects. Frequent side effects include nausea and vomiting, headache, and postural hypotension, particularly on initial use. Less frequently, nasal congestion, digital vasospasm, and CNS effects such as psychosis, hallucinations, nightmares, or insomnia are observed. These adverse effects can be diminished by starting at a low dose (1.25 mg) administered at bedtime with a snack and then slowly increasing the dose as needed by monitoring PRL levels. Patients often develop tolerance to the adverse effects.

Cabergoline. *Cabergoline* is an ergot derivative with a longer $t_{1/2}$ (~65 h), higher affinity, and greater selectivity for the DA D_2 receptor compared to *bromocriptine*. *Cabergoline* undergoes significant first-pass metabolism in the liver.

Cabergoline is the preferred drug for the treatment of hyperprolactinemia because of greater efficacy and lower adverse effects. Therapy is initiated at a dose of 0.25 mg twice a week or 0.5 mg once a week. The dose can be increased to 1.5 to 2 mg two or three times a week as tolerated; the dose should be increased only once every 4 weeks. Doses of 2 mg/week or less normalize PRL levels in 80% of patients. *Cabergoline* induces remission in a significant number of patients with prolactinomas. At higher doses, *cabergoline* is used in some patients with acromegaly alone or in conjunction with SST analogues.

Adverse Effects. Compared to *bromocriptine*, *cabergoline* has a much lower tendency to induce nausea, although it still may cause hypotension and dizziness. *Cabergoline* has been linked to valvular heart disease, an effect proposed to reflect agonist activity at the serotonin $5HT_{2B}$ receptor. However, this effect is seen primarily at the high doses used in patients being treated for Parkinson disease and is not seen in the conventionally used doses (≤2 mg/week) for patients with prolactinomas.

Quinagolide. *Quinagolide* is a nonergot D_2 receptor agonist with a $t_{1/2}$ of about 22 h. *Quinagolide* is administered once daily at doses of 0.1 to 0.5 mg/day. It is not approved for use in the U.S. but has been used in the European Union and Canada.

Treatment of Growth Hormone Deficiency

Somatropin

Replacement therapy is well established in GH-deficient children (Richmond and Rogol, 2016) and is gaining wider acceptance for GH-deficient adults (He and Barkan, 2020). Humans do not respond to GH from nonprimate species. In the past, when GH for therapeutic use was purified from human cadaver pituitaries, GH availability was limited and ultimately linked to the transmission of Creutzfeldt-Jakob disease. Currently, human GH is produced by recombinant DNA technology. *Somatropin* refers to the many GH preparations whose sequences match that of native GH.

Pharmacokinetics. As a peptide hormone, GH is administered subcutaneously with a bioavailability of 70%. Although the circulating $t_{1/2}$ of GH is only 20 min, its biological $t_{1/2}$ is considerably longer, and once-daily administration is sufficient.

Indications for Treatment. Deficiency in children is a well-accepted cause of short stature. With the advent of essentially unlimited supplies of recombinant GH, therapy has been extended to children with other conditions associated with short stature despite adequate GH production, including Turner syndrome, Noonan syndrome, Prader-Willi syndrome, chronic renal insufficiency, children born small for gestational age, and children with idiopathic short stature (i.e., >2.25 standard deviations below mean height for age and sex but with normal laboratory indices of GH levels). Severely affected GH-deficient adults may benefit from GH replacement therapy. The FDA also has approved GH therapy for AIDS-associated wasting and for malabsorption associated with the short-bowel syndrome (based on the finding that GH stimulates the adaptation of GI epithelial cells). Adults considered for GH treatment should have organic etiologies for the GH deficiency and must demonstrate low GH production in response to standardized stimulation tests or have at least three other pituitary hormone deficiencies.

Contraindications. GH is contraindicated for promotion of growth in pediatric patients with closed epiphyses. GH should not be used in patients with acute critical illness due to complications after open heart or abdominal surgery, multiple accidental trauma, or acute respiratory failure. GH also should not be used in patients who have any evidence of active malignancy. Other contraindications include proliferative retinopathy or severe nonproliferative diabetic retinopathy. GH therapy for Prader-Willi syndrome with a diagnosis of GH deficiency must be carefully supervised. Sudden death has been observed when GH was given to children with Prader-Willi syndrome who were severely obese or who had severe respiratory impairment. GH treatment should be avoided in patients with known hypersensitivity.

Therapeutic Uses. In GH-deficient children, *somatropin* typically is administered in a dose of 25 to 50 μg/kg per day subcutaneously in the evening; higher daily doses (e.g., 50–67 μg/kg) are employed for patients with Noonan syndrome or Turner syndrome, who have partial GH resistance. In children with overt GH deficiency, measurement of serum IGF-1 levels sometimes is used to monitor initial response and compliance. Long-term response is monitored by close evaluation of height, sometimes in conjunction with measurements of serum IGF-1 levels. GH is continued until the epiphyses are fused and may be extended into the transition period from childhood to adulthood. Children with idiopathic rather than organic GH deficiency need retesting after growth has ceased before continuing GH treatment as adults; many with this diagnosis will have normal GH levels on stimulation testing as adults.

Benefits of GH treatment in GH-deficient adults include a decrease in fat mass and increases in muscle mass, exercise capacity, energy, bone mineral density, and quality of life. For adults, a typical starting dose is 150 to 300 μg/day (these doses may vary depending on brand product), with higher doses used in younger patients transitioning from pediatric therapy. Either an elevated serum IGF-1 level or persistent side effects mandates a decrease in dose; conversely, the dose can be increased (typically by 100–200 μg/day) if serum IGF-1 has not reached the normal range after 2 months of GH therapy. Because estrogen inhibits GH action, women taking oral, but not transdermal, estrogen may require larger GH doses to achieve the target IGF-1 level.

Adverse Effects. In children, GH therapy is associated with remarkably few side effects. Rarely, patients develop intracranial hypertension with papilledema, visual changes, headache, nausea, or vomiting. Because of this, funduscopic examination is recommended at the initiation of therapy and at periodic intervals thereafter. The consensus is that GH should not be administered in the first year after treatment of pediatric tumors, including leukemia, or during the first 2 years after therapy for medulloblastomas or ependymomas. Because an increased incidence of type 2 diabetes mellitus has been reported, fasting glucose levels should be followed periodically during therapy. Finally, too-rapid growth may be associated with slipped epiphyses or scoliosis.

Side effects associated with the initiation of GH therapy in adults (peripheral edema, carpal tunnel syndrome, arthralgias, and myalgias) occur most frequently in older or obese patients and generally respond to a decrease in dose. Estrogens (e.g., birth control medications and estrogen supplements) inhibit GH action so that a larger dose is needed to maintain the same IGF-1 level. GH therapy can increase the metabolic inactivation of cortisol in the liver.

Drug Interactions. The effects of estrogen on GH therapy were noted above. This effect is much less marked with transdermal estrogen preparations. Recent studies suggested that GH therapy can increase the metabolic inactivation of glucocorticoids in the liver. Thus, GH may precipitate adrenal insufficiency in patients with occult secondary adrenal insufficiency or in patients receiving replacement doses of glucocorticoids. This has been attributed to the inhibition of the type 1 isozyme of steroid 11β-hydroxysteroid dehydrogenase, which normally converts inactive cortisone into the active 11-hydroxy derivative cortisol (see Figure 50–6). GH treatment may decrease insulin sensitivity. Therefore, the dose of insulin and/or other hypoglycemic agents may need to be adjusted when GH therapy is initiated.

Somapacitan

Somapacitan, approved by the FDA in 2020, is a human GH analogue designed with a 1.2-kDa albumin-binding moiety. This moiety extends the half-life and reduces the clearance of *somapacitan* by allowing reversible binding to endogenous albumin. This modification makes possible once-weekly administration instead of the standard daily injection.

Pharmacokinetics. As a modified form of GH, greater than 99% of *somapacitan* is bound to plasma proteins. A maximum concentration after initial subcutaneous injection is reached in 4 to 24 h, with steady-state concentrations achieved within 2 weeks of administration. The plasma $t_{1/2}$ is 2 to 3 days. The improved pharmacokinetics of *somapacitan* over GH make *somapacitan* the first human GH therapy that is administered to patients only once a week.

Therapeutic Uses. *Somapacitan* is approved in the U.S. only for adults with GH deficiency with similar outcomes to native GH. Administration follows the same indications and contraindications for GH in adults. The typical starting dose is 1.5 mg weekly. This dose is increased in increments of 0.5 to 1.5 mg (weekly dose no higher than 8 mg) until the desired clinical response and serum IGF-1 concentrations are achieved.

Adverse Effects. Side effects from *somapacitan* were reported in less than 2% of treated patients. The most common adverse effects of *somapacitan* include back and joint pain, indigestion, sleep disorder, dizziness, tonsillitis, peripheral edema, vomiting, adrenal insufficiency, hypertension, increase in blood creatine phosphokinase, weight gain, and anemia. Less common adverse effects reported are arthralgia and dyspepsia.

Insulin-like Growth Factor 1

Based on the hypothesis that GH predominantly acts via increases in IGF-1 (see Figure 46–2), IGF-1 has been developed for therapeutic use (Cohen et al., 2014). Recombinant human IGF-1 (*mecasermin*) and a combination of recombinant human IGF-1 with its binding protein, IGFBP-3 (*mecasermin rinfabate*), are FDA-approved. The latter formulation was subsequently discontinued for use in short stature due to patent issues, although it remains available for other conditions, such as severe insulin resistance, muscular dystrophy, and HIV-related adipose redistribution syndrome.

Mecasermin is administered by subcutaneous injection, and absorption is virtually complete. IGF-1 is bound by six proteins: a ternary complex that includes IGFBP-3 and the acid labile subunit. This ternary complex accounts for more than 80% of the circulating IGF-1 and prolongs the $t_{1/2}$ of IGF-1 to about 6 h. Both the liver and kidney metabolize IGF-1.

Therapeutic Uses. *Mecasermin* is FDA-approved for patients with impaired growth secondary to mutations in the GHR or postreceptor signaling pathway, patients who develop antibodies against GH that interfere with its action, and patients with IGF-1 gene defects that lead to primary IGF-1 deficiency. Typically, the starting dose is 40 to 80 μg/kg twice daily by subcutaneous injection, with a maximum of 120 μg/kg per dose twice daily. In patients with impaired growth secondary to GH deficiency or with idiopathic short stature, *mecasermin* stimulates linear growth but is less effective than conventional therapy using recombinant GH.

Adverse Effects. Side effects of *mecasermin* include hypoglycemia and lipohypertrophy. To diminish the frequency of hypoglycemia, *mecasermin* should be administered shortly before or after a meal or snack. Lymphoid tissue hypertrophy, including enlarged tonsils, also is seen and may require surgical intervention. Other adverse effects are similar to those associated with GH therapy.

Contraindications. *Mecasermin* should not be used for growth promotion in patients with closed epiphyses. It should not be given to patients with active or suspected neoplasia and should be stopped if evidence of neoplasia develops.

Growth Hormone–Releasing Hormone

Tesamorelin. *Tesamorelin* is a synthetic N-terminally modified form of human GHRH that is resistant to degradation by dipeptidyl peptidase 4 and therefore has a prolonged duration of action. Although *tesamorelin* increases the levels of GH and IGF-1, its clinical effects are primarily to reduce visceral fat accumulation, with minimal effects on insulin resistance. *Tesamorelin* is FDA-approved for treatment of HIV-associated lipodystrophy but not for GH deficiency (Spooner and Olin, 2012).

Macimorelin. *Macimorelin* is a synthetic GH secretagogue receptor agonist or ghrelin mimetic that is FDA-approved for the diagnosis of GH deficiency. After oral administration of *macimorelin*, maximum serum GH levels of less than 2.8 ng/mL have been established by clinical studies to confirm the presence of adult GH deficiency.

Pituitary Glycoprotein Hormones: TSH and Gonadotropins

The pituitary glycoprotein hormones include *LH*, *FSH*, and *CG*. They are referred to as the gonadotropins because of their actions on the gonads. Together with TSH, they constitute the glycoprotein family of pituitary hormones (see Table 46–2). LH and FSH were named initially based on their actions on the ovary. Appreciation of their roles in male reproductive function came later. LH and FSH are synthesized and secreted by gonadotropes, which make up about 10% of the hormone-secreting cells in the anterior pituitary. CG is produced by the placenta only in primates and horses. GnRH released by a small population of hypothalamic neurons stimulates pituitary gonadotropin production, which is further regulated by feedback effects of the gonadal hormones (Figure 46–6; see Chapters 48 and 49 for details). TSH is discussed in detail in Chapter 47. TSH is measured to establish the diagnosis of thyroid disorders, and recombinant TSH (thyrotropin alfa) is used in the evaluation and treatment of well-differentiated thyroid cancer.

Structure and Function of the Gonadotropins

Each gonadotropic hormone is a glycosylated heterodimer containing a common α subunit and a distinct β subunit that confers receptor specificity (see Table 46–2). The heterogeneity of glycosylation on the subunits produces myriad isoforms of these hormones and may affect receptor binding and signal transduction. Terminal sialate residues seem to increase plasma half-lives of these gonadotropins (Mullen et al., 2013). Among the gonadotropin β subunits, that of CG is most divergent because it contains a carboxy-terminal extension of 30 amino acids and extra carbohydrate residues that prolong its $t_{1/2}$. The longer $t_{1/2}$ of hCG has some clinical relevance for its use in assisted reproductive technology.

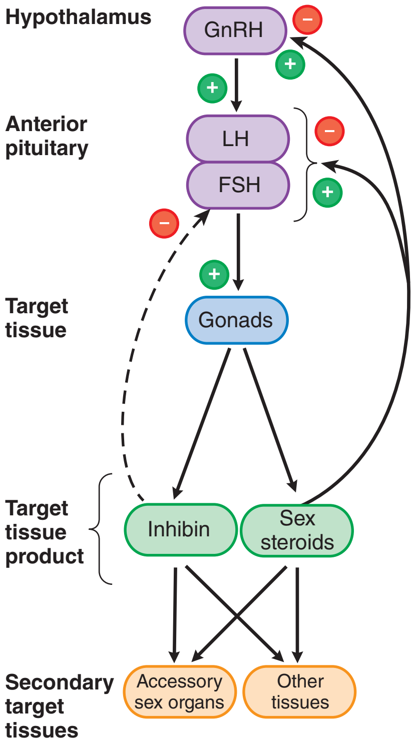

Figure 46–6 *The hypothalamic-pituitary-gonadal axis.* A single hypothalamic-releasing factor, GnRH, controls the synthesis and release of both gonadotropins (LH and FSH) in males and females. Gonadal steroid hormones (androgens, estrogens, and progesterone) exert feedback inhibition at the level of the pituitary and the hypothalamus. However, these feedback effects are dependent on sex, concentration, and time; the preovulatory surge of estrogen also can exert a stimulatory effect at the level of the pituitary and the hypothalamus. Inhibins, a family of polypeptide hormones produced by the gonads, specifically inhibit FSH secretion by the pituitary.

Physiology of the Gonadotropins

In women, FSH stimulates the growth of developing ovarian follicles and induces the expression of LH receptors on theca and granulosa cells. FSH also regulates the expression of aromatase in granulosa cells, thereby stimulating the production of *estradiol*. LH acts on the theca cells to stimulate the *de novo* synthesis of *androstenedione*, the major precursor of ovarian estrogens in premenopausal women (see Figure 48–1). LH also is required for the rupture of the dominant follicle during ovulation and for the synthesis of progesterone by the corpus luteum. *In men*, the actions of FSH and LH are less complex. FSH acts on the Sertoli cells to stimulate the production of proteins and nutrients required for sperm maturation. LH acts on testicular Leydig cells to stimulate the *de novo* synthesis of androgens, primarily *testosterone*, from cholesterol.

Regulation of Gonadotropin Synthesis and Secretion

The predominant regulator of gonadotropin synthesis and secretion is the hypothalamic peptide GnRH, a decapeptide with blocked amino and carboxyl termini derived by proteolytic cleavage of a precursor peptide with 92 amino acids. GnRH is produced by a small population of neurons in the medial preoptic area of the hypothalamus. These neurons are unique in their developmental origin, arising from the olfactory placode mid-gestation and migrating to their final location prior to formation of the cribriform plate.

Regulation of Pulsatile Secretion. Gonadotropin-releasing hormone secretion is pulsatile and varies in amplitude and frequency across mammalian species. Secretion is dynamically modulated by sex steroid feedback. Although some evidence indicates that GnRH neurons themselves are inherently pulsatile, developmental regulation of GnRH release through the onset of puberty and the modulation of GnRH secretion throughout the ovulatory cycle is largely governed by neurons in the arcuate nucleus, preoptic area, and periventricular area of the third ventricle that co-express kisspeptin, neurokinin B, and dynorphin. Expression of these hormones exhibits strong sex differences and is sensitive to sex steroid feedback. GnRH neurons express GPR54, the kisspeptin receptor, and ultimately, kisspeptin action governs GnRH secretion. At birth, the reproductive hypothalamic-pituitary-gonadal axis is active but becomes quiescent postpartum. The axis remains so until shortly before puberty. At that time, CNS inhibition decreases and the amplitude and frequency of GnRH pulses increase, particularly during sleep. As puberty progresses, the GnRH pulses increase further in amplitude and frequency until the normal adult pattern is established. The precise mediator gating the advancement to puberty is not well understood, but multiple lines of evidence suggest endocrine and metabolic mediators of body mass, adiposity, and energy balance may cumulatively promote reawakening of the axis and establish the rhythmicity of the ovulatory cycle in females and advancement of gonadal maturation in both sexes.

The intermittent release of GnRH is crucial for the proper synthesis and release of the gonadotropins; the continuous administration of GnRH leads to desensitization and downregulation of GnRH receptors on pituitary gonadotropes. LH synthesis and secretion are acutely sensitive to GnRH pulses, whereas FSH, which is secreted through regulated and unregulated pathways, is less so.

Molecular and Cellular Basis of GnRH Action. GnRH signals through a unique GPCR on gonadotropes that lacks the typical caroboxyl-terminal intracellular tail that mediates β-arrestin desensitization of signaling. Activation of the receptor leads to a signaling response that is characterized by robust activation of the $G_{q/11}$-PLC-IP_3-Ca^{2+}, $G_{q/11}$-PLC-PKC-MAP kinase, and NADPH/dual oxidase–reactive oxygen species signaling pathways that are rapidly quenched through dual specificity phosphatase and antioxidant negative feedback (see Chapter 3). Activation results in increased LH and FSH secretion, activation of cap-dependent protein synthesis, and increased expression of glycoprotein hormone subunit genes. The magnitude of the cellular responses to GnRH stimulation is proportional to the amplitude and/or frequency of stimulation, and the gonadotrope is capable of interpreting GnRH pulse patterns, which is essential for proper timing of the female ovulatory cycle. Rapid GnRH pulses generally favor elevated LH secretion and ovulation, whereas slow pulses favor FSH secretion and promote follicular development. The GnRH receptors also are present in the ovary, testis, prostate, and breast, where their physiological significance remains to be determined.

Sex Steroid Regulation of Gonadotropin Production. Gonadal steroids regulate gonadotropin production at the level of the pituitary and the hypothalamus, but effects on the hypothalamus predominate (see Figure 46–6). The feedback effects of gonadal steroids are dependent on sex, concentration, and time. In females, granulosa cell proliferation in the maturing follicle results in increased conversion of steroid precursors to estradiol, which exerts negative feedback on the hypothalamus and pituitary, promoting reduced pulsatile LH secretion. This negative feedback action restrains the neural GnRH pulse generator through upstream inhibition of kisspeptin neurons of the hypothalamic infundibular nucleus (arcuate nucleus in rodents), and this may contribute to the postovulatory decrease in GnRH and LH pulse activity during the luteal phase (estrus in rodents). However, sustained elevated estradiol near the end of the follicular phase (proestrus in rodents) exerts positive feedback effects on a different kisspeptin population in the hypothalamic preoptic area. This estradiol-induced activation of preoptic kisspeptin neurons results in high stimulation of GnRH secretion, thereby stimulating a surge in LH release that triggers ovulation. In men, testosterone inhibits gonadotropin production via actions in the hypothalamus and pituitary, in part through direct actions and in part via its conversion by aromatase to estradiol.

Other Regulators of Gonadotropin Production. Gonadotropin production is also regulated by *inhibins* and *activin.* Inhibins are members of the bone morphogenetic protein family of secreted signaling proteins. *Inhibin A* and *B* are made by granulosa cells in the ovary and Sertoli cells in the testis in response to the gonadotropins and local growth factors. They act directly in the pituitary to inhibit FSH secretion without affecting that of LH. Inhibin A exhibits variation during the menstrual cycle, suggesting that it acts as a dynamic regulator of FSH secretion. Independent of GnRH, activin is a positive regulator of FSH synthesis and secretion. Activin is produced in the placenta, pituitary, and gonads, the latter being the main source of circulating activin. The physiological role of circulating activin is unclear due to its being mostly bound by follistatin, a negative regulator of activin. Activin acts primarily as autocrine/paracrine factors at or near sites of expression, suggesting that the pituitary is the source of bioavailable activin that regulates FSH secretion. In women, activin increases FSH binding and FSH-induced aromatization in the ovarian follicle and enhances LH action in the ovary. In men, activin augments LH action in the testis, increasing androgen production and spermatogenesis.

Molecular and Cellular Basis of Gonadotropin Action

The actions of LH and hCG on target tissues are mediated by the LH receptor; those of FSH are mediated by the FSH receptor. The FSH and LH receptors couple to G_s to activate the adenylyl cyclase–cAMP pathway. At higher ligand concentrations, the agonist-occupied gonadotropin receptors also activate PKC and Ca^{2+} signaling pathways via G_q-mediated effects on $PLC_β$. Most actions of the gonadotropins can be mimicked by cAMP analogues.

Clinical Disorders of the Hypothalamic-Pituitary-Gonadal Axis

Clinical disorders of the hypothalamic-pituitary-gonadal axis can manifest either as alterations in levels and effects of sex steroids (hyper- or hypogonadism) or as impaired reproduction. This section focuses on those conditions that specifically affect the hypothalamic-pituitary components of the axis and those for which gonadotropins are used diagnostically or therapeutically.

Deficient sex steroid production resulting from hypothalamic or pituitary defects is termed *hypogonadotropic hypogonadism* because circulating levels of gonadotropins are either low or undetectable. Hypogonadotropic hypogonadism in some patients results from GnRH receptor mutations. Some of these mutations impair targeting of the GnRH receptor to the plasma membrane of gonadotropes, prompting efforts to develop pharmacological strategies to correct receptor trafficking and restore function (Conn et al., 2007). Many other disorders can impair gonadotropin secretion, including pituitary tumors, genetic disorders such as Kallmann syndrome, infiltrative processes such as sarcoidosis, and functional disorders such as exercise-induced amenorrhea.

In contrast, reproductive disorders caused by processes that directly impair gonadal function are termed *hypergonadotropic* because the impaired production of sex steroids leads to a loss of negative-feedback inhibition, thereby increasing the synthesis and secretion of gonadotropins.

- **Precocious Puberty.** Puberty normally is a sequential process requiring several years over which the GnRH neurons escape CNS inhibition and initiate pulsatile secretion of GnRH. This stimulates the secretion of gonadotropins and gonadal steroids, thus directing the development of secondary sexual characteristics. Normally, the initial signs of puberty (breast development in girls and testes enlargement in boys) do not occur before age 8 in girls or age 9 in boys. The initiation of sexual maturation before this time is termed "precocious." GnRH-dependent excessive secretion of gonadotropins is rare and causes precocious puberty in children. This condition may be due to GnRH-producing hamartomas or other CNS abnormalities, but often no specific abnormality is found. This central precocious puberty must be differentiated from that due to hormone-producing tumors of the gonads, in which case, gonadotropin levels will be low. GnRH-independent precocious puberty results from peripheral production of sex steroids in a manner not driven by pituitary gonadotropins. Etiologies include adrenal or gonadal tumors, activating mutations of the LH receptor in boys, and congenital adrenal hyperplasia. Synthetic GnRH analogues play important roles in the diagnosis and treatment of GnRH-dependent precocious puberty (see further discussion). In contrast, drugs that interfere with the production of sex steroids, including *ketoconazole* and aromatase inhibitors, are used in patients with GnRH-independent precocious puberty (Shulman et al., 2008), with varying success.

- **Sexual Infantilism.** The converse of precocious puberty is a failure to initiate the processes of pubertal development at the normal time. This can reflect defects in the GnRH neurons or gonadotropes (secondary hypogonadism) or primary dysfunction in the gonads. In either case, induction of sexual maturation using sex steroids (estrogen followed by estrogen/progesterone in females, testosterone in males) is standard therapy. This suffices to direct sex differentiation in the normal manner. If fertility is the goal, then therapy with either GnRH or gonadotropins is needed to stimulate appropriate germ cell maturation.
- **Infertility.** Infertility, or a failure to conceive after 12 months of unprotected intercourse, is seen in up to 10% to 15% of couples and is increasing in frequency as women choose to delay childbearing. When the infertility is due to impaired synthesis or secretion of gonadotropins (hypogonadotropic hypogonadism), various pharmacological approaches are employed. In contrast, when infertility results from intrinsic processes affecting the gonads, pharmacotherapy generally is less effective. Therapeutic approaches to male infertility are described further in this chapter; strategies for female infertility are described in Chapter 48.

Treatment and Diagnosis of Gonadal Disorders

GnRH and Its Synthetic Agonist Analogues

A synthetic peptide comprising the native sequence of GnRH has been used both diagnostically and therapeutically in human reproductive disorders. In addition, several GnRH analogues with structural modifications have been synthesized and brought to market (Table 46–3).

GnRH Congeners

Synthetic agonist congeners of GnRH have longer half-lives than native GnRH. After a transient stimulation of gonadotropin secretion, they downregulate the GnRH receptor and inhibit gonadotropin secretion. The available GnRH agonists contain substitutions of the native sequence at position 6 that protect against proteolysis and substitutions at the carboxyl terminus that improve receptor-binding affinity. Compared to GnRH, these analogues exhibit enhanced potency and prolonged duration of action (see Table 46–3).

Pharmacokinetics. The myriad formulations of GnRH agonists provide for diverse applications, including relatively short-term effects (e.g., assisted reproduction technology) and more prolonged action (e.g., depot forms that inhibit gonadotropin secretion in GnRH-dependent precocious puberty). The rates and extents of absorption vary considerably. The intranasal formulations have bioavailability (~4%) that is considerably lower than that of the parenteral formulations, which include products for implantation and injection (subcutaneous and intramuscular).

Clinical Uses. The depot form of the GnRH agonist *leuprolide* has been used diagnostically to differentiate between GnRH-dependent and GnRH-independent precocious puberty. *Leuprolide depot* (3.75 mg) is injected subcutaneously, and serum LH is measured 2 h later. A plasma LH level of more than 6.6 mIU/mL is diagnostic of GnRH-dependent (central) disease. Clinically, the various GnRH agonists are used to achieve pharmacological castration in disorders that respond to reduction in gonadal steroids (Fuqua, 2013). A clear indication is in children with GnRH-dependent precocious puberty, whose premature sexual maturation can be arrested with minimal side effects by chronic administration of a depot form of a GnRH agonist (Li et al., 2014).

TABLE 46–3 ■ STRUCTURES OF GONADOTROPIN-RELEASING HORMONE AND GNRH ANALOGUES

GNRH CONGENER	AMINO ACID RESIDUE										DOSAGE FORMS
	1	2	3	4	5	6	7	8	9	10	
Agonists											
GnRH	PyroGlu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly-NH_2	IV, SC
Goserelin	—	—	—	—	—	D-Ser(tBu)	—	—	—	AzGly-NH_2	SC implant
Nafarelin	—	—	—	—	—	D-Nal	—	—	—	—	IN
Triptorelin	—	—	—	—	—	D-Trp	—	—	—	—	IM depot
Buserelin[a]	—	—	—	—	—	D-Ser(tBu)	—	—	Pro-NHEt		IN, SC
Deslorelin[a]	—	—	—	—	—	D-Trp	—	—	Pro-NHEt		IM, SC, depot
Histrelin	—	—	—	—	—	D-His(Bzl)	—	—	Pro-NHEt		SC implant
Leuprolide	—	—	—	—	—	D-Leu	—	—	Pro-NHEt		IM, SC, depot
Antagonists											
Cetrorelix	Ac-D-Nal	D-Cpa	D-Pal	—	—	D-Cit	—	—	—	D-Ala-NH_2	SC
Degarelix	Ac-D-Nal	D-Cpa	D-Pal	—	D-Aph (L-Hor)	D-Aph(Cbm)	—	Lys(iPr)	—	D-Ala-NH_2	SC
Ganirelix	Ac-D-Nal	D-Cpa	D-Pal	—	—	D-hArg$(Et)_2$	—	D-hArg$(Et)_2$	—	D-Ala-NH_2	SC

Ac, acetyl; Aph, aminophenyl alanine; Bzl, benzyl; AzGly, azaglycyl; Cbm, carbamoyl; Cpa, chlorophenylalanyl; D-Nal, 3-(2-naphthyl)-D-alanyl; $EtNH_2$, *N*-ethylamide; hArg$(Et)_2$, ethyl homoarginine; Hor, hydroorotyl; Lys(iPr), isopropyl-lysyl; Pal, 3-pyridylalanyl; tBu, t butyl. A dash (—) denotes amino acid identity with GnRH. IM, intramuscular; IN, intranasal; IV, intravenous; SC, subcutaneous.

[a] Not available in the U.S.

Long-acting GnRH agonists are used for palliative therapy of hormone-responsive tumors (e.g., prostate or breast cancer), generally in conjunction with agents that block steroid biosynthesis or action to avoid transient increases in hormone levels (see Chapters 50 and 73). The GnRH agonists also are used to suppress steroid-responsive conditions such as endometriosis, uterine fibroids, acute intermittent porphyria, and priapism. They also have been evaluated off label for their potential to preserve follicles in women undergoing therapy with cytotoxic drugs for cancer treatment, although efficacy in this setting has not been established. Depot preparations can be administered subcutaneously or intramuscularly monthly or every 3 months. The long-lasting GnRH agonists have been used to avoid a premature LH surge, and thus ovulation, in various ovarian stimulation protocols for *in vitro* fertilization.

Adverse Effects. The long-acting agonists generally are well tolerated, and side effects are those that would be predicted to occur when gonadal steroidogenesis is inhibited (e.g., hot flashes and decreased bone density in both sexes, vaginal dryness and atrophy in women, and erectile dysfunction in men). Because of these effects, therapy in non–life-threatening diseases such as endometriosis or uterine fibroids generally is limited to 6 months. GnRH agonists are contraindicated in pregnant women.

Formulations and Indications.

Leuprolide. *Leuprolide* is formulated in multiple doses for injection: subcutaneous (1 mg/day), subcutaneous depot (7.5 mg/month; 22.5 mg/3 months; 30 mg/4 months; 45 mg/6 months), and intramuscular depot (3.75 mg/month; 11.25 mg/3 months). It is approved for endometriosis, uterine fibroids, advanced prostate cancer, and precocious puberty. For endometriosis, *leuprolide* once-monthly injections (3.75 mg) or 3-month injections (11.25 mg) are also copackaged in combination with once-daily *norethindrone* (a steroidal progestin) 5-mg tablets for oral administration. Pediatric formulations of *leuprolide* also are approved for central precocious puberty.

Goserelin. *Goserelin* is formulated as a subcutaneous implant (3.6 mg/month; 10.8 mg/12 weeks). It is approved for endometriosis, for use as an endometrial-thinning agent prior to endometrial ablation for dysfunctional uterine bleeding, and for advanced prostate and breast cancer.

Histrelin. *Histrelin* is formulated as a subcutaneous implant (50 mg/12 months). It is approved for central precocious puberty and advanced prostate cancer.

Nafarelin. *Nafarelin* is formulated as a nasal spray (200 μg/spray). It is approved for endometriosis (400 μg/d) and central precocious puberty (1600 μg/d).

Triptorelin. *Triptorelin* is formulated for depot intramuscular injection (3.75 mg/month; 11.25 mg/12 weeks; 22.5 mg/24 weeks) and approved for advanced prostate cancer. *Buserelin* and *deslorelin* are not available in the U.S.

GnRH Antagonist Analogues

Ganirelix and Cetrorelix. *Ganirelix acetate* and *cetrorelix acetate* are FDA-approved to suppress the LH surge and thus prevent premature ovulation in ovarian-stimulation protocols as part of assisted reproduction technology (see Chapter 48).

Both GnRH antagonists are formulated for subcutaneous administration. Bioavailability exceeds 90% within 1 to 2 h, and the $t_{1/2}$ varies depending on the dose. Once-daily administration suffices for therapeutic effect. Hypersensitivity reactions, including anaphylaxis, have been noted in postmarketing surveillance, some with the initial dose. When used in conjunction with gonadotropin injections for assisted reproduction, the effects of estrogen withdrawal (e.g., hot flashes) are not seen. GnRH antagonists are contraindicated in pregnant women.

Cetrorelix is also used off label for endometriosis and uterine fibroids, both of which are estrogen dependent. As antagonists rather than agonists, these drugs do not transiently increase gonadotropin secretion and sex steroid biosynthesis.

Degarelix. *Degarelix acetate* is FDA-approved for treatment of advanced prostate cancer. *Degarelix* suppresses testosterone levels to 50 ng/dL or less (i.e., medical castration) and lowers prostate-specific antigen more rapidly than GnRH agonists without an initial testosterone surge (Shore, 2013).

Degarelix forms a depot gel at the site of injection and is released in a biphasic pattern with a median plasma $t_{1/2}$ of 42 days for the starting dose and 28 days for the maintenance dose. *Degarelix* is distributed throughout total body water, is 90% protein bound, and is degraded by proteolysis via the hepatobiliary system. Degraded protein fragments are eliminated in the feces, and unmetabolized drug is eliminated via the kidneys.

Adverse Effects. Adverse effects include injection site reactions, hot flashes, weight gain, increases in transaminase and γ-glutamyltransaminase levels, prolonged QT interval, and decreased bone mineral density.

Dosage and Route of Administration. *Degarelix* is administered subcutaneously in the abdomen, with the site of injection varied on a regular basis. The starting dose is 240 mg administered as two injections of 120 mg on each side of the abdomen, followed by a maintenance dose of 80 mg every 28 days.

Natural and Recombinant Gonadotropins

The gonadotropins are used for both diagnosis and therapy in reproductive endocrinology. For further discussion of the uses of gonadotropins in female reproduction, see Chapter 48.

The original gonadotropin preparations for clinical therapy were prepared from human urine and included *chorionic gonadotropin*, obtained from the urine of pregnant women, and *menotropins*, obtained from the urine of postmenopausal women. Because of their relatively low purity, these gonadotropins were administered intramuscularly to decrease the incidence of hypersensitivity reactions. Subsequently, urine-derived preparations were developed with sufficient purity to be administered subcutaneously. Highly purified preparations of human gonadotropins now are prepared using recombinant DNA technology and exhibit less batch-to-batch variation. This technology is being used to produce forms of gonadotropins with increased half-lives or higher clinical efficacy. One such "designer" gonadotropin, FSH-CTP, contains the β subunit of FSH fused to the carboxy-terminal extension of hCG, thereby considerably increasing the $t_{1/2}$ of the recombinant protein. In clinical trials, FSH-CTP has been shown to stimulate follicle maturation *in vivo* when injected weekly (Macklin et al., 2006).

Preparations

Follicle-Stimulating Hormone

Follicle-stimulating hormone has long been a mainstay of regimens for either ovarian stimulation or *in vitro* fertilization. The original *menotropins* formulations contained roughly equal amounts of FSH and LH, as well as a number of other urinary proteins, and were administered intramuscularly to diminish local reactions. *Urofollitropin*, prepared by immunoconcentration of FSH with monoclonal antibodies, is pure enough to be administered subcutaneously. The amount of LH contained in such preparations is diminished considerably.

Recombinant FSH is prepared by expressing cDNAs encoding the α and β subunits of human FSH in mammalian cell lines, yielding products whose glycosylation pattern mimics that of FSH produced by gonadotropes. The two available recombinant FSH preparations, *follitropin alfa* and *follitropin beta*, differ slightly in their carbohydrate structures: Both are more pure and exhibit less interbatch variability than do preparations purified from urine; thus, they can be administered subcutaneously. The relative advantages (i.e., efficacy, lower frequency of side effects such as ovarian hyperstimulation) of recombinant FSH versus urine-derived gonadotropins have not been definitively established (van Wely et al., 2011).

Human Chorionic Gonadotropin

The hCG used clinically originally came from the urine of pregnant women. Several urine-derived preparations are available; all of them are administered intramuscularly due to local reactions. Recombinant hCG (*choriogonadotropin alfa*) is the predominant preparation used clinically.

Recombinant Human LH

Menotropins contain considerable LH activity, thereby providing any LH activity that is needed to promote follicle maturation. Traditionally, LH was not used for ovulation induction because hCG produced identical effects via the LH receptor and had a longer $t_{1/2}$. Human LH produced using recombinant DNA technology and designated *lutropin alfa* has been discontinued from the U.S. market but is available elsewhere.

Diagnostic Uses

Pregnancy Testing

During pregnancy, the placenta produces significant amounts of hCG, which can be detected in maternal urine. Over-the-counter pregnancy kits containing antibodies specific for the unique β subunit of hCG qualitatively assay for the presence of hCG and can detect pregnancy within a few days after a woman's first missed menstrual period. Quantitative measurements of plasma hCG concentration by radioimmunoassay can indicate whether pregnancy is proceeding normally and can help to detect the presence of an ectopic pregnancy, hydatidiform mole, or choriocarcinoma. Such assays also are used to follow the therapeutic response of malignancies that secrete hCG, such as germ cell tumors.

Timing of Ovulation

Ovulation occurs about 36 h after the onset of the LH surge. Therefore, urinary concentrations of LH, as measured with an over-the-counter ELISA kit, can be used to predict the time of ovulation. Urine LH levels are measured every 12 to 24 h, beginning on day 10 to 12 of the menstrual cycle (assuming a 28-day cycle), to detect the rise in LH and estimate the time of ovulation. This estimate facilitates the timing of sexual intercourse to optimize the chance of achieving pregnancy.

Localization of Endocrine Disease

Measurements of plasma LH and FSH levels with β-subunit–specific ELISA kits are useful in the diagnosis of several reproductive disorders. Low or undetectable levels of LH and FSH are indicative of hypogonadotropic hypogonadism and suggest hypothalamic or pituitary disease, whereas high levels of gonadotropins suggest primary gonadal diseases. A plasma FSH level of 10 to 12 mIU/mL or greater on day 3 of the menstrual cycle is associated with reduced fertility. Elevated FSH levels also are diagnostic of menopause in women with amenorrhea in the appropriate age range.

Human CG can be used to stimulate testosterone production and thus to assess Leydig cell function in males suspected of having primary hypogonadism (e.g., in delayed puberty). A diminished response to multiple injections of hCG indicates Leydig cell failure; a normal response suggests a hypothalamic-pituitary disorder and normal Leydig cells.

Therapeutic Uses

Male Infertility

In men with impaired fertility secondary to gonadotropin deficiency (hypogonadotropic hypogonadism), gonadotropins can establish or restore fertility (Farhat et al., 2010). Treatment typically is initiated with hCG (1500–2000 IU intramuscularly or subcutaneously) three times per week until the plasma testosterone levels indicate full induction of steroidogenesis. Thereafter, the dose of hCG is reduced to 2000 IU twice a week or 1000 IU three times a week. If spermatogenesis does not occur with hCG alone, then recombinant FSH (typical dose of 150 IU) is added to fully induce spermatogenesis.

The most common side effect of gonadotropin therapy in males is gynecomastia, which occurs in up to a third of patients and presumably reflects increased production of estrogens due to the induction of aromatase. Maturation of the prepubertal testes typically requires treatment for more than 6 months, and optimal spermatogenesis in some patients may require treatment for up to 2 years. Once spermatogenesis has been initiated, ongoing treatment with hCG alone usually is sufficient to support sperm production.

Cryptorchidism

Cryptorchidism, the failure of one or both testes to descend into the scrotum, affects up to 3% of full-term male infants and becomes less prevalent with advancing postnatal age. Cryptorchid testes have defective spermatogenesis and are at increased risk for developing germ cell tumors. Hence, the current approach is to reposition the testes as early as possible, typically at 1 year of age but definitely before 2 years of age. The local actions of androgens stimulate descent of the testes; thus, hCG has been used by some to induce testicular descent if the cryptorchidism is not secondary to anatomical blockage. Therapy usually consists of injections of hCG (3000 IU/m^2 body surface area) intramuscularly every other day for six doses.

Posterior Pituitary Hormones: Oxytocin and Vasopressin

The neurohypophyseal hormones oxytocin and arginine vasopressin are cyclic nonapeptides that differ by only two amino acids (Figure 46–7). The physiology and pharmacology of vasopressin are presented in Chapter 29.

Physiology of Oxytocin

Oxytocin is best known for its female roles in parturition and breastfeeding but is also implicated in regulation of the autonomic nervous system. In males, oxytocin seems incidental and modulates sperm motility and testosterone production. It is synthesized as a larger precursor peptide in magnocellular neurons whose cell bodies reside in the paraventricular nucleus and, to a lesser extent, the supraoptic nucleus in the hypothalamus. The precursor peptide is rapidly cleaved to active oxytocin and neurophysin I, packaged into secretory granules as an oxytocin-neurophysin complex, and secreted from nerve endings that terminate primarily in the posterior pituitary gland (neurohypophysis). In addition, oxytocinergic neurons project to regions of the hypothalamus, olfactory nucleus, cortex, limbic system, brainstem, cerebellum, and spinal cord. Other sites of oxytocin synthesis include the luteal cells of the ovary, the endometrium, the placenta, Leydig cells of the testis, and nonreproductive tissues; however, the physiologic significance of extraneuronal oxytocin is not known (Jurek and Neumann, 2018).

Oxytocin acts via a specific GPCR (OXTR) closely related to the V_{1a} and V_2 vasopressin receptors. In the human myometrium, oxytocin binds OXTR on uterine myocytes resulting in the coupling of $G_{q/11}$, activation of the PLC_{β}-IP_3-Ca^{2+} pathway, and enhanced activation of voltage-sensitive Ca^{2+} channels to stimulate uterine muscle contraction (Figure 46–8). Oxytocin also increases uterine prostaglandin production, which further stimulates contraction. In human mammary tissue, oxytocin stimulates contraction of myoepithelial cells through OTXR to eject milk, known as the letdown reflex.

Regulation of Oxytocin Secretion

Stimuli for oxytocin secretion include sensory input arising from cervical dilation, vaginal stretch, suckling at the breast, and infant crying.

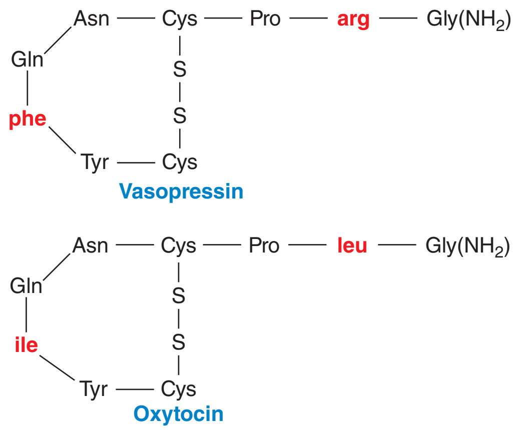

Figure 46–7 *The structures of vasopressin and oxytocin.* Vasopressin and oxytocin are cyclic nonapeptides that differ from each other by two amino acids (red).

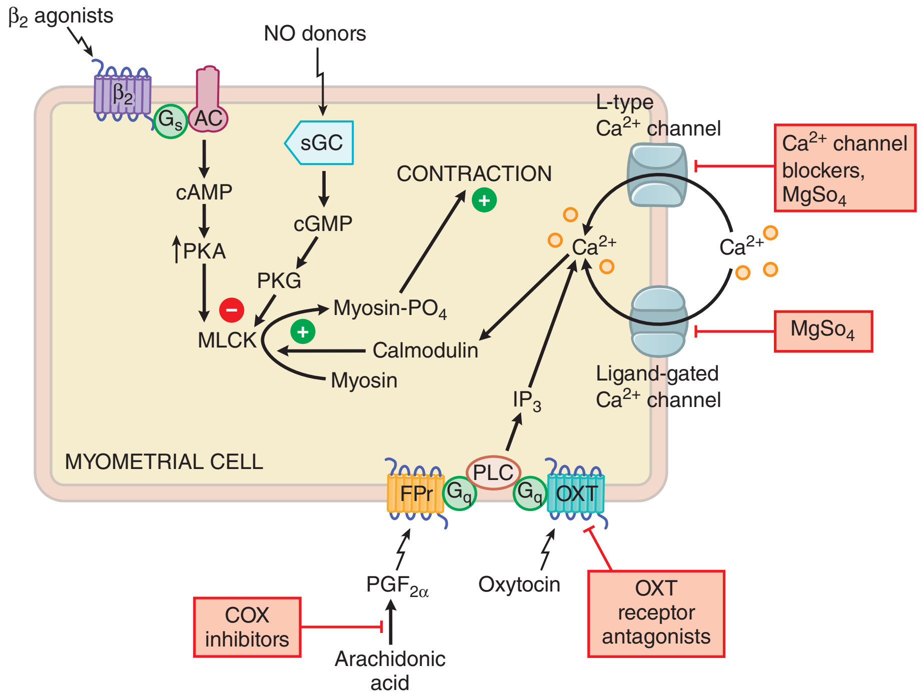

Figure 46–8 *Sites of action of oxytocin and tocolytic drugs in the uterine myometrium.* The elevation of cellular Ca^{2+} promotes contraction via the Ca^{2+}/calmodulin–dependent activation of myosin light chain kinase (MLCK). Relaxation is promoted by the elevation of cyclic nucleotides (cAMP and cGMP) and their activation of protein kinases, which cause phosphorylation/inactivation of MLCK. Pharmacological manipulations to reduce myometrial contraction include the following: (1) inhibiting Ca^{2+} entry (Ca^{2+} channel blockers, Mg$_2$ SO$_4$); (2) reducing mobilization of intracellular Ca^{2+} by antagonizing GPCR-mediated activation of the G$_q$-PLC-IP$_3$-Ca^{2+} pathway (with antagonists of the FPr and OXT receptors) or reducing production of the FPr agonist PGF$_{2\alpha}$ (with COX inhibitors); and (3) enhancing relaxation by elevating cellular cAMP (with β$_2$ adrenergic agonists that activate G$_s$-AC) and cGMP (with NO donors that stimulate soluble guanylyl cyclase).

Increases in circulating oxytocin in women in labor are difficult to detect, partly because of the pulsatile nature of oxytocin secretion and partly because of the activity of circulating oxytocinase. Nevertheless, increased oxytocin in maternal circulation is detected in the second stage of labor, likely triggered by sustained distension of the cervix and vagina.

Estradiol stimulates oxytocin secretion, whereas the ovarian polypeptide *relaxin* inhibits its release. The inhibitory effect of relaxin appears to be the net result of a direct effect on oxytocin-producing cells and indirect action mediated by endogenous opiates. Other factors that primarily affect vasopressin secretion also have some impact on oxytocin release: Pain, dehydration, hemorrhage, and hypovolemia stimulate release, whereas ethanol inhibits release. Although peripheral actions of oxytocin appear to play no significant role in the response to dehydration, hemorrhage, or hypovolemia, oxytocin may participate in the central regulation of blood pressure. Pharmacological doses of oxytocin can inhibit free water clearance by the kidney through arginine vasopressin–like activity at vasopressin V$_2$ receptors. As judged by the effects of intravenously administered oxytocin during labor induction, the plasma $t_{1/2}$ of oxytocin is about 13 min.

Unlike the *negative-feedback* regulation of the other pituitary hormones, oxytocin release is augmented by *positive-feedback* regulation. This positive feedback is most evident in parturition with oxytocin-induced contractions activating mechanoreceptors that signal for more oxytocin release. For this reason, uterine hyperstimulation is an adverse effect of intravenous administration of oxytocin to augment labor that should be avoided.

Sites of Oxytocin Action

Uterus

During the third trimester of pregnancy, spontaneous uterine contractions increase progressively until the sharp rise in frequency that constitutes the initiation of labor. Oxytocin regulates the frequency and force of uterine contractions. Uterine responsiveness to oxytocin roughly parallels the increase in spontaneous activity and is highly dependent on estrogen, which increases the expression of the OXTRs.

Because of difficulties associated with the measurement of oxytocin levels and because loss of pituitary oxytocin apparently does not compromise labor and delivery, the physiological role of oxytocin in pregnancy is debated. Exogenous oxytocin can enhance rhythmic contractions at any time, but an 8-fold increase in uterine sensitivity to oxytocin occurs in the last half of pregnancy and is accompanied by a 30-fold increase in OXTR numbers. Progesterone antagonizes the stimulatory effect of oxytocin *in vitro*, and refractoriness to progesterone in late pregnancy may contribute to the normal initiation of human parturition.

Breast

Oxytocin plays an important physiological role in milk ejection. Stimulation of the breast through suckling or mechanical manipulation induces oxytocin secretion, causing contraction of the myoepithelium that surrounds alveolar channels in the mammary gland. This action forces milk from the alveolar channels into large collecting sinuses, where it is available to the suckling infant.

Brain

Studies in rodents have implicated oxytocin as an important CNS regulator of social behavior and affiliation, a central role of oxytocin that remains to be established in humans. Research on the impact of oxytocin in humans has generated the Social Salience Hypothesis (Shamay-Tsoory and Abu-Akel, 2016). The Social Salience Hypothesis reconciles multiple effects of oxytocin on the CNS including enhancement of affiliative prosocial behaviors such as trust and bonding, attenuation of stress, and the regulation of cooperation and conflict among humans in a group social setting such as recognition, fear, or anxiety. In this framework, oxytocin is thought to modulate the response to social cues through interaction with dopaminergic neurons.

Brain regions innervated by oxytocinergic neurons include the amygdala, hypothalamus, hippocampus, and midbrain. Oxytocin treatment showed decreased activation of the amygdala, midbrain, and striatum in response to stressful stimuli, implicating oxytocin as a potential intervention for mental conditions regulated by the hormone (Baumgartner et al., 2008; Huber et al., 2005). The possible therapeutic benefit of drugs that manipulate CNS oxytocin on mental conditions with perturbations in oxytocin signaling, such as social phobia, addiction, and autism, is an exciting area of ongoing investigation (Bowen and Neumann, 2017; Romano et al., 2016).

Clinical Use of Oxytocin

Despite heightened interest in using oxytocin nasal spray to treat social disorders, oxytocin is used therapeutically only to induce or augment labor and to treat or prevent postpartum hemorrhage. In 2007, oxytocin was added to a list of drugs "bearing a heightened risk of harm," although it is widely used. In the U.S., the FDA-approved label contains this notice:

> *Elective induction of labor is defined as the initiation of labor in a pregnant individual who has no medical indications for induction. Since the available data are inadequate to evaluate the benefits-to-risks considerations, Pitocin is not indicated for elective induction of labor.*

Induction of Labor

Induction of labor is indicated when the perceived risk of continued pregnancy to the mother or fetus exceeds the risks of pharmacological induction. Oxytocin is the drug of choice for induction of labor for women with a suitably ripened cervix (see Chapter 41 for a discussion of the role of prostaglandins in cervical ripening). It is administered by intravenous infusion of a diluted solution, has a $t_{1/2}$ of 12 to 15 min, and achieves a steady-state uterine response after about 30 min. Both a high-dose protocol (starting with an infusion of 6 mU/min) and low-dose protocols (starting with an infusion dose of 0.5–2 mU/min) have been used (American College of Obstetricians and Gynecologists, 2009). Oxytocin at high doses activates the vasopressin V_2 receptor and has antidiuretic effects. Vasodilating actions of oxytocin also have been noted that may provoke hypotension and reflex tachycardia. Deep anesthesia may exaggerate the hypotensive effect of oxytocin by preventing the reflex tachycardia.

Augmentation of Dysfunctional Labor

Oxytocin also is used when spontaneous labor is not progressing at an acceptable rate. To augment hypotonic contractions, an infusion rate of 10 mU/min typically is sufficient. As with labor induction, potential complications of uterine overstimulation include trauma of the mother or fetus due to forced passage through an incompletely dilated cervix, uterine rupture, and compromised fetal oxygenation due to decreased uterine perfusion.

Prevention and Treatment of Postpartum Hemorrhage

Oxytocin (10 units IM) is given immediately after delivery to help maintain uterine contractions and tone. Alternatively, oxytocin (20 units) is diluted in 1 L of intravenous solution (yielding a concentration of 20 mU/mL) and infused at a rate of 10 mU/min until the uterus is contracted. The infusion rate then is reduced to 1 to 2 mU/min until the mother is ready for transfer to the postpartum unit.

Tocolytic Therapy for Established Preterm Labor

Inhibition of uterine contractions of preterm labor, or *tocolysis*, has been a focus of therapy (see Figure 46–8). Although tocolytic agents delay delivery in approximately 80% of women, they neither prevent premature births nor improve adverse fetal outcomes, such as respiratory distress syndrome. Specific tocolytic agents include β adrenergic receptor agonists, $MgSO_4$, Ca^{2+} channel blockers, COX inhibitors, NO donors, and the oxytocin receptor antagonist *atosiban*. *Atosiban* is widely used in Europe but is not FDA-approved in the U.S. Chapter 48 presents additional information on tocolytic therapy.

Drug Facts for Your Personal Formulary: *Pituitary-Related Drugs*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Pituitary Hormones (Recombinant)		
Growth hormone • Somatropin • Somapacitan (GH analogue, adult only)	• Stimulating growth in childhood • GH replacement in GH-deficient adults	• Given by daily (somatropin) or weekly (somapacitan) SC injection to stimulate body growth, primarily through stimulation of IGF-1. As growth ceases, test for GH deficiency to determine if GH should be continued into adulthood. • Given only to adults with GH deficiency proven by GH stimulation tests or known organic childhood GH deficiency and low IGF-1 levels on testing of GH treatment. • Treatment in adults decreases fat mass, increases muscle mass, increases bone mass, and improves quality of life.
Oxytocin	• Augmentation of labor • Management of postpartum hemorrhage	• Administered by intravenous infusion. • Hyperstimulating the uterus should be avoided during augmentation of labor. • May provoke hypotension and reflex tachycardia.
Other Peptide Hormones		
Human chorionic gonadotropin	• Testing of Leydig cell function • Female/male infertility • Cryptorchidism in children	• Stimulates LH receptor, stimulating ovulation in female and causing increased testicular testosterone production in male. • Induces testicular descent in children with cryptorchidism.
Tesamorelin	• Treatment of HIV-associated lipodystrophy	• N-terminally modified version of human GHRH with primary effect of reducing visceral and other body fat in patients with HIV lipodystrophy.
Macimorelin	• Diagnosis of adult GH deficiency	• Orally available synthetic mimetic of ghrelin that stimulates GH secretion.
Insulin-like growth factor 1 (mecasermin)	• Treatment of children with mutations in the GH receptor or transduction mechanisms mediating GH action or IGF-1 gene defects	• Adverse effects include hypoglycemia and lipohypertrophy.
Gonadotropin-releasing hormone agonist analogues • Goserelin • Histrelin • Leuprolide • Nafarelin • Triptorelin	• Endometriosis • Diagnosis and treatment of precocious puberty • Palliative treatment of hormone-responsive tumors (prostate and breast cancer)	• Prolonged stimulation of the GnRH receptor by analogues results in downregulation of those receptors with decreased gonadotropin secretion.

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Drug Facts for Your Personal Formulary: *Pituitary-Related Drugs* (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Gonadotropin-releasing hormone antagonist analogues • Ganirelix • Cetrorelix • Degarelix	• Suppression of gonadotropin secretion and used in conjunction with exogenous gonadotropins for assisted reproduction • Palliative treatment of advanced prostate cancer (degarelix)	• Antagonism at the GnRH receptor results in decreased gonadotropin secretion without initial LH surge as seen with agonist analogues.
Somatostatin Analogues: Act on somatostatin receptors to reduce hormone secretion		
Octreotide	• Acromegaly	• Long-acting release form is the standard type; given monthly.
Lanreotide	• Acromegaly	• Long-acting release form is the only available standard type; given monthly.
Pasireotide	• Acromegaly • Cushing disease	• Short-acting subcutaneous form is the only version FDA-approved for Cushing disease. • LAR form given monthly is the only version FDA-approved for acromegaly. • Additional adverse effects include significant hyperglycemia in many patients.
Dopamine Agonists: Act on dopamine receptors (D_2) to decrease prolactin secretion and prolactinoma size		
Bromocriptine	• Treatment of hyperprolactinemia • Reduction in size of prolactinomas • Treatment of Parkinson disease	• An ergot derivative that must be given 1–2 times daily. • Common adverse effects include nausea, vomiting, headache, and postural hypotension.
Cabergoline	• Treatment of hyperprolactinemia • Reduction in size of prolactinomas • Parkinson disease • Acromegaly	• A long-acting ergot derivative given once or twice weekly. • Has greater efficacy and tolerability than bromocriptine and may be active in patients who do not respond to bromocriptine. • At high doses used in patients with Parkinson disease; it cross-reacts at the $5HT_{2B}$ receptor, causing cardiac valve abnormalities (not seen when used for patients with prolactinomas).
Quinagolide	• Treatment of hyperprolactinemia • Reduction in size of prolactinomas	• Not available in the U.S.
Hormone Receptor Blockers		
Pegvisomant	• Treatment of acromegaly	• Blocks GH receptor and thus the activity of high GH levels and the generation of IGF-1 in acromegaly. Given by subcutaneous injections daily alone or weekly in combination with somatostatin analogues.

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Chapter 47

Thyroid and Antithyroid Drugs

Ronald J. Koenig and Gregory A. Brent

THYROID HORMONES

- Chemistry of Thyroid Hormones
- Biosynthesis of Thyroid Hormones
- Quantitative Aspects of Thyroid Hormone Metabolism
- Activation and Inactivation of Thyroid Hormone by Deiodination in Peripheral Tissues
- Transport of Thyroid Hormones in the Blood
- Degradation and Excretion of Thyroid Hormones
- Factors Regulating Thyroid Hormone Secretion
- Transport of Thyroid Hormones Into and Out of Cells
- Mediation of Effects by Nuclear Receptors
- Nongenomic Effects of Thyroid Hormone

MAJOR CLINICAL EFFECTS OF THYROID HORMONES

- Growth and Development
- Skeleton
- Thermogenesis
- Cardiovascular System
- Lipid Metabolism

DISORDERS OF THYROID FUNCTION

- Thyroid Hypofunction
- Thyroid Hyperfunction
- Thyroid Function Tests

THYROID HORMONE PREPARATIONS

- Levothyroxine
- Liothyronine
- T_4/T_3 Combination Preparations
- Therapeutic Uses of Thyroid Hormone
- Adverse Effects of Thyroid Hormone
- Drug Interactions
- Investigational Uses of Thyroid Hormone Analogues

ANTITHYROID DRUGS AND OTHER THYROID INHIBITORS

- Antithyroid Drugs
- Ionic Inhibitors
- Iodine
- Radioactive Iodine

CHEMOTHERAPY OF THYROID CANCER

- Papillary and Follicular Thyroid Carcinomas
- Anaplastic Thyroid Carcinoma
- Medullary Thyroid Carcinoma

Thyroid hormone is essential for normal development, especially of the CNS. In the adult, thyroid hormone maintains metabolic homeostasis and influences the functions of virtually all organ systems. Thyroid hormone contains iodine, which must be supplied by nutritional intake. The thyroid gland contains large stores of thyroid hormone in the form of *thyroglobulin*. These stores maintain adequate systemic concentrations of thyroid hormone despite significant variations in iodine availability and nutritional intake. The thyroidal secretion is predominantly the prohormone T_4, which is converted in the liver and other tissues to supply the plasma with the active form, T_3. Local activation of T_4 also occurs in target tissues (e.g., brain and pituitary) and is increasingly recognized as an important regulatory step in thyroid hormone action. Similarly, local deactivation of T_3 is an important regulatory step. Serum concentrations of thyroid hormones are precisely regulated by the pituitary hormone *TSH* in a negative-feedback system. The predominant actions of thyroid hormone are mediated via nuclear TRs that modulate the transcription of specific genes.

Overt *hyperthyroidism* and *hypothyroidism*, thyroid hormone excess and deficiency, respectively, are associated with numerous clinical manifestations. Milder disease often has a subtler clinical presentation and may be identified based solely on abnormal biochemical tests of thyroid function. Maternal and neonatal hypothyroidism, due to iodine deficiency, remains a major preventable cause of intellectual disability worldwide (Zimmermann, 2009). Treatment of the hypothyroid patient consists of thyroid hormone replacement (Biondi and Wartofsky, 2014). Treatments for hyperthyroidism include antithyroid drugs to decrease hormone synthesis and secretion, destruction of the gland by the administration of radioactive iodine, and surgical removal (Brent, 2008). In most patients, disorders of thyroid function can be either cured or controlled.

Likewise, thyroid malignancies are most often localized and resectable (Haugen et al., 2016; Haugen and Sherman, 2013). Metastatic disease often responds to *radioiodine* treatment but may become highly aggressive. *Radioiodine*-refractory, progressive thyroid cancers may respond to targeted chemotherapies, such as tyrosine kinase inhibitors.

Thyroid Hormones

The thyroid gland produces two fundamentally different types of hormones. The thyroid follicle produces the iodothyronine hormones T_4 and T_3. The thyroid's parafollicular cells produce *calcitonin*, a peptide with 32 amino acids, which is not an important endogenous hormone but can be useful as a therapeutic agent in hypercalcemia and osteoporosis (see Chapter 52). Figures 47–1 and 47–2 show the structures of the thyroid hormones and their pathways of synthesis, storage, and release.

Chemistry of Thyroid Hormones

The principal hormones of the thyroid gland are the iodine-containing amino acid derivatives of thyronine (see Figure 47–1). Following the isolation and the chemical identification of T_4, it was generally thought that all the hormonal activity of thyroid tissue could be accounted for by its content of T_4. However, careful studies revealed that crude thyroid preparations possessed greater calorigenic activity than could be accounted for by their T_4 content. The presence of a "second" thyroid hormone was debated, but T_3 was finally detected, isolated, and synthesized by Gross and Pitt-Rivers in 1952. T_3 has a much higher affinity for the nuclear thyroid hormone receptor (TR) compared with T_4 and is much more potent biologically on a molar basis. The subsequent demonstration of T_3 production from T_4 in athyreotic humans led to the practice of effective replacement in hypothyroidism with *levothyroxine* only.

Abbreviations

CYP: cytochrome P450
Dio1, Dio2, and Dio3: deiodinase types 1, 2, and 3
DIT: diiodotyrosine
ERK: extracellular signal-regulated kinase
GPCR: G protein-coupled receptor
HOI: hypoiodous acid
IGF-1: insulin-like growth factor 1
IP_3: inositol 1,4,5-trisphosphate
KISS: potassium iodide (KI) saturated solution
LDL: low-density lipoprotein
MAP kinase: mitogen-activated protein kinase
MCT: monocarboxylic acid transporter
MEK: MAP kinase kinase
MHC: myosin heavy chain, isoform α or β
MIT: monoiodotyrosine
MTC: medullary thyroid carcinoma
NIS: sodium iodide symporter
NO: nitric oxide
NTRK: gene family coding for neutrophic tyrosine receptor kinases (TRKs)
OATP1C1: solute carrier organic anion transporter family, member 1C1
PLC: phospholipase C
RAIU: radioactive iodine uptake
RET: *rearranged during transfection* tyrosine protein kinase
rT_3: reverse T_3
T_3: 3,5,3′-triiodothyronine
T_4: thyroxine
TBG: thyroxine-binding globulin
TR: thyroid hormone receptor
TRH: thyrotropin-releasing hormone
Triac: 3,5,3′-triiodothyroacetic acid, tiratricol
TRKs: tropomyosin receptor kinases, a family of tyrosine protein kinases encoded by NTRK genes
TSH: thyroid-stimulating hormone, thyrotropin

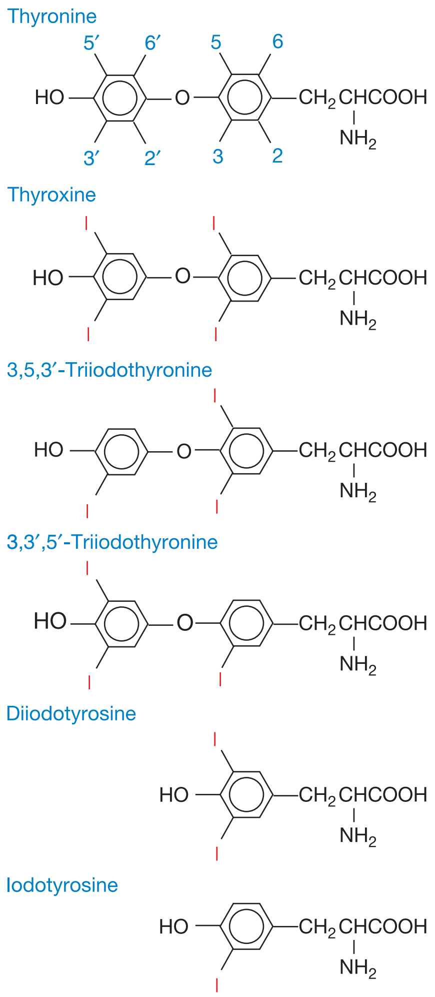
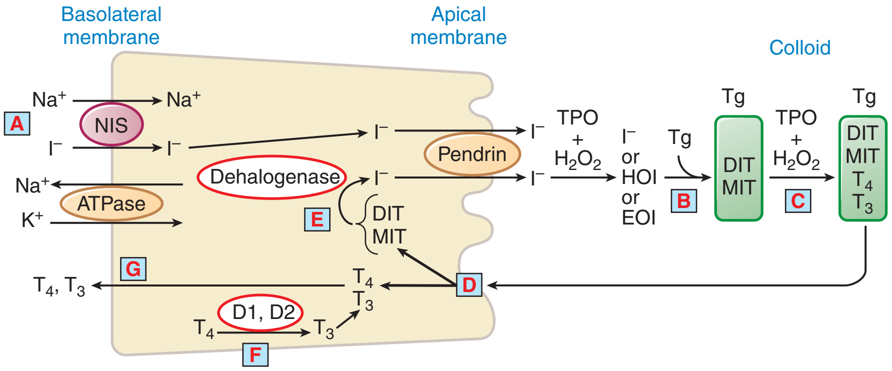

Thyronine
5′
6′
5
6
3′
2′
3
2
HO
O
$CH_2CHCOOH$
NH_2
Thyroxine
I
3,5,3′-Triiodothyronine
3,3′,5′-Triiodothyronine
Diiodotyrosine
Iodotyrosine

Figure 47–1 *Thyronine, thyroid hormones, and precursors.*


Basolateral membrane
Apical membrane
Colloid
A
Na^+
NIS
Na^+
I^-
I^-
Na^+
ATPase
K^+
Dehalogenase
E
DIT
MIT
Pendrin
I^-
TPO + H_2O_2
I^- or HOI or EOI
Tg
B
DIT MIT
Tg
TPO + H_2O_2
C
Tg
DIT MIT T_4 T_3
D
G
T_4, T_3
T_4 T_3
D1, D2
T_4
T_3
F

	METABOLIC STEP	INHIBITOR
A	Iodine transport	ClO_4^-, SCN
B	Iodination	PTU, MMI
C	Coupling	PTU, MMI
D	Colloid Resorption	Colchicine, Li^+, I^-
E	Deiodination of DIT + MIT	Dinitrotyrosine
F	Deodination of T_4	PTU
G	Secretion	I^-

Figure 47–2 *Major pathways of thyroid hormone biosynthesis, storage as colloid, and release* MMI, methimazole; PTU, propylthiouracil; SCN, thiocyanate; TPO, thyroid peroxidase; D#, deiodinase 1, 2, or 3; DIT, diiodotyrosine; MIT, monoiodotyrosine; NIS, sodium iodide symporter; HOI, hypoiodous acid, and EOI, the enzyme-linked species; Tg; thyroglobulin.

Biosynthesis of Thyroid Hormones

The thyroid hormones are synthesized and stored as amino acid residues of *thyroglobulin*, a complex glycoprotein made up of two apparently identical subunits and constituting the vast majority of the thyroid follicular colloid. The thyroid gland is unique in storing great quantities of hormone precursor in this way, and extracellular thyroglobulin is proportional to the thyroid mass. The major steps in the synthesis, storage, release, and interconversion of thyroid hormones are summarized in Figure 47–2 and described next.

HISTORICAL PERSPECTIVE

The thyroid is named for the Greek word for "shield shaped," from the shape of the nearby tracheal cartilage. The gland was first recognized as an organ of importance when thyroid enlargement was observed to be associated with changes in the eyes and heart in the condition we now call *hyperthyroidism*. Parry saw his first patient in 1786 but did not publish his findings until 1825. Graves reported the disorder in 1835, Basedow in 1840. Hypothyroidism was described later, in 1874, when Gull associated atrophy of the gland with the symptoms characteristic of *hypothyroidism*. The term *myxedema* was applied to the clinical syndrome in 1878 by Ord, in the belief that the characteristic thickening of the subcutaneous tissues was due to excessive formation of mucus. In 1891, Murray first treated a case of hypothyroidism by injecting an extract of sheep thyroid gland, later shown to be fully effective when given by mouth. The successful treatment of thyroid deficiency by administering thyroid extract was an important step toward modern endocrinology.

Extirpation experiments to elucidate the function of the thyroid were at first misinterpreted because of the simultaneous removal of the parathyroids. However, Gley's research on the parathyroid glands in the late 19th century allowed the functional differentiation of these two endocrine glands. The structure of parathyroid hormone, however, was not reported until the early 1970s. Calcitonin was discovered in 1961, demonstrating that the thyroid gland produced a second hormone.

Uptake of Iodide

Iodine ingested in the diet reaches the circulation in the form of iodide ion (I^-). Normally, the I^- concentration in the blood is very low (0.2–0.4 µg/dL; ~15–30 nM). The thyroid actively transports the ion via a specific membrane-bound protein, termed sodium iodide symporter (NIS) (Kogai and Brent, 2012; Portulano et al., 2014). As a result, the ratio of $[I^-]_{\text{thyroid}}$ to $[I^-]_{\text{plasma}}$ is usually between 20 and 50 and can exceed 100 when the gland is stimulated. Iodide transport is inhibited by a number of ions, such as thiocyanate and perchlorate. TSH stimulates NIS gene expression and promotes insertion of NIS protein into the membrane in a functional configuration. Thus, decreased stores of thyroid iodine enhance iodide uptake by increasing TSH, and the administration of iodide can reverse this situation by decreasing NIS protein expression. Iodine is accumulated by other tissues, including the salivary glands, gut, and lactating breast, and it is all mediated by a single NIS gene. Individuals with congenital NIS gene mutations have absent or defective iodine concentration in all tissues known to concentrate iodine.

Oxidation and Iodination

Transport of iodine from the thyroid follicular cell to the colloid is facilitated by the apical transporter *pendrin*. The oxidation of iodide to its active form is accomplished by *thyroid peroxidase*. The reaction results in the formation of monoiodotyrosine (MIT) and diiodotyrosine (DIT) residues in thyroglobulin, a process referred to as *organification of iodine*, just prior to its extracellular storage in the lumen of the thyroid follicle.

Formation of Thyroxine and Triiodothyronine From Iodotyrosines

The remaining synthetic step is the coupling of two DIT residues to form T_4 or of an MIT and a DIT residue to form T_3. These oxidative reactions also are catalyzed by *thyroid peroxidase*. Intrathyroidal and secreted T_3 are also generated by the 5′-deiodination of T_4.

Synthesis and Secretion of Thyroid Hormones

Because T_4 and T_3 are synthesized and stored within thyroglobulin, proteolysis is an important part of the secretory process. This process is initiated by endocytosis of colloid from the follicular lumen at the apical surface of the cell, with the participation of a thyroglobulin receptor, *megalin*. This "ingested" thyroglobulin appears as intracellular colloid droplets, which apparently fuse with lysosomes containing the requisite proteolytic enzymes. TSH enhances the degradation of thyroglobulin by increasing the activity of lysosomal *thiol endopeptidases*, which selectively cleave thyroglobulin, yielding hormone-containing intermediates that subsequently are processed by exopeptidases. The liberated hormones then exit the cell primarily as T_4 along with some T_3. The T_3 secreted by the thyroid derives partly from T_3 within mature thyroglobulin and partly from deiodination of T_4 (Figure 47–3), which also occurs peripherally (Figure 47–4).

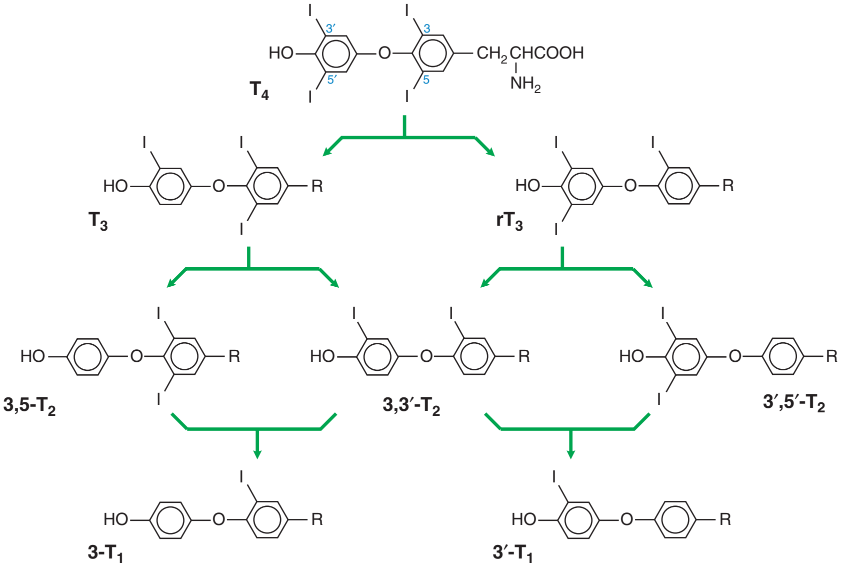


Figure 47–3 *Pathways of iodothyronine deiodination.*

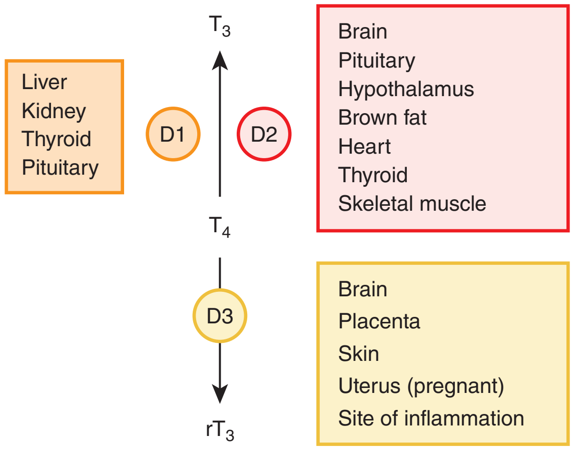


Figure 47–4 *Peripheral T_4 to T_3 conversion by different deiodinase enzymes (D1, D2, D3).*

Quantitative Aspects of Thyroid Hormone Metabolism

Selected quantitative aspects of thyroid hormone metabolism are provided in Table 47–1. The non–protein-bound (free) fraction of T_4 is much smaller than that of T_3 primarily because thyroxine-binding globulin (TBG) has a 20-fold lower dissociation constant for T_4, discussed below.

Activation and Inactivation of Thyroid Hormone by Deiodination in Peripheral Tissues

Although T_3 is secreted by the thyroid, metabolism of T_4 by 5′-deiodination of the outer ring in the peripheral tissues accounts for approximately 80% of circulating T_3; in contrast, 5′-deiodination of the inner ring produces the metabolically inactive 3,3′,5′-triiodothyronine (reverse T_3 [rT_3]; see Figure 47–3). Under normal conditions, approximately 40% of T_4 is converted to each of T_3 and rT_3, and approximately 20% is metabolized via other pathways, such as glucuronidation in the liver and excretion in the bile. T_3 has a much higher affinity for the nuclear TR compared with T_4 and is much more potent biologically on a molar basis.

There are three *iodothyronine deiodinases*, produced by the genes *DIO1*, *DIO2*, and *DIO3* (Marsili et al., 2011). Dio1 and Dio2 convert T_4 to T_3. Dio1 is a plasma membrane protein expressed primarily in the liver and kidney and also in the thyroid and pituitary (see Figure 47–4). It is upregulated in hyperthyroidism, downregulated in hypothyroidism, and inhibited by the antithyroid drug *propylthiouracil*. Dio2 is expressed primarily in the CNS (including the pituitary and hypothalamus) and brown adipose tissue, also in the thyroid, and at low levels in skeletal muscle, heart, and other tissues. The activity of Dio2 is unaffected by *propylthiouracil*. Dio2 localizes to the endoplasmic reticulum, which facilitates access of Dio2-generated T_3 to the nucleus. Organs that express Dio2 use the locally generated T_3 in addition to plasma T_3 and therefore may have a relatively high fractional occupancy of TRs by T_3. T_4 induces ubiquitination and degradation of the Dio2 enzyme. This results in suppressed levels of Dio2 in hyperthyroidism and elevated levels in hypothyroidism, thus helping to maintain T_3 homeostasis. In addition, T_3 mildly represses Dio2 expression at the transcriptional level. It has been difficult to determine the relative contributions of Dio1 and Dio2 to the plasma T_3 in humans, but current best estimates are that Dio1 accounts for approximately one-third and Dio2 for approximately two-thirds of the circulating T_3 in euthyroid individuals. Due to the positive regulation of Dio1 by T_3 and negative regulation of Dio2 by T_4 and T_3, it is thought that Dio2 accounts for more than two-thirds of the plasma T_3 in hypothyroid individuals and that Dio1 accounts for the majority of the plasma T_3 in hyperthyroid individuals.

TABLE 47–1 ■ SELECTED QUANTITATIVE ASPECTS OF THYROID HORMONE METABOLISM

	T_4	T_3
Daily production	~80–100 μg (~100–130 nmol)	~30–40 μg (~45–60 nmol)
Plasma concentration, total hormone	~5–12 μg/dL (~60–150 nmol/L)	~80–175 ng/dL (~1.2–2.7 nmol/L)
Plasma concentration, free hormone	~0.8–2.0 ng/dL (~10–25 pmol/L)	~2–4 pg/mL (~3–6 pmol/L)
Plasma free fraction (non–protein bound)	~0.02%	~0.3%
Intracellular fraction (excluding thyroid gland)	~15%	~65%
Volume of distribution	10 L	40 L
Plasma $t_{1/2}$	~7 days	~20 h
Fractional turnover	~10%/day	~56%/day

The Dio2 polymorphism Thr92Ala is associated with reduced enzyme activity, primarily by inducing endoplasmic reticulum stress and accumulation of inactive enzyme in the trans-Golgi. In several small studies, the Dio2 Thr92Ala polymorphism has been associated with biochemical or clinical consequences (Jonklaas et al., 2021). For example, it has been associated with increased body mass index and insulin resistance. It also has been associated with lower circulating free T_3 levels in hypothyroid patients taking *levothyroxine* and with decreased psychological well-being and greater improvement in well-being following therapy with T_4/T_3 combination compared with T_4 alone. However, other studies have not confirmed these findings. The significance of Dio2 polymorphisms in human health and disease remains to be clarified.

Dio3 catalyzes inner ring or 5-deiodination, the main inactivating pathway of T_3 metabolism; Dio1 performs this function to some extent. Dio3 is found at highest levels in the CNS and placenta and also is expressed in skin and pregnant uterus. Dio3 can be induced locally by inflammation and hypoxia and is highly expressed in certain tumors. Both Dio2 and Dio3 are expressed in time- and spatially restricted patterns during development, in which they play important roles by regulating local levels of T_3.

The three deiodinases contain the rare amino acid *selenocysteine* in their active sites. Incorporation of selenocysteine into the growing peptide chain is a complex process involving multiple proteins. Mutations in one such protein, SECIS binding protein 2, are associated with abnormal circulating thyroid hormone levels.

Transport of Thyroid Hormones in the Blood

Iodine in the circulation is normally present in several forms, with 95% as organic iodine and about 5% as iodide. Most (90%–95%) organic iodine is T_4; T_3 represents a relatively minor fraction (~5%). The thyroid hormones are transported in the blood in strong but noncovalent association with several plasma proteins.

Thyroxine-binding globulin is the major carrier of thyroid hormones. It is a glycoprotein (mass of ~63,000 Da) that binds one molecule of T_4 per molecule of protein with a very high affinity (K_d is ~10^{-10} M); T_3 is bound less avidly. T_4, but not T_3, also is bound by *transthyretin* (thyroxine-binding prealbumin), a retinol-binding protein. This protein is present in higher concentration than is TBG and binds T_4 with a K_d about 10^{-7} M. Albumin can also bind T_4 when the more avid carriers are saturated, but its physiological importance is unclear. Binding of thyroid hormones to plasma proteins protects the hormones from metabolism and excretion, resulting in their long half-lives in the circulation. The free (unbound) hormone is a small percentage (~0.02% of T_4 and ~0.3% of T_3) of the total hormone in plasma. The differential binding affinities for serum proteins also contribute to establishing the 10- to 100-fold differences in circulating hormone concentrations and half-lives of T_4 and T_3.

Essential to understanding the regulation of thyroid function is the "free hormone" concept: Only the unbound hormone has metabolic

TABLE 47–2 ■ IMPORTANT FACTORS THAT ALTER BINDING OF THYROXINE TO THYROXINE-BINDING GLOBULIN

INCREASE BINDING	DECREASE BINDING
Drugs	
Estrogens, tamoxifen	Corticosteroids, androgens
Selective estrogen receptor modulators	L-Asparaginase, furosemide
Methadone, heroin	Salicylates, mefenamic acid
Clofibrate, 5-fluorouracil	Antiseizure medications (phenytoin, carbamazepine)
Systemic factors	
Liver disease, porphyria	Acute and chronic illness
HIV infection	Inheritance
Inheritance	

activity. Because of the high degree of binding of thyroid hormones to plasma proteins, changes in either the concentrations of these proteins or the affinities of the hormone-protein interactions have major effects on the total serum hormone levels. Certain drugs and a variety of pathological and physiological conditions can alter both the binding of thyroid hormones to plasma proteins and the amounts of these proteins (Table 47–2).

Degradation and Excretion of Thyroid Hormones

T_3 and T_4 not only can be deiodinated but also can be metabolized by ether cleavage, conjugation, and oxidative decarboxylation (Figure 47–5). T_4 is eliminated slowly from the body, with a $t_{1/2}$ of 6 to 8 days. In hyperthyroidism, the $t_{1/2}$ is shortened to 3 to 4 days, whereas in hypothyroidism, it may be 9 to 10 days. In conditions associated with increased binding to TBG, such as pregnancy, clearance is retarded. The opposite effect is observed when binding to protein is inhibited by certain drugs (see Table 47–2). T_3, which is less avidly bound to protein, has a $t_{1/2}$ of about 18 to 24 h.

The liver is the major site of nondeiodinative degradation of thyroid hormones; T_4 and T_3 are conjugated with glucuronic and sulfuric acids and excreted in the bile. Some thyroid hormone is liberated by hydrolysis of the conjugates in the intestine and reabsorbed. A portion of the conjugated material reaches the colon unchanged, where it is hydrolyzed and eliminated in feces as the free compounds.

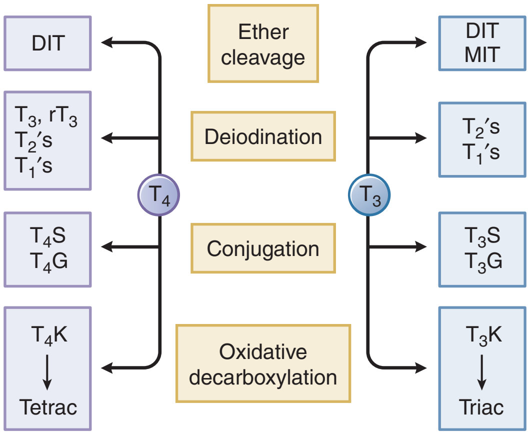


Figure 47–5 *Pathways of metabolism of T_4 and T_3.* rT_3, reverse T_3; T_3G, T_3 glucuronide; T_4G, T_4 glucuronide; T_3K, T_3 pyruvic acid; T_4K, T_4 pyruvic acid; Tetrac, tetraiodothyroacetic acid; Triac, 3,5,3′-triiodothyroacetic acid; T_3S, T_3 ulfate: T_4S, T_4 sulfate.

Factors Regulating Thyroid Hormone Secretion

Thyrotropin is a glycoprotein hormone that consists of an α subunit, common to pituitary glycoproteins such as gonadotropins, and a unique β subunit. TSH is secreted in a pulsatile manner and in a circadian pattern (levels are slightly higher during sleep at night) that is the inverse of the cortisol circadian pattern, reflecting the fact that cortisol reduces TSH secretion. TSH secretion is controlled by the hypothalamic peptide *TRH* and by the concentration of free thyroid hormones in the circulation. Increased thyroid hormone inhibits transcription of both the TRH gene and the genes encoding the α and β subunits of TSH, which suppresses the secretion of TSH and causes the thyroid to become inactive and regress. Any decrease in the normal rate of thyroid hormone secretion by the thyroid evokes an enhanced secretion of TSH. Additional mechanisms mediating the effect of thyroid hormone on TSH secretion appear to be a reduction in TRH secretion by the hypothalamus and a reduction in the number of TRH receptors on pituitary cells (Figure 47–6).

Thyrotropin-Releasing Hormone

Thyrotropin-releasing hormone stimulates the release of preformed TSH from secretory granules and also stimulates the subsequent synthesis of both α and β subunits of TSH. TRH is a tripeptide (L-pyroglutamyl-L-histidyl-L-proline amide) synthesized by the hypothalamus and released into the hypophyseal-portal circulation, where it interacts with TRH receptors on thyrotropes in the anterior pituitary. The binding of TRH to its receptor, a GPCR, stimulates the G_q-PLC-IP_3-Ca^{2+} pathway and activates protein kinase C, ultimately stimulating the synthesis and release of TSH. Two TRH receptors have now been identified, TRH-R1 and TRH-R2, and there are receptor-selective TRH analogues. Somatostatin, dopamine, and glucocorticoids inhibit TRH-stimulated TSH secretion.

Actions of TSH on the Thyroid

TSH increases the synthesis and secretion of thyroid hormone. These effects follow the binding of TSH to its receptor (a GPCR) on the plasma membrane of thyroid cells. Binding of TSH to its receptor stimulates the

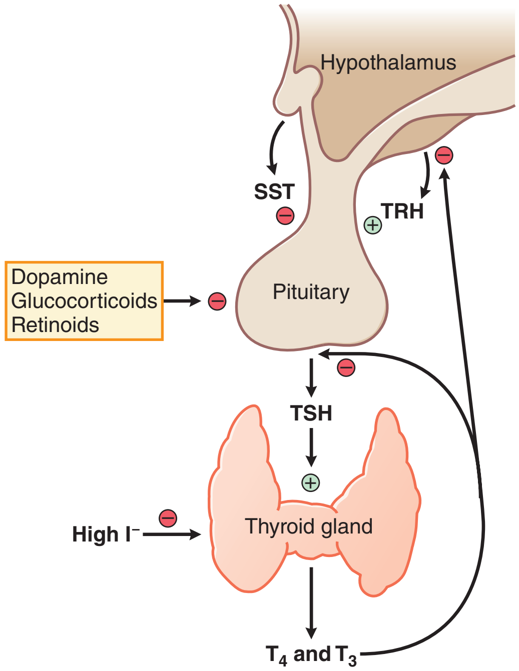


Figure 47–6 *Regulation of thyroid hormone secretion.* Myriad neural inputs influence hypothalamic secretion of thyrotropin-releasing hormone (TRH). TRH stimulates release of TSH from the anterior pituitary; TSH stimulates the synthesis and release of the thyroid hormones T_3 and T_4. T_3 and T_4 feed back to inhibit the synthesis and release of TRH and TSH. Somatostatin (SST) can inhibit TRH action, as can dopamine and high concentrations of glucocorticoids. Low levels of I^- are required for T_4 synthesis, but high levels inhibit T_4 synthesis and releas

G_s-adenylyl cyclase–cyclic AMP pathway. Higher concentrations of TSH activate the G_q-PLC pathway. Inactivating and activating mutations of the TSH receptor have been described and can result in thyroid dysfunction.

Iodine and Thyroid Function

Adequate iodine intake is essential for normal thyroid hormone production. When iodine intake is low, thyroid hormone production is reduced, TSH is secreted in excess, and the thyroid becomes hyperplastic and hypertrophic. The enlarged and stimulated thyroid becomes remarkably efficient at extracting the residual traces of iodide from the blood, developing an iodine gradient that may be 10 times normal; in mild-to-moderate iodine deficiency, the thyroid usually succeeds in producing sufficient hormone and preferentially secreting T_3. In more severe iodine deficiency, adult hypothyroidism or congenital iodine deficiency syndrome may occur. High levels of iodine inhibit T_4 synthesis and release. In some areas of the world, simple or nontoxic goiter is prevalent because of insufficient dietary iodine. The addition of iodate to table salt (NaCl) provides a convenient iodine supplement. In the U.S., iodized salt provides 100 μg of iodine per gram. The recommended daily allowances for iodine are from 90 to 120 μg for children, 150 μg for adults, 220 μg for pregnancy, and 290 μg for lactation (Public Health Committee of the American Thyroid Association et al., 2006). Vegetables, meat, and poultry contain minimal amounts of iodine, whereas dairy products and fish are relatively high in iodine.

Iodized Salt

Iodine has been used empirically for the treatment of iodine-deficiency goiter for 150 years; however, its modern use evolved from extensive studies using iodine to prevent goiter in schoolchildren in Akron, Ohio, where endemic iodine-deficiency goiter was prevalent. The success of these experiments led to the adoption of iodine prophylaxis and therapy in many regions throughout the world where iodine-deficiency goiter was endemic. The most practical method for providing small supplements of iodine for large segments of the population is the addition of iodide or iodate to table salt; iodate is now preferred. The use of iodized salt is required by law in some countries, but in others, such as the U.S., the use is optional.

Transport of Thyroid Hormones Into and Out of Cells

The passage of thyroid hormones across the cell membrane is mediated by specific transporters (Groeneweg et al., 2020), the most well-documented of which are MCT8 and MCT10, OATP1C1, and SLC17A4. MCT8 is widely expressed, including in multiple cell types and regions of the brain. MCT8 mutations cause Allan-Herndon-Dudley syndrome, characterized by severe neurodevelopmental defects that are likely due to impaired entry of T_4 and T_3 into the brain. Individuals with this syndrome also tend to have low circulating free T_4 and elevated free T_3 levels, with some clinical evidence of thyrotoxicosis. The T_3 metabolite triac is a biologically active thyroid hormone that does not require MCT8 for cell entry (it is present naturally in serum at very low levels). In a phase II trial of patients with MCT8 deficiency, therapy with triac (*tiratricol*) improved signs of hyperthyroidism, although the patients were beyond the age where improvement in neurodevelopment might be feasible.

OATP1C1 preferentially transports T_4 over T_3 and is highly expressed in the brain. In mice, OATP1C1 plays an important role in the transport of T_4 across the blood-brain barrier. A single patient with a homozygous OATP1C1 mutation and a severe neurodevelopmental phenotype has been described. This patient showed clinical improvement when treated with triac, which does not require OATP1C1 for cell entry.

MCT10 transports T_3 more effectively than T_4 and is widely expressed. SLC17A4 transports T_3 and T_4 and is expressed in liver, kidney, intestine, and pancreas. However, the importance of MCT10 and SLC17A4 in thyroid hormone transport into or out of cells in humans remains to be determined, and mutations causing abnormal patient phenotypes have not been described.

Mediation of Effects by Nuclear Receptors

Thyroid hormone action is mediated largely by the binding of T_3 to TRs, which are members of the nuclear receptor superfamily of transcription factors (Brent, 2012). This superfamily includes the receptors for steroid hormones, vitamin D, retinoic acid, and a variety of small-molecule metabolites such as certain fatty acids and bile acids, as well as a number of "orphan receptors" (see Chapter 3).

The TRs have the classic nuclear receptor structure: an amino terminal domain, a centrally located zinc finger DNA-binding domain, and a ligand-binding domain that occupies the carboxyl terminal half of the protein. T_3 binds to TRs with about 10-fold greater affinity than does T_4, and T_4 is not thought to be biologically active in normal physiology. TRs bind to specific DNA sequences (thyroid hormone response elements) in the promoter/regulatory regions of target genes. The transcription of most target genes is repressed by unliganded TRs and induced in response to the binding of T_3. In the unliganded state, the TR ligand-binding domain interacts with a corepressor complex that includes *histone deacetylases* and other proteins. The binding of T_3 causes replacement of the corepressor complex by a coactivator complex that includes *histone acetyltransferases*, *methyltransferases*. Other thyroid hormone target genes, such as those encoding TRH and the TSH subunits, are negatively regulated by T_3. The mechanism is not well defined, but these genes tend to be induced by the unliganded TR in addition to being repressed by T_3.

Two genes encode TRs: *THRA* and *THRB*. *THRA* encodes the receptor TRα1. TRα1 is expressed in most cell types, but its major activities are in the regulation of heart rate, body temperature, skeletal muscle function, and the development of bone and small intestine. Patients with *THRA* mutations have been described with short stature, bony abnormalities, and chronic constipation, along with normal circulating TSH and low-normal T_4 levels, a syndrome termed *resistance to thyroid hormone alpha* (Onigata and Szinnai, 2014).

The *THRB* gene has two promoters that lead to the production of TRβ1 and TRβ2. These receptors have unique amino terminal domains but otherwise are identical. TRβ1 is ubiquitous; TRβ2 has a highly restricted pattern of expression, including the pituitary and hypothalamus. TRβ1 mediates specific effects in liver metabolism (including the hypocholesterolemic effect of T_3); TRβ2 has roles in the negative feedback by T_3 on hypothalamic TRH and pituitary TSH and in the development of retinal cones and the inner ear. Mutations in *THRB* cause the syndrome of *resistance to thyroid hormone beta*. This syndrome is associated with goiter, tachycardia, impaired hearing, attention-deficit/hyperactivity disorder, and other abnormalities. Laboratory evaluation typically shows elevated levels of T_4 and T_3 with TSH in the mid to upper portion of the reference range or frankly elevated (Onigata and Szinnai, 2014).

Nongenomic Effects of Thyroid Hormone

Although nuclear receptors are generally thought of as DNA-binding transcription factors, nuclear receptors, such as TRs, also are found outside the nucleus where they can exert biological effects via rapid nongenomic mechanisms (Hones et al., 2017). A truncated form of TRα1 is palmitoylated and localizes to the plasma membrane, where it causes rapid T_3-dependent NO production as well as activation of ERK and Akt signaling. Full-length TRα1 and TRβ1 reportedly associate in a T_3-dependent manner with the p85α subunit of PI3K (phosphoinositide 3-kinase), resulting in activation of Akt (protein kinase B). It is interesting to note that T_3 administration causes rapid vasodilation, which might be explained by the aforementioned generation of NO. Perhaps the strongest *in vivo* evidence for the relevance of noncanonical actions of T_3 comes from studies in mice in which the endogenous *Thra* and *Thrb* genes were mutated to yield TR proteins incapable of binding DNA (Hones et al., 2017). In these mice, T_3 could still regulate heart rate, body temperature, and blood glucose and triglyceride concentrations. There also is some evidence for nongenomic actions of thyroid hormone via a plasma membrane receptor within integrin αVβ3. This putative receptor binds extracellular T_4 in preference to T_3, resulting in activation of MAP kinase. The importance of this putative receptor in thyroid hormone physiology and pathophysiology remains uncertain.

Major Clinical Effects of Thyroid Hormones

Most cells and organs are responsive to thyroid hormone. As such, thyroid hormone regulates many physiological processes, only a few of which will be mentioned here.

Growth and Development

Perhaps the most dramatic example of thyroid hormone action is amphibian metamorphosis, in which the tadpole transforms into a frog. The development of limbs, lungs, and other features required for terrestrial life, as well as regression of the tail, are driven by T_3.

Thyroid hormone plays a critical role in brain development by mechanisms that are incompletely understood (Abduljabbar and Afifi, 2012). The absence of thyroid hormone during the period of active neurogenesis (up to 6 months postpartum) leads to irreversible intellectual disability and is accompanied by multiple morphological alterations in the brain. These severe morphological alterations result from disturbed neuronal migration, deranged axonal projections, and decreased synaptogenesis. Thyroid hormone supplementation begun during the first 2 weeks of postnatal life can prevent the development of these morphological changes. The extensive defects in growth and development in untreated congenital hypothyroidism vividly illustrate the pervasive effects of thyroid hormones in normal individuals.

Severe hypothyroidism in infancy (congenital iodine deficiency syndrome) can be *endemic* (caused by extreme environmental iodine deficiency) or *sporadic* (a consequence of abnormal thyroid development or a defect in the synthesis of thyroid hormone). The affected child usually appears normal at birth but subsequently manifests decreased growth with short extremities, intellectual disability, and listlessness. Other manifestations include puffy face, enlarged tongue, dry and doughy skin, slow heart rate, constipation, and decreased body temperature. For treatment to be fully effective, the diagnosis must be made shortly after birth and T_4 treatment initiated long before these clinical manifestations are apparent. In regions of endemic iodine deficiency, iodine replacement is best instituted before pregnancy. Screening of newborn infants for deficient thyroid function is carried out in the U.S. and in most industrialized countries.

Skeleton

Childhood hypothyroidism results in decreased linear growth, delayed bone age, and epiphyseal dysgenesis (Wojcicka et al., 2013). That these effects are mediated through TRα1 is demonstrated by the similar short stature, delayed ossification, and skeletal dysplasia that characterize subjects with *THRA* gene mutations. Childhood thyrotoxicosis also can cause decreased final height, but this is due to rapid bone maturation with premature fusion of the epiphyseal growth plates. In the adult, thyrotoxicosis accelerates bone turnover and can increase the risk of osteoporosis, especially in concert with postmenopausal estrogen deficiency.

Thermogenesis

Thyroid hormone is necessary for both obligatory thermogenesis (the heat resulting from vital processes) and facultative or adaptive thermogenesis (Mullur et al., 2014). Only a few organs, including the brain, gonads, and spleen, are unresponsive to the thermogenic effects of T_3. Obligatory thermogenesis is the result of T_3 making most biological processes thermodynamically less efficient for the sake of producing heat. It is likely that multiple mechanisms contribute to this effect, such as the induction of futile cycling, changes in mitochondrial energetics, stimulation of the Na^+/K^+ gradient across the cell membrane, and stimulation of the Ca^{2+} gradient between the cytoplasm and the sarcoplasmic reticulum. Thermogenesis is highly sensitive to thyroid hormone around the physiological range: Small changes in *levothyroxine* replacement doses may significantly alter resting energy expenditure in the hypothyroid patient. The capacity of T_3 to stimulate thermogenesis has evolved along with ancillary effects to support this action, such as the stimulation of appetite and lipogenesis.

Cardiovascular System

Hyperthyroid patients have tachycardia, increased stroke volume, increased cardiac index, cardiac hypertrophy, decreased peripheral vascular resistance, and increased pulse pressure (Grais and Sowers, 2014). Hyperthyroidism is a relatively common cause of atrial fibrillation. Hypothyroid patients have bradycardia, decreased cardiac index, pericardial effusion, increased peripheral vascular resistance, decreased pulse pressure, and elevation of mean arterial pressure.

T_3 regulates myocardial gene expression primarily through TRα1, which is expressed at a higher level in cardiomyocytes than TRβ. T_3 increases the speed of diastolic relaxation (lusitropic effect) by inducing expression of the sarcoplasmic reticulum ATPase SERCA2 and decreasing phospholamban, a SERCA2 inhibitor. T_3 increases the force of myocardial contraction (inotropic effect) in part by inducing expression of the ryanodine receptor-channel (RyR2), the calcium channel of the sarcoplasmic reticulum. T_3 induces the gene encoding the α isoform of myosin heavy chain (MHC) and decreases expression of MHCβ. Because MHCα endows the myosin holoenzyme with greater ATPase activity, this is one mechanism by which T_3 enhances the velocity of contraction. The chronotropic effect of T_3 is mediated at least in part by increases in the pacemaker ion current I_f in the sinoatrial node. Several proteins that comprise the I_f channel are induced by T_3, including the hyperpolarization-activated, cyclic nucleotide–gated cation channels HCN2 and HCN4. T_3 also has a vasodilating effect on vascular smooth muscle. The mechanism is multifactorial and appears to include rapid nongenomic stimulation of endothelial cell NO production, which contributes to the decreased systemic vascular resistance and increased cardiac output of hyperthyroidism.

Lipid Metabolism

Thyroid hormone stimulates the expression of hepatic LDL receptors and reduces apolipoprotein B levels through non-LDL receptor pathways (Mullur et al., 2014), such that hypercholesterolemia is a characteristic feature of hypothyroidism.

Disorders of Thyroid Function

Thyroid Hypofunction

Hypothyroidism, known as *myxedema* when severe, is the most common disorder of thyroid function. Worldwide, hypothyroidism resulting from iodine deficiency remains a common problem. In nonendemic areas where iodine is sufficient, chronic autoimmune thyroiditis (Hashimoto thyroiditis) accounts for most cases. This disorder is characterized by circulating antibodies directed against thyroid peroxidase and, sometimes, against thyroglobulin. These conditions are examples of *primary hypothyroidism,* failure of the thyroid gland itself. *Central hypothyroidism* occurs much less often and results from diminished stimulation of the thyroid by TSH because of pituitary failure (*secondary hypothyroidism*) or hypothalamic failure (*tertiary hypothyroidism*). Hypothyroidism present at birth (*congenital hypothyroidism*) is an important preventable cause of intellectual disability in the world.

Common symptoms of hypothyroidism include fatigue, lethargy, cold intolerance, mental slowness, depression, dry skin, constipation, mild weight gain, fluid retention, muscle aches and stiffness, irregular menses, and infertility. Common signs include goiter (primary hypothyroidism only), bradycardia, delayed relaxation phase of the deep tendon reflexes, cool and dry skin, hypertension, nonpitting edema, and facial puffiness. Deficiency of thyroid hormone during the first few months of life causes feeding problems, failure to thrive, constipation, and sleepiness. Impairment of mental development is irreversible if not treated promptly. Childhood hypothyroidism impairs linear growth and bone maturation. Because the signs and symptoms of hypothyroidism are nonspecific, diagnosis requires the finding of an elevated serum TSH level and reduced or low-normal serum free T_4 or, in cases of central hypothyroidism, only a decreased serum free T_4.

Thyroid Hyperfunction

Thyrotoxicosis is a condition caused by elevated concentrations of circulating free thyroid hormones. Increased thyroid hormone production is the most common cause, with the common link of TSH receptor stimulation and increased iodine uptake by the thyroid gland, as established by the measurement of the percentage uptake of ^{123}I in a 24-h radioactive iodine uptake (RAIU) test.

TSH receptor stimulation is either the result of TSH receptor stimulating antibodies in Graves disease or somatic activating TSH receptor mutations in autonomously functioning nodules or a toxic goiter. In contrast, thyroid inflammation or destruction resulting in excess "leak" of thyroid hormones or excess exogenous thyroid hormone intake results in a low 24-h RAIU test. The term *subclinical hyperthyroidism* is defined as those with a subnormal serum TSH and normal concentrations of free T_4 and T_3. Atrial arrhythmias, excess cardiac mortality, and excessive bone loss have been associated with this profile of thyroid function tests.

Graves disease is the most common cause of high RAIU thyrotoxicosis, accounting for 60% to 90% of cases, depending on age and geographic region. Graves disease is an autoimmune disorder characterized by increased thyroid hormone production, diffuse goiter, and IgG antibodies that bind to and activate the TSH receptor. As with most types of thyroid dysfunction, women are affected more than men, with a ratio ranging from 5:1 to 7:1. While Graves disease may occur at any age, it is more common between the ages of 20 and 50. Graves disease is commonly associated with other autoimmune diseases. The characteristic exophthalmos associated with Graves disease is an infiltrative ophthalmopathy and is considered an autoimmune-mediated inflammation of the periorbital connective tissue and extraocular muscles. Toxic uninodular/multinodular goiter accounts for 10% to 40% of cases of hyperthyroidism and is more common in older patients. A low RAIU value is seen in the destructive thyroiditides and in thyrotoxicosis in patients taking excessive doses of thyroid hormone.

Most of the signs and symptoms of thyrotoxicosis stem from the excessive production of heat, increased motor activity, and increased sensitivity to catecholamines produced by the sympathetic nervous system. The skin is flushed, warm, and moist; the muscles are weak and tremulous; the heart rate is rapid, the heartbeat is forceful, and the arterial pulses are prominent and bounding. Increased expenditure of energy gives rise to increased appetite and, if intake is insufficient, to loss of weight. There also may be insomnia, difficulty in remaining still, anxiety and apprehension, intolerance to heat, and increased frequency of bowel movements. Angina, arrhythmias, and heart failure may be present in older patients. Older patients may experience lessened manifestations of sympathetic nervous system stimulation and reduced symptoms compared to younger individuals, sometimes referred to as "apathetic hyperthyroidism." Some individuals may show extensive muscular wasting as a result of thyroid myopathy. The most severe form of hyperthyroidism is thyroid storm (see section that follows on therapeutic uses of antithyroid drugs).

Thyroid Function Tests

Measurement of the total hormone concentration in plasma may not give an accurate picture of the activity of the thyroid gland; total hormone concentration changes with alterations in amount and affinity of TBG in plasma. Although equilibrium dialysis of undiluted serum and radioimmunoassay for free T_4 in the dialysate represent the gold standard for determining free T_4 concentrations, this assay is costly and typically unavailable in routine clinical laboratories. The most common assays used for estimating the free T_4 and free T_3 concentrations employ labeled analogues of these iodothyronines in chemiluminescence and enzyme-linked immunoassays. These assays are subject to influences of altered serum-binding proteins, nonthyroid disease states, acute illnesses, and other drugs. In individuals with normal pituitary function, serum measurement of TSH is the thyroid function test of choice because pituitary secretion of TSH is sensitively regulated in response to circulating concentrations of thyroid hormones. TSH is suppressed in patients with thyrotoxicosis and elevated in those with primary hypothyroidism, and these changes generally precede abnormalities in free T_4 and free T_3.

Thyroid Hormone Preparations

Synthetic preparations of the sodium salts of the natural isomers of T_4 and T_3 are used for thyroid hormone therapy (Biondi and Wartofsky, 2014).

Levothyroxine

Chemistry and Mechanism of Action

The chemistry and mechanism of action are identical to those of endogenous T_4 and have been presented above.

ADME

Levothyroxine sodium is available in tablets and liquid-filled capsules for oral administration and as a lyophilized powder for injection. Absorption of *levothyroxine* occurs in the stomach and small intestine and is incomplete (~80% of the tablet dose is absorbed). Absorption is slightly increased when the hormone is taken on an empty stomach, and it is associated with less variability in TSH levels when taken this way regularly. Patients with gastrointestinal problems that result in poor absorption of tablet formulations may achieve better absorption with liquid-filled capsules. Serum T_4 peaks 2 to 4 h after oral ingestion, but changes are barely discernible with once-daily dosing due to the plasma $t_{1/2}$ of about 7 days. Given this long $t_{1/2}$, omission of one day's dose has only marginal effects on the serum TSH and free T_4, but to maintain consistent dosing, the patient should be instructed to take a double dose the next day. Additional ADME data for *levothyroxine* are the same as for endogenous T_4 and have been presented above. If patients cannot take oral medications or intestinal absorption is in question, *levothyroxine* may be given intravenously once daily at a dose of about 80% of the patient's daily oral requirement. For any given serum TSH, the serum T_4/T_3 ratio is slightly higher in patients taking *levothyroxine* than in patients with endogenous thyroid function due to the fact that about 20% of circulating T_3 normally is supplied by direct thyroidal secretion (Gullo et al., 2011).

Liothyronine

Chemistry and Mechanism of Action

The chemistry and mechanism of action are identical to those of endogenous T_3 and have been presented above.

ADME

Liothyronine sodium is the salt of T_3 and is available in tablets and in an injectable form. *Liothyronine* absorption is nearly 100%, with peak serum levels 2 to 4 h following oral ingestion. Additional ADME data for *liothyronine* are the same as for endogenous T_3 and have been presented above. On a weight basis, the required daily dose of *liothyronine* (given three times a day to achieve steady serum T_3 levels) is about one-third that of *levothyroxine* to achieve an equivalent TSH level in hypothyroid patients (Celi et al., 2011). However, normalization of circulating TSH requires an almost 2-fold higher serum T_3 compared with *levothyroxine* therapy because negative feedback on TSH normally relies in part on the local generation of T_3 from circulating T_4.

Liothyronine may be used occasionally for thyroid hormone replacement when a rapid onset of action is desired, such as in the rare presentation of myxedema coma, or if rapid termination of action is desired, such as when preparing a patient with thyroid cancer for ^{131}I therapy. *Liothyronine* is less desirable than *levothyroxine* for chronic replacement therapy due to the requirement for more-frequent dosing (plasma $t_{1/2}$ is ~20 h), higher cost, and transient elevations of serum T_3 concentrations above the normal range. Also, organs that express Dio2 use the locally generated T_3 in addition to plasma T_3; hence, there is theoretical concern that these organs will not maintain physiological intracellular T_3 levels in the absence of plasma T_4.

T_4/T_3 Combination Preparations

A mixture of *levothyroxine* and *liothyronine* around 4:1 by weight, and desiccated thyroid preparations with a similar T_4:T_3 ratio, also are available. A 60-mg (1-grain) desiccated thyroid tablet is approximately equivalent to 65 μg of *levothyroxine* in TSH-lowering capacity.

Therapeutic Uses of Thyroid Hormone

The major indications for the therapeutic use of thyroid hormone are for hormone replacement therapy in patients with hypothyroidism and for TSH suppression therapy in patients with thyroid cancer.

Thyroid Hormone Replacement Therapy in Hypothyroidism

Levothyroxine is the hormone of choice for thyroid hormone replacement therapy due to its consistent potency and prolonged duration of action (Jonklaas et al., 2014). This therapy relies on Dio1 and Dio2 to convert T_4 to T_3 to maintain a steady serum level of free T_3.

The average daily adult full replacement dose of *levothyroxine* is 1.7 μg/kg body weight (0.8 μg/lb). Dosing should generally be based on lean body mass. The goal of therapy is to normalize the serum TSH (in primary hypothyroidism) or free T_4 (in secondary or tertiary hypothyroidism) and to relieve symptoms of hypothyroidism. In primary hypothyroidism, generally it is sufficient to follow TSH without free T_4. A patient with mild primary hypothyroidism will achieve a normal TSH with substantially less than a full replacement dose, but as the endogenous thyroid function declines, the dose will need to be increased. In individuals older than 60 years and those with known or suspected cardiac disease or with areas of autonomous thyroid function, institution of therapy at a subreplacement dose of *levothyroxine* (12.5–50 μg/day) is appropriate. The dose can be increased by 25 μg/day every 6 weeks until the TSH is normalized. Follow-up blood tests typically are done about 6 weeks after any dosage change due to the 1-week plasma $t_{1/2}$ of T_4. The vast majority of controlled trials do not support the hypothesis that combination therapy with T_4 plus T_3 provides a better therapeutic response than does T_4 alone (Jonklaas et al., 2021), although for unclear reasons, occasionally patients say they feel better when taking combination therapies, such as desiccated thyroid. A double-blind crossover study of hypothyroid patients comparing *levothyroxine* and desiccated thyroid, maintaining the same reference range TSH, found that those who preferred desiccated thyroid had lost weight on this preparation. Monotherapy with *levothyroxine* most closely mimics normal physiology and generally is recommended (Jonklaas et al., 2014).

Although *levothyroxine* monotherapy is the recommended therapy in hypothyroidism, it is worth noting that this does not precisely replicate thyroid gland secretion because 20% of circulating T_3 normally derives directly from the thyroid. The published studies comparing T_4/T_3 combination therapy with T_4 alone have significant limitations, and it remains possible that there exists a currently undefined subgroup of individuals for whom combination therapy would result in an improved clinical outcome (Jonklaas et al., 2021). For example, the published comparison trials have not focused on hypothyroid patients who feel unwell while taking *levothyroxine* despite clearly euthyroid TSH and free T_4 levels, nor have there been large trials focused on individuals with the Dio2 Thr92Ala polymorphism, which was discussed earlier. An ideally designed test of T_4/T_3 combination therapy would be difficult to conduct at this time because there are no long-acting preparations of *liothyronine* and there are no T_4/T_3 combination preparations that match the approximately 11:1 T_4:T_3 ratio of thyroid gland secretion. Nevertheless, it should be emphasized that the vast majority of hypothyroid patients feel well on *levothyroxine* monotherapy and the published outcomes from trials of T_4/T_3 combination therapy provide little evidence for benefit of combination therapy over T_4 alone.

Hypothyroidism During Pregnancy

Due to the increased serum concentration of TBG induced by estrogen, the expression of Dio3 by the placenta, and the small amount of transplacental passage of T_4 from mother to fetus, a higher dose of *levothyroxine* is usually required in pregnant patients. Overt hypothyroidism during pregnancy is associated with increased risk of miscarriage, fetal distress, preterm delivery, and impaired psychoneural and motor development in the progeny. Even mild maternal hypothyroidism may have subtle adverse effects. As part of prepregnancy planning, the dose of *levothyroxine* should be adjusted to maintain the TSH in the lower portion of the reference range. Women should increase their *levothyroxine* dose by about 30% as soon as pregnancy is confirmed, thus anticipating the increased need (Jonklaas et al., 2014). Being proactive instead of reactive is recommended since avoiding maternal hypothyroidism is important, and the approximate 7-day plasma T_4 $t_{1/2}$ means that it takes many weeks for free T_4 and TSH to reach new steady states after a *levothyroxine* dosage change. In patients taking a single *levothyroxine* tablet daily, the 30% dosage increase can be achieved by taking two extra tablets per week. The serum TSH is measured 4 to 6 weeks later, and the *levothyroxine* dose is further adjusted with the goal of maintaining the TSH in the lower portion of the reference range. Subsequent dosage adjustments are based on serum TSH, measured 4 to 6 weeks after each adjustment. TSH should be monitored frequently through the first 20 weeks' gestation when the dose adjustment is usually maximal and then less often. The *levothyroxine* dosage should revert to the prepregnancy level the day after delivery, with a follow-up TSH checked about 6 weeks later.

Isolated *hypothyroxinemia* during pregnancy, defined by a low serum free T_4 concentration and normal serum TSH concentration, has been associated in a few studies with adverse neurocognitive development in the offspring. There are currently insufficient studies to recommend routine treatment of isolated hypothyroxinemia in pregnancy. Evaluation and treatment are further complicated by the influence of the elevated serum-binding proteins in pregnancy and lower values obtained for free T_4 by the analogue method, especially in the second and third trimesters. There is some debate as to whether standard laboratory analogue free T_4 assays or total T_4 combined with an assessment of protein binding, such as T_3 uptake, more accurately reflects the true free T_4 during pregnancy. TSH remains the best test during pregnancy to evaluate thyroid status and response to treatment.

Myxedema Coma

Myxedema coma is a rare syndrome that represents the extreme expression of severe, long-standing hypothyroidism. Common precipitating factors include infection, congestive heart failure, and medical noncompliance. Myxedema coma occurs most often in elderly patients during the winter months. Cardinal features of myxedema coma are *hypothermia*, *respiratory depression*, and *decreased consciousness*.

Intravenous administration of thyroid hormone is advised (Jonklaas et al., 2014). Therapy with *levothyroxine* is begun with a loading dose of 200 to 400 μg followed by a daily full replacement dose or slightly less in the very elderly or in patients with cardiac disease (typically 50–100 μg/day intravenously). Some clinicians recommend adding *liothyronine* (10 μg intravenously followed by 2.5–10 μg every 8 h) until the patient is stable and conscious. Other important aspects of therapy include ventilatory support, passive warming with blankets, correction of hyponatremia, and treatment of the precipitating cause. Treatment with intravenous glucocorticoids is recommended until coexisting adrenal insufficiency is excluded.

Congenital Hypothyroidism

Success in the treatment of congenital hypothyroidism depends on the age at which therapy is started and the speed with which hypothyroidism is corrected. If therapy is instituted within the first 2 weeks of life, normal physical and intellectual development can be achieved (Cherella and Wassner, 2020).

To rapidly normalize the serum T_4 concentration in the congenitally hypothyroid infant, an initial daily dose of *levothyroxine* of 10 to 15 μg/kg is recommended (Cherella and Wassner, 2020; Jonklaas et al., 2014). The *levothyroxine* is administered orally as crushed tablets mixed with breast milk or water. Because rapid normalization is even more important in infants with severe hypothyroidism, doses on the higher side of the above range are preferred in those infants. There are mixed reports as to whether brand name and generic *levothyroxine* are bioequivalent in infants with severe congenital hypothyroidism, which has led some experts to recommend against generic *levothyroxine* in this situation. The biochemical goal is to achieve a free T_4 in the upper half of the reference range and a TSH in the lower half, although some infants maintain a high

TSH that seems to reflect mis-set feedback regulation. Laboratory evaluations of TSH and free T_4 are performed approximately every 2 weeks until the laboratory goals have been achieved, then every 1 to 3 months for the first year of life, every 2 to 4 months until 3 years of age, and every 3 to 12 months from age 3 years until the end of growth. Monitoring should be more frequent if the results are abnormal or compliance is in doubt. Soy formula may impair *levothyroxine* absorption, necessitating a dosage increase.

Thyroid Hormone Replacement in Thyroid Cancer

The mainstays of therapy for well-differentiated thyroid cancer (papillary, follicular) are surgical thyroidectomy, *radioiodine* (discussed in material that follows), and *levothyroxine* to maintain a low TSH (Haugen et al., 2016). The rationale for TSH suppression is that TSH is a growth factor for thyroid cancer, but there are no randomized controlled trials that addressed the optimal TSH target range. A reasonable approach is to adjust the *levothyroxine* dose to maintain a low-normal TSH value in patients without persistent disease and at low risk for recurrence, a mildly subnormal TSH value (~0.1 mU/L) in patients at high risk for recurrence, and a more subnormal TSH level (<0.1 mU/L) for patients with persistent disease. The benefits of TSH suppression need to be weighed against the risks, including osteoporosis and atrial fibrillation.

Thyroid Nodules

Nodular thyroid disease is the most common endocrinopathy. Thyroid nodules usually are asymptomatic, although they can cause neck discomfort, dysphagia, and a choking sensation. As with other forms of thyroid disease, nodules are more frequent in women. Exposure to ionizing radiation, especially in childhood, increases the rate of nodule development. Approximately 5% of thyroid nodules that come to medical attention are malignant. Most patients with thyroid nodules are euthyroid, which should be confirmed by TSH measurement. The most useful diagnostic procedures generally are ultrasound imaging and a fine-needle aspiration biopsy. The use of *levothyroxine* to suppress TSH in euthyroid individuals with thyroid nodules cannot be recommended as a general practice. However, if the TSH is elevated, it is appropriate to administer *levothyroxine* to bring the TSH into the lower portion of the reference range.

Adverse Effects of Thyroid Hormone

Adverse effects of thyroid hormone generally occur only on overtreatment and are similar to the consequences of hyperthyroidism. An excess of thyroid hormone can increase the risk of atrial fibrillation, especially in the elderly, and can increase the risk of osteoporosis, especially in postmenopausal women.

Drug Interactions

Table 47–3 lists drugs and other factors that may influence *levothyroxine* dosage requirements.

Investigational Uses of Thyroid Hormone Analogues

Thyroid hormone analogues that bind TRβ in preference to TRα1 could in principle regulate thyroid hormone–dependent processes in liver or other TRβ-dependent organs without causing cardiac side effects. Several relatively TRβ-specific agonists have been developed with this goal in mind (Zucchi, 2020). TRβ-specific agonists were tested but are not currently being pursued as therapies for hypercholesterolemia. However, the TRβ-specific agonist *resmetirom* is under investigation as therapy for nonalcoholic fatty liver disease.

The T_3 metabolite triac does not depend on the thyroid hormone transporters MCT8 and OATP1C1 for entry into cells. As described above, triac is being studied as a potential therapy for Allan-Herndon-Dudley syndrome, which is caused by MCT8 deficiency, and has been used to treat one patient with an OATP1C1 mutation.

TABLE 47–3 ■ IMPORTANT FACTORS INFLUENCING ORAL LEVOTHYROXINE THERAPY

Drugs and other factors that may increase levothyroxine dosage requirements
Impaired levothyroxine absorption Aluminum-containing antacids, proton pump inhibitors, sucralfate Bile acid sequestrants (cholestyramine, colestipol, colesevelam) Calcium carbonate (effect generally small), phosphate binders (lanthanum carbonate, sevelamer) Chromium picolinate, raloxifene, iron salts Orlistat, kayexalate, simethicone Food, soy products (effect generally very small), lactose intolerance (single case report)
Increased thyroxine metabolism, CYP3A4 induction Rifampin, carbamazepine, phenytoin, sertraline, phenobarbital
Impaired $T_4 \rightarrow T_3$ conversion Amiodarone, glucocorticoids, beta blockers
Mechanisms uncertain or multifactorial Estrogen, pregnancy, lovastatin, simvastatin, ethionamide, Tyr kinase inhibitors
Drugs and other factors that may decrease levothyroxine dosage requirements
Advancing age (>65 years), androgen therapy in women
Drugs that may decrease TSH without changing free T_4 in levothyroxine-treated patients
Metformin

Antithyroid Drugs and Other Thyroid Inhibitors

Myriad compounds are capable of interfering, directly or indirectly, with the synthesis, release, or action of thyroid hormones (Tables 47–3 and 47–4). Several types are clinically useful:

- Antithyroid drugs, which interfere directly with the synthesis of thyroid hormones
- Ionic inhibitors, which block the iodide transport mechanism
- High concentrations of iodine, which decrease release of thyroid hormones from the gland and also may decrease hormone synthesis
- Radioactive iodine, which damages the thyroid gland with ionizing radiation

TABLE 47–4 ■ SOME AGENTS THAT DISRUPT THYROID HORMONE SYNTHESIS, RELEASE, AND METABOLISM

MECHANISM	AGENT
Iodide uptake	Perchlorate, fluoroborate, thiocyanate, nitrate
Organification of iodine	Thionamides (propylthiouracil, methimazole, carbimazole), thiocyanate, sulfonamides
Coupling reaction	Sulfonamides, thionamides
Hormone release	Li^+ salts, iodide
Peripheral iodothyronine deiodination	Propylthiouracil, amiodarone, oral cholecystographic agents
Accelerated hepatic metabolism	Phenobarbital, rifampin, carbamazepine, phenytoin, sertraline, bexarotene

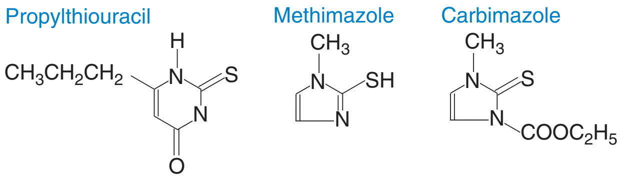


Figure 47–7 *Structures of antithyroid drugs of the thioamide type.*

Adjuvant therapy with drugs that have no specific effects on thyroid hormone synthesis is useful in controlling the peripheral manifestations of thyrotoxicosis, including inhibitors of the peripheral deiodination of T_4 to T_3, β adrenergic receptor antagonists, and Ca^{2+} channel blockers.

Antithyroid Drugs

The antithyroid drugs with clinical utility are the *thioureylenes*, which belong to the family of thioamides. *Propylthiouracil* is the prototype (Figure 47–7).

HISTORICAL PERSPECTIVE

Studies on the mechanism of the development of goiter began with the observation that rabbits fed a diet composed largely of cabbage often developed goiters. This result was probably due to the presence of precursors of the thiocyanate ion in cabbage leaves. Later, two pure compounds were shown to produce goiter: *sulfaguanidine*, a sulfanilamide antimicrobial used to treat enteric infections, and *phenylthiourea*. Investigation of the effects of thiourea derivatives revealed that rats became hypothyroid despite hyperplastic changes in their thyroid glands that were characteristic of intense thyrotropic stimulation. After treatment was begun, no new hormone was made, and the goitrogen had no visible effect on the thyroid gland following hypophysectomy or the administration of thyroid hormone. This suggested that the goiter was a compensatory change resulting from the induced state of hypothyroidism and that the primary action of the compounds was to inhibit the formation of thyroid hormone. The therapeutic possibilities of such agents in hyperthyroidism were evident, and the substances so used became known as *antithyroid drugs*.

Mechanism of Action

Antithyroid drugs inhibit the formation of thyroid hormones by interfering with the incorporation of iodine into tyrosyl residues of thyroglobulin; they also inhibit the coupling of these iodotyrosyl residues to form iodothyronines (see Figure 47–2). These drugs are thought to inhibit the peroxidase enzyme. Inhibition of hormone synthesis results in the depletion of stores of iodinated thyroglobulin as the protein is hydrolyzed and the hormones are released into the circulation. In addition to blocking hormone synthesis, *propylthiouracil* partially inhibits the peripheral deiodination of T_4 to T_3. *Methimazole* does not have this effect; this provides a rationale for the choice of *propylthiouracil* over other antithyroid drugs in the treatment of severe hyperthyroid states or of thyroid storm (Angell et al., 2015).

ADME

The antithyroid compounds currently used in the U.S. are *methimazole* (1-methyl-2-mercaptoimidazole) and, for limited indications, *propylthiouracil* (6-*n*-propylthiouracil). In Europe, *carbimazole*, a carbethoxy derivative of *methimazole*, is available, and its antithyroid action is due to its conversion to *methimazole* after absorption (see Figure 47–7). Pharmacological properties of *methimazole* and *propylthiouracil* are shown in Table 47–5.

A small dose of *methimazole*, 0.5 mg, decreases the organification of iodine in the thyroid gland, but a single dose of 10 to 25 mg is needed to extend the inhibition to 24 h. Absorption of effective amounts of *propylthiouracil* occurs within 20 to 30 min of an oral dose; the duration of action is brief. The effect of a dose of 100 mg of *propylthiouracil* begins to wane in 2 to 3 h; even a 500-mg dose is completely inhibitory for only 6 to 8 h.

TABLE 47–5 ■ PHARMACOKINETIC FEATURES OF ANTITHYROID DRUGS

	PROPYLTHIOURACIL	METHIMAZOLE
Plasma protein binding	~75%	Nil
Plasma $t_{1/2}$	75 min	~4–6 h
Volume of distribution	~0.4 L/kg	~0.7 L/kg
Concentrated in thyroid	Yes	Yes
Metabolism of drug during illness		
Severe liver disease	Normal	Decreased
Severe kidney disease	Normal	Normal
Dosing frequency	1–4 times daily	Once or twice daily
Transplacental passage	Low	Low
Levels in breast milk	Low	Low

The plasma $t_{1/2}$ of *methimazole* is 4 to 6 h; the $t_{1/2}$ of *propylthiouracil* is about 75 min. The drugs are concentrated in the thyroid, and *methimazole*, derived from the metabolism of *carbimazole*, accumulates after *carbimazole* is administered. Drugs and metabolites appear largely in the urine.

Therapeutic Uses

The antithyroid drugs are used in the treatment of hyperthyroidism in the following ways:

- As definitive treatment to control the disorder in anticipation of a spontaneous remission in Graves disease, or as long-term therapy in patients who do not undergo spontaneous remission and prefer medication rather than *radioiodine* treatment or surgery
- In conjunction with radioactive iodine, to hasten recovery while awaiting the effects of radiation
- To control the disorder in preparation for surgical treatment

Methimazole is the drug of choice for Graves disease; it is effective when given as a single daily dose, has improved adherence, and is less toxic than *propylthiouracil*, especially having a reduced risk of the very rare but devastating complication of liver failure. *Methimazole* has a relatively long plasma and intrathyroidal $t_{1/2}$, as well as a long duration of action. The usual starting dose for *methimazole* is 15 to 40 mg/day. The usual starting dose of *propylthiouracil* is 100 mg every 8 h. When doses greater than 300 mg daily are needed, further subdivision of the time of administration to every 4 to 6 h is occasionally helpful. Once euthyroidism is achieved, usually within 12 weeks, the dose of antithyroid drug can be reduced, but not stopped, lest an exacerbation of Graves disease occur.

Response to Treatment

The thyrotoxic state usually improves within 3 to 6 weeks after the initiation of antithyroid drugs. The clinical response is related to the dose of antithyroid drug, the size of the goiter, and pretreatment serum T_3 concentration. The rate of response is determined by the quantity of stored hormone, the rate of turnover of hormone in the thyroid, the $t_{1/2}$ of the hormone in the periphery, and the completeness of the block in synthesis imposed by the dosage given. Hypothyroidism may develop as a result of overtreatment. After treatment is initiated, patients should be examined and thyroid function tests (serum free T_4 and total or free T_3 concentrations) measured every 2 to 4 months. Once euthyroidism is established, follow-up every 4 to 6 months is reasonable. Control of the hyperthyroidism is usually associated with a decrease in goiter size and normalization of serum TSH concentration. When this occurs, the dose of the antithyroid drug should be significantly decreased to avoid hypothyroidism.

Untoward Reactions

The incidence of side effects from *propylthiouracil* and *methimazole* as currently used is relatively low. Fulminant hepatic necrosis is an exceedingly rare but potentially devastating complication of therapy with *propylthiouracil* that can result in death or the need for liver transplantation. It is estimated to occur in 1 in 10,000 adults or 1 in 2000 children taking this drug. Agranulocytosis is a serious reaction that occurs in 0.1% to 0.5% of patients taking either *propylthiouracil* or *methimazole*, usually in the first few weeks or months of therapy but sometimes later. Because agranulocytosis usually occurs rapidly and is not associated with a gradual reduction in granulocyte count, periodic prospective monitoring of granulocyte count is not generally helpful. Patients should be instructed to immediately report the development of sore throat or fever and should discontinue their antithyroid drug and obtain a granulocyte count. Agranulocytosis is reversible on discontinuation of the offending drug, and the administration of recombinant human granulocyte colony-stimulating factor may hasten recovery. Mild granulocytopenia, if noted, may be due to thyrotoxicosis or may be the first sign of this dangerous drug reaction; frequent leukocyte counts are then required.

The most common reaction is a mild urticarial papular rash that often subsides spontaneously without interrupting treatment but sometimes requires administration of an antihistamine and corticosteroids and changing to another antithyroid drug. Other less frequent complications are pain and stiffness in the joints, paresthesias, headache, nausea, skin pigmentation, and loss of hair. Drug fever, hepatitis, and nephritis are rare, although abnormal liver function tests are not infrequent with higher doses of *propylthiouracil*. Although vasculitis was previously thought to be a rare complication, antineutrophilic cytoplasmic antibodies have been reported to occur in about 50% of patients receiving *propylthiouracil* and rarely with *methimazole*. Hypoprothrombinemia and bleeding have also been reported with *methimazole* treatment.

Thyrotoxicosis in Pregnancy

Thyrotoxicosis occurs in about 0.2% of pregnancies and is caused most frequently by Graves disease. Antithyroid drugs are the treatment of choice; radioactive iodine is clearly contraindicated. Both *propylthiouracil* and *methimazole* cross the placenta equally, and both have been used safely in the pregnant patient. *Methimazole* is usually avoided in the first trimester in favor of *propylthiouracil* due to *methimazole*-associated embryopathy, and then *methimazole* is used for the remainder of the pregnancy due to the concern for *propylthiouracil*-associated liver failure in pregnancy. The more recent recognition of *propylthiouracil*-associated embryopathy from exposure in the first trimester, especially those defects that are not apparent at birth and detected later, such as in the urinary collecting system, has challenged this approach. Guidelines now recommend limiting use of antithyroid drugs as much as possible in the first trimester (Alexander et al., 2017). The antithyroid drug dosage should be minimized to keep the serum free T_4 index in the upper half of the normal range or slightly elevated. As pregnancy progresses, Graves disease often improves. Relapse or worsening of Graves disease is common after delivery, and patients should be monitored closely. *Methimazole* in nursing mothers, up to 20 mg daily, reportedly has no effect on thyroid function in the infant; *propylthiouracil* is thought to partition into breast milk even less than *methimazole*.

Adjuvant Therapy

Several drugs that have no intrinsic antithyroid activity are useful in the symptomatic treatment of thyrotoxicosis.

The *β adrenergic receptor antagonists* (see Chapter 14) are effective in antagonizing the sympathetic/adrenergic effects of thyrotoxicosis—thereby reducing the tachycardia, tremor, and stare—and relieving palpitations, anxiety, and tension. Either *propranolol*, 20 to 40 mg four times daily, or *atenolol*, 50 to 100 mg daily, is usually given initially.

The *Ca^{2+} channel blockers* (*diltiazem*, 60–120 mg four times daily) can be used to control tachycardia and decrease the incidence of supraventricular tachyarrhythmias. Usually, only short-term treatment with β adrenergic receptor antagonists or Ca^{2+} channel blockers is required (2–6 weeks), and it should be discontinued once the patient is euthyroid.

Immunotherapy has been used for Graves hyperthyroidism and ophthalmopathy. The B-lymphocyte–depleting agent *rituximab*, when used with *methimazole*, prolongs remission of Graves disease. The IGF-1 receptor blocker *teprotumumab* has shown remarkable effects in patients with advanced ophthalmopathy, with almost 80% of treated patients showing improvement (see Chapter 74).

Thyroid Storm

Thyroid storm is an uncommon but life-threatening complication of thyrotoxicosis in which a severe form of the disease is usually precipitated by an intercurrent medical problem. It occurs in untreated or partially treated thyrotoxic patients. Precipitating factors associated with thyrotoxic crisis include infections, stress, trauma, thyroidal or nonthyroidal surgery, diabetic ketoacidosis, labor, heart disease, and, rarely, radioactive iodine treatment.

Clinical features are similar to those of thyrotoxicosis but more exaggerated. Cardinal features include fever (temperature usually >38.5°C) and tachycardia out of proportion to the fever. Nausea, vomiting, diarrhea, agitation, and confusion are frequent presentations. Coma and death may ensue in up to 20% of patients. Thyroid function abnormalities are similar to those found in uncomplicated hyperthyroidism. Therefore, thyroid storm is primarily a clinical diagnosis.

Treatment includes supportive measures such as intravenous fluids, antipyretics, cooling blankets, and sedation. Antithyroid drugs are given in large doses. *Propylthiouracil* is preferred over *methimazole* because it also inhibits Dio1, thus impairing peripheral conversion of T_4 to T_3. Oral iodides are used after the first dose of an antithyroid drug has been administered. Treatment of the underlying precipitating illness is essential.

Ionic Inhibitors

The *ionic inhibitors* are substances that interfere with the concentration of iodide by the thyroid gland. These agents are anions that resemble iodide: *thiocyanate*, *perchlorate*, and *fluoroborate*, all monovalent hydrated anions of a size similar to that of iodide.

Thiocyanate differs from the rest qualitatively; it is not concentrated by the thyroid gland but in large amounts may inhibit the organification of iodine. *Perchlorate* is 10 times as active as *thiocyanate*. *Perchlorate* (ClO_4^-) blocks the entrance of iodide into the thyroid by competitively inhibiting the NIS and itself can be transported by NIS into the thyroid gland. The various NIS inhibitors (*perchlorate*, *thiocyanate*, and *nitrate*) are additive in inhibiting iodine uptake. *Perchlorate* can be used to control hyperthyroidism; however, when given in excessive amounts (2–3 g daily), it has caused fatal aplastic anemia. *Perchlorate* in doses of 750 mg daily has been used in the treatment of Graves disease, although it is not available in North America.

Perchlorate can be used to "discharge" inorganic *iodide* from the thyroid gland in a diagnostic test of iodide organification. Other ions, selected on the basis of their size, also have been found to be active; *fluoroborate* (BF_4^-) is as effective as *perchlorate*.

Lithium decreases secretion of T_4 and T_3, which can cause overt hypothyroidism in some patients taking Li^+ for the treatment of mania (see Chapter 19).

Iodine

Iodide is the oldest remedy for disorders of the thyroid gland. In high concentration, *iodide* can influence several of the important functions of the thyroid gland. *Iodide* limits its own transport and acutely and transiently inhibits the synthesis of iodotyrosines and iodothyronines (the *Wolff-Chaikoff effect*) (Pramyothin et al., 2011). An important clinical effect of high $[I^-]_{plasma}$ is inhibition of the release of thyroid hormone. This action is rapid and efficacious in severe thyrotoxicosis. The effect is exerted directly on the thyroid gland and can be demonstrated in the euthyroid subject as well as in the hyperthyroid patient.

Response to Iodine in Hyperthyroidism

The response to iodine in patients with hyperthyroidism is often striking and rapid: Release of thyroid hormone into the circulation is rapidly blocked, and its synthesis is mildly decreased. In the thyroid gland, vascularity is reduced, the gland becomes much firmer, the cells become smaller, and colloid reaccumulates in the follicles as iodine concentration increases. The maximal effect occurs after 10 to 15 days of continuous therapy. *Iodide* therapy usually does not completely control the manifestations of hyperthyroidism, and the beneficial effect disappears. The uses of *iodide* in the treatment of hyperthyroidism are in the preoperative period in preparation for thyroidectomy and, in conjunction with antithyroid drugs and *propranolol*, in the treatment of thyrotoxic crisis.

Another use of *iodide* is to protect the thyroid from radioactive iodine fallout following a nuclear accident or military exposure. Because the uptake of radioactive iodine is inversely proportional to the serum concentration of stable iodine, the administration of 30 to 100 mg of iodine daily will markedly decrease the thyroid uptake of radioisotopes. Strong iodine solution (Lugol solution) consists of 5% iodine and 10% potassium iodide, yielding a dose of about 8 mg of iodine per drop. *Potassium iodide saturated solution (KISS)* also is available, containing 50 mg per drop. Typical doses include 16 to 36 mg (2–6 drops) of Lugol solution or 50 to 100 mg (1–2 drops) of KISS three times a day. Potassium iodide products (Thyroshield, various generics) are available over the counter to take in the event of a radiation emergency and block the uptake of *radioiodine* into the thyroid gland. The adult dose is 2 mL (130 mg) every 24 h, as directed by public health officials.

Euthyroid patients with a history of a wide variety of underlying thyroid disorders may develop iodine-induced hypothyroidism when exposed to large amounts of iodine present in many commonly prescribed drugs (Table 47–6), and these patients do not escape from the acute Wolff-Chaikoff effect (Pramyothin et al., 2011).

Untoward Reactions

Occasional individuals show a marked sensitivity to iodine. Angioedema is the prominent symptom, and laryngeal edema may lead to suffocation. Multiple cutaneous hemorrhages may be present; manifestations of the serum sickness type of hypersensitivity (e.g., fever, arthralgia, lymph node enlargement, and eosinophilia) may appear. Thrombotic thrombocytopenic purpura and fatal periarteritis nodosa attributed to hypersensitivity to *iodide* also have been described.

The severity of symptoms of chronic intoxication with *iodide* (*iodism*) is related to the dose. The symptoms start with an unpleasant brassy taste and burning in the mouth and throat as well as soreness of the teeth and gums. Increased salivation, coryza, sneezing, and irritation of the eyes with swelling of the eyelids commonly occur. Mild iodism simulates a "head cold." Excess transudation into the bronchial tree may lead to pulmonary edema. In addition, the parotid and submaxillary glands may become enlarged and tender, and the syndrome may be mistaken for mumps parotitis. Skin lesions are common and vary in type and intensity. Rarely, severe and sometimes fatal eruptions (ioderma) may occur after the prolonged use of *iodides*. The lesions are bizarre; they resemble those caused by bromism and generally involute quickly when *iodide* is withdrawn. Symptoms of gastric irritation are common, and diarrhea, which is sometimes bloody, may occur. Fever, anorexia, and depression may be present. The symptoms of iodism disappear within a few days after stopping the administration of *iodide*. Renal excretion of I^- can be increased by procedures that promote Cl^- excretion (e.g., osmotic diuresis, chloruretic diuretics, and salt loading). These procedures may be useful when the symptoms of iodism are severe.

TABLE 47–6 ■ IODIDE CONTENT OF COMMONLY USED DRUGS AND COMPOUNDS

DRUGS	IODINE CONTENT
Oral or local	
Amiodarone	75 mg/200 mg tablet
Iodoquinol (diiodohydroxyquin)	134 mg/tablet
Echothiophate iodide ophthalmic solution	5–41 µg/drop
Iodoquinol	134 mg/tablet
Idoxuridine ophthalmic solution	18 µg/drop
Lugol solution	5–6 mg/drop
KI, saturated solution (KISS)	38 mg/drop
Topical antiseptics	
Clioquinol cream	12 mg/g
Povidone-iodine	10 mg/mL
Radiographic contrast agents	
Diatrizoate meglumine sodium	370 mg/mL
Iothalamate	320 mg/mL
Ioxaglate	370 mg/mL
Iopamidol	370 mg/mL
Iohexol	350 mg/mL
Ioxilan	370 mg/mL

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Radioactive Iodine

The primary isotopes used for the diagnosis and treatment of thyroid disease are ^{123}I and ^{131}I. ^{123}I is primarily a short-lived γ-emitter with a $t_{1/2}$ of 13 h and is used in diagnostic studies. ^{124}I has been used successfully with positron emission tomographic/computed tomographic scanning for more precise dosimetry in high-risk thyroid cancer (Jentzen et al., 2014). ^{131}I has a $t_{1/2}$ of 8 days and emits both γ rays and β particles. More than 99% of its radiation is expended within 56 days. ^{131}I is used therapeutically for thyroid destruction of an overactive or enlarged thyroid and in thyroid cancer for thyroid ablation and treatment of metastatic disease.

The chemical behavior of the radioactive isotopes of iodine is identical to that of the stable isotope, ^{127}I. ^{131}I is rapidly and efficiently trapped by the thyroid, incorporated into the iodoamino acids, and deposited in the colloid of the follicles, from which it is slowly liberated. Thus, the destructive β particles originate within the follicle and act almost exclusively on the parenchymal cells of the thyroid, with little or no damage to surrounding tissue. The γ radiation passes through the tissue and can be quantified by external detection. The effects of the radiation depend on the dosage. With properly selected doses of ^{131}I, it is possible to destroy the thyroid gland completely without detectable injury to adjacent tissues.

Therapeutic Uses

Radioactive iodine finds its widest use in the treatment of hyperthyroidism and in the diagnosis of disorders of thyroid function. The clearest indication for radioactive iodine treatment is hyperthyroidism in older patients and in those with heart disease. Radioactive iodine also is an effective treatment when Graves disease has persisted or recurred after subtotal thyroidectomy and when prolonged treatment with antithyroid drugs has not led to remission. Finally, radioactive iodine is effective in patients with toxic nodular goiter. Sodium iodide ^{131}I is provided as capsules containing carrier-free ^{131}I suitable for oral administration. Sodium iodide ^{123}I is available for scanning procedures.

Hyperthyroidism

Radioactive iodine is a valuable alternative or adjunctive treatment of hyperthyroidism (Ross, 2011). Stable *iodide* (nonradioactive) may preclude treatment and imaging with radioactive iodine for weeks after the stable *iodide* has been discontinued. In patients exposed to stable *iodide*, a 24-h *radioiodine* measurement of a tracer dose of ^{123}I should be performed before ^{131}I administration to ensure there is sufficient uptake to accomplish the desired ablation. The optimal dose of ^{131}I, expressed as the amount taken up, varies in different laboratories from 80 to 150 µCi per gram of thyroid tissue. The usual total dose is 4 to 15 mCi with a recommended target of delivering 8 mCi to the thyroid gland based on the 24-h *radioiodine* uptake (Alexander and Larsen, 2002; Brent, 2008).

Beginning a few weeks after treatment, the symptoms of hyperthyroidism gradually abate over a period of 2 to 3 months. If therapy has been inadequate, the necessity for further treatment is apparent within 6 to 12 months. It is not uncommon, however, for the serum TSH to remain low for several months after ^{131}I therapy. Thus, assessing radioactive iodine failure based on TSH concentrations alone may be misleading and should always be accompanied by determination of free T_4 and usually serum T_3 concentrations. Depending to some extent on the dosage schedule adopted, 80% of patients are cured by a single dose, about 20% require two doses, and a very small fraction require three or more doses before the disorder is controlled. β Adrenergic antagonists, antithyroid drugs, or both can be used to hasten the control of hyperthyroidism.

Advantages

With radioactive iodine treatment, the patient is spared the risks and discomfort of surgery. The cost is low, hospitalization is not required in the U.S., and patients can participate in their customary activities during the entire procedure, although there are recommendations to limit exposure in young children.

Disadvantages

The chief consequence of the use of radioactive iodine is the high incidence of delayed hypothyroidism. Although cancer death rate is not increased after *radioiodine* therapy, some studies suggest a small but significant increase in specific types of cancer, including stomach, kidney, and breast. This finding is especially significant because these tissues all express the iodine transporter NIS and may thus be especially susceptible to effects of radioactive iodine. Radioactive iodine treatment can induce a radiation thyroiditis, with release of preformed T_4 and T_3 into the circulation. In most patients, this is asymptomatic, but in some, there can be worsening of symptoms of hyperthyroidism; rarely, cardiac manifestations (e.g., atrial fibrillation or ischemic heart disease); and very rarely, thyroid storm. Pretreatment with antithyroid drugs should reduce or eliminate this complication.

The main contraindication for the use of ^{131}*I therapy is pregnancy*. After the first trimester, the fetal thyroid will concentrate the isotope and thus suffer damage; even during the first trimester, radioactive iodine is best avoided because there may be adverse effects of radiation on fetal tissues. In addition, the use of *radioiodine* to treat hyperthyroidism in children is controversial due to theoretical concern about causing neoplastic changes in the thyroid gland or other organs. Data are insufficient to resolve this issue, as the number of children who have been treated with *radioiodine* is relatively small. Many clinics decline to treat younger patients and reserve radioactive iodine for patients older than 25 to 30 years.

Thyroid Carcinoma

Because most well-differentiated thyroid carcinomas accumulate very little iodine, stimulation of iodine uptake with TSH is required to treat metastases effectively (Haugen et al., 2016; Haugen and Sherman, 2013). Endogenous TSH stimulation is promoted by withdrawal of thyroid hormone replacement therapy in patients previously treated with near-total or total thyroidectomy. A typical protocol, due to the *levothyroxine* $t_{1/2}$ of approximately 7 days, is to withhold therapy for about 4 weeks to achieve sufficient stimulation of endogenous TSH, usually greater than 30 mIU/L. *Liothyronine*, which has a much shorter $t_{1/2}$, can be prescribed during the first half of *levothyroxine* withdrawal to minimize symptoms of hypothyroidism. An ablative dose of ^{131}I ranging from 30 to 150 mCi or more is administered, and a repeat total-body scan is obtained several days to 1 week later.

Recombinant thyrotropin alpha (recombinant human TSH) can be used instead of thyroid hormone withdrawal to prepare a patient for *radioiodine* ablation of thyroid remnant tissue or to test the capacity of thyroid tissue, both normal and malignant, to take up radioactive iodine and to secrete thyroglobulin.

Chemotherapy of Thyroid Cancer

This field is rapidly evolving as new therapies directed at thyroid cancer driver genes are developed and as increased experience helps refine best practices for the use of these agents (Cabanillas et al., 2019). The current thyroid cancer chemotherapies are kinase inhibitors, which are discussed in greater detail in Chapter 71. It is important to note that *levothyroxine* dosage requirements often increase in patients taking protein tyrosine kinase inhibitors; therefore, TSH levels should be monitored carefully.

Papillary and Follicular Carcinomas

The majority of thyroid cancers derive from the thyroid follicular cells and are classified histologically as papillary or follicular carcinomas. Most of these carcinomas are adequately treated by surgery, *radioiodine*, and *levothyroxine* to suppress TSH. However, a small fraction progress despite these therapies, in which case systemic therapy with kinase inhibitors may be appropriate.

The choice of systemic therapies ideally is guided by somatic mutation testing of the primary tumor or a metastasis. Patients with tumors that contain *NTRK* or *RET* driver mutations (fusion genes with a variety of partners) can be treated with specific small-molecule inhibitors such as *larotrectinib* or *entrectinib* for *NTRK* fusions or *selpercatinib* or *pralsetinib* for *RET* fusions. However, these driver mutations are relatively uncommon in thyroid cancer.

In the absence of *NTRK* or *RET* driver mutations, therapy can be given with a multikinase inhibitor such as *lenvatinib* or *sorafenib*. Although these drugs are relatively promiscuous, vascular endothelial growth factor receptors are an important target. These antiangiogenic multikinase inhibitors can prolong progression-free survival, but complete responses are rare. There are no head-to-head trials comparing various multikinase inhibitors, but *lenvatinib* is commonly considered first line due to the relative strength of its randomized trial outcomes data.

The most frequent driver mutation in papillary thyroid cancer is *BRAF* V600E. *Vemurafenib* and *dabrafenib* specifically inhibit this kinase and have efficacy in *BRAF*-mutated papillary thyroid cancer, but available data do not suggest a clear benefit over *lenvatinib*. Several papillary and follicular cancer driver mutations (including *BRAF* V600E, *NTRK* and *RET* fusions, and *RAS* mutations) activate the MAP kinase pathway. Treatment of these cancers for several weeks with *dabrafenib* or *vemurafenib* (if *BRAF* V600E is present) and/or a MEK inhibitor such as *trametinib* or *selumetinib* has the potential to restore *radioiodine* uptake and facilitate *radioiodine* therapy in some of these tumors (redifferentiation therapy). The situations in which this therapy is most likely to be of benefit have yet to be defined.

Anaplastic Thyroid Cancer

Anaplastic thyroid carcinomas are undifferentiated tumors derived from thyroid follicular cells. This disease is highly aggressive with an average life expectancy of only approximately 6 months from the time of diagnosis. If feasible, local disease may be treated with surgery and external beam radiotherapy. Ideally, the approach to chemotherapy should be informed by tumor mutation profiling. The combination of *dabrafenib* plus *trametinib* is FDA-approved for anaplastic carcinomas that harbor *BRAF* V600E.

Medullary Thyroid Carcinoma

A minor fraction of thyroid cancers originates from the parafollicular cells that produce calcitonin. These tumors, denoted MTCs, can occur sporadically or as part of the autosomal dominant multiple endocrine neoplasia type 2 syndrome. Germline mutations in *RET* underlie nearly all inherited medullary carcinomas, and somatic *RET* mutations are common in sporadic MTCs. Because they derive from parafollicular cells, MTCs are not responsive to *radioiodine* or TSH suppression. The first-line therapy for MTC is surgery. RET-driven MTCs that progress despite surgery can be treated with a selective RET kinase inhibitor (*selpercatinib* or *pralsetinib*). These agents appear to have better safety profiles than multikinase inhibitors and may be more efficacious. However, the multikinase inhibitors *vandetanib* and *cabozantinib* also have efficacy and can be prescribed without regard for *RET* gene mutational status.

Drug Facts for Your Personal Formulary: *Thyroid and Antithyroid Drugs*

Drug	Therapeutic Uses	Clinical Pharmacology and Tips
Thyroid Hormone Preparations: Replace T_4 or T_3 normally produced by the thyroid		
Levothyroxine (T_4)	• Hypothyroidism • TSH suppression in thyroid cancer	• Plasma $t_{1/2}$ ~1 week • Deiodinases convert circulating T_4 to the bioactive hormone T_3 • Dosage generally needs to increase during pregnancy • Congenital hypothyroidism requires rapid diagnosis and correction to allow normal physical and mental development • Overtreatment can lead to osteoporosis and atrial fibrillation
Liothyronine (T_3)	• When rapid onset of action is desired (sometimes for myxedema coma) • When rapid termination of action is desired (preparing patients with thyroid cancer for radioiodine therapy)	• Plasma $t_{1/2}$ ~18–24 h • Multiple daily doses needed to achieve needed C_{Pss} • Levothyroxine generally preferred over liothyronine for the long-term therapy of hypothyroidism
Desiccated thyroid and T_4–T_3 mixtures	• Generally not a preferred therapy, although occasional hypothyroid patients say they feel better than when taking levothyroxine	• Mixture of levothyroxine and liothyronine (~4:1 by weight) • Supplies a relative excess of T_3 compared to normal thyroidal secretion, which is ~11:1 T_4 to T_3 by weight • No convincing evidence of greater efficacy than levothyroxine alone
Antithyroid Drugs: Thionamides: Interfere with incorporation of iodine into tyrosyl residues and inhibit iodotyrosyl-coupling reactions		
Methimazole	• Reduce thyroid hormone production	• Carbimazole (available in Europe) converted to methimazole after absorption • Long intrathyroidal $t_{1/2}$ allows once-daily dosing for most patients • Preferred antithyroid drug • Do not use in first trimester of pregnancy due to embryopathy
Propylthiouracil	• Reduce thyroid hormone production • At high doses, reduces T_4 to T_3 conversion	• Major concern is liver toxicity; rare but more commonly seen in children and pregnancy • Main indications are for thyroid storm due to action on reducing T_4 to T_3 conversion and in the first trimester of pregnancy, although it has been associated with congenital defects that are not initially detected at birth
Antithyroid Drugs: Ionic Inhibitors: Inhibit iodine uptake by antagonizing the NIS		
Perchlorate	• Primarily used to enhance the response to thioamides in refractory Graves disease (e.g., that associated with amiodarone)	• Not available commercially; must be specialty compounded
Antithyroid Drugs: Iodide: Acute reduction in thyroid hormone		
Lugol solution	• Acutely reduces the secretion and synthesis of thyroid hormone	• "Escape" from thyroid inhibition after 7–10 days • Strictly contraindicated in pregnancy
KISS: potassium iodide saturated solution (or SSKI)	• Acutely reduces the secretion and synthesis of thyroid hormone	• "Escape" from thyroid inhibition after 7–10 days • Strictly contraindicated in pregnancy
Antithyroid Drugs: Radioactive Iodine: Used to destroy hyperfunctioning thyroid tissue		
^{131}I	• Effective for permanent treatment of Graves disease and toxic nodule or toxic goiter • Destruction of iodide-avid thyroid cancer	• Highly effective for permanent cure to hyperthyroidism • Effective treatment of hyperthyroidism usually results in permanent hypothyroidism and lifelong requirement for levothyroxine replacement • Absolutely contraindicated in pregnancy • Treatment of thyroid cancer requires TSH stimulation (endogenous or exogenous)
Recombinant Human TSH Agonist for the TSH Receptor		
Thyrotropin alpha	• Stimulates radioiodine uptake and thyroglobulin release in patients with thyroid cancer after thyroidectomy • Prepares patients for radioiodine ablation of thyroid remnants after thyroidectomy for thyroid cancer	• Allows assessment of residual or recurrent thyroid cancer without stopping levothyroxine and becoming clinically hypothyroid • Allows radioiodine therapy of thyroid remnants without stopping levothyroxine and becoming clinically hypothyroid
Thyroid Cancer Chemotherapeutics: TRK Inhibitors: Used when surgery, ^{131}I, TSH suppression, and external beam radiotherapy are inadequate		
Larotrectinib Entrectinib	• Systemic therapy of follicular cell–derived thyroid cancers with *NTRK* fusion gene driver mutations	• *NTRK* fusion genes are uncommon in thyroid cancer • Limited data suggest better tolerated and more efficacious than multikinase inhibitors when an *NTRK* fusion gene is present • See Chapter 71 for a more general discussion of these drugs

Drug Facts for Your Personal Formulary: *Thyroid and Antithyroid Drugs* (*continued*)

Drug	Therapeutic Uses	Clinical Pharmacology and Tips
Thyroid Cancer Chemotherapeutics: RET Inhibitors: Used when surgery, ^{131}I, TSH suppression, and external beam radiotherapy are inadequate		
Selpercatinib Pralsetinib	• Systemic therapy of thyroid cancers with *RET* driver mutations (papillary cancers with *RET* fusion genes and medullary cancers with *RET* point mutations)	• *RET* driver mutations (fusion genes) are uncommon in papillary thyroid cancer • *RET* driver mutations (point mutations) are common in sporadic medullary cancer and nearly universal in inherited medullary cancers (multiple endocrine neoplasia type 2) • Since medullary cancers are not derived from follicular cells, ^{131}I and TSH suppression are ineffective • Limited data suggest better tolerated and more efficacious than multikinase inhibitors when a *RET* driver mutation is present • See Chapter 71 for a more general discussion of these drugs
Thyroid Cancer Chemotherapeutics: *BRAF* V600E Inhibitors: Used when surgery, ^{131}I, TSH suppression, and external beam radiotherapy are inadequate		
Vemurafenib Dabrafenib	• Systemic therapy of thyroid cancers with *BRAF* V600E driver mutation • May be useful as redifferentiation therapy to induce radioiodine uptake in non–iodine-avid thyroid cancers with *BRAF* V600E	• Although *BRAF* V600E is the most common driver mutation in papillary cancer, BRAF inhibitors have not been shown to be clearly superior to multikinase inhibitors • The role of redifferentiation therapy is uncertain • See Chapter 71 for a more general discussion of these drugs
Thyroid Cancer Chemotherapeutics: Multikinase Inhibitors: Used when surgery, ^{131}I, TSH suppression, and external beam radiotherapy are inadequate		
Lenvatinib Sorafenib	• Systemic therapy of follicular cell–derived thyroid cancers without regard to driver mutation status	• Inhibit multiple kinases including vascular endothelial growth factor receptors • Although there are no head-to-head trials, existing data suggest lenvatinib is more efficacious • Toxicities are common and can limit drug dosage or usage • See Chapter 71 for a more general discussion of these drugs
Vandetanib Cabozantinib	• Systemic therapy of medullary thyroid cancer without regard to driver mutation status	• Inhibit multiple kinases including vascular endothelial growth factor receptors and RET • Since medullary cancers are not derived from follicular cells, ^{131}I and TSH suppression are ineffective • See Chapter 71 for a more general discussion of these drugs
Thyroid Cancer Chemotherapeutics: MEK Inhibitors		
Trametinib Selumetinib	• Trametinib is FDA-approved in combination with dabrafenib to treat anaplastic cancers containing *BRAF* V600E • May be useful as redifferentiation therapy (with or without BRAF inhibition) to induce radioiodine uptake in non–iodine-avid thyroid cancers	• The role of redifferentiation therapy and the effectiveness of MEK inhibitors are uncertain • See Chapter 71 for a more general discussion of these drugs

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Chapter 48

Estrogens, Progestins, and the Female Reproductive Tract

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ESTROGENS
- Chemistry and Synthesis
- Physiological Actions
- Estrogen Receptors
- Pharmacology

SELECTIVE ESTROGEN RECEPTOR MODULATORS AND ANTIESTROGENS
- Selective Estrogen Receptor Modulators: Tamoxifen, Raloxifene, and Toremifene
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- Estrogen Synthesis Inhibitors

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ANTIPROGESTINS AND PROGESTERONE RECEPTOR MODULATORS
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THERAPEUTIC USES OF ESTROGENS AND PROGESTINS
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DRUG THERAPY IN ENDOMETRIOSIS, HIRSUTISM, GENDER TRANSITION, AND HYPOACTIVE SEXUAL DESIRE DISORDER
- Endometriosis
- Hirsutism
- Gender Transition
- Hypoactive Sexual Desire Disorder

Estrogens and *progestins* are endogenous hormones that produce numerous physiological actions. In women, these include developmental effects, neuroendocrine actions involved in the control of ovulation, the cyclical preparation of the reproductive tract for fertilization and implantation, and major actions on mineral, carbohydrate, protein, and lipid metabolism. Estrogens also have important actions in males, including effects on bone, spermatogenesis, and behavior. Well-characterized receptors for each hormone mediate biological actions in both the unliganded and the liganded states.

The most common uses of estrogens and progestins are for contraception and menopausal hormone therapy (MHT) in women, but the specific compounds and dosages used in these two settings differ substantially. Antiestrogens are used in the treatment of hormone-responsive breast cancer and infertility. Selective estrogen receptor modulators (SERMs) that display tissue-selective agonist or antagonist activities are useful to prevent breast cancer and osteoporosis. The main use of antiprogestins has been for medical abortion.

Several naturally occurring and synthetic environmental chemicals mimic, antagonize, or otherwise affect the actions of estrogens in experimental test systems. The precise effect of these agents on humans is unknown but is an area of active investigation.

Estrogens

Chemistry and Synthesis

Chemistry

Many steroidal and nonsteroidal compounds, some of which are shown in Table 48–1 and Figure 48–1, possess estrogenic activity. Estrogens interact with two receptors of the nuclear receptor superfamily, termed ERα and ERβ. The most potent naturally occurring estrogen in humans, for both ERα- and ERβ-mediated actions, is *17β-estradiol*, followed by *estrone* and *estriol*. Each contains a phenolic A ring with a hydroxyl group at carbon 3 and a β-OH or ketone in position 17 of ring D.

The phenolic A ring is the principal structural feature responsible for selective high-affinity binding to both receptors. Most alkyl substitutions on the A ring impair binding, but substitutions on ring C or D may be tolerated. Ethinyl substitutions at the C17 position greatly increase oral potency by inhibiting first-pass hepatic metabolism. Models for the ligand-binding sites of both ERs have been determined from structure-activity relationships and structural analysis (Pike et al., 2000). Selective ligands for ERα and ERβ are available for experimental studies but are not yet used therapeutically (Harrington et al., 2003).

Biosynthesis

Steroidal estrogens arise from androstenedione or testosterone (Figure 48–1) by aromatization of the A ring. The reaction is catalyzed by aromatase (CYP19), which uses NADPH and molecular oxygen as cosubstrates. A ubiquitous flavoprotein, NADPH–cytochrome P450 reductase, also is essential. Both proteins are localized in the endoplasmic reticulum of ovarian granulosa cells, testicular Sertoli and Leydig cells, adipose stroma, placental syncytiotrophoblasts, preimplantation blastocysts, bone, various brain regions, and many other tissues (Simpson et al., 2002).

The ovaries are the principal source of circulating estrogen in premenopausal women, with estradiol the main secretory product. Ovarian estradiol production is traditionally thought to require two cell types: theca cells and granulosa cells. The gonadotropin luteinizing hormone (LH) acts via receptors that couple to the G_s-adenylyl cyclase–cyclic AMP

Abbreviations

AF: activation function
CEE: conjugated equine estrogens
CHD: coronary heart disease
ER: estrogen receptor
ERα: estrogen receptor α
ERβ: estrogen receptor β
ERE: estrogen response element
FP: PGF$_{2\alpha}$ receptor
FSH: follicle-stimulating hormone
GABA: γ-aminobutyric acid
GnRH: gonadotropin-releasing hormone
hCG: human chorionic gonadotropin
HDL: high-density lipoprotein
HERS: Heart and Estrogen/Progestin Replacement Study
HRT: hormone replacement therapy
HSDD: hypoactive sexual desire disorder
HSP: heat shock protein
IGF: insulin-like growth factor
IUD: intrauterine device
IUS: intrauterine system
IVF: *in vitro* fertilization
LDL: low-density lipoprotein
LH: luteinizing hormone
LNg: levonorgestrel, as in LNg-IUS
LNg14 or 20: LNg, 14 or 20 μg/24 h
LPA: lipoprotein A
MHT: menopausal hormone therapy
MPA: medroxyprogesterone acetate
NE: norepinephrine
OHSS: ovarian hyperstimulation syndrome
OPG: osteoprotegrin
PCOS: polycystic ovary syndrome
PG: prostaglandin
PR: progesterone receptor
PRE: progesterone response element
PRM: progesterone receptor modulators
SERM: selective estrogen receptor modulator
SHBG: sex hormone–binding globulin
WHI: Women's Health Initiative

pathway to increase cholesterol (the precursors of all steroids) transport into the mitochondria of cells, where androgen precursors are produced. Follicle-stimulating hormone (FSH) then stimulates CYP19 production and activity in the granulosa cells, which converts the androgen precursors to estrogens. Notably, theca cells of the ovary contain a form of 17β-hydroxysteroid dehydrogenase (type I) that favors the production of testosterone and estradiol from androstenedione and estrone, respectively. However, in the liver, another form of this enzyme (type II) favors oxidation of circulating estradiol to estrone (Peltoketo et al., 1999), and both of these steroids are then converted to estriol (Figure 48–1). All three of these estrogens are excreted in the urine along with their glucuronide and sulfate conjugates.

In postmenopausal women, the principal source of circulating estrogen is adipose tissue stroma, where estrone is synthesized from dehydroepiandrosterone secreted by the adrenals. In men, estrogens are produced by the testes, but extragonadal production by aromatization of circulating C19 steroids (e.g., androstenedione and dehydroepiandrosterone) accounts for most circulating estrogens (Simpson, 2003).

HISTORY Hormones in the Female Reproductive System

The hormonal nature of the ovarian control of the female reproductive system was firmly established in 1900 by Knauer, when he found that ovarian transplants prevented the symptoms of gonadectomy, and by Halban, who showed that normal sexual development and function occurred when glands were transplanted. In 1923, Allen and Doisy devised a bioassay for ovarian extracts based on the vaginal smear of the rat. Frank and associates in 1925 detected an active sex principle in the blood of sows in estrus, and Loewe and Lange discovered in 1926 that a female sex hormone varied in the urine of women throughout the menstrual cycle. The excretion of estrogen in the urine during pregnancy also was reported by Zondek in 1928 and enabled Butenandt and Doisy in 1929 to crystallize an active substance.

Early investigations indicated that the ovary secretes two substances. Beard had postulated in 1897 that the corpus luteum serves a necessary function during pregnancy, and Fraenkel showed in 1903 that destruction of the corpora lutea in pregnant rabbits caused abortion. Several groups then isolated progesterone from mammalian corpora lutea in the 1930s.

In the early 1960s, pioneering studies by Jensen and colleagues suggested the presence of intracellular receptors for estrogens in target tissues. This was the first demonstration of receptors of the steroid/thyroid superfamily and provided techniques to identify receptors for the other steroid hormones. A second estrogen receptor (ER) was identified in 1996 and was termed ERβ to distinguish it from the receptor identified earlier, termed ERα. Two protein isoforms, A and B, of the progesterone receptor (PR) arise from a single gene by transcription initiation from different promoters.

Estrogens may be locally produced from androgens by the actions of aromatase or from estrogen conjugates by hydrolysis. Such local production of estrogens could play a causal or promotional role in the development of certain diseases, such as breast cancer, because mammary tumors contain both aromatase and hydrolytic enzymes. Estrogens also may be produced from androgens via aromatase in the CNS and other tissues and exert local effects near their production site (e.g., in bone, they affect bone mineral density).

The placenta uses fetal dehydroepiandrosterone and its 16α-hydroxyl derivative to produce large amounts of estrone and estriol. Human urine during pregnancy is thus an abundant source of natural estrogens. Pregnant mare's urine is the source of *conjugated equine estrogens*, which have been widely used therapeutically for many years.

Physiological Actions

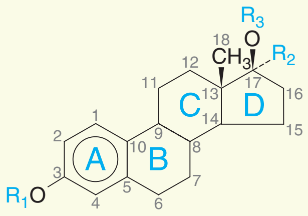
Developmental Actions

Estrogens are largely responsible for pubertal changes in girls and secondary sexual characteristics. Estrogens cause growth and development of the vagina, uterus, and fallopian tubes and contribute to breast enlargement. They also contribute to molding the body contours, shaping the skeleton, and causing the pubertal growth spurt of the long bones and epiphyseal closure. Growth of axillary and pubic hair, pigmentation of the genital region, and the regional pigmentation of the nipples and areolae that occur after the first trimester of pregnancy are also estrogenic actions. Androgens may also play a secondary role in female sexual development (see Chapter 49).

Estrogens appear to play important developmental roles in males. In boys, estrogen deficiency diminishes the pubertal growth spurt and delays skeletal maturation and epiphyseal closure so that linear growth continues into adulthood. Estrogen deficiency in men leads to macroorchidism and elevated gonadotropins and testosterone levels and may

TABLE 48–1 ■ STRUCTURAL FORMULAS OF SELECTED ESTROGENS

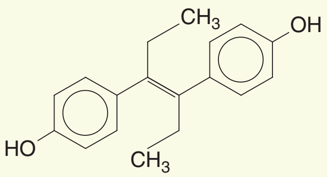
STEROIDAL ESTROGENS


R3
18
O
12
CH3
R2
11
17
C
13
D
16
1
14
9
15
2
10
A
B
8
3
7
R1O
5
4
6

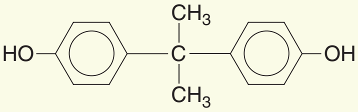
Derivative	*R_1*	*R_2*	*R_3*
Estradiol	—H	—H	—H
Estradiol valerate	—H	—H	$-\overset{O}{\overset{\|}{C}}(CH_2)_3CH_3$
Ethinyl estradiol	—H	—C≡CH	—H
Mestranol	—CH_3	—C≡CH	—H
Estrone sulfate	—SO_3H	—[a]	=O[a]
Equilin[b]	—H	—[a]	=O[a]

NONSTEROIDAL COMPOUNDS WITH ESTROGENIC ACTIVITY

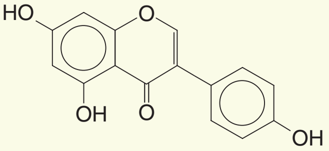
Diethylstilbestrol


CH3
OH
HO
CH3

Bisphenol A


CH3
HO
C
OH
CH3

Genistein



[a] Designates C17 Ketone.

[b] Also contains 7,8 double bond.

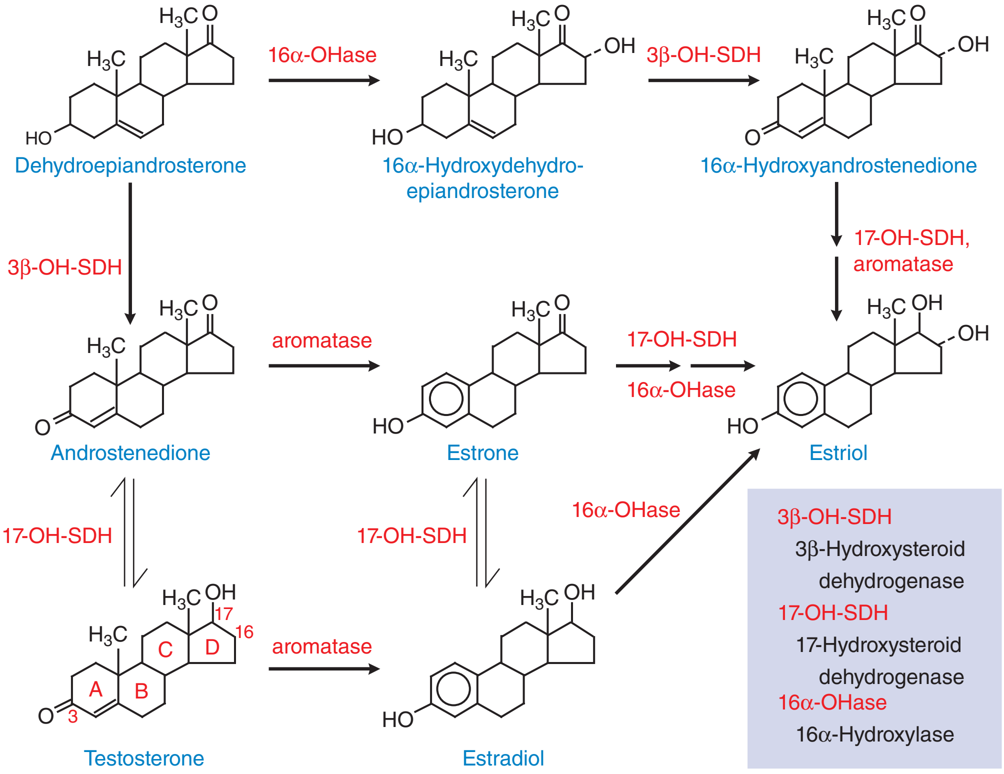

H3C O
H3C
HO
Dehydroepiandrosterone
16α-OHase
H3C O
H3C
OH
HO
16α-Hydroxydehydro-epiandrosterone
3β-OH-SDH
H3C O
H3C
OH
O
16α-Hydroxyandrostenedione
3β-OH-SDH
17-OH-SDH, aromatase
H3C O
H3C
O
Androstenedione
aromatase
H3C O
HO
Estrone
17-OH-SDH
16α-OHase
H3C OH
OH
HO
Estriol
17-OH-SDH
17-OH-SDH
16α-OHase
H3C OH
H3C
17
16
C
D
A
B
O
3
Testosterone
aromatase
H3C OH
HO
Estradiol
3β-OH-SDH
3β-Hydroxysteroid dehydrogenase
17-OH-SDH
17-Hydroxysteroid dehydrogenase
16α-OHase
16α-Hydroxylase

Figure 48–1 *The biosynthetic pathway for the estrogens.*

also affect carbohydrate and lipid metabolism and fertility in some individuals (Grumbach and Auchus, 1999).

Neuroendocrine Control of the Menstrual Cycle

A neuroendocrine cascade involving the hypothalamus, pituitary, and ovaries controls the menstrual cycle (Figure 48–2). A neuronal oscillator, or "clock," in the hypothalamus fires at intervals that coincide with bursts of gonadotropin-releasing hormone (GnRH) release into the hypothalamic-pituitary portal vasculature (see Chapter 46). GnRH interacts with its cognate receptor on pituitary gonadotropes to cause release of LH and FSH. The frequency of the GnRH pulses, which varies in the different phases of the menstrual cycle, controls the relative synthesis of the unique β subunits of FSH and LH.

The gonadotropins (LH and FSH) regulate the growth and maturation of the graafian follicle in the ovary and the ovarian production of estrogen and progesterone, which exert feedback regulation on the pituitary and hypothalamus. Because the release of GnRH is intermittent, LH and FSH secretion is pulsatile. The pulse *frequency* is determined by the neural clock (Figure 48–2), termed the *hypothalamic GnRH pulse generator* (Knobil, 1981), but the amount of gonadotropin released in each pulse (i.e., the pulse *amplitude*) is largely controlled by the actions of estrogens and progesterone on the pituitary. The intermittent, *pulsatile* nature of hormone release is essential for the maintenance of normal ovulatory menstrual cycles because constant infusion of GnRH results in cessation of gonadotropin release and ovarian steroid production (see Chapter 46). The neuropeptide kisspeptin 1, which is released from kisspeptin neurons located in the arcuate nucleus and elsewhere in the hypothalamus, regulates GnRH pulsatility through its G protein-coupled receptor, GPR54, expressed in GnRH neurons (Figure 48–2). Estrogen reduces kisspeptin production in kisspeptin neurons within the acuate nucleus, while neurokinin B through the neurokinin 3 receptor stimulates kisspeptin secretion from the same kisspeptin neurons. Inactivating mutations in GPR54 have been associated with hypogonadotropic hypogonadism (Seminara, 2006), as have inactivating mutations in the kisspeptin gene (Topaloglu et al., 2012). In contrast, activating mutations of the kisspeptin gene can lead to central precocious puberty (Silveira et al., 2010). Finally, mutations in neurokinin B or its receptor have also been associated with pubertal failure (Topaloglu et al., 2009).

Although the precise mechanism that regulates the timing of GnRH release (i.e., pulse frequency) is unclear, hypothalamic cells appear to

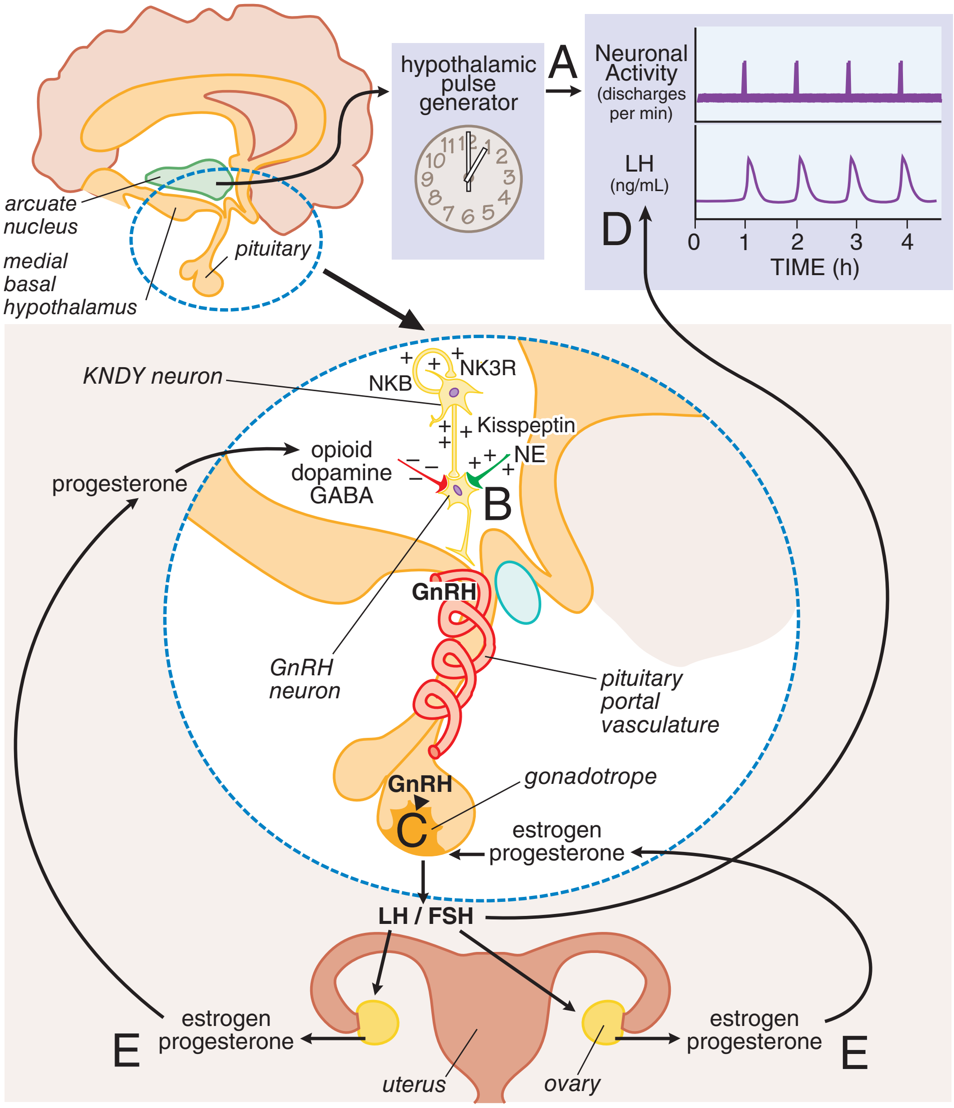


Figure 48–2 *Neuroendocrine control of gonadotropin secretion in females.* The hypothalamic pulse generator located in the arcuate nucleus of the hypothalamus functions as a neuronal "clock" that fires at regular hourly intervals (**A**). This results in the periodic release of GnRH from GnRH-containing neurons into the hypothalamic-pituitary portal vasculature (**B**). GnRH neurons (**B**) receive inhibitory input from opioid, dopamine, and γ-aminobutyric acid (GABA) neurons and stimulatory input from noradrenergic neurons. The pulses of GnRH trigger the intermittent release of LH and FSH from pituitary gonadotropes (**C**), resulting in the pulsatile plasma profile (**D**). FSH and LH regulate ovarian production of estrogen and progesterone, which exert feedback controls (**E**). (See text and Figure 48–3 for additional details.)

have an intrinsic ability to release GnRH episodically. The overall pattern of GnRH release likely is regulated by the interplay of intrinsic mechanism(s) and extrinsic synaptic inputs from opioid, catecholamine, GABAergic, and kisspeptin neurons (Figure 48–2). Ovarian steroids, primarily progesterone, regulate the frequency of GnRH release, but the cellular and molecular mechanisms of this regulation are not well established.

At puberty, the pulse generator is activated and establishes cyclic profiles of pituitary and ovarian hormones. Although the mechanism of activation is not entirely established, it may involve increases in circulating insulin-like growth factor (IGF)-1 and leptin levels, the latter acting to inhibit neuropeptide Y in the arcuate nucleus to relieve an inhibitory effect on GnRH neurons.

Figure 48–3 provides a schematic diagram of the profiles of gonadotropin and gonadal steroid levels in the menstrual cycle. The "average" plasma levels of LH throughout the cycle are shown in panel A of Figure 48–3; insets illustrate the pulsatile patterns of LH during the proliferative and secretory phases in more detail. The average LH levels are similar throughout the early (follicular) and late (luteal) phases of the cycle, but the frequency and amplitude of the LH pulses are quite different in the two phases. This characteristic pattern of hormone secretions results from complex positive- and negative-feedback mechanisms (Hotchkiss and Knobil, 1994).

In the early follicular phase of the cycle, (1) the pulse generator produces bursts of neuronal activity with a frequency of about one per hour that correspond with pulses of GnRH secretion; (2) these cause a corresponding pulsatile release of LH and FSH from pituitary gonadotropes; and (3) FSH in particular causes the graafian follicle to mature and secrete estrogen. The effects of estrogens on the pituitary are inhibitory at this time and cause the amount of LH and FSH released from the pituitary to

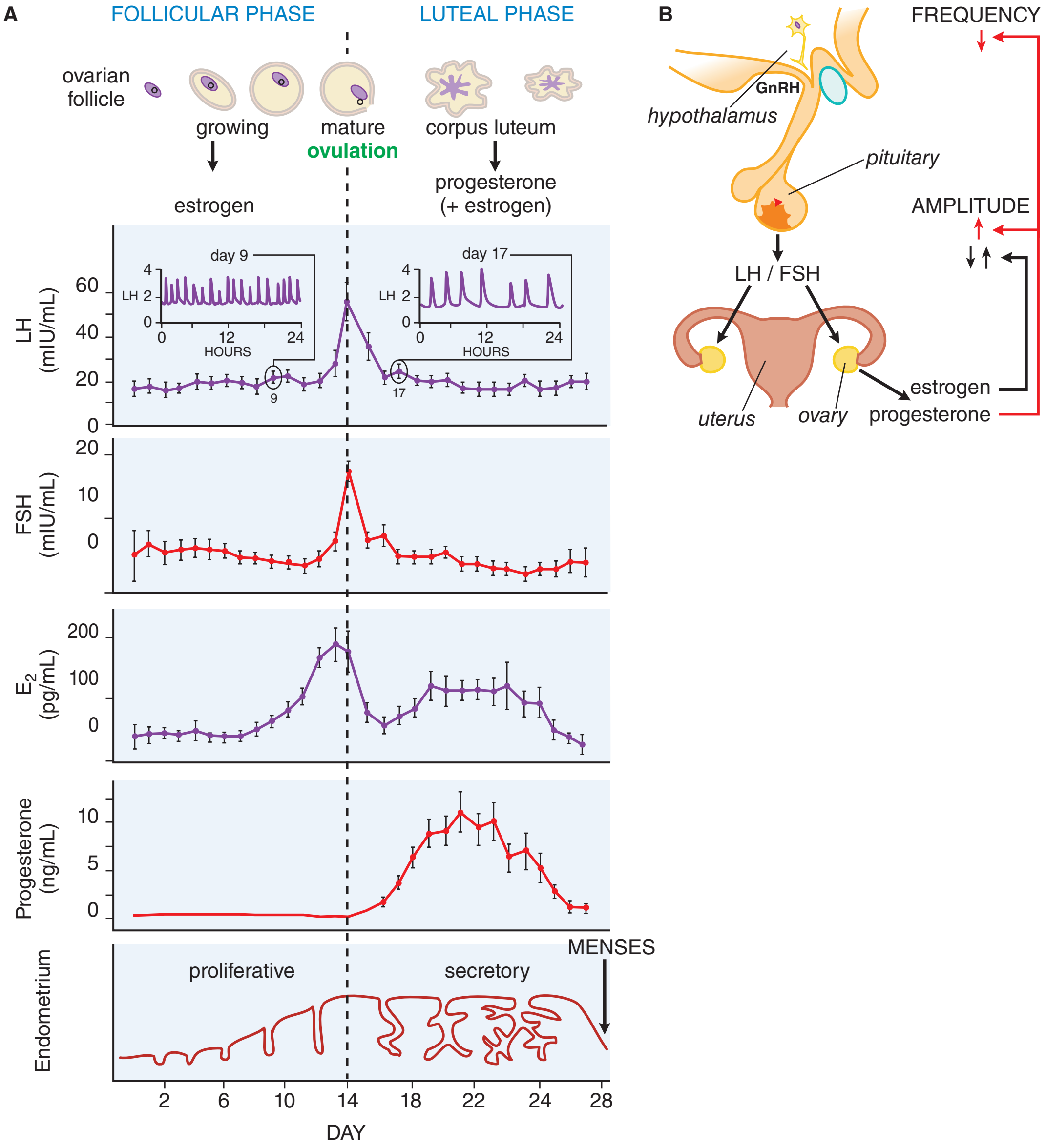


Figure 48–3 *Hormonal relationships of the human menstrual cycle.* **A.** Average daily values of LH, FSH, estradiol (E_2), and progesterone in plasma samples from women exhibiting normal 28-day menstrual cycles. Changes in the ovarian follicle (top) and endometrium (bottom) also are illustrated schematically. Frequent plasma sampling reveals pulsatile patterns of gonadotropin release. Characteristic profiles are illustrated schematically for the follicular phase (day 9, inset on left) and luteal phase (day 17, inset on right). Both the frequency (number of pulses per hour) and amplitude (extent of change of hormone release) of pulses vary throughout the cycle. (Modified from and reproduced with permission from Thorneycroft IH, et al. *Am J Obstet Gynecol,* **1971**, *111*:947–951. Copyright © Elsevier.) **B.** Major regulatory effects of ovarian steroids on hypothalamic-pituitary function. Estrogen decreases the amount of FSH and LH released (i.e., gonadotropin pulse amplitude) during most of the cycle and triggers a surge of LH release only at midcycle. Progesterone decreases the frequency of GnRH release from the hypothalamus and thus decreases the frequency of plasma gonadotropin pulses. Progesterone also increases the amount of LH released (i.e., the pulse amplitude) during the luteal phase of the cycle.

decline (i.e., the amplitude of the LH pulse decreases), so gonadotropin levels gradually fall (Figure 48–3). *Inhibin,* produced by the ovary, exerts negative feedback to selectively decrease serum FSH (see Chapter 46). Activin and follistatin, two other peptides released from the ovary, may also regulate FSH production and secretion to a lesser extent, although their levels do not vary appreciably during the menstrual cycle.

At midcycle, serum estradiol rises above a threshold level of 150 to 200 pg/mL for about 36 h. This sustained elevation of estrogen no longer inhibits gonadotropin release but exerts a brief positive-feedback effect on the pituitary to trigger the preovulatory surge of LH and FSH. This effect involves both a yet uncharacterized estrogen-induced rise in kisspeptin and a change in pituitary responsiveness to GnRH.

The midcycle surge in gonadotropins stimulates follicular rupture and ovulation within 1 to 2 days. The ruptured follicle then develops into the corpus luteum, which produces large amounts of progesterone and lesser amounts of estrogen under the influence of LH during the second half of the cycle. In the absence of pregnancy, the corpus luteum ceases to function, steroid levels drop, and menstruation occurs. When steroid levels drop, the pulse generator reverts to a firing pattern characteristic of the follicular phase, the entire system then resets, and a new ovarian cycle occurs.

Regulation of the frequency and amplitude of gonadotropin secretions by steroids may be summarized as follows: Estrogens act primarily on the pituitary to control the amplitude of gonadotropin pulses, and they may also contribute to the amplitude of GnRH pulses secreted by the hypothalamus.

In the follicular phase of the cycle, estrogens inhibit gonadotropin release but then have a brief midcycle stimulatory action that increases the amount released and causes the LH surge. Progesterone, acting on the hypothalamus, exerts the predominant control of the frequency of LH release. It decreases the firing rate of the hypothalamic pulse generator, an action thought to be mediated largely via inhibitory opioid neurons (containing PRs) that synapse with GnRH neurons. Progesterone also exerts a direct effect on the pituitary to oppose the inhibitory actions of estrogens and thus enhance the amount of LH released (i.e., to increase the amplitude of the LH pulses). These steroid feedback effects, coupled with the intrinsic activity of the hypothalamic GnRH pulse generator, lead to relatively frequent LH pulses of small amplitude in the follicular phase of the cycle and less-frequent pulses of larger amplitude in the luteal phase. Studies in knockout mice indicated that ERα (Hewitt and Korach, 2003) and PR-A (Conneely et al., 2002) mediate the major actions of estrogens and progestins, respectively, on the hypothalamic-pituitary axis.

When the ovaries are removed or cease to function, there is overproduction of FSH and LH, which are excreted in the urine. Measurement of urinary or plasma LH is valuable to assess pituitary function and the effectiveness of therapeutic doses of estrogen.

Effects of Cyclical Gonadal Steroids on the Reproductive Tract

The cyclical changes in estrogen and progesterone production by the ovaries regulate corresponding events in the fallopian tubes, uterus, cervix, and vagina. Physiologically, these changes prepare the uterus for implantation, and the proper timing of events in these tissues is essential for pregnancy. If pregnancy does not occur, the endometrium is shed as the menstrual discharge.

The uterus is composed of an *endometrium* and a *myometrium*. The endometrium contains an epithelium lining the uterine cavity and an underlying stroma; the myometrium is the smooth muscle component responsible for uterine contractions. These cell layers and the fallopian tubes, cervix, and vagina display a characteristic set of responses to both estrogens and progestins. The changes typically associated with menstruation occur largely in the endometrium (Figure 48–3).

The *luminal surface* of the endometrium is a layer of simple columnar epithelial secretory and ciliated cells that is continuous with the openings of numerous glands that extend through the underlying stroma to the myometrial border. Fertilization normally occurs in the fallopian tubes, so ovulation, transport of the fertilized ovum through the fallopian tube, and preparation of the endometrial surface must be temporally coordinated for successful implantation.

The *endometrial stroma* is a highly cellular connective tissue layer containing a variety of blood vessels that undergo cyclic changes associated with menstruation. The predominant cells are fibroblasts, but macrophages, lymphocytes, and other resident and migratory cell types also are present.

Menstruation marks the start of the menstrual cycle. During the follicular (or proliferative) phase of the cycle, estrogen begins the rebuilding of the endometrium by stimulating proliferation and differentiation. An important response to estrogen in the endometrium and other tissues is induction of the PR, which enables cells to respond to this hormone during the second half of the cycle.

In the luteal (or secretory) phase of the cycle, elevated progesterone limits the proliferative effect of estrogens on the endometrium by stimulating differentiation. Major effects include stimulation of epithelial secretions important for implantation of the blastocyst and the characteristic growth of the endometrial blood vessels seen at this time. These effects are mediated by PR-A in animal models (Conneely et al., 2002). Progesterone is thus important in preparation for implantation and for the changes that take place in the uterus at the implantation site (i.e., the decidual response). There is a narrow "window of implantation," spanning days 19 to 24 of the endometrial cycle, when the epithelial cells of the endometrium are receptive to blastocyst implantation. If implantation occurs, human chorionic gonadotropin (hCG) (see Chapter 46), produced initially by the trophoblast and later by the placenta, interacts with the LH receptor of the corpus luteum to maintain steroid hormone synthesis during the early stages of pregnancy. Later, the placenta becomes the major site of estrogen and progesterone synthesis.

Estrogens and progesterone have important effects on the fallopian tube, myometrium, and cervix. In the fallopian tube, estrogens stimulate proliferation and differentiation, whereas progesterone inhibits these processes. Also, estrogens increase and progesterone decreases tubal muscular contractility, which affects transit time of the ovum to the uterus. Estrogens increase the amount of cervical mucus and its water content to facilitate sperm penetration of the cervix, whereas progesterone generally has opposite effects. Estrogens favor rhythmic contractions of the uterine myometrium, and progesterone diminishes contractions. These effects are physiologically important and may also play a role in the action of some contraceptives.

Notably, during implantation of a fertilized ovum into the uterus, there is a strong increase in uteroplacental blood flow. In part, this is caused by spiral arteries invading into the placenta and main uterine artery. This is initially driven by the strong increase in maternal serum estrogen that stimulates nitric oxide formation and subsequent vasodilation. Estrogen stimulates vascular endothelial growth factor and placental growth factor impacting endothelial cell proliferation. Estrogens binding to the estrogen receptors that are expressed in cytotrophoblast cells and in the uterine artery wall stimulate uterine vasculature remodeling. When not present, as occurs in preeclampsia, lower estrogen levels result in altered uterine arterial remodeling, limiting blood flow increases and restricting the growth of the fetus (Mandala, 2020).

Metabolic Effects

Estrogens affect many tissues and have many metabolic actions in humans and animals. Many nonreproductive tissues, including bone, vascular endothelium, liver, CNS, immune system, GI tract, and heart, express low levels of both ERs, and the ratio of ERα to ERβ varies in a cell-specific manner. The effects of estrogens on selected aspects of mineral, lipid, carbohydrate, and protein metabolism are particularly important for understanding their pharmacological actions.

Estrogens have positive effects on bone mass (Riggs et al., 2002). Bone is continuously remodeled at sites called *bone-remodeling units* by the resorptive action of osteoclasts and the bone-forming action of osteoblasts (see Chapter 52). Estrogens directly regulate osteoblasts and increase osteocyte survival by inhibiting apoptosis (Kousteni et al., 2002; Levin, 2008). However, a major effect of estrogens is to decrease the number and activity of osteoclasts. Much of the action of estrogens on osteoclasts appears to be mediated by altering cytokine (both paracrine and autocrine) signals from osteoblasts. Estrogens also increase osteoblast production of the cytokine osteoprotegerin (OPG), a soluble, non–membrane-bound member of the tumor necrosis factor superfamily. OPG acts as a "decoy" receptor that antagonizes the binding of OPG-ligand (OPG-L) to its receptor (termed *RANK*, or *receptor activator of NF-κB*) and prevents the differentiation of osteoclast precursors to mature osteoclasts. Estrogens increase osteoclast apoptosis, either directly or by increasing OPG. Estrogens affect bone growth and epiphyseal closure in both sexes. The importance of estrogen in the male skeleton is illustrated by a man with a completely defective ER who had osteoporosis, unfused epiphyses, increased bone turnover, and delayed bone age (Smith et al., 1994).

Estrogens slightly elevate serum triglycerides and slightly reduce total serum cholesterol levels. They increase high-density lipoprotein (HDL) levels and decrease the levels of low-density lipoprotein (LDL) and lipoprotein A (see Chapter 37). This beneficial alteration of the ratio of HDL to LDL is an attractive but unproven effect of estrogen therapy in postmenopausal women. At relatively high concentrations, estrogens have antioxidant activity and may inhibit the oxidation of LDL by affecting superoxide dismutase. Estrogen actions on the vascular wall include increased production of NO, which occurs within minutes via a mechanism involving activation of Akt (protein kinase B) and induction of NO synthase (Simoncini et al., 2000). All of these changes promote vasodilation and retard atherogenesis. Estrogens also promote endothelial cell growth while inhibiting the proliferation of vascular smooth muscle cells.

The presence of ERs in the liver suggests that the beneficial effects of estrogen on lipoprotein metabolism are due partly to direct hepatic actions. Estrogens also alter bile composition by increasing cholesterol secretion and decreasing bile acid secretion. This leads to increased saturation of bile with cholesterol and appears to be the basis for increased gallstone formation in some women receiving estrogens. In general, estrogens increase plasma levels of cortisol-binding globulin, thyroxine-binding globulin, and sex hormone–binding globulin (SHBG), which binds both androgens and estrogens.

Estrogens alter a number of metabolic pathways that affect the clotting cascade (Mendelsohn and Karas, 1999). Systemic effects include changes in hepatic production of plasma proteins. Estrogens cause a small increase in coagulation factors II, VII, IX, X, and XII, and they decrease the anticoagulation factors protein C, protein S, and antithrombin III (see Chapter 36). Fibrinolytic pathways also are affected, and several studies of women treated with estrogen alone or estrogen with a progestin have demonstrated decreased levels of plasminogen activator inhibitor 1 (PAI-1) protein with a concomitant increase in fibrinolysis (Koh et al., 1997). Thus, estrogens increase both coagulation and fibrinolytic pathways, and imbalance in these two opposing activities may cause adverse effects.

Estrogen Receptors

Estrogens exert their effects by interaction with receptors that are members of the superfamily of nuclear receptors. The two ER genes are located on separate chromosomes: *ESR1* encodes ERα, and *ESR2* encodes ERβ. Both ERs are estrogen-dependent nuclear transcription factors that have different tissue distributions and transcriptional regulatory effects on a wide number of target genes (Hanstein et al., 2004). Both ERα and ERβ exist as multiple mRNA isoforms due to differential promoter use and alternative splicing (reviewed by Kos et al., 2001; Lewandowski et al., 2002). The two human ERs are 44% identical in overall amino acid sequence and share the domain structure common to members of this family. There are significant differences between the two receptor isoforms in the ligand-binding domains and in both transactivation domains. Human ERβ does not appear to contain a functional activation function (AF)-1 domain. The receptors appear to have different biological functions and respond differently to various estrogenic compounds (Kuiper et al., 1997). However, their high homology in the DNA-binding domains suggests that both receptors recognize similar DNA sequences and hence regulate many of the same target genes.

Estrogen receptor (ER) α is expressed most abundantly in the female reproductive tract—especially the uterus, vagina, and ovaries—as well as in the mammary gland, the hypothalamus, endothelial cells, and vascular smooth muscle. *ERβ* is expressed most highly in the prostate and ovaries, with lower expression in lung, brain, bone, and vasculature. Many cells express both ERα and ERβ, which can form either homo- or heterodimers. Both forms of ER are expressed on breast cancers, although ERα is believed to be the predominant form responsible for growth regulation (see Chapter 73). When coexpressed with ERα, ERβ can inhibit ERα-mediated transcriptional activation in many cases (Hall and McDonnell, 1999). Polymorphic variants of ER have been identified, but attempts to correlate specific polymorphisms with the frequency of breast cancer (Han et al., 2003), bone mass (Kurabayashi et al., 2004), endometrial cancer (Weiderpass et al., 2000), or cardiovascular disease (Herrington and Howard, 2003) have led to contradictory results.

A cloned G protein-coupled estrogen receptor, GPER (originally called GPR30), also appears to interact with estrogens in some cell systems (Filardo et al., 2021), and its participation in the rapid effects of estrogen is an attractive idea. There may be interaction/crosstalk between membrane-associated ERα and membrane-localized GPER; GPER has also been reported to exist in intracellular compartments. Whether GPER plays a physiological role in estrogen signaling *in vivo* remains an unsettled question (Levin, 2008; Leeb-Lundberg, 2009; Luo and Liu, 2020; Barton et al., 2018). GPER knock-out mice do not display reproductive dysfunction but have a variety of altered physiological functions consistent with the mediation by GPER of some modulatory effects of estrogens (Prossnitz and Hathaway, 2015). A variety of GPER-selective agonists and antagonists have been characterized (Prossnitz and Arterburn, 2015). A GPER-selective agonist is in early clinical trials for anti-tumor effects ± pembrolizumab (Filardo et al, 2021).

Mechanism of Action

Both ERs are ligand-activated transcription factors that increase or decrease the transcription of target genes (Figure 48–4). After entering the cell by passive diffusion through the plasma membrane, the hormone binds to an ER in the nucleus. In the nucleus, the ER is present as an inactive monomer bound to heat shock protein (HSP) 90, and on binding estrogen, a change in ER conformation dissociates the HSPs and causes receptor dimerization, which increases the affinity and the rate of receptor binding to DNA (Cheskis et al., 1997). Homodimers of ERα or ERβ and ERα/ERβ heterodimers can be produced depending on the receptor complement in a given cell. The concept of ligand-mediated changes in ER conformation is central to understanding the mechanism of action of estrogen agonists and antagonists. The ER dimer binds to estrogen receptor elements (EREs), typically located in the promoter region of target genes. The ER/DNA complex recruits a cascade of coactivator and other proteins to the promoter region of target genes (Figure 48–4B) and allows the proteins that make up the general transcription apparatus to assemble and initiate transcription.

Besides coactivators and corepressors, both ERα and ERβ can interact physically with other transcription factors, such as Sp1 (Saville et al., 2000) or AP-1 (Paech et al., 1997), and these protein-protein interactions provide an alternate mechanism of action. In these circumstances, ER-ligand complexes interact with Sp1 or AP-1 that is already bound to its specific regulatory element, such that the ER complex does not interact directly with an ERE. This may explain how estrogens are able to regulate genes that lack a consensus ERE. Responses to agonists and antagonists mediated by these protein-protein interactions also are ER isoform and promoter specific. For example, 17β-estradiol induces transcription of a target gene controlled by an AP-1 site in the presence of an ERα/AP-1 complex but inhibits transcription in the presence of an ERβ/AP-1 complex. Conversely, antiestrogens are potent activators of ERβ/AP-1 but not of ERα/AP-1 complexes.

Other signaling systems may activate nuclear ER by ligand-independent mechanisms. Phosphorylation of ERα at serine 118 by MAPK activates the receptor (Kato et al., 1995). Similarly, PI3K-activated Akt directly phosphorylates ERα, causing ligand-independent activation of estrogen target genes (Simoncini et al., 2000). This provides a means of crosstalk between membrane-bound receptor pathways (i.e., EGF/IGF-1) that activate MAPK and the nuclear ER.

Some ERs are located on the plasma membrane of cells. These ERs are encoded by the same genes that encode ERα and ERβ but are transported to the plasma membrane and reside mainly in caveolae

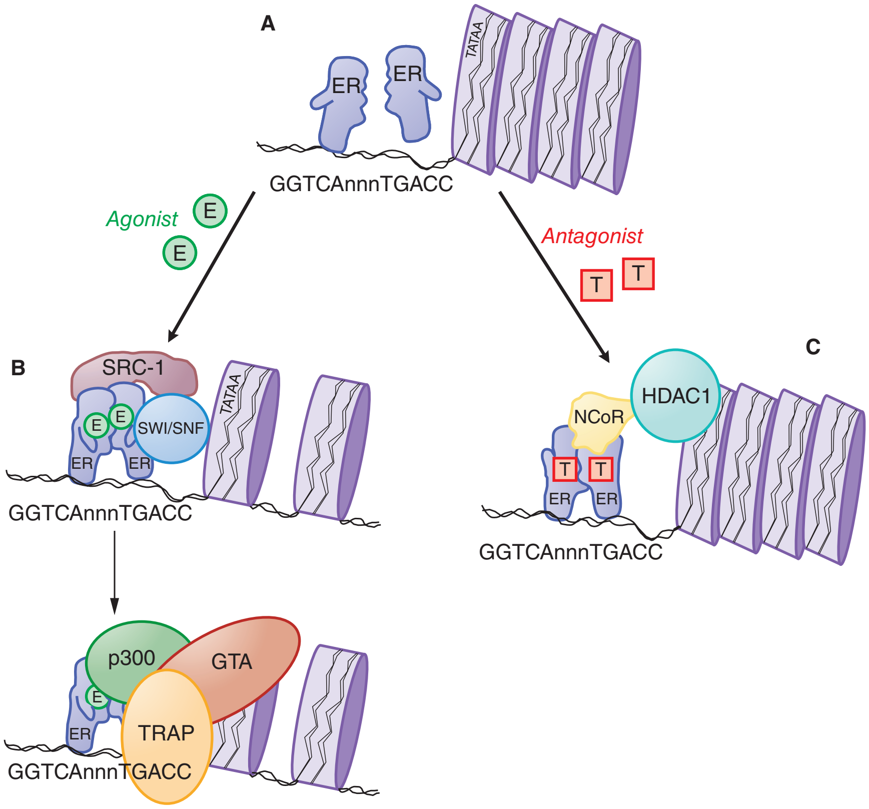


Figure 48–4 *Molecular mechanism of action of nuclear ER.* **A.** Unliganded ER exists as a monomer within the nucleus. **B.** Agonists such as 17β-estradiol (E) bind to the ER and cause a ligand-directed change in conformation that facilitates dimerization and interaction with specific ERE sequences in DNA. The ER-DNA complex recruits coactivators such as SWI/SNF that modify chromatin structure and coactivators such as SRC-1, which has histone acetyltransferase activity that further alters chromatin structure. This remodeling facilitates the exchange of the recruited proteins such that other coactivators (e.g., p300 and the TRAP complex) associate on the target gene promoter and proteins that comprise the general transcription apparatus (GTA) are recruited, with subsequent synthesis of mRNA. **C.** Antagonists such as tamoxifen (T) also bind to the ER but produce a different receptor conformation. The antagonist-induced conformation also facilitates dimerization and interaction with DNA, but a different set of proteins called corepressors, such as nuclear hormone receptor corepressor (NcoR), are recruited to the complex. NcoR further recruits proteins such as histone deacetylase I (HDAC1) that act on histones to stabilize nucleosome structure and prevent interaction with the GTA.

(Pedram et al., 2006). Translocation to the membrane by all sex steroid receptors is mediated by palmitoylation of a 9–amino acid motif in the respective E domains of the receptors (Levin, 2008). Membrane-localized ERs mediate the rapid activation of some proteins such as MAPK (phosphorylated in several cell types) and the rapid increase in cyclic AMP caused by the hormone. The finding that MAPK is activated by estradiol provides an additional level of crosstalk and complexity in estrogen signaling.

Pharmacology

ADME

Various estrogens are available for oral, parenteral, transdermal, or topical administration. Given the lipophilic nature of estrogens, absorption generally is good with the appropriate preparation. Aqueous or oil-based esters of estradiol are available for intramuscular injection, ranging in frequency from every week to once per month. Conjugated estrogens are available for intravenous or intramuscular administration. Transdermal patches that are changed once or twice weekly deliver estradiol continuously through the skin. Preparations are available for topical use in the vagina or for application to the skin. For many therapeutic uses, estrogen preparations are available in combination with a progestin. All estrogens are labeled with precautionary statements urging the prescribing of the lowest effective dose and for the shortest duration consistent with the treatment goals and risks for each individual patient.

Oral administration is common and may use estradiol, conjugated estrogens, esters of estrone and other estrogens, and *ethinyl estradiol* (*in combination with a progestin*). Estradiol is available in nonmicronized and micronized preparations. The micronized formulations yield a large surface for rapid absorption to partially overcome low absolute oral bioavailability due to first-pass metabolism (Fotherby, 1996). Addition of the ethinyl substituent at C17 (ethinyl estradiol) inhibits first-pass hepatic metabolism. Other common oral preparations contain conjugated equine estrogens, which are primarily the sulfate esters of estrone, equilin, and other naturally occurring compounds; *esterified esters*; or mixtures of synthetic conjugated estrogens prepared from plant-derived sources. These are hydrolyzed by enzymes present in the lower gut that remove the charged sulfate groups and allow absorption of estrogen across the intestinal epithelium. In another oral preparation, *estropipate*, estrone is solubilized as the sulfate and stabilized with *piperazine*. Due largely to differences in metabolism, the potencies of various oral preparations differ widely; ethinyl estradiol, for example, is much more potent than conjugated estrogens.

A number of foodstuffs and plant-derived products, largely from soy and wild yams, are available as unapproved marketed nonprescription items and often are touted as providing benefits similar to those from compounds with established estrogenic activity. These products may contain flavonoids such as genistein (Table 48–1) that display estrogenic activity in laboratory tests, albeit generally much less than that of estradiol. In theory, these preparations could produce appreciable estrogenic effects, but their efficacy at relevant doses has not been established in human trials (Fitzpatrick, 2003).

Administration of estradiol via transdermal patches provides slow, sustained release of the hormone, systemic distribution, and more constant blood levels than oral dosing. Estradiol is also available as a topical emulsion applied to the upper thigh and calf or as a gel applied once daily to the arm. The transdermal route does not lead to the high levels of the drug that occur in the portal circulation after oral administration, and it is thus expected to minimize hepatic effects of estrogens (e.g., effects on hepatic protein synthesis, lipoprotein profiles, and triglyceride levels).

When dissolved in oil and injected, esters of estradiol are well absorbed. Preparations available for intramuscular injection include *estradiol valerate* or *estradiol cypionate* and may be absorbed over several weeks following a single intramuscular injection.

Preparations of estradiol or conjugated estrogen creams are available for topical administration to the vagina. These are effective locally, but systemic effects also are possible due to significant absorption. A 3-month vaginal ring may be used for slow release of estradiol, and tablets are also available for vaginal use (*Vagifem* and generics).

Estradiol, ethinyl estradiol, and other estrogens are extensively bound to plasma proteins. Estradiol and other naturally occurring estrogens are bound mainly to SHBG and to a lesser degree to serum albumin. In contrast, ethinyl estradiol is bound extensively to serum albumin but not SHBG. Due to their size and lipophilic nature, unbound estrogens distribute rapidly and extensively.

Variations in estradiol metabolism occur and depend on the stage of the menstrual cycle, menopausal status, and several genetic polymorphisms (Herrington and Klein, 2001). In general, the hormone undergoes rapid hepatic biotransformation, with a plasma $t_{1/2}$ measured in minutes. Estradiol is converted primarily by 17β-hydroxysteroid dehydrogenase to estrone, which undergoes conversion by 16α-hydroxylation and 17-keto reduction to estriol, the major urinary metabolite. A variety of sulfate and glucuronide conjugates also are excreted in the urine. Lesser amounts of estrone or estradiol are oxidized to the 2-hydroxycatechols by CYP3A4 in the liver and by CYP1A in extrahepatic tissues or to 4-hydroxycatechols by CYP1B1 in extrahepatic sites, with the 2-hydroxycatechol formed to a greater extent. The 2- and 4-hydroxycatechols are largely inactivated by catechol-*O*-methyl transferases. However, smaller amounts may be converted by CYP- or peroxidase-catalyzed reactions to yield semiquinones or quinones that are capable of forming DNA adducts or of generating (via redox cycling) reactive oxygen species (ROSs) that could oxidize DNA bases (Yue et al., 2003).

Estrogens also undergo enterohepatic recirculation via (1) sulfate and glucuronide conjugation in the liver, (2) biliary secretion of the conjugates into the intestine, and (3) hydrolysis in the gut (largely by bacterial enzymes) followed by reabsorption.

Many other drugs and environmental agents (e.g., cigarette smoke) act as inducers or inhibitors of the various enzymes that metabolize estrogens and thus have the potential to alter their clearance. Consideration of the impact of these factors on efficacy and untoward effects is increasingly important with the decreased doses of estrogens currently employed for both menopausal hormone therapy (MHT) and contraception.

Ethinyl estradiol is cleared much more slowly than estradiol due to decreased hepatic metabolism, and the elimination-phase $t_{1/2}$ in various studies ranges from 13 to 27 h. Unlike estradiol, the primary route of biotransformation of ethinyl estradiol is via 2-hydroxylation and subsequent formation of the corresponding 2- and 3-methyl ethers. *Mestranol*, another semisynthetic estrogen and a component of some combination oral contraceptives, is the 3-methyl ether of ethinyl estradiol. In the body, it undergoes rapid hepatic demethylation to ethinyl estradiol, which is its active form (Fotherby, 1996).

Selective Estrogen Receptor Modulators and Antiestrogens

By altering the conformation of the two different ERs and thereby changing interactions with coactivators and corepressors in cell-specific and promoter-specific contexts, ligands may have a broad spectrum of activities from purely antiestrogenic in all tissues, to partially estrogenic in some tissues with antiestrogenic or no activities in others, to purely estrogenic activities in all tissues. The elucidation of these concepts has been a major breakthrough in estrogen pharmacology and should permit the rational design of drugs with selective patterns of estrogenic activity (Smith and O'Malley, 2004).

Selective Estrogen Receptor Modulators: Tamoxifen, Raloxifene, and Toremifene

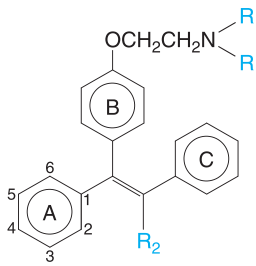
Selective estrogen receptor modulators, or SERMs, are compounds with tissue-selective actions. The pharmacological goal of these drugs is to produce beneficial estrogenic actions in certain tissues (e.g., bone, brain, and liver) during post-MHT but antagonist activity in tissues such as breast and endometrium, where estrogenic actions (e.g., carcinogenesis) might be deleterious. Currently approved drugs in the U.S. in this class are *tamoxifen citrate*, *raloxifene hydrochloride*, and *toremifene*, which is chemically related and has similar actions to *tamoxifen*. *Tamoxifen* and *toremifene* are used for the treatment of breast cancer, and *raloxifene* is used primarily for the prevention and treatment of osteoporosis and to reduce the risk of invasive breast cancer in high-risk postmenopausal women. They are considered in detail in Chapter 73.

Antiestrogens: Clomiphene and Fulvestrant

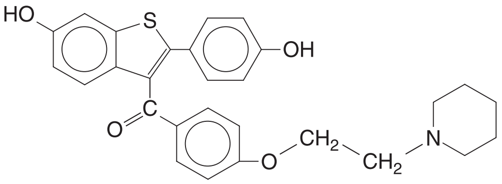
The antiestrogen compounds are distinguished from the SERMs in that they are pure antagonists in all tissues studied. *Clomiphene* is approved for the treatment of infertility in anovulatory women, and *fulvestrant* is used for the treatment of breast cancer in women with disease progression after *tamoxifen*.

Chemistry

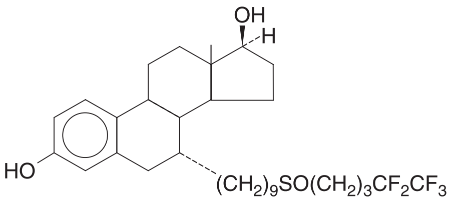
The structures of the *trans*-isomer of *tamoxifen*, and of *raloxifene*, *trans*-clomiphene (enclomiphene), and *fulvestrant* are as follows:



	ENCLOMIPHENE	TAMOXIFEN
R_1:	$-CH_2CH_3$	$-CH_3$
R_2:	$-Cl$	$-CH_2CH_3$



RALOXIFENE



FULVESTRANT (ICI 182, 780)

Tamoxifen is a triphenylethylene with the same stilbene nucleus as *diethylstilbestrol*; compounds of this class display a variety of estrogenic and antiestrogenic activities. In general, the *trans* conformations have antiestrogenic activity, whereas the *cis* conformations display estrogenic activity. However, the pharmacological activity of the *trans* compound depends on the species, target tissue, and gene. Hepatic metabolism produces primarily *N*-desmethyltamoxifen, which has affinity for ER comparable to that of *tamoxifen*, and lesser amounts of the highly active 4-hydroxy metabolite, which has a 25- to 50-times higher affinity for both

ERα and ERβ than does *tamoxifen* (Kuiper et al., 1997). *Tamoxifen* is marketed as the pure *trans*-isomer. *Toremifene* is a triphenylethylene with a chlorine substitution at the R2 position.

Raloxifene is a polyhydroxylated nonsteroidal compound with a benzothiophene core. *Raloxifene* binds with high affinity for both ERα and ERβ (Kuiper et al., 1997).

Clomiphene citrate is a triphenylethylene; its two isomers, zuclomiphene (*cis* clomiphene) and enclomiphene (*trans* clomiphene), are a weak estrogen agonist and a potent antagonist, respectively. *Clomiphene* binds to both ERα and ERβ, but the individual isomers have not been examined (Kuiper et al., 1997).

Fulvestrant is a 7α-alkylamide derivative of estradiol that interacts with both ERα and ERβ (Van Den Bemd et al., 1999).

Pharmacological Effects

All of these agents bind to the ligand-binding pocket of both ERα and ERβ and competitively block estradiol binding. However, the conformation of the ligand-bound ERs is different with different ligands (Smith and O'Malley, 2004), and this has two important mechanistic consequences. The distinct ER-ligand conformations recruit different coactivators and corepressors onto the promoter of a target gene by differential protein-protein interactions at the receptor surface. The tissue-specific actions of SERMs thus can be explained in part by the distinct conformation of the ER when occupied by different ligands, in combination with different coactivator and corepressor levels in different cell types that together affect the nature of ER complexes formed in a tissue-selective fashion.

Tamoxifen. *Tamoxifen* exhibits antiestrogenic, estrogenic, or mixed activity depending on the species and target gene measured. In clinical tests or laboratory studies with human cells, the drug's activity depends on the tissue and end point measured. For example, *tamoxifen* inhibits the proliferation of cultured human breast cancer cells and reduces tumor size and number in women (Jaiyesimi et al., 1995), and yet it stimulates proliferation of endometrial cells and causes endometrial thickening (Lahti et al., 1993). The drug has an antiresorptive effect on bone, and in humans, it decreases total cholesterol, LDL, and lipoprotein A but does not increase HDL and triglycerides (Love et al., 1994). *Tamoxifen* treatment causes a 2- to 3-fold increase in the relative risk of deep vein thrombosis and pulmonary embolism and a roughly 2-fold increase in endometrial carcinoma (Smith, 2003). *Tamoxifen* produces hot flashes and other adverse effects, including cataracts and nausea. Due to its agonist activity in bone, it does not increase the incidence of fractures when used in this setting.

The conformation of ERs, especially in the AF-2 domain, determines whether a coactivator or a corepressor will be recruited to the ER-DNA complex (Smith and O'Malley, 2004). *Tamoxifen* induces a conformation that permits the recruitment of the corepressor to both ERα and ERβ, in contrast to 17α-estradiol, which induces a conformation that recruits coactivators to the receptor. The agonist activity of *tamoxifen* seen in tissues such as the endometrium is mediated by the ligand-independent AF-1 transactivation domain of ERα; because ERβ does not contain a functional AF-1 domain, *tamoxifen* does not activate ERβ (McInerney et al., 1998).

Raloxifene. *Raloxifene* is an estrogen agonist in bone, where it exerts an antiresorptive effect. The drug also acts as an estrogen agonist in reducing total cholesterol and LDL, but it does not increase HDL or normalize PAI-1 in postmenopausal women (Walsh et al., 1998). Studies indicated that *raloxifene* has an antiproliferative effect on ER-positive breast tumors and significantly reduces the risk of ER-positive but not ER-negative breast cancer (Cummings et al., 1999). *Raloxifene* does not alleviate the vasomotor symptoms associated with menopause. Adverse effects include hot flashes and leg cramps and a 3-fold increase in deep vein thrombosis and pulmonary embolism (Cummings et al., 1999).

Raloxifene acts as a partial agonist in bone but does not stimulate endometrial proliferation in postmenopausal women. Presumably this is due to some combination of differential expression of transcription factors in the two tissues and the effects of this SERM on ER conformation. *Raloxifene* induces a configuration in ERα that is distinct from that of *tamoxifen*-ERβ (Tamrazi et al., 2003), suggesting that a different set of coactivators/corepressors may interact with ER-*raloxifene* compared with ER-*tamoxifen*.

Fulvestrant. *Fulvestrant* is antiestrogenic. In clinical trials, it is efficacious in treating *tamoxifen*-resistant breast cancers (Robertson et al., 2003). *Fulvestrant* binds to ERα and ERβ with high affinity comparable to estradiol but represses transactivation. It also increases dramatically the intracellular proteolytic degradation of ERα while apparently protecting ERβ from degradation (Van Den Bemd et al., 1999). This effect on ERα protein levels may explain *fulvestrant*'s efficacy in *tamoxifen*-resistant breast cancer.

Clomiphene. *Clomiphene* increases gonadotropin secretion and stimulates ovulation. It increases the amplitude of LH and FSH pulses without changing pulse frequency (Kettel et al., 1993). This suggests that the drug is acting largely at the pituitary level to block inhibitory actions of estrogen on gonadotropin release from the gland or is somehow causing the hypothalamus to release larger amounts of GnRH per pulse.

The most prominent effect of *clomiphene* in women was enlargement of the ovaries and the drug-induced ovulation in many patients with amenorrhea, polycystic ovary syndrome (PCOS), and dysfunctional bleeding with anovulatory cycles. Thus, *clomiphene*'s major pharmacological use is to induce ovulation in women with a functional hypothalamic-hypophyseal-ovarian system and adequate endogenous estrogen production. In some cases, *clomiphene* is used in conjunction with human gonadotropins (see Chapter 46) to induce ovulation.

ADME

Tamoxifen is given orally, and peak plasma levels are reached within 4 to 7 h. It has two elimination phases with $t_{1/2}$ of 7 to 14 h and 4 to 11 days. Due to the prolonged $t_{1/2}$, 3 to 4 weeks of treatment are required to reach steady-state plasma levels. *Tamoxifen* is metabolized in humans by multiple hepatic CYPs, some of which it also induces (Sridar et al., 2002). In humans and other species, 4-hydroxytamoxifen is produced via hepatic metabolism, and this compound is considerably more potent than the parent drug as an antiestrogen. The major route of elimination from the body involves *N*-demethylation and deamination. The drug undergoes enterohepatic circulation, and excretion is primarily in the feces as conjugates of the deaminated metabolite. Polymorphisms affect the rate of *tamoxifen* metabolism to its more potent 4-hydroxy metabolite and may affect its therapeutic activity in breast cancer (see Chapter 73).

Raloxifene is absorbed rapidly after oral administration and has an absolute bioavailability of about 2%. The drug has a $t_{1/2}$ of about 28 h and is eliminated primarily in the feces after hepatic glucuronidation.

Clomiphene is well absorbed following oral administration, and the drug and its metabolites are eliminated primarily in the feces and to a lesser extent in the urine. The long plasma $t_{1/2}$ (5–7 days) is due largely to plasma-protein binding, enterohepatic circulation, and accumulation in fatty tissues.

Fulvestrant is administered monthly by intramuscular depot injections. Plasma concentrations reach maximal levels in 7 days and are maintained for a month. Numerous metabolites are formed *in vivo*, possibly by pathways similar to endogenous estrogen metabolism, but the drug is eliminated primarily (90%) via the feces in humans.

Therapeutic Uses

Breast Cancer. *Tamoxifen* is highly efficacious in the treatment of breast cancer. It is used alone for palliation of advanced breast cancer in women with ER-positive tumors, and it is now indicated as the hormonal treatment of choice for both early and advanced breast cancer in women of all ages (Jaiyesimi et al., 1995). Response rates are about 50% in women with ER-positive tumors. *Tamoxifen* increases disease-free survival and overall survival; treatment for 5 years reduces cancer recurrence by 50% and death by 27% and is more efficacious than shorter 1- to 2-year treatment periods. *Tamoxifen* reduces the risk of developing contralateral breast cancer and is approved for primary prevention of breast cancer in women at high risk, in whom it causes a 50% decrease in the development of new tumors. Prophylactic treatment should be limited to 5 years because effectiveness decreases thereafter. The most frequent side effect is hot flashes. *Tamoxifen* has estrogenic activity in the uterus, increases the risk of endometrial cancer by 2- to 3-fold, and also causes a comparable increase in the risk of thromboembolic disease that leads to serious risks for women receiving anticoagulant therapy (Smith, 2003) and women with a history of deep vein thrombosis or stroke.

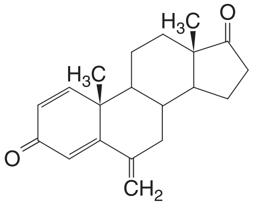
Toremifene has therapeutic actions similar to *tamoxifen*, and *fulvestrant* may be efficacious in women who become resistant to *tamoxifen*. Untoward effects of *fulvestrant* include hot flashes, GI symptoms, headache, back pain, and pharyngitis.

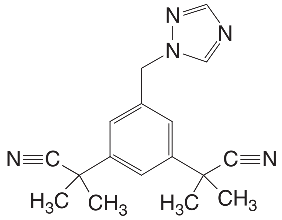
Osteoporosis. *Raloxifene* reduces the rate of bone loss and may increase bone mass at certain sites. In a large clinical trial, *raloxifene* increased spinal bone mineral density by more than 2% and reduced the rate of vertebral fractures by 30% to 50% but did not significantly reduce nonvertebral fractures (Delmas et al., 2002; Ettinger et al., 1999). *Raloxifene* does not appear to increase the risk of developing endometrial cancer. The drug has beneficial actions on lipoprotein metabolism, reducing both total cholesterol and LDL; however, HDL is not increased. Adverse effects include hot flashes, deep vein thrombosis, and leg cramps.

Infertility. *Clomiphene citrate* is a potent antiestrogen that primarily is used for treatment of anovulation in the setting of an intact hypothalamic-pituitary axis and adequate estrogen production (e.g., PCOS) or to induce superovulation in women with unexplained infertility. By inhibiting the negative-feedback effects of estrogen at hypothalamic and pituitary levels, *clomiphene* increases FSH levels and thereby enhances follicular maturation. The drug is relatively inexpensive, is orally active, and requires less-extensive monitoring than do other fertility protocols. However, the drug may exhibit untoward effects, including ovarian hyperstimulation, increased incidence of multiple births, ovarian cysts, hot flashes, and blurred vision. Prolonged use (e.g., ≥12 cycles) may increase the risk of ovarian cancer. The drug should not be administered to pregnant women due to reports of teratogenicity in animals, but there is no evidence of this when the drug has been used to induce ovulation.

Experimental SERM-Estrogen Combinations. There is considerable interest in MHT using combinations of a pure estrogen agonist (e.g., estradiol) with a SERM that has predominantly antagonist activity in the breast and endometrium but does not distribute to the CNS. The strategy is to obtain the beneficial actions of the agonist (e.g., prevention of hot flashes and bone loss) while the SERM blocks unwanted agonist action at peripheral sites (e.g., proliferative effects in breast and endometrium) but does not enter the brain to cause hot flashes. Animal studies have been encouraging (Labrie et al., 2003), but clinical efficacy and safety of this approach remain to be established.

Estrogen Synthesis Inhibitors


EXEMESTANE


ANASTROZOLE

Continual administration of GnRH agonists prevents ovarian synthesis of estrogens but not their peripheral synthesis from adrenal androgens (see Chapter 46). *Aminoglutethimide* inhibits aromatase activity, but its use is limited by its lack of selectivity and its side effects (sedation). It was discontinued in the U.S. in 2008.

The recognition that locally produced as well as circulating estrogens may play a significant role in breast cancer has greatly stimulated interest in the use of aromatase inhibitors to selectively block production of estrogens (see Figure 73–3). Both steroidal (e.g., *exemestane*) and nonsteroidal (e.g., *anastrozole*, *letrozole*, and *vorozole*) agents are available. Steroidal, or type I, agents are substrate analogues that act as suicide inhibitors to irreversibly inactivate aromatase, whereas the nonsteroidal, or type II, agents interact reversibly with the heme groups of CYPs (Haynes et al., 2003). *Exemestane*, *letrozole*, and *anastrozole* are currently approved in the U.S. for the treatment of breast cancer.

As discussed in Chapter 73, these agents may be used as first-line treatment of breast cancer or as second-line drugs after *tamoxifen*. They are highly efficacious and actually superior to *tamoxifen* in adjuvant use for postmenopausal women (Coombes et al., 2004), and they are indicated either following *tamoxifen* for 2 to 5 years or as initial agents. They have the added advantage of not increasing the risk of uterine cancer or venous thromboembolism. Because they dramatically reduce circulating as well as local levels of estrogens, they produce hot flashes. They lack the beneficial effect of *tamoxifen* to maintain bone density and thus are often administered with bisphosphonates. Their effects on plasma lipids remain to be established.

Progestins

HISTORY Progestins

Corner and Allen originally isolated a hormone in 1933 from the corpora lutea of sows and named it *progestin*. The next year, several European groups independently isolated the crystalline compound and called it *luteo-sterone*, unaware of the previous name. This difference in nomenclature was resolved in 1935 at a garden party in London given by Sir Henry Dale, who helped persuade all parties that the name *progesterone* was a suitable compromise.

Two major advances overcame the early difficulties and expense of obtaining progesterone from animal sources. The first was the synthesis of progesterone by Russel Marker from the plant product diosgenin in the 1940s, which provided a relatively inexpensive and highly pure product. The second was the synthesis of 19-nor compounds, the first orally active progestins, in the early 1950s by Carl Djerassi, who synthesized *norethindrone* at Syntex, and Frank Colton, who synthesized the isomer *norethynodrel* at Searle. These advances led to the development of effective oral contraceptives.

Chemistry

Compounds with biological activities similar to those of progesterone are referred to as progestins, progestational agents, progestagens, progestogens, gestagens, or gestogens. The progestins (Figure 48–5) include the naturally occurring hormone progesterone, 17α-acetoxyprogesterone derivatives in the pregnane series, 19-nortestosterone derivatives in the estrane series, and norgestrel and related compounds in the gonane series. *Medroxyprogesterone acetate* (MPA) and *megestrol acetate* are C21 steroids in the pregnane family with selective activity very similar to that of progesterone itself. MPA and oral micronized progesterone are widely used with estrogens for MHT and other situations in which a selective progestational effect is desired. Furthermore, depot MPA is used as a long-acting injectable contraceptive. The 19-nortestosterone derivatives (estranes) were developed for use as progestins in oral contraceptives, and although their predominant activity is progestational, they exhibit androgenic and other activities. The gonanes are another family of "19-nor" compounds, containing an ethyl rather than a methyl substituent in the 13 position. They have diminished androgenic activity relative to the estranes. These two classes of 19-nortestosterone derivatives are the

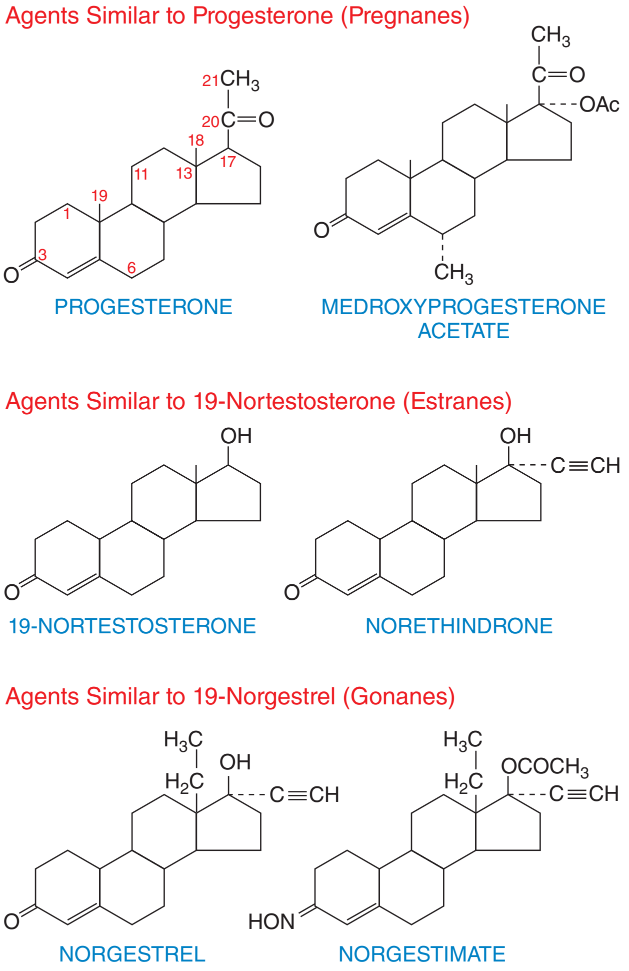

Agents Similar to Progesterone (Pregnanes)
21CH3
20C=O
18
17
11
13
19
1
3
6
O
PROGESTERONE
CH3
C=O
OAc
O
CH3
MEDROXYPROGESTERONE ACETATE
Agents Similar to 19-Nortestosterone (Estranes)
OH
O
19-NORTESTOSTERONE
OH
C≡CH
O
NORETHINDRONE
Agents Similar to 19-Norgestrel (Gonanes)
H3C
H2C
OH
C≡CH
O
NORGESTREL
H3C
H2C
OCOCH3
C≡CH
HON
NORGESTIMATE

Figure 48–5 *Structural features of various progestins.*

progestational components of most oral and some long-acting injectable contraceptives. The remaining oral contraceptives contain a class of progestins derived from *spironolactone* (e.g., *drospirenone*) that have antimineralocorticoid and antiandrogenic properties.

The structural features of several progestins are shown in Figure 48–5. Unlike the ER, which requires a phenolic A ring for high-affinity binding, the PR favors a Δ^4–3-one A-ring structure in an inverted 1β,2α-conformation. Other steroid hormone receptors also bind this nonphenolic A-ring structure, although the optimal conformation differs from that for the PR. Thus, some synthetic progestins (especially the 19-nor compounds) display limited binding to glucocorticoid, androgen, and mineralocorticoid receptors, a property that probably accounts for some of their nonprogestational activities. The spectrum of activities of these compounds is highly dependent on specific substituent groups, especially the nature of the C17 substituent in the D ring, the presence of a C19 methyl group, and the presence of an ethyl group at position C13.

Biosynthesis and Secretion

Progesterone is secreted by the ovary, mainly from the corpus luteum, during the second half of the menstrual cycle (Figure 48–3). LH, acting via its G protein-coupled receptor, stimulates progesterone secretion during the normal cycle.

After fertilization, the trophoblast secretes hCG into the maternal circulation, which then stimulates the LH receptor to sustain the corpus luteum and maintain progesterone production. During the second or third month of pregnancy, the developing placenta begins to secrete estrogen and progesterone in collaboration with the fetal adrenal glands, and thereafter, the corpus luteum is not essential to continued gestation. Estrogen and progesterone continue to be secreted in large amounts by the placenta up to the time of delivery.

Physiological Actions

Neuroendocrine Actions

Progesterone produced in the luteal phase of the cycle has several physiological effects, including decreasing the frequency of GnRH pulses. This progesterone-mediated decrease in GnRH pulse frequency is critical for suppressing gonadotropin release and resetting the hypothalamic-pituitary-gonadal axis to transition from the luteal back to the follicular phase. Furthermore, GnRH suppression is the major mechanism of action of progestin-containing contraceptives.

Reproductive Tract. Progesterone decreases estrogen-driven endometrial proliferation and leads to the development of a secretory endometrium (Figure 48–3), and the abrupt decline in progesterone at the end of the cycle is the main determinant of the onset of menstruation. If the duration of the luteal phase is artificially lengthened, either by sustaining luteal function or by treatment with progesterone, decidual changes in the endometrial stroma similar to those seen in early pregnancy can be induced. Under normal circumstances, estrogen antecedes and accompanies progesterone in its action on the endometrium and is essential to the development of the normal menstrual pattern.

Progesterone also influences the endocervical glands, and the abundant watery secretion of the estrogen-stimulated structures is changed to a scant viscid material. As noted previously, these and other effects of progestins decrease penetration of the cervix by sperm.

The estrogen-induced maturation of the human vaginal epithelium is modified toward the condition of pregnancy by the action of progesterone, a change that can be detected in cytological alterations in the vaginal smear. If the quantity of estrogen concurrently acting is known to be adequate, or if it is ensured by giving estrogen, the cytological response to a progestin can be used to evaluate its progestational potency.

Progesterone is important for the maintenance of pregnancy. Progesterone suppresses menstruation and uterine contractility.

Mammary Gland. Development of the mammary gland requires both estrogen and progesterone. During pregnancy and to a minor degree during the luteal phase of the cycle, progesterone, acting with estrogen, brings about a proliferation of the acini of the mammary gland. Toward the end of pregnancy, the acini fill with secretions, and the vasculature of the gland notably increases; however, only after the levels of estrogen and progesterone decrease at parturition does lactation begin.

During the normal menstrual cycle, mitotic activity in the breast epithelium is very low in the follicular phase and then peaks in the luteal phase. This pattern is due to progesterone, which triggers a *single* round of mitotic activity in the mammary epithelium. This effect is transient because continued exposure to the hormone is rapidly followed by arrest of growth of the epithelial cells. Importantly, progesterone may be responsible for the increased risk of breast cancer associated with estrogen-progestin use in postmenopausal women, although controlled studies with only progestin have not been performed (Anderson et al., 2004; Rossouw et al., 2002).

CNS. During a normal menstrual cycle, an increase in basal body temperature of about 0.6°C (1°F) may be noted at midcycle; this correlates with ovulation. This increase is due to progesterone, but the exact mechanism of this effect is unknown. Progesterone also increases the ventilatory response of the respiratory centers to carbon dioxide and leads to reduced arterial and alveolar P_{CO_2} in the luteal phase of the menstrual cycle and during pregnancy. Progesterone also may have depressant and hypnotic actions in the CNS, possibly accounting for reports of drowsiness after hormone administration. This potential untoward effect may be abrogated by giving progesterone preparations at bedtime, which may even help some patients sleep.

Metabolic Effects. Progestins have numerous metabolic actions. Progesterone itself increases basal insulin levels and the rise in insulin after carbohydrate ingestion, but it does not normally alter glucose tolerance.

However, long-term administration of more potent progestins, such as *norgestrel*, may decrease glucose tolerance. Progesterone stimulates lipoprotein lipase activity and seems to enhance fat deposition. Progesterone and analogues such as MPA have been reported to increase LDL and cause either no effects or modest reductions in serum HDL levels. The 19-norprogestins may have more pronounced effects on plasma lipids because of their androgenic activity.

Medroxyprogesterone acetate decreases the favorable HDL increase caused by conjugated estrogens during postmenopausal hormone replacement, but it does not significantly affect the beneficial effect of estrogens to lower LDL. In contrast, micronized progesterone does not significantly alter beneficial estrogen effects on either HDL or LDL profiles (Writing Group for the PEPI Trial, 1995); the *spironolactone* derivative *drospirenone* may have advantageous effects on the cardiovascular system due to its antiandrogenic and antimineralocorticoid activities. Progesterone also may diminish the effects of aldosterone in the renal tubule and cause a decrease in sodium reabsorption that may increase mineralocorticoid secretion from the adrenal cortex.

Pharmacology

Mechanism of Action

A single gene encodes two isoforms of the progesterone receptor (PR), PR-A and PR-B. The first 164 N-terminal amino acids of PR-B are missing from PR-A; this occurs by use of two distinct estrogen-dependent promoters in the PR gene (Giangrande and McDonnell, 1999). The ratios of the individual isoforms vary in reproductive tissues as a consequence of tissue type, developmental status, and hormone levels. Both PR-A and PR-B have AF-1 and AF-2 transactivation domains, but the longer PR-B also contains an additional AF-3 that contributes to its cell- and promoter-specific activity. Because the ligand-binding domains of the two PR isoforms are identical, there is no difference in ligand binding. In the absence of ligand, PR is present primarily in the nucleus in an inactive monomeric state bound to HSP90, HSP70, and p59. When receptors bind progesterone, the HSPs dissociate, and the receptors are phosphorylated and subsequently form dimers (homo- and heterodimers) that bind with high selectivity to PREs located on target genes (Giangrande and McDonnell, 1999). Transcriptional activation by PR occurs primarily via recruitment of coactivators such as steroid-receptor coactivator 1 (SRC-1), NcoA-1, or NcoA-2 (Collingwood et al., 1999). The receptor-coactivator complex then favors further interactions with additional proteins, such as CBP and p300, which mediate other processes, including histone acetylase activity. Histone acetylation causes remodeling of chromatin that increases the accessibility of general transcriptional proteins, including RNA polymerase II, to the target promoter.

The biological activities of PR-A and PR-B are distinct and depend on the target gene. In most cells, PR-B mediates the stimulatory activities of progesterone; PR-A strongly inhibits this action of PR-B and is also a transcriptional inhibitor of other steroid receptors (McDonnell and Goldman, 1994). Current data suggest that coactivators and corepressors interact differentially with PR-A and PR-B (e.g., the corepressor SMRT binds much more tightly to PR-A than to PR-B) (Giangrande et al., 2000), and this may account, at least in part, for the differential activities of the two isoforms. Female PR-A knockout mice are infertile, with impaired ovulation and defective decidualization and implantation. Several uterine genes appear to be regulated exclusively by PR-A, including calcitonin and amphiregulin (Mulac-Jericevic et al., 2000), and the antiproliferative effect of progesterone on the estrogen-stimulated endometrium is lost in PR-A knockout mice. In contrast, knockout studies suggested that PR-B is largely responsible for mediating hormone effects in the mammary gland (Mulac-Jericevic et al., 2003).

Certain effects of progesterone, such as increased Ca^{2+} mobilization in sperm, can be seen in as little as 3 min (Blackmore, 1999) and are therefore considered transcription independent. Similarly, progesterone can promote oocyte maturation (meiotic resumption) independent of transcription (Hammes, 2004).

ADME

Progesterone undergoes rapid first-pass metabolism, but high-dose (e.g., 100–200 mg) preparations of micronized progesterone are available for oral use. Although the absolute bioavailability of these preparations is low (Fotherby, 1996), efficacious plasma levels nevertheless may be obtained. Progesterone also is available in oil solution for injection, as a vaginal gel, and as a vaginal insert for assisted reproductive technology.

Esters such as MPA are available for intramuscular or subcutaneous administration, and MPA and *megestrol acetate* may be used orally. The 19-nor steroids have good oral activity because the ethinyl substituent at C17 significantly slows hepatic metabolism. Implants and depot preparations of synthetic progestins are available in many countries for release over very long periods of time (see section on contraceptives).

In the plasma, progesterone is bound by albumin and corticosteroid-binding globulin but is not appreciably bound to SHBG. 19-Nor compounds, such as *norethindrone*, *norgestrel*, and *desogestrel*, bind to SHBG and albumin, and esters such as MPA bind primarily to albumin. Total binding of all these synthetic compounds to plasma proteins is extensive, 90% or less, but the proteins involved are compound specific.

The elimination $t_{1/2}$ of progesterone is about 5 min, and the hormone is metabolized primarily in the liver to hydroxylated metabolites and their sulfate and glucuronide conjugates, which are eliminated in the urine. A major metabolite specific for progesterone is pregnane-3α,20α-diol; its measurement in urine and plasma is used as an index of endogenous progesterone secretion. The synthetic progestins have much longer $t_{1/2}$ (e.g., ~ 7 h for *norethindrone*, 16 h for *norgestrel*, 12 h for *gestodene*, and 24 h for MPA). The metabolism of synthetic progestins is thought to be primarily hepatic, and elimination is generally via the urine as conjugates and various polar metabolites.

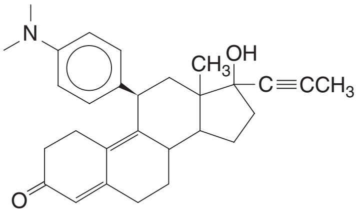
Antiprogestins and Progesterone Receptor Modulators

The first report of an antiprogestin, RU 38486 (often referred to as RU-486) or *mifepristone*, appeared in 1981; this drug is available for the termination of pregnancy (Christin-Maitre et al., 2000). In 2010, the FDA approved *ulipristal acetate*, a partial agonist at the progesterone receptor, for emergency contraception. Antiprogestins also have several other potential applications, including to prevent conception, to induce labor, and to treat uterine leiomyomas, endometriosis, meningiomas, and breast cancer (Spitz and Chwalisz, 2000).

Mifepristone

Chemistry

Mifepristone is a derivative of the 19-norprogestin *norethindrone* containing a dimethyl-aminophenol substituent at the 11β position. It effectively competes with both progesterone and glucocorticoids for binding to their respective receptors. *Mifepristone* is considered a progesterone receptor modulator (PRM) due to its context-dependent activity. Another widely studied antiprogestin is *onapristone* (or ZK 98299), which is similar in structure to *mifepristone* but contains a methyl substituent in the 13α rather than 13β orientation. More selective PRMs, such as *asoprisnil*, are being studied experimentally (DeManno et al., 2003).



MIFEPRISTONE

Pharmacological Effects

Mifepristone acts primarily as a competitive receptor antagonist for both PRs, although it may have some agonist activity in certain contexts. In contrast, *onapristone* appears to be a pure progesterone antagonist. PR complexes of both compounds antagonize the actions of progesterone-PR complexes and appear to preferentially recruit corepressors (Leonhardt and Edwards, 2002).

When administered in the early stages of pregnancy, *mifepristone* causes decidual breakdown by blockade of uterine PRs. This leads to detachment of the blastocyst, which decreases hCG production. This in turn causes a decrease in progesterone secretion from the corpus luteum, which further accentuates decidual breakdown. Decreased endogenous progesterone coupled with blockade of PRs in the uterus increases uterine prostaglandin (PG) levels and sensitizes the myometrium to their contractile actions. *Mifepristone* also causes cervical softening, which facilitates expulsion of the detached blastocyst.

Mifepristone can delay or prevent ovulation depending on the timing and manner of administration. These effects are due largely to actions on the hypothalamus and pituitary rather than the ovary, although the mechanisms are unclear.

If administered for one or several days in the mid- to late-luteal phase, *mifepristone* impairs the development of a secretory endometrium and produces menses. PR blockade at this time is the pharmacological equivalent of progesterone withdrawal, and bleeding normally ensues within several days and lasts for 1 to 2 weeks after antiprogestin treatment.

Mifepristone also binds to glucocorticoid and androgen receptors and exerts antiglucocorticoid and antiandrogenic actions. A predominant effect in humans is blockade of the feedback inhibition by cortisol of adenocorticotropic hormone secretion from the pituitary, thus increasing both corticotropin and adrenal steroid levels in the plasma.

ADME

Mifepristone is orally active with good bioavailability. Peak plasma levels occur within several hours, and the drug is slowly cleared, with a plasma $t_{1/2}$ of 20 to 40 h. In plasma, it is bound by α_1-acid glycoprotein, which contributes to the drug's long $t_{1/2}$. Metabolites are primarily the mono- and didemethylated products (thought to have pharmacological activity) formed via CYP3A4. The drug undergoes hepatic metabolism and enterohepatic circulation; metabolic products are found predominantly in the feces (Jang and Benet, 1997).

Therapeutic Uses

Mifepristone, in combination with *misoprostol* or other PGs, is available for the termination of early pregnancy. When *mifepristone* is used to produce a medical abortion, a PG is given 48 h after the antiprogestin to further increase myometrial contractions and ensure expulsion of the detached blastocyst. Intramuscular *sulprostone*, intravaginal *gemeprost*, and oral *misoprostol* have been used. The success rate with such regimens is greater than 90% among women with pregnancies of 49 days' duration or less. The most severe untoward effect is vaginal bleeding, which most often lasts 8 to 17 days but is only rarely (0.1% of patients) severe enough to require blood transfusions. High percentages of women also have experienced abdominal pain and uterine cramps, nausea, vomiting, and diarrhea due to the PG. Women receiving chronic glucocorticoid therapy should not be given *mifepristone* because of its antiglucocorticoid activity. In fact, due to its high affinity for the glucocorticoid receptor, high doses of *mifepristone* can result in adrenal insufficiency, and *mifepristone* is FDA-approved for the management of the excess glucocorticoid production seen in Cushing syndrome.

Ulipristal

Chemistry

Ulipristal, a derivative of 19-norprogesterone, functions as a selective progesterone receptor modulator, acting as a partial agonist at PRs. Unlike *mifepristone*, *ulipristal* appears to be a relatively weak glucocorticoid antagonist.

Pharmacological Effects

In high doses, *ulipristal* has antiproliferative effects in the uterus; however, its most relevant actions to date involve its capacity to inhibit ovulation. *Ulipristal*'s antiovulatory actions likely occur due to progesterone regulation at many levels, including inhibition of LH release through the hypothalamus and pituitary and inhibition of LH-induced follicular rupture within the ovary.

A 30-mg dose of *ulipristal* can inhibit ovulation when taken up to 5 days after intercourse. *Ulipristal* can block ovarian rupture at or even just after the time of the LH surge, confirming that at least some of its effects are directly in the ovary.

Therapeutic Uses

Ulipristal acetate is licensed in the E.U. and the U.S. as an emergency contraceptive. Studies comparing *ulipristal* to *levonorgestrel* (progesterone-only emergency contraception) demonstrate that *ulipristal* is at least as effective when taken up to 72 h after unprotected sexual intercourse. In addition, *ulipristal* remains effective up to 120 h (5 days) after intercourse, making *ulipristal* a more versatile emergency contraceptive than *levonorgestrel*, which does not work well beyond 72 h after unprotected intercourse. The most severe side effect in clinical trials using *ulipristal* has been a self-limited headache and some abdominal pain.

Perspective: Too Many People?

The incredible growth of the earth's human population stands out as one of the fundamental events of the last two centuries. The Old Testament dictum "be fruitful and multiply" (Genesis 9:1) has been followed too religiously by readers and nonreaders of the Bible alike. In 1798, Malthus started a great controversy by opposing the prevailing view of unlimited progress for humankind by making two postulates and a conclusion. Malthus postulated "that food is necessary for the existence of man" and that sexual attraction between female and male is necessary and likely to persist because "toward the extinction of the passion between the sexes, no progress whatever has hitherto been made," barring "individual exceptions." Malthus concluded that "the power of populations is infinitely greater than the power of the earth to produce subsistence for man," producing a "natural inequality" that would someday loom "insurmountable in the way to perfectibility of society."

Malthus was right: Passion between the sexes persists, and the power of populations is very great indeed, so much so that our sheer numbers have increased to the point that they are straining Earth's capacity to supply food, energy, and raw materials and to absorb the detritus of its human burden. Marine fisheries are being depleted, forests and aquifers are disappearing, and the atmosphere is accumulating greenhouse gases from combustion of the fossil fuels that provide the energy needs of 7 billion people, up from 1 billion in Malthus's day. Perhaps some of the blame can be laid at the feet of medical science: Advances in public health and medicine have led to a significant decline in mortality and an increased life expectancy. However, medical science has also begun to assume a portion of the responsibility for overpopulation and its adverse effects. To this end, drugs in the form of hormones and their analogues have been developed to control human fertility.

Therapeutic Uses of Estrogens and Progestins

Hormonal Contraception

Types of Hormonal Contraceptives

Combination Oral Contraceptives. The most frequently used agents in the U.S. are combination oral contraceptives containing both an estrogen and a progestin. These agents come in a variety of formulations and strengths (Table 48–2). Their theoretical efficacy generally is considered

TABLE 48–2 ■ FORMULATIONS OF REPRESENTATIVE ORAL CONTRACEPTIVES

PRODUCT	FORMULATION	
COMBINATION[a] MONOPHASIC	**ESTROGEN (μg)**	**PROGESTIN (mg)**
Ethinyl estradiol/desogestrel	25	0.15
Ethinyl estradiol/drospirenone (formulated with and without 28 tabs containing 0.451 mg levomefolate calcium)	30	3
Ethinyl estradiol/levonorgestrel (formulated with and without 28 tabs containing 36.5 mg ferrous bisglycinate)	20	0.1
Ethinyl estradiol/levonorgestrel	30	0.15
Ethinyl estradiol/levonorgestrel (21 tabs also containing 75 mg ferrous fumerate)	30	0.3
Ethinyl estradiol/norgestrel	30	0.3
Ethinyl estradiol/norethindrone	20[b]	1
	30[b]	1.5
	35[c]	0.4
	35	0.5
	35	1
Ethinyl estradiol/norgestimate	35	0.25
	35	0.215
	25	0.215
	25	0.25
COMBINATION BIPHASIC	**ESTROGEN (μg)**	**PROGESTIN (mg)**
Ethinyl estradiol/desogestrel (note: 28-day packs contain 2 inert pills to be taken on days 22 and 23)	20	0.15 (21 tabs)
	10	0 (5 tabs)
Ethinyl estradiol/norethindrone acetate (Lo Loestrin Fe) 28-day packs are copackagd with 2 tablets containing 75 mg ferrous fumarate	10	1 (24 tabs)
	10	0 (2 tabs)
COMBINATION TRIPHASIC	**ESTROGEN (μg)**	**PROGESTIN (mg)**
Ethinyl estradiol/desogestrel	25	0.1 (7 tabs)
	25	0.125 (7 tabs)
	25	0.15 (7 tabs)
Ethinyl estradiol/levonorgestrel	20	1 (7 tabs)
	30	1 (7 tabs)
	35	1 (7 tabs)
Ethinyl estradiol/norethindrone	35	0.5 (7 tabs)
	35	0.75 (7 tabs)
	35	1 (7 tabs)
Ethinyl estradiol/norethindrone	35	0.5 (7 tabs)
	35	1 (9 tabs)
	35	0.5 (5 tabs)
Ethinyl estradiol/norethindrone	20	1 (7 tabs)
	30	1 (7 tabs)
	35	1 (7 tabs)
COMBINATION ESTROPHASIC	**ESTROGEN (μg)**	**PROGESTIN (mg)**
Ethinyl estradiol/norethindrone	20	1 (5 tabs)
	30	1 (7 tabs)
	35	1 (9 tabs)

(Continued)

TABLE 48–2 ■ FORMULATIONS OF REPRESENTATIVE ORAL CONTRACEPTIVES (*CONTINUED*)

PRODUCT	FORMULATION	
COMBINATION EXTENDED CYCLE	**ESTROGEN (µg)**	**PROGESTIN (mg)**
Ethinyl estradiol/drospirenone (formulated with and without 28 tabs containing 0.451 mg levomefolate calcium)	20	3 (24 tabs)
Ethinyl estradiol/levonorgestrel	30	0.15 (84 tabs)
	10	0 (7 tabs)
Ethinyl estradiol/levonorgestrel	20	0.1 (84 tabs)
	10	0 (7 tabs)
Ethinyl estradiol/levonorgestrel	20	0.15 (42 tabs)
	25	0.15 (21 tabs)
	30	15 (21 tabs)
	10	0 (7 tabs)
Ethinyl estradiol/norethindrone, copackagd with 4 tablets containing 75 mg ferrous fumarate (the 20/1 formulation is also available as chewable tablets and soft gelatin capsules)	25	0.8 (24tabs)
	20	1 (24 tabs)
Estetrol/drospirenone (FDA-approved April 15, 2021)	14.2 mg	3 (24 tabs)
PROGESTIN ONLY	**ESTROGEN (µg)**	**PROGESTIN (mg)**
Drospirenone (SLYND)	—	4 (24 tabs packaged with 4 inactive tabs)

Unless otherwise indicated, the products are packaged with 21 active (hormone-containing) pills and 7 placebo tablets. For formulations that differ from this standard (e.g., multiphasic pills, extended-cycle formulations), the number of tablets of each pill strength are indicated. Some formulations also contain iron to diminish the risk of iron deficiency anemia; these are not listed separately here.

[a] Combination formulations contain both an estrogen and a progestin.

[b] Packaged with and without seven tablets containing 75 mg ferrous fumarate.

[c] Available as a chewable tablet.

to be 99.9%. In practice, the 1-year failure rates of oral contraceptives are somewhat greater than 0.1% (Table 48–3). Combination oral contraceptives are available in many formulations. Almost all contain ethinyl estradiol as the estrogen and a 17α-alkyl-19-nortestosterone derivative as the progestin. Monophasic, biphasic, or triphasic pills are generally provided in 28-day packs, with the pills for the last 7 days containing only inert ingredients. For the monophasic agents, fixed amounts of the estrogen and progestin are present in each pill, which is taken daily for 21 days, followed by a 7-day "pill-free" period. The biphasic and triphasic preparations provide two or three different pills containing varying amounts of active ingredients, to be taken at different times during the 21-day cycle. This reduces the total amount of steroids administered and more closely approximates the estrogen-to-progestin ratios that occur during the menstrual cycle. With these preparations, predictable menstrual bleeding generally occurs during the 7-day "off" period each month. However, several oral contraceptives are now available whereby progestin withdrawal is only induced every 3 months or not at all.

TABLE 48–3 ■ ONE-YEAR FAILURE RATE WITH VARIOUS FORMS OF CONTRACEPTION

BIRTH CONTROL METHOD	FAILURE (*Perfect Use*)	RATE (%) (*Typical Use*)
Combination oral contraceptive pills	0.3	8
Progestin-only minipill	0.5	8
Depo-Provera	0.3	3
Copper intrauterine device	0.6	0.8
Progestin intrauterine device	0.2	0.2
Implanon	0.05	0.05
Ortho Evra	0.3	8
NuvaRing	0.3	8
Condoms/diaphragms	2	15
Spermicides	18	9
Tubal ligation	0.5	0.5
Vasectomy	0.1	0.15
None	85	85

The estrogen content of current preparations ranges from 20 to 35 µg. The dose of progestin is more variable because of differences in potency of the compounds used.

A transdermal preparation of *levonorgestrel* and *ethinyl estradiol* is marketed for reproductive-age women with a body mass index less than 30 kg/m^2 as a weekly application to the buttock, abdomen, or upper torso for the first 3 consecutive weeks followed by a patch-free week for each 28-day cycle. A similar 3-week-on/1-week-off cycle is employed for a disposable intravaginal ring containing *ethinyl estradiol* and *etonogestrel* and a 1-year durable intravaginal ring containing *ethinyl estradiol* and *segesterone acetate*.

Progestin-Only Contraceptives.

Several agents are available for progestin-only contraception, with theoretical efficacies of 99%. Specific preparations include the "minipill"; low doses of progestins (e.g., 350 µg of *norethindrone*) taken daily without interruption; subdermal implants of 68 mg of *etonogestrel* for contraception lasting 3 years; and crystalline suspensions of MPA for injection of 104 mg (SC) or 150 mg (IM) of drug.

Intrauterine Devices. Three doses of *levonorgestrel*-releasing intrauterine systems (IUSs) are available in the U.S. The LNg20 contains 52 mg of *levonorgestrel* (LNg), which is initially released at a rate of 20 µg/day and declines gradually to 10 to 14 µg/day after 5 years. A smaller LNg IUS is available for women with a small uterine cavity or cervical stenosis and may result in less pain with insertion. The LNg14 contains 13.5 mg of *levonorgestrel*, which is initially released at a rate of 14 µg/day and declines to 5 µg/day after 3 years. A third device, also smaller, contains 19.5 mg of *levonorgestrel* and similarly last 3 years. A copper IUD, TCu380A, is also available in the U.S. It contains 380 mm^2 of copper and is approved for 10-year use. The TCu380A may be preferred over an LNg IUS in women who desire long-term contraception and wish to avoid exogenous hormones and hormonal side effects and can also be used as an emergency contraceptive.

Mechanism of Action

Combination Oral Contraceptives. Combination oral contraceptives act by preventing ovulation. Direct measurements of plasma hormone levels indicate that LH and FSH levels are suppressed, a midcycle surge of LH is absent, endogenous steroid levels are diminished, and ovulation does not occur. Although either component alone can be shown to exert these effects in certain situations, the combination synergistically decreases plasma gonadotropin levels and suppresses ovulation more consistently than either alone.

Hormonal Contraception: A Brief History

Around the beginning of the 20th century, a number of European scientists, including Beard, Prenant, and Loeb, developed the concept that secretions of the corpus luteum suppressed ovulation during pregnancy. The Austrian physiologist Haberlandt then produced temporary sterility in rodents in 1927 by feeding them ovarian and placental extracts—a clear example of an oral contraceptive. In 1937, Makepeace and colleagues demonstrated that pure progesterone blocked ovulation in rabbits, and Astwood and Fevold found a similar effect in rats in 1939.

In the 1950s, Pincus, Garcia, and Rock found that progesterone and 19-norprogestins prevented ovulation in women. Ironically, this finding grew out of their attempts to treat infertility with estrogen-progestin combinations. The initial findings were that these treatments effectively blocked ovulation in most women. However, concern about cancer and other possible side effects of the estrogen they used (i.e., *diethylstilbestrol*) led to the use of a progestin alone in their studies. One of the compounds used was *norethynodrel*, and early batches of this compound were contaminated with a small amount of *mestranol*. When *mestranol* was removed, it was noted that treatment with pure *norethynodrel* led to increased breakthrough bleeding and less-consistent inhibition of ovulation. *Mestranol* was thus reincorporated into the preparation, and this combination was employed in the first large-scale clinical trial of combination oral contraceptives.

Clinical studies in the 1950s in Puerto Rico and Haiti established the virtually complete contraceptive success of the *norethynodrel/mestranol* combination. In early 1961, Enovid (*norethynodrel* plus *mestranol*; no longer marketed in the U.S.) was the first "Pill" approved by the FDA for use as a contraceptive agent in the U.S.; this was followed in 1962 by approval for Ortho-Novum (*norethindrone* plus *mestranol*). By 1966, numerous preparations using either *mestranol* or *ethinyl estradiol* with a 19-norprogestin were available. In the 1960s, the progestin-only minipill and long-acting injectable preparations were developed and introduced.

Millions of women began using oral contraceptives, and frequent reports of untoward effects began appearing in the 1970s. The recognition that these side effects were dose dependent and the realization that estrogens and progestins synergistically inhibited ovulation led to the reduction of doses and the development of low-dose or second-generation contraceptives. The increasing use of biphasic and triphasic preparations throughout the 1980s further reduced steroid dosages; it may be that currently used doses are the lowest that will provide reliable contraception. In the 1990s, the "third-generation" oral contraceptives, containing progestins with reduced androgenic activity (e.g., *norgestimate* and *desogestrel*), became available in the U.S. after being used in Europe. A variety of contraceptive formulations are currently available, including pills, injections, skin patches, subdermal implants, vaginal rings, and intrauterine devices (IUDs) that release hormones.

Given the multiple actions of estrogens and progestins on the hypothalamic-pituitary-ovarian axis during the menstrual cycle, several effects probably contribute to the blockade of ovulation.

Hypothalamic actions of steroids play a major role in the mechanism of oral contraceptive action. Progesterone diminishes the frequency of GnRH pulses. Because the proper frequency of LH pulses is essential for ovulation, this effect of progesterone likely plays a major role in the contraceptive action of these agents.

Multiple pituitary effects of both estrogen and progestin components are thus likely to contribute to oral contraceptive action. Oral contraceptives seem likely to decrease pituitary responsiveness to GnRH. Estrogens also suppress FSH release from the pituitary during the follicular phase of the menstrual cycle, and this effect seems likely to contribute to the lack of follicular development in oral contraceptive users. The progestin component may also inhibit the estrogen-induced LH surge at midcycle. Other effects may contribute to a minor extent to the extraordinary efficacy of oral contraceptives. Transit of sperm, the egg, and fertilized ovum are important to establish pregnancy, and steroids are likely to affect transport in the fallopian tube. In the cervix, progestin effects also are likely to produce a thick, viscous mucus to reduce sperm penetration and in the endometrium to produce a state that is not receptive to implantation. However, it is difficult to assess quantitatively the contributions of these effects because the drugs block ovulation.

Progestin-Only Contraceptives. Progestin-only pills and *levonorgestrel* implants are highly efficacious but block ovulation in only 60% to 80% of cycles. Their effectiveness is thought to be due largely to a thickening of cervical mucus, which decreases sperm penetration, and to endometrial alterations that impair implantation; such local effects account for the efficacy of IUDs that release progestins. Depot injections of MPA are thought to exert similar effects, but they also yield plasma levels of drug high enough to prevent ovulation in virtually all patients, presumably by decreasing the frequency of GnRH pulses.

Intrauterine Devices. While the contraceptive benefit of the LNg IUS is attributed to the progestin-mediated effects of thickening of cervical mucous and endometrial alterations, the contraceptive mechanism of the copper IUD is related to an inflammatory reaction within the endometrium that impairs sperm viability, motility, and fertilization.

Untoward Effects

Combination Oral Contraceptives. Untoward effects of early hormonal contraceptives fell into several major categories: adverse cardiovascular effects, including hypertension, myocardial infarction, hemorrhagic or ischemic stroke, and venous thrombosis and embolism; breast, hepatocellular, and cervical cancers; and several endocrine and metabolic effects. The current consensus is that low-dose preparations pose minimal health risks in women who have no predisposing risk factors, and these drugs also provide many beneficial health effects (Burkman et al., 2004).

Cardiovascular Effects. The question of cardiovascular side effects has been reexamined for low-dose oral contraceptives (Burkman et al., 2004). For nonsmokers without other risk factors such as hypertension or diabetes, there is no significant increase in the risk of myocardial infarction or stroke. There is a 28% increase in relative risk for venous thromboembolism, but the estimated absolute increase is very small because the incidence of these events in women without other predisposing factors is low (e.g., roughly half that associated with the risk of venous thromboembolism in pregnancy). The risk is significantly increased in women who smoke or have other factors that predispose to thrombosis or thromboembolism (Castelli, 1999). Postmarketing epidemiological studies indicated that women using transdermal contraceptives have a higher-than-expected exposure to estrogen and are at increased risk for the development of venous thromboembolism. Early high-dose combination oral contraceptives caused hypertension in 4% to 5% of normotensive women and increased blood pressure in 10% to 15% of those with preexisting hypertension. This incidence is much lower with newer low-dose preparations, and most reported changes in blood pressure are not significant. Estrogens increase serum HDL and decrease LDL levels, and progestins tend to have the opposite effect. Recent studies of several low-dose preparations have not found significant changes in total serum cholesterol or lipoprotein profiles, although slight increases in triglycerides have been reported.

Cancer. Given the growth-promoting effects of estrogens, there has been a long-standing concern that oral contraceptives might increase the incidence of endometrial, cervical, ovarian, breast, and other cancers. These concerns were further heightened in the late 1960s by reports of endometrial changes caused by sequential oral contraceptives, which have since been removed from the market in the U.S. However, it is now clear that there is *not* a widespread association between oral contraceptive use and cancer (Burkman et al., 2004; Westhoff, 1999).

Epidemiological evidence suggests that combined oral contraceptive use may increase the risk of cervical cancer by about 2-fold but only in long-term (>5 years) users with persistent human papillomavirus infection (Moodley, 2004).

There have been reports of increases in the incidence of hepatic adenoma and hepatocellular carcinoma in oral contraceptive users. Current estimates indicate there is about a doubling in the risk of liver cancer after 4 to 8 years of use. However, these are rare cancers, and the absolute increases are small.

The major present concern about the carcinogenic effects of oral contraceptives is focused on breast cancer. The risk of breast cancer in women of childbearing age is very low, and current oral contraceptive users in this group have only a very small increase in relative risk of 1.1 to 1.2, depending on other variables. This small increase is not substantially affected by duration of use, dose or type of component, age at first use, or parity. Importantly, 10 years after discontinuation of oral contraceptive use, there is no difference in breast cancer incidence between past users and never users. In addition, breast cancers diagnosed in women who have ever used oral contraceptives are more likely to be localized to the breast and thus easier to treat (Westhoff, 1999).

Combination oral contraceptives decrease the incidence of endometrial cancer by 50%, an effect that lasts 15 years after the pills are stopped. This is thought to be due to the inclusion of a progestin, which opposes estrogen-induced proliferation, throughout the entire 21-day cycle of administration. These agents also decrease the incidence of ovarian cancer. There are accumulating data that oral contraceptive use decreases the risk of colorectal cancer (Fernandez et al., 2001).

Metabolic and Endocrine Effects. The effects of sex steroids on glucose metabolism and insulin sensitivity are complex (Godsland, 1996) and may differ among agents in the same class (e.g., the 19-norprogestins). Early studies with high-dose oral contraceptives generally reported impaired glucose tolerance; these effects have decreased as steroid dosages have been lowered, and current low-dose combination contraceptives may even improve insulin sensitivity. Similarly, the high-dose progestins in early oral contraceptives did raise LDL and reduce HDL levels, but modern low-dose preparations do not produce unfavorable lipid profiles (Sherif, 1999). There also have been periodic reports that oral contraceptives increase the incidence of gallbladder disease, but any such effect appears to be weak and limited to current or very long-term users (Burkman et al., 2004).

The estrogenic component of oral contraceptives may increase hepatic synthesis of a number of serum proteins, including those that bind thyroid hormones, glucocorticoids, and sex steroids. Although physiological feedback mechanisms generally adjust hormone synthesis to maintain normal "free" hormone levels, these changes can affect the interpretation of endocrine function tests that measure *total* plasma hormone levels and may necessitate dose adjustment in patients receiving thyroid hormone replacement.

The *ethinyl estradiol* present in oral contraceptives appears to cause a dose-dependent increase in several serum factors known to increase coagulation. However, in healthy women who do not smoke, there also is an increase in fibrinolytic activity, which exerts a countereffect so that overall there is a minimal effect on hemostatic balance. This compensatory effect is diminished in smokers (Fruzzetti, 1999).

Miscellaneous Effects. Nausea, edema, and mild headache occur in some individuals, and more severe migraine headaches may be precipitated by oral contraceptive use in a smaller fraction of women. Some patients may experience breakthrough bleeding during the 21-day cycle when the active pills are being taken. Withdrawal bleeding may fail to occur in a small fraction of women during the 7-day off period, thus causing confusion about a possible pregnancy. Acne and hirsutism are thought to be mediated by the androgenic activity of the 19-norprogestins.

Progestin-Only Contraceptives. Episodes of irregular, unpredictable spotting and breakthrough bleeding are the most frequently encountered untoward effect and the major reason women discontinue use of all three types of progestin-only contraceptives. With time, the incidence of these bleeding episodes decreases.

No evidence indicates that the progestin-only minipill preparations increase thromboembolic events, which are thought to be related to the estrogenic component of combination preparations. Acne may be a problem because of the androgenic activity of *norethindrone*-containing preparations. These preparations may be attractive for nursing mothers because they do not decrease lactation as do products containing estrogens.

Headache is the most reported untoward effect of depot MPA. Mood changes and weight gain also have been reported, but controlled clinical studies of these effects are not available. Many studies have found decreases in HDL levels and increases in LDL levels, and there have been several reports of decreased bone density. These effects may be due to reduced endogenous estrogens because depot MPA is particularly effective in lowering gonadotropin levels. Because of the time required to completely eliminate the drug, the contraceptive effect of this agent may remain for 6 to 12 months after the last injection.

Progesterone-only medications have been associated with decreased bone mineral density, as noted by a black-box warning in the product label. Teenagers and younger women who have not achieved maximal bone density may be particularly at risk, although the data suggest that bone density returns to pretreatment levels quickly after drug cessation.

Implants of *etonogestrel*, one of the most effective contraceptives available, may be associated with implant site reactions and changes in menstrual bleeding pattern but do not induce significant bone loss.

Intrauterine Devices. Intrauterine devices are generally well tolerated, although complications related to the device and side effects related to the progestin can occur. Expulsion of the device is greatest in the first year and has been reported in 3% to 6% of women with an LNg20 and in 3.2% of women with an LNg14. Malposition of the device, extending into the myometrium or the endocervical canal, occurs in 10% of women and is associated with difficult placement, uterine distortion, and obesity. For malposition that is symptomatic (e.g., pain or postinsertion bleeding extending beyond 3 months), displaced through the uterine serosa, or visible in the cervical canal, the device should be removed or replaced. Not all malpositioned devices need to be removed, as this condition is often asymptomatic and does not compromise the contraceptive efficacy of the device.

Uterine perforation at the time of IUD insertion complicates approximately 1 in 1000 insertions. Symptoms of perforation may include pelvic pain and bleeding, although perforations are often asymptomatic. Surgical removal of the perforated IUD is preferred to minimize serious complications related to adhesions or perforation into the bowel, bladder, or blood vessels.

Pelvic inflammatory disease is infrequent at the time of insertion (1–10 per 1000 women undergoing insertion) and after insertion (1.4 women per 1000 women after insertion). Infections at the time of insertion or 1 month after insertion are generally related to new sexually transmitted infections. The LNg20 is associated with less risk of pelvic inflammatory disease due to thickening of the cervical mucus. Oral antibiotic therapy may be attempted, and worsening infections should be treated with intravenous antibiotics and IUD removal.

Ectopic and intrauterine pregnancies rarely occur with an IUD *in situ*. Intrauterine pregnancies with an IUD *in situ* are at increased risk for adverse pregnancy outcomes if the IUD is left in place or removed. The decision to leave the IUD in place or remove it in pregnancy should be individualized based on the women's obstetrical history, the trimester when it is diagnosed, and the anticipated difficulty of removing the IUD.

While device-related complications are infrequent, side effects related to the progestin are common. Irregular bleeding in the first 3 to 6 months after insertion and amenorrhea at 1 year after insertion are common. Complaints of side effects such as hirsutism, acne, weight change, nausea, headache, mood change, and breast tenderness are related to systemic effects of *levonorgestrel* and are the most common reason for discontinuation (~12% of women with the LNg20). The copper IUD may be an alternative for women who discontinue the LNg IUS due to hormonal side effects, but it is associated with intermenstrual bleeding and increased volume of bleeding.

Contraindications

Modern oral contraceptives are considered generally safe in most healthy women; *however, these agents can contribute to the incidence and severity of cardiovascular, thromboembolic, or malignant disease, particularly if other risk factors are present.* Contraindications for combination oral contraceptive use are the following: the presence or history of thromboembolic disease, cerebrovascular disease, myocardial infarction, coronary artery disease, or congenital hyperlipidemia; known or suspected carcinoma of the breast or carcinoma of the female reproductive tract; abnormal undiagnosed vaginal bleeding; known or suspected pregnancy; and past or present liver tumors or impaired liver function. *The risk of serious cardiovascular side effects is particularly marked in women more than 35 years of age who smoke heavily*; even low-dose oral contraceptives are contraindicated in such patients.

Other relative contraindications include migraine headaches, hypertension, diabetes mellitus, obstructive jaundice of pregnancy or prior oral contraceptive use, and gallbladder disease. If elective surgery is planned, many physicians recommend discontinuation of oral contraceptives for several weeks to a month to minimize the possibility of thromboembolism after surgery.

Progestin-only contraceptives are contraindicated in the presence of undiagnosed vaginal bleeding, benign or malignant liver disease, and known or suspected breast cancer.

The Centers for Disease Control and Prevention U.S. medical eligibility criteria list all progestin-containing IUDs as category 2 for history of venous thromboembolism, which means a condition for which the advantages of using the method generally outweigh risks. The contraindications to IUDs are severe uterine distortion, active pelvic infection, and unexplained abnormal uterine bleeding. The copper IUD should be avoided in women with Wilson disease or a copper allergy.

Noncontraceptive Health Benefits

Oral contraceptives significantly reduce the incidence of ovarian and endometrial cancer within 6 months of use, and the incidence is decreased 50% after 2 years of use. Depot MPA injections also reduce very substantially the incidence of uterine cancer. This protective effect persists for up to 15 years after oral contraceptive use is discontinued. These agents also decrease the incidence of ovarian cysts and benign fibrocystic breast disease.

Oral contraceptives have major benefits related to menstruation in many women. These include more regular menstruation, reduced menstrual blood loss and less iron-deficiency anemia, and decreased frequency of dysmenorrhea. There also is a decreased incidence of pelvic inflammatory disease and ectopic pregnancies, and endometriosis may be ameliorated. Some women also may obtain these benefits with progestin-only contraceptives. There are suggestions that MPA may improve hematological parameters in women with sickle cell disease (Cullins, 1996).

IUDs such as the LNg20 reduce dysmenorrhea and menstrual blood loss. One year after insertion, 30% to 40% of women experience amenorrhea. The LNg20 can also be used to prevent and treat endometrial hyperplasia, although close monitoring is necessary, as endometrial adenocarcinoma has occurred in LNg20 users. While LNg14 is effective at preventing pregnancy, less is known regarding the noncontraceptive benefits of the LNg14. Even without considering the additional health benefits of these agents, fertility regulation by contraceptives is substantially safer than pregnancy or childbirth for most women.

Postcoital Contraception

Postcoital (or emergency) contraception is indicated for use in cases of mechanical failure of barrier devices or in circumstances of unprotected intercourse (Cheng et al., 2008). Because it is less effective than standard oral contraceptive regimens, it is not intended as a routine method of contraception. The mechanisms of action of the postcoital contraceptives are not fully understood, but their efficacy clearly cannot be accounted for solely by the inhibition of ovulation. Other potential mechanisms of action include effects on gamete function and survival and on implantation. These agents do not affect established pregnancies.

Both the copper IUD and the *levonorgestrel* 52-mg IUD are more effective than oral emergency contraceptive agents and can provide ongoing pregnancy prevention (Turok et al., 2021). The copper IUD or *levonorgestrel* 52-mg IUD can be inserted within 5 days after an unprotected act of sexual intercourse after a negative pregnancy test.

Selective PRMs such as *ulipristal* are approved as an emergency contraceptive, effective up to 120 h after unprotected intercourse. *Mifepristone* in oral doses ranging from 10 to 50 mg when taken within 5 days after unprotected intercourse can also be used but is not FDA-approved.

Plan B ONE STEP, which contains a single 1.5-mg tablet of *levonorgestrel*, can be taken as emergency contraception within 72 h after unprotected sex. Plan B ONE STEP and similar generic forms are available over the counter and without a prescription.

Termination of Pregnancy

If contraception is not used or fails, either *mifepristone* (RU-486) or *methotrexate* (50 mg/m^2 intramuscularly or orally) can be used to terminate an unwanted pregnancy in settings outside surgical centers. A PG then is administered to stimulate uterine contractions and expel the detached conceptus; in the U.S., PGs used include *dinoprostone* (PGE$_2$) administered vaginally or the PGE$_1$ analogue *misoprostol* given orally or vaginally. PGs used in other countries include the PGE$_2$ analogue *sulprostone* and the PGE$_1$ analogue *gemeprost*.

Mifepristone (600 mg) is FDA-approved for pregnancy termination within 49 days after the start of a woman's last menstrual period. The synthetic PGE$_1$ analogue *misoprostol* (400 μg) is administered orally 48 h later; vaginal administration is at least as effective but is not FDA-approved. Complete abortion using this procedure exceeds 90%; when termination of pregnancy fails or is incomplete, surgical intervention is required. Other published regimens include lower doses of *mifepristone* (200 or 400 mg) and different time intervals between the mifepristone and *misoprostol*. Finally, repeated doses of *misoprostol* alone (e.g., 800 μg vaginally or sublingually every 3 h or every 12 h for three doses) also have been effective in settings where *mifepristone* is unavailable. Vaginal bleeding follows pregnancy termination and typically lasts from 1 to 2 weeks but rarely (in 0.1% of patients) is severe enough to require blood transfusion. A high percentage of women also experience abdominal pain and uterine cramps, nausea and vomiting, and diarrhea secondary to the PG. Myocardial ischemia and infarction have been reported in association with *sulprostone* and *gemeprost*.

Because *mifepristone* carries a risk of serious, and sometimes fatal, infections and bleeding following its use for medical abortion, a black-box warning has been added to the product labeling. Fulminant septic shock associated with *Clostridium sordellii* infections may result and is attributable to the combined effects of uterine infection and inhibition of glucocorticoid action by *mifepristone* (Cohen et al., 2007). Patients who develop symptoms and signs of infection, especially marked leukocytosis even without fever, should be treated aggressively with antibiotics effective against anaerobic organisms such as *C. sordellii* (e.g., *penicillin*, *ampicillin*, a macrolide, *clindamycin*, a tetracycline, or *metronidazole*).

Induction of Sexual Maturation

Estrogen Treatment in the Failure of Ovarian Development

In several conditions (e.g., Turner syndrome), the ovaries do not develop, and puberty does not occur. Therapy with estrogen at the appropriate time replicates the events of puberty, and androgens (see Chapter 49) or growth hormone (see Chapter 46) may be used concomitantly to promote normal growth. Although estrogens and androgens promote bone growth, they also accelerate epiphyseal fusion, and their premature use can thus result in shorter ultimate height.

Types of estrogens used and the treatment regimens may vary by country or individual preference. Examples include conjugated estrogens, 0.3 to 1.25 mg; micronized 17β-estradiol, 0.5 to 2.0 mg; ethinyl estradiol, 5 to 20 μg; and transdermal 17β-estradiol, 25 to 50 μg. To achieve optimal breast development, treatment typically is initiated with a low dose of estrogen (e.g., conjugated estrogens at a starting dosage of 0.3 mg/day or *ethinyl estradiol* at 5 μg/day) starting in patients between ages 10 and 12 years or immediately if the diagnosis is made after this age. After 3 to 6 months, the dosage is increased (e.g., 0.9–1.25 mg/day of conjugated estrogens or 20 μg/day of *ethinyl estradiol*). Once this is achieved, a progestin (e.g., *medroxyprogesterone*, 10 mg/day, or micronized progesterone, 200–400 μg/day) for 12 days each cycle is added to the regimen to optimize breast development and permit cyclical menses, thereby avoiding endometrial hyperplasia and its consequent risk of uterine cancer. Once menses are established, many clinicians will switch to a standard low-dose oral contraceptive pill or even may use an extended-cycle formulation.

Short stature, a universal feature of nonmosaic Turner syndrome, usually is treated with human growth hormone, often together with an androgen such as *oxandrolone* (see Chapter 49). Initiating treatment with human growth hormone and an androgen and delaying the onset of estrogen therapy generally produces better growth response. Doses for growth hormone treatment in this context are higher than those in growth hormone–deficient children (e.g., 67 μg/kg per day; see Chapter 46 for further discussion of growth hormone replacement therapy).

Induction of Ovulation

Infertility (i.e., the failure to conceive after 1 year of unprotected sex) affects about 10% to 15% of couples in developed nations and is increasing in incidence as more women choose to delay childbearing until later in life. The cause of infertility is attributed primarily to the woman in approximately one-third of cases, to the man in approximately one-third, and to both in approximately one-third.

Anovulation accounts for about 50% of female infertility and is a major focus of pharmacological interventions used to achieve conception. Although a history of regular cyclic bleeding is strong presumptive evidence for ovulation, assessment of urine LH levels with an ovulation predictor kit or measurement of the serum progesterone levels during the luteal phase provides more definitive information. Evaluation of anovulation may uncover PCOS, thyroid disorders, hyperprolactinemia, or hypogonadism, but the cause is often idiopathic.

A number of approaches have been used to stimulate ovulation in anovulatory women. Often, a stepwise approach is taken, initially using simpler and less expensive treatments, followed by more complex and expensive regimens if initial therapy is unsuccessful.

Clomiphene

Clomiphene citrate was reviewed previously in this chapter. A typical regimen is 50 mg/day orally for 5 consecutive days starting between days 2 and 5 of the cycle in women who have spontaneous uterine bleeding or following a bleed induced by progesterone withdrawal in women who do not. If this regimen fails to induce ovulation, the dose of *clomiphene* is increased, first to the FDA-approved maximum of 100 mg/day and possibly to higher levels of 150 or 200 mg/day. Although *clomiphene* is effective in inducing ovulation in perhaps 75% of women, successful pregnancy ensues in only 40% to 50% of those who ovulate. This has been attributed to *clomiphene*'s inhibition of estrogen action on the endometrium, resulting in an environment that is not optimal for fertilization or implantation.

Aromatase Inhibitors

Aromatase inhibitors (e.g., *letrozole*, 2.5–7.5 mg/day for 5 days, typically starting on day 3 of the cycle) induce follicle development by inhibiting estrogen biosynthesis, thus decreasing estrogen negative feedback and increasing FSH levels and follicle development. In comparing *letrozole* and *clomiphene* for ovulation induction in women with PCOS and infertility, *letrozole* was associated with a higher pregnancy and live birth rate (Legro et al., 2014). *Letrozole* is associated with fewer estrogen deprivation side effects (hot flashes, mood change) and possibly fewer multifetal gestations than *clomiphene*.

Gonadotropins

The preparations of gonadotropins available for clinical use are detailed in Chapter 46. Gonadotropins are indicated for ovulation induction in anovulatory women with hypogonadotropic hypogonadism secondary to hypothalamic or pituitary dysfunction. Gonadotropins also are used to induce ovulation in women with polycystic ovary syndrome (PCOS) who do not respond to *clomiphene*.

Given the marked increases in maternal and fetal complications associated with multifetal gestation, the goal of ovulation induction in anovulatory women is to induce the formation and ovulation of a single dominant follicle. Generally, the increased risks of twin gestation will be accepted if two follicles are present.

As shown in Figure 48–6, a typical regimen for ovulation induction is to administer 75 IU of FSH daily in a "low-dose, step-up protocol." The dose is titrated based on the rise in estradiol and the growth rate of follicles as determined by estradiol levels and transvaginal ultrasonography. If three or more mature follicles are induced, gonadotropin therapy can be canceled, and barrier contraception can be used to prevent pregnancy, thereby avoiding multifetal pregnancy.

To complete follicular maturation and induce ovulation, chorionic gonadotropin (5000–10,000 IU) or choriogonadotropin alfa is given 1 day after the last dose of gonadotropin. Fertilization of the oocyte(s) at 36 h after choriogonadotropin administration then is attempted, by either intercourse or intrauterine insemination.

Gonadotropin induction also is used for ovarian stimulation in conjunction with *in vitro* fertilization (IVF) (Figure 48–6). In this setting, larger doses of FSH (typically 225–300 IU/day) are administered to induce the maturation of multiple (ideally at least 5 and up to 20) oocytes that can be retrieved for IVF. To prevent the LH surge and subsequent premature luteinization of the ovarian follicles, gonadotropins typically are administered in conjunction with a GnRH agonist or a GnRH antagonist. The length of the IVF protocol is predicated by the initial flare of gonadotropin secretion that occurs in response to the GnRH agonists. In the long protocol, the agonist is started in the luteal phase of the previous cycle (generally on cycle day 21) and then maintained until the time of choriogonadotropin injection to induce ovulation. Alternatively, in the "flare" protocol, the GnRH agonist is started on cycle day 2 (immediately after the start of menses), and gonadotropin injections are added 1 day later. In the GnRH antagonist "short protocol," the antagonist can be used to inhibit endogenous LH secretion and is typically started after follicular recruitment is initiated. Current regimens include daily injection in a dose of 0.25 mg (*ganirelix* or *cetrorelix*) starting on the fifth or sixth day of gonadotropin stimulation or a single dose of 3 mg of *cetrorelix* administered on day 8 or 9 of the late follicular phase. Adequate follicle maturation typically takes 8 to 12 days after gonadotropin therapy is initiated.

Using either the long or short protocols, choriogonadotropin (at typical doses of 5000–10,000 IU of urine-derived product or 250 μg of choriogonadotropin alfa) is given to induce final oocyte development, and the mature eggs are retrieved from the preovulatory follicles at 36 h thereafter. The ova are retrieved by transvaginal ultrasound-guided aspiration and

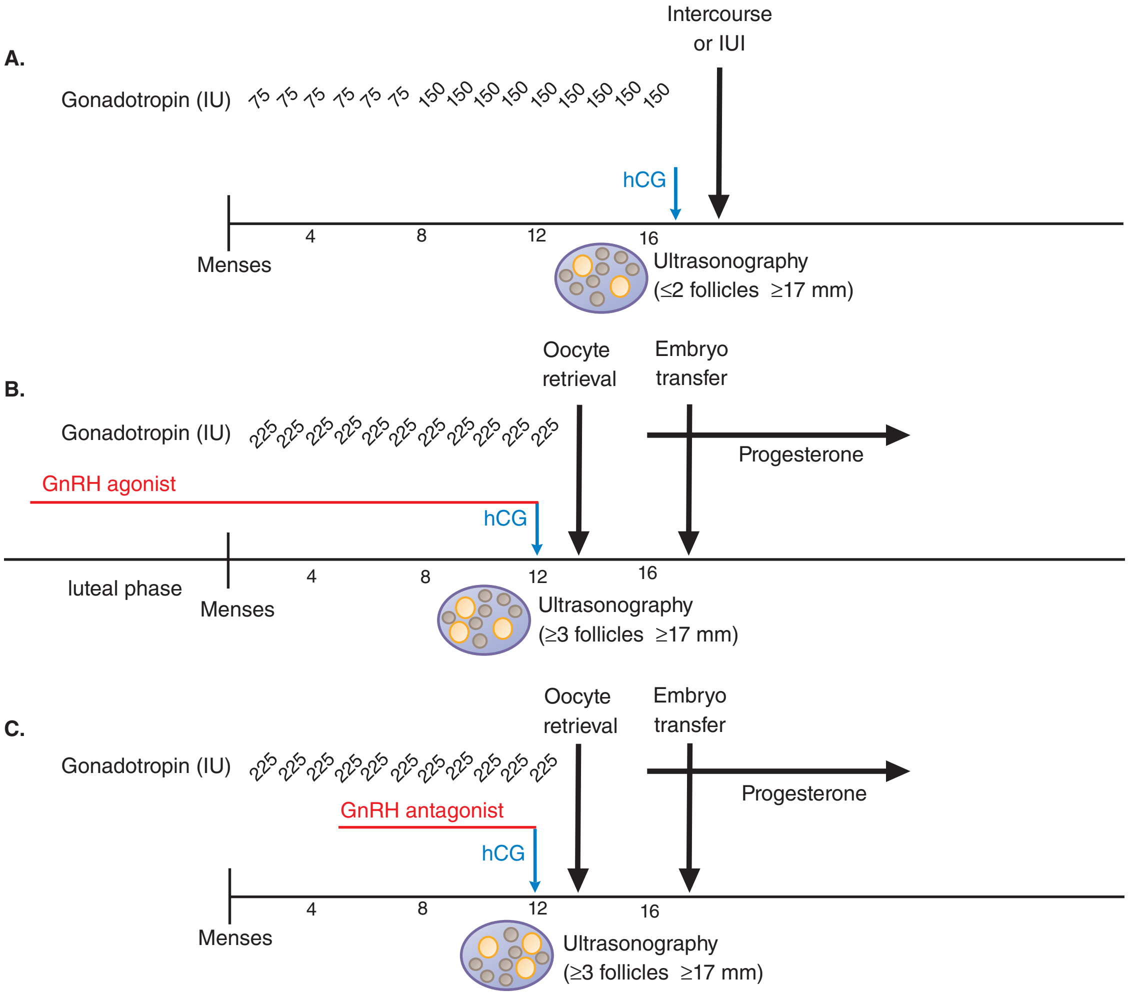

A.
Gonadotropin (IU)
75 75 75 75 75 75 150 150 150 150 150 150 150 150 150
Intercourse or IUI
hCG
4
8
12
16
Menses
Ultrasonography (≤2 follicles ≥17 mm)
B.
Oocyte retrieval
Embryo transfer
Gonadotropin (IU)
225 225 225 225 225 225 225 225 225 225 225
Progesterone
GnRH agonist
hCG
luteal phase
Menses
4
8
12
16
Ultrasonography (≥3 follicles ≥17 mm)
C.
Oocyte retrieval
Embryo transfer
Gonadotropin (IU)
225 225 225 225 225 225 225 225 225 225 225
Progesterone
GnRH antagonist
hCG
Menses
4
8
12
16
Ultrasonography (≥3 follicles ≥17 mm)

Figure 48–6 *Idealized regimens using exogenous gonadotropins for induction of fertility.* **A.** Step-up regimen for ovulation induction. After menses, daily injections of gonadotropin (75 IU) are started. Follicle maturation is assessed by serial measurement of plasma estradiol and follicle size, as discussed in the text. If an inadequate response is seen, the dose of gonadotropin is increased to 112 or 150 IU/day. When one or two follicles have achieved a diameter of 17 mm or greater, final follicle maturation and ovulation are induced by injection of choriogonadotropin. Fertilization then is achieved at 36 h after choriogonadotropin injection by intercourse or intrauterine insemination. If more than two mature follicles are seen, the cycle is terminated, and barrier contraception is used to avoid triplets or higher degrees of multifetal gestation. **B.** Long protocol for ovarian hyperstimulation using a gonadotropin-releasing hormone (GnRH) agonist to inhibit premature ovulation, followed by *in vitro* fertilization (IVF). After the GnRH agonist has inhibited endogenous secretion of gonadotropins, therapy with exogenous gonadotropins is initiated. Follicle maturation is assessed by serial measurements of plasma estradiol and follicle size by ultrasonography. When three or more follicles are 17 mm or larger in diameter, then ovulation is induced by injection of choriogonadotropin. At 32 to 36 h after the choriogonadotropin injection, the eggs are retrieved and used for IVF. Exogenous progesterone is provided to promote a receptive endometrium, followed by embryo transfer at 3 to 5 days after fertilization. **C.** Protocol for ovarian hyperstimulation in an IVF protocol using a GnRH antagonist. The cycle duration is shorter because the GnRH antagonist does not induce a transient flare of gonadotropin secretion that might disrupt the timing of the cycle, but many other elements of the cycle are analogous to those in **B**.

fertilized *in vitro* with sperm (IVF) or by intracytoplasmic sperm injection; one or two embryos then are transferred to the uterus 3 to 5 days after fertilization or are cryopreserved for a frozen embryo transfer.

Because of the inhibitory effects of GnRH agonists or antagonists on pituitary gonadotropes, the secretion of LH that normally sustains the corpus luteum after ovulation does not occur. Repeated injections of choriogonadotropin, while sustaining the corpus luteum, may increase the risk of ovarian hyperstimulation syndrome (OHSS). Thus, standard IVF regimens typically provide exogenous progesterone replacement to support the fetus until the placenta acquires the biosynthetic capacity to take over this function; regimens include progesterone in oil (50–100 mg/day intramuscularly) or micronized progesterone (180–300 mg twice daily vaginally). Vaginal preparations containing 100 or 90 mg of micronized progesterone are approved for administration two or three times daily as part of IVF.

Aside from the attendant complications of multifetal gestation, the major side effect of gonadotropin treatment is OHSS. This potentially life-threatening event is believed to result from increased ovarian secretion of substances that increase vascular permeability and is characterized by rapid accumulation of fluid in the peritoneal cavity, thorax, and even the pericardium. Symptoms and signs include abdominal pain or distention, nausea and vomiting, diarrhea, dyspnea, oliguria, and marked ovarian enlargement on ultrasonography. OHSS can lead to hypovolemia, electrolyte abnormalities, acute respiratory distress syndrome, thromboembolic events, and hepatic dysfunction.

In an effort to minimize OHSS in at-risk patients, the FSH can be withheld for a day or two ("coasting"). The rationale for this approach is that larger follicles become relatively gonadotropin independent and thus will continue to mature, while the smaller follicles undergo atresia in response to gonadotropin deprivation. Alternatively, an endogenous LH surge can be induced with a GnRH agonist during a GnRH antagonist short protocol, which nearly eliminates the incidence of OHSS by avoiding the use of choriogonadotropin to trigger oocyte maturation.

The potential deleterious effects of gonadotropins are debated. Some studies have suggested that gonadotropins are associated with an increased risk of ovarian cancer, but this conclusion is controversial (Brinton et al., 2005)

Insulin Sensitizers

Polycystic ovary syndrome affects 4% to 7% of women of reproductive age and is the most frequent cause of anovulatory infertility. Inasmuch as patients with PCOS often exhibit hyperinsulinemia and insulin resistance, insulin sensitizers such as *metformin* have been evaluated for their effects on ovulation and fertility. Although several small trials suggested that *metformin* increased ovulation relative to placebo in patients with PCOS, a trial failed to demonstrate a significant effect of *metformin* on fertility (Legro et al., 2007); *metformin* was less effective than *clomiphene* in inducing ovulation, promoting conception, or improving live birth rates, and there was no benefit of combining *metformin* with *clomiphene* on live births, except possibly in women resistant to *clomiphene*. Thus, except in women who exhibit glucose intolerance, the consensus is that *metformin* generally should not be used for fertility induction in women with PCOS (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2008).

Thiazolidinediones also have been evaluated for their ability to induce ovulation in patients with PCOS but are not used for this indication given an increased risk of congestive heart failure and myocardial ischemia.

Drug Therapy in Obstetrics

Pregnancy-Induced Hypertension/Preeclampsia

Hypertension affects up to 10% of pregnant women in the U.S. Hypertension that precedes pregnancy or manifests before 20 weeks of gestation is believed to overlap considerably in pathogenesis with essential hypertension. These patients appear to be at increased risk for gestational diabetes and need careful monitoring. In contrast, pregnancy-induced hypertension, or preeclampsia, generally presents after 20 weeks of gestation as a new-onset hypertension with proteinuria (>300 mg of urinary protein/24 h); preeclampsia is thought to involve placenta-derived factors that affect vascular integrity and endothelial function in the mother, thus causing peripheral edema, renal and hepatic dysfunction, and in severe cases, seizures. Chronic hypertension is an established risk factor for preeclampsia. The consensus panel recommended initiation of drug therapy in women with a diastolic blood pressure greater than 105 mmHg or a systolic blood pressure greater than 160 mmHg. If severe preeclampsia ensues, with marked hypertension and evidence of end-organ damage, then termination of the pregnancy by delivery of the baby is the treatment of choice, provided that the fetus is sufficiently mature to survive outside the uterus. If the baby is very preterm, then hospitalization and pharmacotherapy may be employed in an effort to permit further fetal maturation *in utero.*

Several drugs commonly used for hypertension in nonpregnant patients (e.g., angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists) should not be used in pregnant women due to unequivocal evidence of adverse fetal effects. Many experts will convert the patient to the centrally acting α adrenergic agonist *α-methyldopa* (250 mg twice daily) (former FDA category B: no evidence of fetal risk in animal studies; no well-controlled studies in pregnant women), which rarely is used for hypertension in nonpregnant patients. Other drugs with reasonable evidence of safety also may be used, including the combination α_1-selective, β-nonselective adrenergic antagonist *labetalol* (100 mg twice daily) and the Ca^{2+} channel blocker *nifedipine* (30 mg once daily).

If severe preeclampsia or impending labor requires hospitalization, blood pressure can be controlled acutely with *hydralazine* (5 or 10 mg IV or IM, with repeated dosing at 20-min intervals depending on blood pressure response) or *labetalol* (20 mg IV, with dose escalation to 40 mg at 10 min if blood pressure control is inadequate). In addition to receiving drugs for blood pressure control, women with severe preeclampsia or who have CNS manifestations (e.g., headache, visual disturbance, or altered mental status) are treated as inpatients with *magnesium sulfate*, based on its documented efficacy in seizure prevention and lack of adverse effects on the mother or baby. Such treatment also should be considered for postpartum women with CNS manifestations: Approximately 20% of episodes of eclampsia occur in women more than 48 h after delivery.

Prevention or Arrest of Preterm Labor

Scope of the Problem and Etiology

Preterm birth, defined as delivery before 37 weeks of gestation, occurs in more than 10% of pregnancies in the U.S. and is increasing in frequency; it is associated with significant complications, such as neonatal respiratory distress syndrome, pulmonary hypertension, and intracranial hemorrhage.

Although incompletely understood, risk factors for preterm labor include multifetal gestation, premature rupture of the membranes, intrauterine infection, and placental insufficiency. The more premature the baby, the greater the risk of complications, prompting efforts to prevent or interrupt preterm labor.

The therapeutic objective in preterm labor is to delay delivery so that the mother can be transported to a regional facility specializing in the care of premature babies and supportive agents can be administered; such supportive treatments include glucocorticoids to stimulate fetal lung maturation (see Chapter 50) and antibiotics (e.g., *erythromycin*, *ampicillin*) to diminish the frequency of neonatal infection with group B β-hemolytic *Streptococcus* (see Chapters 58 and 60). Based on concerns over deleterious effects of antibiotic therapy, it is essential that antibiotics are not administered indiscriminately to all women thought to have preterm labor, but rather be reserved for those with premature rupture of the membranes and evidence of infection.

Prevention of Preterm Labor: Progesterone Therapy

Progesterone levels in some species diminish considerably in association with labor, whereas administration of progesterone inhibits the secretion of proinflammatory cytokines and delays cervical ripening. Thus, progesterone and its derivatives have long been advocated to diminish the onset of preterm labor in women at increased risk due to previous preterm delivery. Despite considerable controversy, recent randomized trials have revived interest in this approach. While *hydroxyprogesterone caproate* at a dose of 250 mg administered weekly by intramuscular injection has been shown to reduce preterm birth by about one-third in women with a prior preterm singleton birth, the Progestin's Role in Optimizing Neonatal Gestation trial showed no difference in the rate of preterm birth with this therapy when compared to inert oil placebo among women with a prior spontaneous preterm birth in a singleton pregnancy, possibly due to differences in the study populations (Blackwell et al., 2020; Meis et al., 2003). Vaginal administration of progesterone (200 mg each night) has been shown to reduce preterm birth in women with midtrimester cervical shortening by ultrasound examination. The role of progesterone for prevention of preterm birth in multiple gestations is controversial.

Tocolytic Therapy for Established Preterm Labor

Inhibition of uterine contractions of preterm labor, or *tocolysis*, has been a focus of therapy (Simhan and Caritis, 2007). Although tocolytic agents delay delivery in about 80% of women, they neither prevent premature births nor improve adverse fetal outcomes such as respiratory distress syndrome.

Specific tocolytic agents include β adrenergic receptor agonists, $MgSO_4$, Ca^{2+} channel blockers, COX inhibitors, oxytocin receptor antagonists, and NO donors. The mechanisms of action of these agents are illustrated in Figure 48–7.

The β adrenergic receptor agonists relax the myometrium by activating the cyclic AMP-PKA signaling cascade that phosphorylates and inactivates MLCK, a key enzyme in uterine contraction. *Ritodrine*, a selective β_2 agonist, was specifically developed as a uterine relaxant and remains the only tocolytic drug to have gained FDA approval; it was voluntarily withdrawn from the U.S. market. *Terbutaline*, which is FDA-approved for asthma, has been used off label for this purpose and can be administered orally, subcutaneously, or intravenously. *Terbutaline* may delay births, but only during the first 48 h of treatment, and is associated with a number of adverse maternal effects, including tachycardia, hypotension, and pulmonary edema.

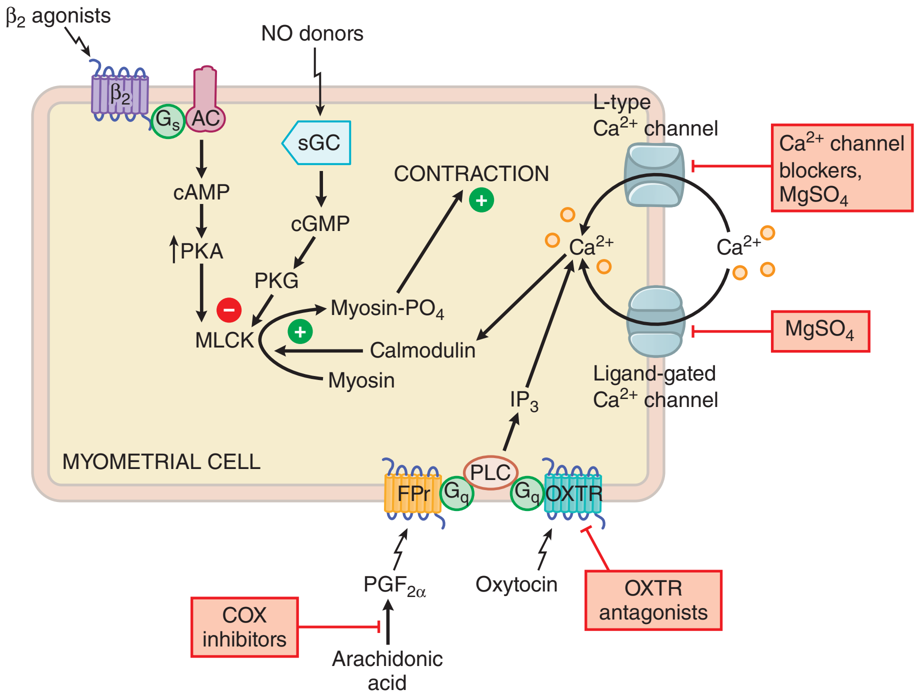


Figure 48–7 *Sites of action of tocolytic drugs in the uterine myometrium.* The elevation of cellular Ca^{2+} promotes contraction via the Ca^{2+}/calmodulin-dependent activation of MLCK. Relaxation is promoted by the elevation of cyclic nucleotides (cAMP and cGMP) and their activation of protein kinases, which cause phosphorylation/inactivation of MLCK. Pharmacological manipulations to reduce myometrial contraction include the following: (1) inhibiting Ca^{2+} entry (Ca^{2+} channel blockers, Mg_2SO_4); (2) reducing mobilization of intracellular Ca^{2+} by antagonizing G protein-coupled receptor-mediated activation of the G_q-PLC-IP_3-Ca^{2+} pathway (with antagonists of the $PGF_{2\alpha}$ and oxytocin receptors, FPr and OXTR) or reducing production of the FPr agonist, $PGF_{2\alpha}$ (with COX inhibitors); and (3) enhancing relaxation by elevating cellular cAMP (with β_2 adrenergic agonists that activate G_s-AC) and cellular cGMP (with NO donors that stimulate sGC). Note that pharmacological activators of sGC (e.g., *riociguat*) are contraindicated in pregnancy (see Chapter 35). AC, adenylyl cyclase; COX, cyclooxygenase; FPr, the PGF_2 receptor; OXTR, the oxytocin receptor; PLC, phospholipase C; sGC, soluble guanylyl cyclase.

Similarly, Ca^{2+} channel blockers inhibit the influx of Ca^{2+} through depolarization-activated, voltage-sensitive Ca^{2+} channels in the plasma membrane, thereby preventing the activation of MLCK and the stimulation of uterine contraction. *Nifedipine*, the Ca^{2+} channel blocker used most commonly for this purpose, can be administered parenterally or orally. Relative to β_2 adrenergic agonists, *nifedipine* is more likely to improve fetal outcomes and less likely to cause maternal side effects.

Based on the role of PGs in uterine contraction, COX inhibitors (e.g., *indomethacin*) have been used to inhibit preterm labor, and some data suggest that they may reduce the number of preterm births. Because they also can inhibit platelet function and induce closure *in utero* of the ductus arteriosus, these inhibitors should not be employed in term pregnancies (or in pregnancies beyond 32 weeks of gestation, when the risk of severe complications of prematurity is relatively lower). Short courses of treatment (<72 h) pose less risk for impaired circulation in the fetus.

Despite numerous clinical trials, the superiority of any one therapy has not been established, and none of the drugs has been shown definitively to improve fetal outcome.

Initiation of Labor

Labor induction is indicated when the perceived risk of continued pregnancy to the mother or fetus exceeds the risks of delivery or pharmacological induction.

Prostaglandins and Cervical Ripening

Prostaglandins play key roles in parturition (see Chapter 41). Thus, PGE_1, PGE_2, and $PGF_{2\alpha}$ are used to facilitate labor by promoting ripening and dilation of the cervix. They can be administered either orally or via local administration (either vaginally or intracervically). The ability of certain PGs to stimulate uterine contractions also makes them valuable agents in the therapy of postpartum hemorrhage.

Available preparations include *dinoprostone* (PGE_2), which is FDA-approved to facilitate cervical ripening. *Dinoprostone* is formulated as a gel for intracervical administration via syringe in a dose of 0.5 mg or as a vaginal insert (pessary) in a dose of 10 mg; the latter is designed to release active PGE_2 at a rate of 0.3 mg/h for up to 12 h and should be removed at the onset of labor or 12 h after insertion. No more than three doses should be used in a 24-h period. *Dinoprostone* should not be used in women with a history of asthma, glaucoma, or myocardial infarction. The major adverse effect is uterine hyperstimulation, which may be reversed more rapidly using the vaginal insert by removing it with the attached tape.

Misoprostol, a synthetic derivative of PGE_1 (see Chapter 41), is used off label either orally or vaginally to induce cervical ripening; typical doses are 100 μg (orally) or 25 μg (vaginally). An advantage of *misoprostol* in this setting is its considerably lower cost. Adverse effects include uterine hyperstimulation and, rarely, uterine rupture. *Misoprostol* should be discontinued for at least 3 h before initiating *oxytocin* therapy.

Oxytocin

The structure and physiology of *oxytocin* are discussed in Chapter 46. This section presents therapeutic uses of *oxytocin* in obstetrics, which include the induction of labor, the augmentation of labor that is not progressing, and the prophylaxis or treatment of postpartum hemorrhage. Although widely used, *oxytocin* recently was added to a list of drugs "bearing a heightened risk of harm" (Clark et al., 2009), and its role and specific application to most deliveries in the U.S. remain open to debate. Thus, careful review of the appropriate indications for *oxytocin* administration and attention to the dose and progress of labor during induction are essential.

Labor Induction. *Oxytocin* is the drug of choice for labor induction; for this purpose, it is administered by intravenous infusion of a diluted solution, preferably via an infusion pump. Current protocols start with an *oxytocin* dose of 6 mIU/min, followed by advancement of dose as needed, up to 40 mIU/min. Uterine hyperstimulation should be avoided; however, if it occurs, as evidenced by too-frequent contractions (more than five contractions in a 10-min interval) or the development of uterine tetany, the

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oxytocin infusion should be discontinued immediately. Because the $t_{1/2}$ of intravenous *oxytocin* is relatively short (12–15 min), the hyperstimulatory effects of *oxytocin* will dissipate rapidly after the infusion is discontinued. Thereafter, the infusion can be reinitiated at a dose of half that at which hyperstimulation occurred and increased cautiously as tolerated.

Because of its structural similarity to vasopressin, *oxytocin* at higher doses activates the vasopressin V_2 receptor and has antidiuretic effects. Particularly if hypotonic fluids (e.g., dextrose in water) are infused too liberally, water intoxication may result in convulsions, coma, and even death. Vasodilating actions of *oxytocin* also have been noted, particularly at high doses, which may provoke hypotension and reflex tachycardia. Deep anesthesia may exaggerate the hypotensive effect of *oxytocin* by preventing the reflex tachycardia.

Augmentation of Dysfunctional Labor. *Oxytocin* also is used when spontaneous labor is not progressing at an acceptable rate. To augment hypotonic contractions in dysfunctional labor, an infusion rate of 10 mIU/min typically is sufficient; doses in excess of 40 mIU/min rarely are effective when lower concentrations fail. As with labor induction, potential complications of uterine overstimulation include trauma of the mother or fetus due to forced passage through an incompletely dilated cervix, uterine rupture, and compromised fetal oxygenation due to decreased uterine perfusion.

Menopause and Hormone Therapy

Menopause refers to the permanent cessation of menstrual periods (i.e., for >12 months) resulting from the loss of ovarian follicular activity; it usually occurs when women are between 45 and 60 years of age. The decline in estradiol levels produces a variety of symptoms and signs, including vasomotor disturbances (hot flashes or flushes), sweating, irritability, sleep disturbances, and atrophy of estrogen-dependent tissue. In addition, postmenopausal women are at increased risk for osteoporosis, bone fractures, and coronary heart disease (CHD) and experience increased memory loss and other cognitive difficulties.

Estrogens

Estrogens are most commonly used to treat vasomotor disturbances ("hot flashes") in postmenopausal women. Other important benefits are amelioration of the effects of urogenital atrophy, a decreased incidence of colon cancer, and prevention of bone loss. A variety of preparations, including oral, transdermal, and vaginal, are available. *Regardless of the specific drug(s) selected, treatment should use the minimum dose and duration for the desired therapeutic end point.*

In postmenopausal women with an *intact uterus*, a progestin is included to prevent endometrial cancer. MPA is used in the U.S., but micronized progesterone is preferred; *norethindrone* and *norgestrel/levonorgestrel* are also commonly used. Women *without a uterus* are administered estrogen alone. Postmenopausal hormone therapy and contraception are the most frequent uses of progestins.

The two major uses of estrogens are for *MHT* and as components of *combination oral contraceptives*, and the pharmacological considerations for their use and the specific drugs and doses used differ in these settings. Historically, conjugated equine estrogens have been the most common agents for postmenopausal use (0.625 mg/day). In contrast, most combination oral contraceptives in current use employ 20 to 35 µg/day of *ethinyl estradiol*. These preparations differ widely in their oral potencies (e.g., a dose of 0.625 mg of conjugated estrogens generally is considered equivalent to 5–10 µg of *ethinyl estradiol*). Thus, the "effective" dose of estrogen used for MHT is less than that in oral contraceptives when one considers potency. Furthermore, over the past two decades, the doses of estrogens employed in both settings have decreased substantially. The untoward effects of the 20- to 35-µg doses now commonly used thus have a lower incidence and severity than those reported in older studies (e.g., with oral contraceptives that contained 50–150 µg of *ethinyl estradiol* or *mestranol*).

Menopausal Hormone Therapy

The established benefits of estrogen therapy in postmenopausal women include amelioration of vasomotor symptoms and the prevention of bone fractures and urogenital atrophy.

Vasomotor Symptoms

The decline in ovarian function at menopause is associated with vasomotor symptoms in most women. The characteristic hot flashes may alternate with chilly sensations, inappropriate sweating, and (less commonly) paresthesias. Treatment with estrogen is specific and is the most efficacious pharmacotherapy for these symptoms (Belchetz, 1994). If estrogen is contraindicated or otherwise undesirable, other options may be considered. MPA may provide some relief of vasomotor symptoms for certain patients, and the α_2 adrenergic agonist *clonidine* diminishes vasomotor symptoms in some women, presumably by blocking the CNS outflow that regulates blood flow to cutaneous vessels. In many women, hot flashes diminish within several years; when prescribed for this purpose, the dose and duration of estrogen use should thus be the minimum necessary to provide relief.

An exciting new approach toward treating vasomotor symptoms involves inhibition of the aforementioned neurokinin 3 receptor. With the postmenopausal absence of estradiol, kisspeptin release from the arcuate nucleus is high, promoting increased GnRH pulsatility and elevated FSH and LH release from the pituitary. This increase in kisspeptin release from the arcuate nucleus may be in part secondary to increased signaling through the neurokinin 3 receptor. Small studies suggest that neurokinin 3 receptor inhibition may improve vasomotor symptoms in peri- or postmenopausal women (Prague et al., 2018).

Osteoporosis

Osteoporosis is a disorder of the skeleton associated with the loss of bone mass (see Chapter 52). The result is thinning and weakening of the bones and an increased incidence of fractures, particularly compression fractures of the vertebrae and minimal-trauma fractures of the hip and wrist. The frequency and severity of these fractures and their associated complications (e.g., death and permanent disability) are a major public health problem, especially as the population continues to age. Osteoporosis is an indication for estrogen therapy, which clearly is efficacious in decreasing the incidence of fractures. However, because of the risks associated with estrogen use, first-line use of other drugs, such as bisphosphonates, should be considered (see Chapter 52). Most fractures in the postmenopausal period occur in women without a prior history of osteoporosis, and estrogens are the most efficacious agents available for prevention of fractures at all sites in such women (Anderson et al., 2004; Rossouw et al., 2002).

Estrogens act primarily to decrease bone resorption; consequently, estrogens are more effective at preventing rather than restoring bone loss (Belchetz, 1994; Prince et al., 1991). Estrogens are most effective if treatment is initiated before significant bone loss occurs, and their maximal beneficial effects require continuous use; bone loss resumes when treatment is discontinued. An appropriate diet with adequate intake of Ca^{2+} and vitamin D and weight-bearing exercise enhance the effects of estrogen treatment.

Vaginal Dryness and Urogenital Atrophy

Loss of tissue lining the vagina or bladder leads to a variety of symptoms in many postmenopausal women (Robinson and Cardozo, 2003). These include dryness and itching of the vagina, dyspareunia, swelling of tissues in the genital region, pain during urination, a need to urinate urgently or often, and sudden or unexpected urinary incontinence. When estrogens are being used solely for relief of vulvar and vaginal atrophy, local administration as a vaginal cream, ring device, or tablets may be considered. *Prasterone* is a steroid vaginal insert with an unclear mechanism of action that is indicated for moderate to severe dyspareunia related to vaginal atrophy, although use has been associated with abnormal cervical Pap tests.

Cardiovascular Disease

The incidence of cardiovascular disease is low in premenopausal women, rising rapidly after menopause, and epidemiological studies consistently showed an association between estrogen use and reduced cardiovascular disease in postmenopausal women. Estrogens produce a favorable lipoprotein profile, promote vasodilation, inhibit the response to vascular injury, and reduce atherosclerosis. However, estrogens promote coagulation and thromboembolic events. Randomized prospective studies unexpectedly have indicated that the incidence of heart disease and stroke in older postmenopausal women treated with conjugated estrogens and a progestin was initially increased, although the trend reversed with time (Grady et al., 2002; Rossouw et al., 2002). Combined estrogen-progestin therapy is associated with a decrease in heart attacks in younger women.

Other Therapeutic Effects

Many other changes occur in postmenopausal women, including a general thinning of the skin; changes in the urethra, vulva, and external genitalia; and a variety of changes, including headache, fatigue, and difficulty concentrating. Chronic lack of sleep created by hot flashes and other vasomotor symptoms may be contributing factors. Estrogen replacement may help alleviate or lessen some of these via direct actions (e.g., improvement of vasomotor symptoms) or secondary effects resulting in an improved feeling of well-being (Belchetz, 1994). The Women's Health Initiative (WHI) demonstrated that a conjugated estrogen in combination with a progestin reduces the risk of colon cancer by roughly one-half in postmenopausal women (Rossouw et al., 2002).

Menopausal Hormone Regimens

In the 1960s and 1970s, there was an increase in *estrogen replacement therapy* (i.e., estrogens alone) in postmenopausal women, primarily to reduce vasomotor symptoms, vaginitis, and osteoporosis. Around 1980, epidemiological studies indicated that this treatment increased the incidence of endometrial carcinoma. This led to the use of *hormone replacement therapy* (HRT), which includes a progestin to limit estrogen-related endometrial hyperplasia. Postmenopausal HRT, when indicated, should include both an estrogen and progestin for women with a uterus (Belchetz, 1994). For women who have undergone a hysterectomy, endometrial carcinoma is not a concern, and estrogen alone avoids the possible deleterious effects of progestins.

Conjugated estrogens and MPA historically have been used most commonly in menopausal hormone regimens, although *estradiol*, *estrone*, and *estriol* have been used as estrogens, and *norethindrone*, *norgestimate*, *levonorgestrel*, *norethisterone*, and *progesterone* also have been widely used (especially in Europe). Various "continuous" or "cyclic" regimens have been used; the latter regimens include drug-free days. An example of a cyclic regimen is as follows: (1) administration of an estrogen for 25 days; (2) the addition of MPA for the last 12 to 14 days of estrogen treatment; and (3) 5 to 6 days with no hormone treatment, during which withdrawal bleeding normally occurs due to breakdown and shedding of the endometrium. Continuous administration of combined estrogen plus progestin does not lead to regular, recurrent endometrial shedding but may cause intermittent spotting or bleeding, especially in the first year of use. Other regimens include a progestin intermittently (e.g., every third month), but the long-term endometrial safety of these regimens remains to be firmly established. Conjugated estrogens plus MPA given as a fixed dose daily and conjugated estrogens given for 28 days plus MPA given for 14 of 28 days are widely used combination formulations. Other combination products available in the U.S. are *ethinyl estradiol* plus *norethindrone acetate*, *estradiol* plus *norethindrone*, *estradiol* and *norgestimate*, and *estradiol* and *drospirenone*. Doses and regimens are usually adjusted empirically based on control of symptoms, patient acceptance of bleeding patterns, or other untoward effects.

Another pharmacological consideration is the route of estrogen administration. Oral administration exposes the liver to higher concentrations of estrogens than does transdermal administration and may increase SHBG, other binding globulins, and angiotensinogen and possibly the cholesterol content of the bile. Transdermal estrogen appears to cause smaller beneficial changes in LDL and HDL profiles (~50% of those seen with the oral route) (Walsh et al., 1994).

Tibolone is widely used in the E.U. for treatment of vasomotor symptoms and prevention of osteoporosis but is not currently approved in the U.S. The parent compound itself is devoid of activity, but it is metabolized in a tissue-selective manner to three metabolites that have predominantly estrogenic, progestogenic, and androgenic activities. The effects of this drug on fractures, breast cancer, and long-term outcomes remain to be established (Modelska and Cummings, 2002).

Finally, a combination of conjugated estrogens and the nonsteroidal SERM *bazedoxifene* can be used for menopausal symptoms. This combination has a neutral effect in the uterus and breast but has been shown to improve hot flashes, reduce vaginal dryness, and prevent (but not treat) bone loss (Parish and Gillespie, 2017).

Regardless of the specific agent or regimen, MHT with estrogens should use the lowest dose and shortest duration necessary to achieve an appropriate therapeutic goal.

Untoward Responses

The use of unopposed estrogen for hormone treatment in postmenopausal women increases the risk of endometrial carcinoma by 5- to 15-fold (Shapiro et al., 1985). This increased risk can be prevented if a progestin is coadministered with the estrogen (Pike et al., 1997), and this is now standard practice.

The association between estrogen or estrogen-progestin use and breast cancer is of great concern. The results of two large randomized clinical trials of estrogen/progestin and estrogen only (i.e., the two arms of WHI) in postmenopausal women clearly established a small but significant increase in the risk of breast cancer in the conjugated equine estrogens (CEE) plus MPA studies (Anderson et al., 2004; Rossouw et al., 2002). In the WHI study, CEE+MPA was associated with an increased relative risk of breast cancer by 25%; the absolute increase in attributable cases of disease was 6 per 1000 women and required 3 or more years of treatment. In women without a uterus who received CEE alone, the relative risk of breast cancer was actually decreased by 23%, and the decrease only narrowly missed reaching statistical significance. Interestingly, the incidence of colon cancer was reduced by 26% in the WHI trial.

The Million Women Study in the U.K. was a cohort study rather than a clinical trial (Beral et al., 2003). It surveyed more than 1 million women; about half had received some type of hormone treatment, and half had never used this type of treatment. Those receiving an estrogen-progestin combination had an increased relative risk of invasive breast cancer of 2, and those receiving estrogen alone had an increased relative risk of 1.3, but the increase in actual attributable cases of the disease was again small.

Both the WHI and Million Women Study data are thus consistent with earlier studies indicating that the progestin component (e.g., *medroxyprogesterone*) in combined HRT plays a major role in this increased risk of breast cancer (Ross et al., 2000; Schairer et al., 2000). Importantly, although long-term data have not accumulated for the WHI trials, the available data suggest that the excess risk of breast cancer associated with menopausal hormone use appears to abate 5 years after discontinuing therapy. Thus, HRT for 5 years or less is often prescribed to mitigate hot flashes and likely has a minimal effect on the risk of breast cancer.

Historically, the carcinogenic actions of estrogens were thought to be related to their trophic effects. However, if catechol estrogens, especially the 4-hydroxycatechols, are converted to semiquinones or quinones prior to "inactivation" by catechol-*O*-methyl transferase, the generation of ROSs may cause direct chemical damage to DNA bases (Yue et al., 2003). In this regard, CYP1B1, which has specific estrogen-4-hydroxylase activity, is present in tissues such as uterus, breast, ovary, and prostate, which often give rise to hormone-responsive cancers.

Metabolic and Cardiovascular Effects

Although they may slightly elevate plasma triglycerides, estrogens themselves generally have favorable overall effects on plasma lipoprotein profiles. However, addition of progestins may reduce the favorable actions of estrogens. Estrogens do increase cholesterol levels in bile and cause a relative 2- to 3-fold increase in gallbladder disease. Currently prescribed doses of estrogens generally do not increase the risk of hypertension, and estrogen engaging the ERβ receptor typically reduces blood pressure.

Postmenopausal women who take HRT have a lower rate of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis development than age-matched men or women not taking HRT. There is also a reduction of the abnormal metabolism in the liver, including excessive liver fat deposition that results in insulin resistance. Furthermore, the development of liver inflammation and fibrosis is attenuated by estrogen in postmenopausal women. The latter likely contributes to preventing progression to hepatocellular carcinoma in some individuals (DiStefano, 2020).

Many studies and clinical trials suggested that estrogen therapy in postmenopausal women would reduce the risk of cardiovascular disease by 35% to 50% (Manson and Martin, 2001). However, two recent randomized clinical trials have not found such protection. The Heart and Estrogen/Progestin Replacement Study (HERS) study followed women with established CHD and found that estrogen plus a progestin increased the relative risk of nonfatal myocardial infarction or CHD death within 1 year of treatment, but there was no overall change at 5 years (Hulley et al., 1998). The HERS II follow-up found no overall change in the incidence of CHD after 6.8 years of the treatment (Grady et al., 2002). In women *without* existing CHD (WHI trials) treated with an estrogen plus progestin, protective effects were seen but only when hormone replacement was initiated within 10 years of menopause (Rossouw et al., 2002).

It is clear that oral estrogens increase the risk of thromboembolic disease in healthy women and in women with preexisting cardiovascular disease (Grady et al., 2000). The increase in absolute risk is small but significant. In the WHI, for example, an estrogen-progestin combination led to an increase in eight attributable cases of stroke per 10,000 older women and a similar increase in pulmonary embolism (Rossouw et al., 2002). The latter was seen mainly in women who concomitantly smoked cigarettes.

Effects on Cognition

Several retrospective studies had suggested that estrogens had beneficial effects on cognition and delayed the onset of Alzheimer's disease (Green and Simpkins, 2000). However, the WHI Memory Study of a group of women 65 years of age or older found that estrogen-progestin therapy was associated with doubling in the number of women diagnosed with probable dementia, and no benefit of hormone treatment on global cognitive function was observed (Rapp et al., 2003; Shumaker et al., 2003).

Estrogen and Parkinson's Disease

Based on many animal studies including in primates, estrogen and progesterone were found to have positive effects of preventing apoptosis of basal ganglia, dopamine-secreting neurons (Morissette et al., 2008). In human studies, there are data that estrogen prevents the development of Parkinson's disease, and less estrogen may contribute to the increased incidence of this disease in men (Vegeto et al., 2019).

Other Potential Untoward Effects

Nausea and vomiting are an initial reaction to estrogen therapy in some women, but these effects may disappear with time and may be minimized by taking estrogens with food or just before sleep. Fullness and tenderness of the breasts and edema may occur but sometimes can be diminished by lowering the dose.

Drug Therapy in Endometriosis, Fibroids Hirsutism, Gender Transition, and Hypoactive Sexual Desire Disorder

Endometriosis

Endometriosis is an estrogen-dependent disorder that results from endometrial tissue ectopically located outside the uterine cavity (Farquhar, 2007). It predominantly affects women during their reproductive years, with a prevalence of 0.5% to 5% in fertile women and 25% to 40% in infertile women. Diagnosis typically is made at laparoscopy, either prompted by unexplained pelvic pain (dysmenorrhea or dyspareunia) or infertility. Although poorly understood, the infertility is thought to reflect involvement of the fallopian tubes with the underlying process and, possibly, impaired oocyte maturation.

Because the proliferation of ectopic endometrial tissue is responsive to ovarian steroid hormones, many symptomatic approaches to therapy aim to produce a relatively hypoestrogenic state. Combination oral contraceptives have been standard first-line treatment of symptoms of endometriosis, and ample evidence from observational trials supports their benefit. The predominant mechanism of action is believed to be suppression of gonadotropin secretion, with subsequent inhibition of estrogen biosynthesis. Progestins (e.g., *medroxyprogesterone, dienogest*) also have been used to promote decidualization of the ectopic endometrial tissue. The *levonorgestrel* IUS, which is approved for contraception, also has been used off label for this indication, as well as for menorrhagia.

Stable GnRH agonists can suppress gonadotropin secretion and thus effect medical castration. Drugs that carry an indication for endometriosis include *leuprolide*, *goserelin*, and *nafarelin*; other GnRH agonists also may be used off label for this purpose (see Chapter 46). Due to significant decreases in bone density and symptoms of estrogen withdrawal, "add-back" therapy with either a low-dose synthetic estrogen (e.g., CEE 0.625–1.25 mg) or a high-dose progestin (e.g., *norethindrone* 5 mg) has been used when the duration of therapy has exceeded 6 months (Olive, 2008). Alternatively, an oral GnRH receptor antagonist, *elagolix*, is effective at improving dysmenorrhea and pelvic pain in women with endometriosis, although *elagolix* is associated with hypoestrogenic adverse effects such as hot flashes, increased serum lipids, and a reduction in bone mineral density (Taylor, 2017).

Danazol, a synthetic androgen that inhibits gonadotropin production via feedback inhibition of the pituitary-ovarian axis, also is FDA-approved for endometriosis therapy; it rarely is used now because of its significant adverse effects, including hirsutism and elevation of hepatic transaminases. In Europe and elsewhere, the antiprogestin *gestrinone* has been employed. *Danazol* has also been to treat severe forms of hereditary angioedema by increasing expression of esterase inhibitors through yet unknown mechanisms.

Hirsutism

Hirsutism, or increased hair growth in the male distribution, affects about 10% of women of reproductive age. It can be a relatively benign, idiopathic process or part of a more severe disorder of androgen excess that includes overt virilization (voice deepening, increased muscle mass, male pattern balding, clitoromegaly) and often results from ovarian or adrenal tumors. Specific etiologies associated with hirsutism include congenital adrenal hyperplasia, PCOS, and Cushing syndrome. After excluding serious pathology such as a steroid-producing malignancy, the treatment largely becomes empirical (Martin et al., 2008).

Pharmacotherapy is directed at decreasing androgen production and action. Initial therapy often involves treatment with combination oral contraceptive pills, which suppress gonadotropin secretion and thus the production of ovarian androgens. The estrogen also increases the concentration of SHBG, thereby diminishing the free concentration of testosterone. The full effect of this suppression may take up to 6 to 9 months. GnRH agonists downregulate gonadotropin secretion and also may be used to suppress ovarian steroid production.

In patients who fail to respond to ovarian suppression, efforts to block androgen action may be effective. *Spironolactone*, a mineralocorticoid receptor antagonist, and *flutamide* (see Chapter 49) inhibit the androgen receptor. In Europe and elsewhere, *cyproterone* (50–100 mg/day) is used as an androgen receptor blocker, often in conjunction with a combination oral contraceptive. *Finasteride*, an inhibitor of the type 2 isozyme of 5α-reductase that blocks the conversion of testosterone to dihydrotestosterone, also is effective. Male offspring of women who become pregnant while taking any of these androgen inhibitors are at risk of impaired virilization secondary to impaired synthesis or action of dihydrotestosterone (fetal risk; contraindicated in pregnancy). The antifungal *ketoconazole*, which inhibits CYP steroid hydroxylases (see Chapters 50 and

61), also can block androgen biosynthesis but may cause liver toxicity. Topical *eflornithine*, an ornithine decarboxylase inhibitor, has been used with some success to decrease the rate of facial hair growth.

Nonpharmacological approaches include bleaching, depilatory treatments (e.g., shaving, treatment with hair-removing chemicals), or methods that remove the entire hair follicle (e.g., plucking, electrolysis, laser ablation).

Gender Transition

In the past 10 to 20 years, sex steroids have been used more frequently in transgender patients. Because no significant clinical trials have been performed, a great deal of variability exists in the approaches taken in both male-to-female and female-to-male transgender patients. In general, younger patients in their early teens are often held from natural puberty through the use of GnRH agonists until the individuals are old and mature enough to be certain of their decision. Once the decision is made, whether the patients are younger or older, the approaches can be myriad, although they follow the same principles: (1) suppress endogenous sex steroid production and (2) promote physical and mental features of the desired gender.

Male-to-Female Transitions

The primary medication used for male-to-female transition is some form of estrogen, whether it be oral *estradiol* (2–6 mg/day), transdermal *estradiol* (0.1–0.4 mg every 24 h), or injectable estrogens such as *estradiol valerate* or *estradiol cypionate* (5–10 mg IM every 2 weeks). Side effects with estrogens, including thrombosis and breast cancer (not really established in male-to-female transgender patients), must be discussed with patients. Target serum estradiol levels are usually in the range of 100 to 200 pg/mL. In many patients, estrogen treatment alone will be sufficient to suppress endogenous androgen production and therefore androgen-mediated effects; however, in patients in whom this is not possible, antiandrogens such as *spironolactone* (100–400 mg/d) can be used. Alternatively, endogenous androgen production can be suppressed with GnRH agonists. The advantage of using antiandrogens or GnRH agonists is that the dosages of *estradiol* can often be significantly lower.

Female-to-Male Transitions

The primary medication used in female-to-male transitions is some form of androgen, whether it be injectable, such as *testosterone enanthate* or *cypionate* (50–100 mg IM per week), or androgen gels (25–100 mg/day of testosterone). Target plasma androgen levels should be in the normal male range (300–500 mg/day). Side effects of excess androgens, including polycythemia and lipid abnormalities, should be discussed and monitored with all patients. In general, these doses of androgens are sufficient to suppress endogenous ovarian steroid hormone production; however, if breakthrough uterine bleeding still occurs, patients can be treated with depot *medroxyprogesterone* (150 mg every 3 months) until bleeding no longer occurs.

Hypoactive Sexual Desire Disorder

Hypoactive sexual desire disorder (HSDD) is characterized by deficient or absent sexual fantasies and desire of sexual activity for an extended period of time that causes marked distress or interpersonal difficulty that cannot be attributed to a comorbid medical condition, problems in the relationship, or a drug or substance side effect. As the underlying cause of decreased sexual desire may be multifactorial, an appropriate treatment plan for HSDD may include interventions that target psychosocial, behavioral, and biological causes, sex therapy, and psychotherapy. *Flibanserin*, a centrally acting daily medication, is an FDA-approved therapy for generalized HSDD in premenopausal women. *Flibanserin* is a $5HT_{1A}$ receptor agonist and a $5HT_{2A}$ receptor antagonist, but the mechanism by which the drug improves sexual desire and related distress is not known. *Flibanserin* has been associated with severe hypotension and syncope when taken with alcohol, which should be avoided (USFDA, 2015/2019). *Bremelanotide*, a self-administered injection 45 min prior to anticipated sexual activity, is another FDA-approved medication for the treatment of generalized HSDD in premenopausal women. *Bremelanotide* activates melanocortin receptors, but the mechanism by which it improves sexual desire and related distress is unknown. It is associated with nausea and increased blood pressure and is not recommended in women at high risk for cardiovascular disease (USFDA, 2019). Hormone therapy may benefit select postmenopausal women. Postmenopausal women with vulvovaginal atrophy and/or dyspareunia will benefit from low-dose local vaginal estrogen therapy or a SERM such as *ospemifene* (USFDA, 2019b). Surgically menopausal and postmenopausal women with HSDD who are low risk for cardiometabolic disease may benefit from short-term testosterone therapy that achieves blood concentrations of testosterone that approximate premenopausal physiological concentrations (Davis et al., 2019).

Drug Facts for Your Personal Formulary: *Estrogens, Progestins, GnRH, Gonadotropins*

Drug	Therapeutic Uses	Major Toxicity and Clinical Pearls
Estrogens		
Steroidal Estrogen and Derivatives Estradiol Estradiol valerate Estradiol cypionate Ethinyl estradiol Mestranol Estrone sulfate **Nonsteroidal Compounds** Diethylstilbestrol	• Menopause hormone therapy • Components of oral contraceptives • Treatment of transgender individuals • Depending on the preparation, may be available for oral, parenteral, transdermal, or topical administration	• Act via ERα and ERβ • Precaution: prescribe the lowest effective dose for the shortest duration consistent with treatment goals and risks for each individual patient • Increased risk of thromboembolism • Potencies of various oral preparations differ due to differences in first-pass metabolism
Selective Estrogen Receptor Modulators		
Tamoxifen	• Treatment of breast cancer • Antiestrogenic, estrogenic, or mixed activity depending on tissue	• Tissue-selective actions on ERs • Beneficial estrogenic actions in bone, brain, and liver during postmenopausal hormone therapy • Antagonist activity in breast and endometrium • Increased risk of thromboembolism • Hot flashes in premenopausal women
Raloxifene	• Treatment of osteoporosis (estrogen agonist in bone) • Reduces total cholesterol and LDL but does not increase HDL • Reduce risk of breast cancer in high-risk postmenopausal women	• Increased risk of thromboembolism • Neutral or antagonistic in uterus
Toremifene	• Treatment of breast cancer	
Bazedoxifene	• Osteoporosis prevention in postmenopausal women	• Increased risk of thromboembolism • Formulated in combination with conjugated estrogens
Ospemifene	• HSDD and dyspareunia treatment	• Increased risk of thromboembolism • Potential thickening of endometrium
Antiestrogens		
Clomiphene	• Treatment of infertility in anovulatory women	• Primarily a receptor antagonist but also has weak agonist activity
Fulvestrant	• Treatment of breast cancer in women with disease progression after tamoxifen • Used in women with resistance to aromatase inhibitors	• Receptor antagonist in all tissues
Estrogen Synthesis Inhibitors		
Aromatase Inhibitors *Steroidal inhibitors* Exemestane *Nonsteroidal inhibitors* Anastrozole Letrozole, vorozole	• Treatment of breast cancer (exemestane, letrozole, and anastrozole approved in the U.S.)	• *Steroidal inhibitors:* substrate analogues that irreversibly inactivate aromatase • *Nonsteroidal inhibitors:* interact reversibly with the heme groups of CYPs • Risk of osteoporosis with long-term use
Progestins		
Pregnanes Progesterone Medroxyprogesterone acetate Megestrol acetate	• Menopause hormone therapy • Contraception • Assisted reproductive technology • Depot MPA used as a long-acting injectable contraceptive	• Formulations: oral, injection (IM, SC), vaginal gel, vaginal insert • Progesterone: rapid first-pass metabolism • MPA and micronized progesterone are available for oral use
Estranes Norethindrone 19-Norethindrone	• Used in oral and injectable contraceptives • Used in combination with estrogen to treat hypogonadism	• 19-Nortestosterone derivatives • Progestational activity but also some androgenic and other activities
Gonanes Norgestrel Norgestimate	• Used in oral and injectable contraceptives	• 19-Nortestosterone derivatives, ethyl rather than methyl group at position 13 • Progestational components of contraceptives

Drug Facts for Your Personal Formulary: *Estrogens, Progestins, GnRH, Gonadotropins (continued)*

Drug	Therapeutic Uses	Major Toxicity and Clinical Pearls
Antiprogestins and Progesterone Receptor Modulators		
Mifepristone (RU 38486)	• Termination of early pregnancy	• Competitive receptor antagonist of both progesterone receptors • May have some agonist activity
Ulipristal acetate	• Emergency contraception	• Partial progesterone receptor agonist
GnRH Agonist and Antagonists		
GnRH agonist Leuprolide	• Controlled ovarian hyperstimulation • Endometriosis • Uterine leiomyomas • Precocious puberty • Menstrual suppression in special circumstance (e.g., thrombocytopenia)	• Initial agonist action ("flare effect") results in increase in FSH and LH • After 1–3 weeks, desensitization and pituitary downregulation result in a hypogonadotropic, hypogonadal state • Risk of osteoporosis with long-term use
GnRH antagonist Cetrorelix, ganirelix Goserelin, buserelin Triptorelin, nafarelin	• Controlled ovarian hyperstimulation	• Competitive GnRH receptor antagonist • Immediate decline in LH and FSH levels • Risk of osteoporosis with long-term use
Gonadotropins		
FSH *Recombinant FSH* Follitropin-alpha Follitropin-beta *Human menopausal menotropins* Menotropins Urofollitropins Highly purified urinary FSH	• Ovulation induction • Controlled ovarian hyperstimulation	• Human menopausal gonadotropin may contain FSH, LH, and hCG and purification results in standardization of the FSH and LH activity • Injectable or intravenous
LH Recombinant LH	• Controlled ovarian hyperstimulation in women with LH deficiency due to hypogonadotropic hypogonadism	• Injectable or intravenous
hCG Recombinant hCG Urinary hCG Highly purified urinary hCG	• Promotes meiotic maturation from prophase I to metaphase II in oocytes	• Injectable or intravenous • Also used to stimulate testosterone and sperm production in men

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Chapter 49

Androgens and the Male Reproductive Tract

Peter J. Snyder

TESTOSTERONE AND OTHER ANDROGENS

SECRETION AND TRANSPORT OF TESTOSTERONE

METABOLISM OF TESTOSTERONE TO ACTIVE AND INACTIVE COMPOUNDS

PHYSIOLOGICAL AND PHARMACOLOGICAL EFFECTS OF ANDROGENS
- Effects That Occur via the Androgen Receptor
- Effects That Occur via the Estrogen Receptor
- Effects of Androgens at Different Stages of Life

CONSEQUENCES OF ANDROGEN DEFICIENCY
- During Fetal Development
- Before Completion of Puberty
- After Completion of Puberty
- In Women

THERAPEUTIC ANDROGEN PREPARATIONS
- Testosterone Esters
- Alkylated Androgens
- Transdermal Delivery Systems
- Selective Androgen Receptor Modulators

THERAPEUTIC USES OF ANDROGENS
- Male Hypogonadism
- Male Senescence
- Female Hypogonadism
- Enhancement of Athletic Performance
- Catabolic and Wasting States
- Angioedema
- Blood Dyscrasias

ANTIANDROGENS
- Inhibitors of Testosterone Secretion
- Inhibitors of Androgen Action

PHARMACOLOGICAL TREATMENT OF ERECTILE DYSFUNCTION
- Erectile Signaling and Erectile Dysfunction
- PDE5 Inhibitors

Testosterone and Other Androgens

In men, *testosterone* is the principal secreted androgen. *Leydig cells* synthesize the majority of testosterone by the pathways shown in Figure 49–1. In women, testosterone also is the principal androgen and is synthesized in the corpus luteum and the adrenal cortex by similar pathways. The testosterone precursors *androstenedione* and *dehydroepiandrosterone* are weak androgens that can be converted peripherally to testosterone.

Secretion and Transport of Testosterone

Testosterone secretion is greater in men than in women at almost all stages of life, a difference that explains many of the other differences between men and women. In the first trimester in utero, the fetal testes begin to secrete testosterone, the principal factor in male sexual differentiation, likely stimulated by *human chorionic gonadotropin* (hCG) secreted by the placenta. By the beginning of the second trimester, the serum testosterone concentration is close to that of midpuberty, about 250 ng/dL (Figure 49–2). Testosterone production then falls by the end of the second trimester, but by birth, the concentration is again about 250 ng/dL, possibly due to stimulation of the fetal Leydig cells by *luteinizing hormone* (LH) from the fetal pituitary gland. The testosterone value falls again in the first few days after birth, but it rises and peaks again at about 250 ng/dL at 2 to 3 months after birth and falls to less than 50 ng/dL by 6 months, where it remains until puberty. During puberty, from about 12 to 17 years of age, the serum testosterone concentration in males increases so that by early adulthood the serum testosterone concentration is 300 to 800 ng/dL in men, compared to 30 to 50 ng/dL in women. The magnitude of the testosterone concentration in the male is responsible for the pubertal changes that further differentiate men from women. As men age, their serum testosterone concentrations gradually decrease, which may contribute to other effects of aging in men.

Luteinizing hormone, secreted by the pituitary gonadotroph cells (see Chapter 46), is the principal stimulus of testosterone secretion in men, perhaps potentiated by *follicle-stimulating hormone* (FSH), also secreted by gonadotrophs. The secretion of LH by gonadotrophs is stimulated by hypothalamic *gonadotropin-releasing hormone* (GnRH); testosterone directly inhibits LH secretion in a negative-feedback loop. LH is secreted in pulses, which occur approximately every 2 h and are greater in magnitude in the morning (Crowley et al., 1985). The pulsatility appears to result from pulsatile secretion of GnRH from the hypothalamus. Testosterone secretion is likewise pulsatile and diurnal, with the highest plasma concentrations occurring at about 8 AM and the lowest at about 8 PM. The morning peaks diminish as men age. *Sex hormone–binding globulin* binds about 38% of circulating testosterone with high affinity, rendering the bound hormone unavailable for biological effects. Albumin binds almost 60% of circulating testosterone with low affinity, leaving about 2% unbound or free. In women, LH stimulates the *corpus luteum* (formed from the follicle after release of the ovum) to secrete testosterone. Under normal circumstances, however, *estradiol* and *progesterone*, not testosterone, are the principal inhibitors of LH secretion in women.

Metabolism of Testosterone to Active and Inactive Compounds

Testosterone has many different effects in tissues, both directly and through its metabolism to *dihydrotestosterone* and *estradiol* (Figure 49–3). The enzyme 5α-reductase catalyzes the conversion of testosterone to dihydrotestosterone. Dihydrotestosterone binds to the *androgen receptor*

Abbreviations

AR: androgen receptor
CYP: cytochrome P450
FSH: follicle-stimulating hormone
GnRH: gonadotropin-releasing hormone
hCG: human chorionic gonadotropin
LH: luteinizing hormone
NO: nitric oxide
PDE5: phosphodiesterase type 5
PKG: protein kinase G
sGC: soluble guanylate cyclase

(AR) with higher affinity than testosterone and activates gene expression more efficiently. Two forms of 5α-reductase have been identified: *type I*, which is found predominantly in nongenital skin, liver, and bone; and *type II*, which is found predominantly in urogenital tissue in men and genital skin in men and women. The enzyme complex aromatase, present in many tissues, catalyzes the conversion of testosterone to estradiol. This conversion accounts for about 85% of circulating estradiol in men; the remainder is secreted directly by the testes (MacDonald et al., 1979). Hepatic metabolism converts testosterone to the biologically inactive compounds androsterone and etiocholanolone (see Figure 49–3). Dihydrotestosterone is metabolized to androsterone, androstanedione, and androstanediol.

Physiological and Pharmacological Effects of Androgens

Testosterone is the principal circulating androgen in men. At least three mechanisms contribute to the varied effects of testosterone:

- Direct binding to the AR
- Conversion in certain tissues to dihydrotestosterone, which also binds to the AR
- Conversion to estradiol, which binds to the estrogen receptor (Figure 49–4)

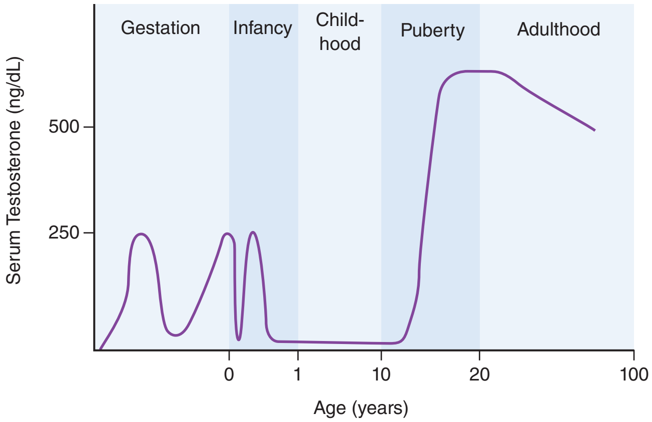


Figure 49–2 *Schematic representation of the serum testosterone concentration from early gestation to old age.*

Effects That Occur via the Androgen Receptor

Testosterone and dihydrotestosterone act as androgens via a single AR, a member of the nuclear receptor superfamily designated as NR3A. The AR has an amino-terminal domain that contains a polyglutamine repeat of variable length, a DNA-binding domain consisting of two Zn finger motifs, and a carboxyterminal ligand-binding domain. The polyglutamine repeat of variable length is unique to the AR; a shorter length appears to increase the receptor's activity.

In the absence of a ligand, the AR is in the cytoplasm associated with a heat shock protein complex. When testosterone or dihydrotestosterone binds to the ligand-binding domain, the AR dissociates from the heat shock protein complex, dimerizes, and translocates to the nucleus. The dimer then binds via the DNA-binding domains to androgen response elements on certain responsive genes. The ligand-receptor complex recruits coactivators and acts as a transcription factor complex, stimulating or repressing expression of those genes (Agoulnik and Weigel, 2008).

Mutations in the hormone- or DNA-binding regions of the AR result in resistance to the action of testosterone, beginning in utero (McPhaul and Griffin, 1999); consequently, male sexual differentiation and pubertal development are incomplete. Other AR mutations occur in patients with spinal and bulbar muscular atrophy, known as *Kennedy disease*. These patients have an expansion of the CAG (cytosine, adenine, guanine) repeat,

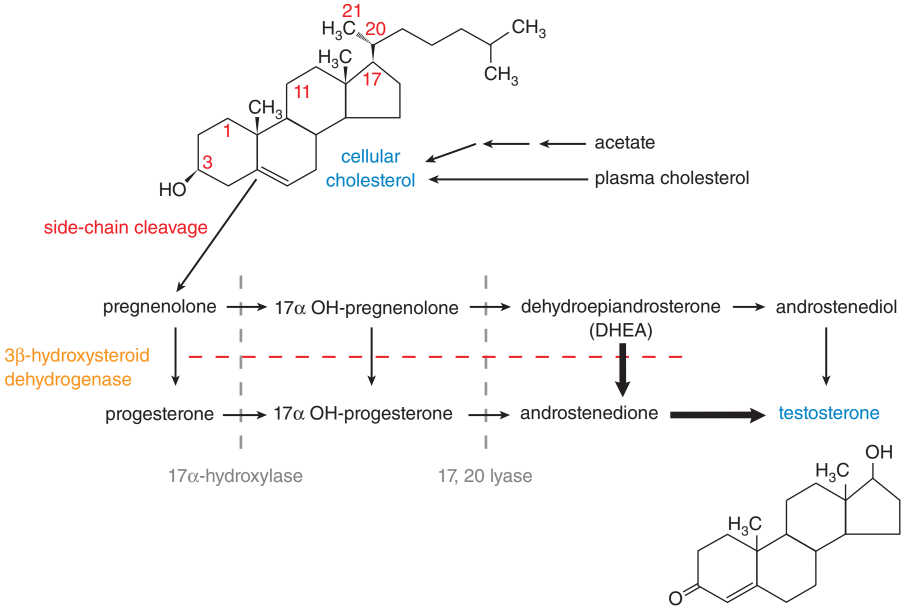


Figure 49–1 *Pathway of testosterone synthesis in Leydig cells of the testes.* In Leydig cells, the 11 and 21 hydroxylases (present in adrenal cortex) are absent, but CYP17 (17α-hydroxylase) is present. Thus, androgens and estrogens are synthesized; corticosterone and cortisol are not formed. Bold arrows indicate favored pathways.

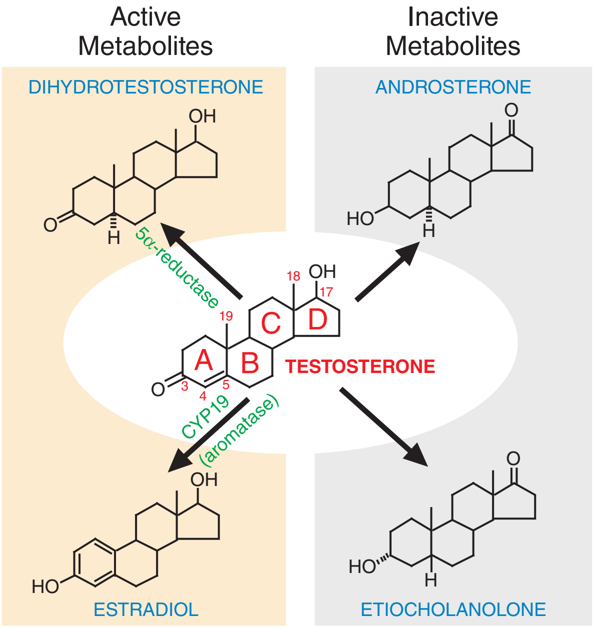

Active Metabolites
Inactive Metabolites
DIHYDROTESTOSTERONE
ANDROSTERONE
5α-reductase
TESTOSTERONE
A
B
C
D
18
19
17
3
4
5
CYP19 (aromatase)
OH
O
H
HO
ESTRADIOL
ETIOCHOLANOLONE

Figure 49–3 *Metabolism of testosterone to its major active and inactive metabolites.*

which codes for glutamine, at the amino terminus of the molecule (Walcott and Merry, 2002). The result is very mild androgen resistance, manifest principally by gynecomastia, and progressively severe motor neuron atrophy (Dejager et al., 2002). The mechanism by which the neuronal atrophy occurs is unknown. Other mutations in AR may explain why metastatic prostate cancer often regresses initially in response to androgen deprivation treatment but then becomes unresponsive to continued deprivation. AR continues to be expressed in androgen-independent prostate cancer, and its signaling remains active. The ligand-independent signaling may result from mutations in the AR gene or changes in AR coregulatory proteins. In some patients resistant to standard androgen deprivation therapy, the tumor responds to further depletion of androgens by inhibitors of adrenal androgen synthesis, such as *abiraterone*.

Effects That Occur via the Estrogen Receptor

Certain effects of testosterone are mediated by its conversion to estradiol, catalyzed by *cytochrome P450* (CYP) type 19 (aromatase). In rare cases of males deficient in CYP19 or the estrogen receptor, the epiphyses do not fuse, and long-bone growth continues indefinitely; such patients are also osteoporotic. Administration of estradiol corrects the bone abnormalities in patients with aromatase deficiency but not in those with an estrogen-receptor defect. Because men have larger bones than women, and bone expresses the AR (Colvard et al., 1989), testosterone also may act on bone via the AR. Administration of estradiol to a male with CYP19 deficiency can increase libido, suggesting that the effect of testosterone on male libido may be mediated by conversion to estradiol (Smith et al., 1994).

Suppression of testosterone production with a GnRH analogue and then replacing testosterone with or without *anastrozole*, an inhibitor of CYP19, also illustrates effects of testosterone that require conversion to estradiol. This paradigm demonstrated that the increase in sexual desire and erectile function and decrease in subcutaneous and abdominal fat require conversion of testosterone to estradiol but that the increase in lean mass and muscle strength do not (Finkelstein et al., 2013).

Effects of Androgens at Different Stages of Life

In Utero

When the fetal testes, stimulated by hCG, begin to secrete testosterone at about the eighth week of gestation, the high local concentration of testosterone around the testes stimulates the nearby Wolffian ducts to differentiate into the male internal genitalia: the epididymis, vas deferens, and seminal vesicles. In the anlage of the external genitalia, testosterone is converted to dihydrotestosterone, which causes the development of the male external genitalia. The increase in testosterone at the end of gestation may result in further phallic growth.

Infancy

The consequences of the increase in testosterone secretion by the testes during the first few months of life are not yet known.

Puberty

Puberty in the male begins at a mean age of 12 years with an increase in the secretion of FSH and LH from the gonadotrophs, stimulated by increased secretion of GnRH from the hypothalamus. The increased secretion of FSH and LH stimulates the testes. The increase in testosterone production by Leydig cells and the effect of FSH on the Sertoli cells stimulate the development of the seminiferous tubules, which eventually produce mature sperm. Increased secretion of testosterone into the systemic circulation affects many tissues simultaneously; the changes in most tissue occur gradually over the course of several years. The phallus enlarges in length and width, the scrotum becomes rugate, and the prostate begins secreting the fluid it contributes to the semen. The skin becomes coarser and oilier

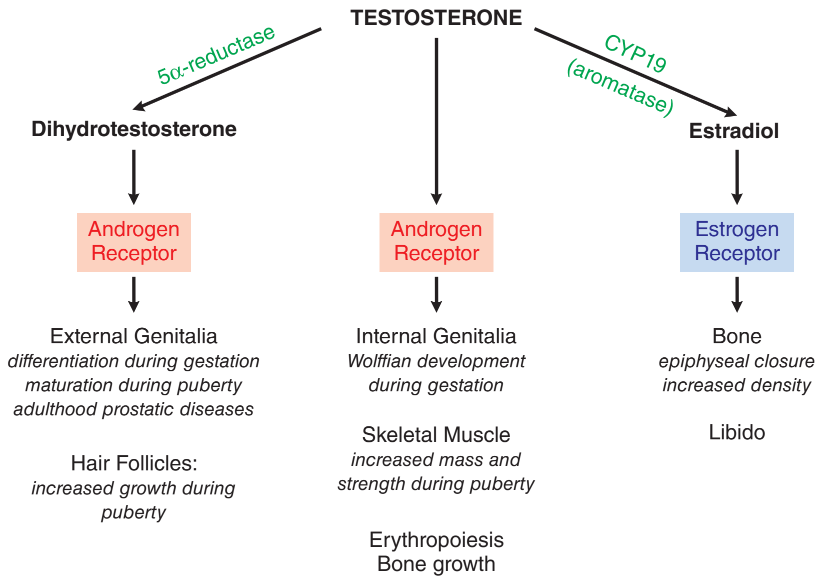

TESTOSTERONE
5α-reductase
CYP19 (aromatase)
Dihydrotestosterone
Estradiol
Androgen Receptor
Androgen Receptor
Estrogen Receptor
External Genitalia
differentiation during gestation
maturation during puberty
adulthood prostatic diseases
Hair Follicles:
increased growth during puberty
Internal Genitalia
Wolffian development during gestation
Skeletal Muscle
increased mass and strength during puberty
Erythropoiesis
Bone growth
Bone
epiphyseal closure
increased density
Libido

Figure 49–4 *Direct effects of testosterone and effects mediated indirectly via dihydrotestosterone or estradiol.*

due to increased sebum production, which contributes to the development of acne. Male differential sexual hair begins to grow, initially pubic and axillary hair, then hair on the lower legs, and finally other body hair and facial hair. Muscle mass and strength, especially of the shoulder girdle, increase, and subcutaneous fat decreases. Epiphyseal bone growth accelerates, resulting in the pubertal growth spurt, but epiphyseal maturation leads eventually to slowing and then cessation of growth. Bone also becomes thicker. Erythropoiesis increases, resulting in higher hematocrit and hemoglobin concentrations in men than boys or women. The larynx thickens, resulting in a lower voice. Libido develops. Other changes may result from the increase in testosterone during puberty; men tend to have a better sense of spatial relations than do women and to exhibit behavior that differs in some ways from that of women, including being more aggressive.

Adulthood

The serum testosterone concentration and the characteristics of the adult man are maintained largely during early adulthood and midlife. One change during this time is the gradual development of male pattern baldness, beginning with recession of hair at the temples or at the vertex.

Two other conditions are of great medical significance. One is benign *prostatic hyperplasia*, which occurs to a variable degree in almost all men, sometimes obstructing urine outflow by compressing the urethra as it passes through the prostate. This development is mediated by the conversion of testosterone to dihydrotestosterone by 5α-reductase II within prostatic cells (Wilson, 1980). The other change is the development of *prostate cancer*. Although no direct evidence suggests that testosterone causes the disease, prostate cancer depends on androgen stimulation. This dependency is the basis of treating metastatic prostate cancer by lowering the serum testosterone concentration or by blocking its action at the receptor.

Senescence

As men age, the serum testosterone concentration gradually declines (see Figure 49–2), and the sex hormone–binding globulin concentration gradually increases, so that by age 80, the total testosterone concentration is about 80% and the free testosterone is about 40% of those at age 20 (Harman et al., 2001). This fall in serum testosterone probably results in several other changes that occur with increasing age in men, including decreases in libido, bone mineral density, and hemoglobin, as suggested by improvements in these parameters when testosterone is increased to normal levels for young men (see Therapeutic Uses of Androgens, below).

Consequences of Androgen Deficiency

The consequences of androgen deficiency depend on the stage of life during which the deficiency first occurs and on the degree of the deficiency.

During Fetal Development

Testosterone deficiency in a male fetus during the first trimester in utero causes incomplete sexual differentiation. Complete deficiency of testosterone secretion results in entirely female external genitalia. Testosterone deficiency at this stage of development also leads to failure of the Wolffian ducts to differentiate into the male internal genitalia, but the Müllerian ducts do not differentiate into the female internal genitalia as long as testes are present and secrete Müllerian inhibitory substance. Similar changes occur if testosterone is secreted normally, but its action is diminished because of an abnormality of the AR or of the 5α-reductase.

Abnormalities of the AR can have quite varied effects. The most severe form results in complete absence of androgen action and a female phenotype; moderately severe forms result in partial virilization of the external genitalia; and the mildest forms permit normal virilization in utero and result only in impaired spermatogenesis in adulthood (McPhaul and Griffin, 1999). Abnormal 5α-reductase results in incomplete virilization of the external genitalia in utero but normal development of the male internal genitalia, which requires only testosterone (Wilson et al., 1993). Testosterone deficiency during the third trimester impairs phallus growth. The result, microphallus, is a common occurrence in boys later discovered to be unable to secrete LH due to abnormalities of GnRH secretion or action. In addition, with testosterone deficiency, the testes fail to descend into the scrotum; this condition, cryptorchidism, occurs commonly in boys whose LH secretion is subnormal (see Chapter 46).

Before Completion of Puberty

When a boy can secrete testosterone normally in utero but loses the capacity to do so before the anticipated age of puberty, the result is failure to complete puberty. All the pubertal changes previously described, including those of the external genitalia, sexual hair, muscle mass, voice, and behavior, are impaired to a degree proportionate to the abnormality of testosterone secretion. In addition, if growth hormone secretion is normal when testosterone secretion is subnormal during the years of expected puberty, the long bones continue to lengthen because the epiphyses do not close. The result is longer arms and legs relative to the trunk. Another consequence of subnormal testosterone secretion during the age of expected puberty is enlargement of glandular breast tissue, called *gynecomastia*.

After Completion of Puberty

When testosterone secretion becomes impaired after puberty (e.g., castration or antiandrogen treatment), regression of the pubertal effects of testosterone depends on both the degree and the duration of testosterone deficiency. When the degree of testosterone deficiency is substantial, libido and energy decrease within a week or two, but other testosterone-dependent characteristics decline more slowly. A clinically detectable decrease in muscle mass in an individual does not occur for several years. A pronounced decrease in hemoglobin will occur within several months. A decrease in bone mineral density can be detected within a year, but an increase in fracture incidence is not likely to occur for many years. Additionally, loss of sexual hair takes many years.

In Women

Loss of androgen secretion in women results in a decrease in sexual hair, but not for many years. Androgens may have other important effects in women, and the loss of androgens (especially with the severe loss of ovarian and adrenal androgens that occurs in panhypopituitarism) may result in decreased libido, energy, muscle mass and strength, and bone mineral density.

Therapeutic Androgen Preparations

Ingestion of testosterone is not an effective means of replacing testosterone deficiency due to its rapid hepatic metabolism following intestinal absorption. Most pharmaceutical preparations of androgens, therefore, are designed to bypass hepatic metabolism of testosterone. Note that all FDA-approved testosterone, *methyltestosterone*, and *oxandrolone* products are Drug Enforcement Administration schedule III controlled substances.

Testosterone Esters

Esterifying a fatty acid to the 17α-hydroxyl group of testosterone creates a compound that is even more lipophilic than testosterone itself. When an ester, such as testosterone enanthate (heptanoate) or cypionate (cyclopentylpropionate) (Table 49–1), is dissolved in oil and administered intramuscularly every 1 to 2 weeks to hypogonadal men, the ester hydrolyzes *in vivo* and results in serum testosterone concentrations that range from higher than normal in the first few days after the injection to low-normal just before the next injection (Figure 49–5). Attempts to decrease the frequency of injections by increasing the amount of each injection result in wider fluctuations and poorer therapeutic outcomes. The undecanoate ester of testosterone, when dissolved in oil and ingested orally, is absorbed into the lymphatic circulation, thus bypassing initial hepatic metabolism. *Testosterone undecanoate* in oil also can be injected and produces stable serum testosterone concentrations for 2 months.

TABLE 49–1 ■ ANDROGENS AVAILABLE FOR THERAPEUTIC USE

Testosterone
Testosterone Esters Testosterone cypionate/enanthate/undecanoate
17α-Alkylated Androgens Methyltestosterone, oxandrolone, stanozolol Fluoxymesterone, danazol

Alkylated Androgens

Several decades ago, chemists found that adding an alkyl group to the 17α position of testosterone retards its hepatic metabolism. Consequently, 17α-alkylated androgens can be administered orally. Table 49–1 lists 17α-alkylated androgens used clinically. However, 17α-alkylated androgens are less androgenic than testosterone and cause hepatotoxicity, whereas native testosterone does not. Some 17α-alkylated androgens show greater anabolic effects than androgenic effects compared to native testosterone in laboratory tests in rats; however, these “anabolic” steroids, so favored by athletes to illicitly improve performance, have not been convincingly demonstrated to have such a differential effect in human beings. Citing potentially serious health risks, the FDA has recommended against the use of body-building products that are marketed as containing steroids or steroid-like substances (FDA, 2017a, 2017b).

Transdermal Delivery Systems

To avoid the “first-pass” inactivation of testosterone by the liver, chemicals called excipients are used to facilitate the absorption of native testosterone across the skin in a controlled fashion. These transdermal preparations provide more stable serum testosterone concentrations than do injections of testosterone esters. Available preparations include gels or a solution applied to the skin or nasal mucosa, a transdermal patch, and a buccal tablet (see Figure 49–5).

Selective Androgen Receptor Modulators

Attempts have been made to develop selective nonsteroidal AR modulators that exhibit desirable effects of testosterone in some tissues (such as muscle and bone) without the undesirable effects in other tissues, such as prostate. Some molecules with these properties have been developed and have been tested in humans, but unlike selective estrogen receptor modulators (see Chapter 48), none is currently available for clinical use.

Therapeutic Uses of Androgens

Male Hypogonadism

The best-established indication for administration of androgens is testosterone replacement of testosterone deficiency in men. Any of the testosterone preparations or testosterone esters described can be used to treat testosterone deficiency.

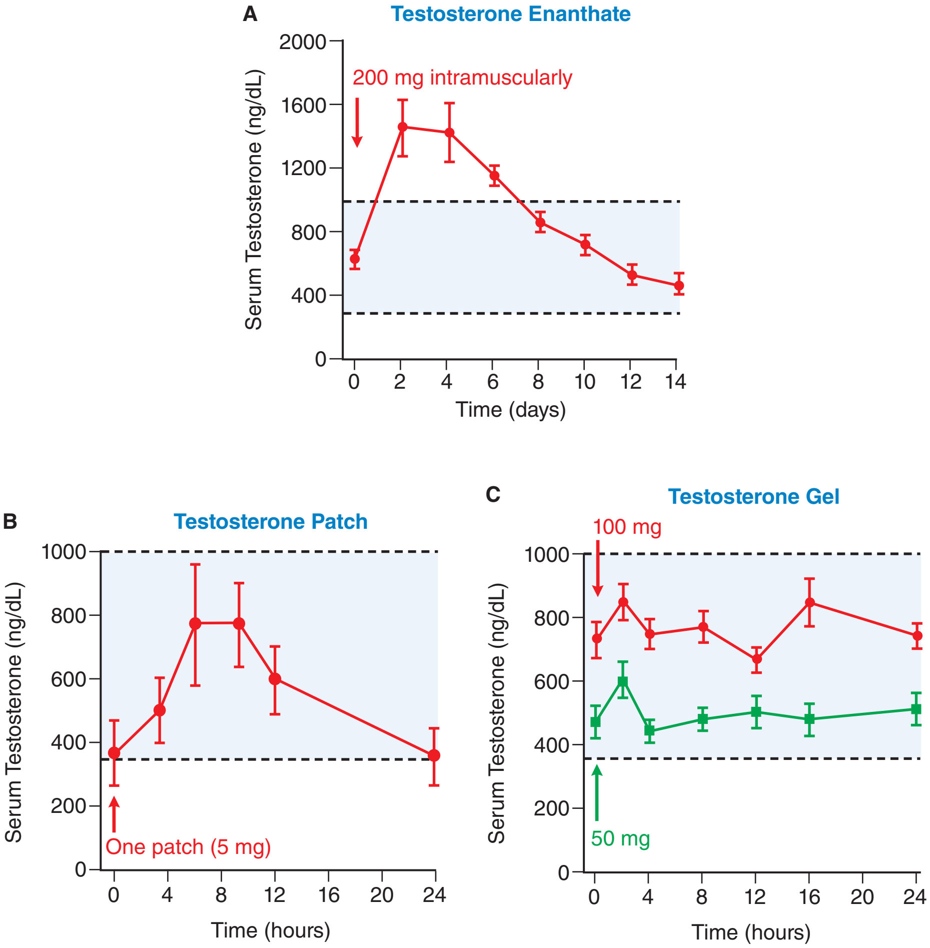


Figure 49–5 *Pharmacokinetic profiles of testosterone preparations during chronic administration to hypogonadal men.* Doses of each were given at time 0. Shaded areas indicate ranges of normal levels (**A.** Data adapted from Snyder and Lawrence, 1980. **B.** Data adapted from Dobs et al., 1999. **C.** Data adapted from Swerdloff et al., 2000.

Monitoring for Efficacy

The goal of administering testosterone to a hypogonadal man is to mimic as closely as possible the normal serum concentration (see Figure 49–5). Therefore, measuring the serum testosterone concentration during treatment is the most important aspect of monitoring testosterone treatment for efficacy. With testosterone gels, the serum testosterone concentration is relatively constant from one application to the next (Swerdloff et al., 2000). When the enanthate or cypionate ester of testosterone is administered once every 2 weeks, the serum testosterone concentration measured midway between doses should be normal; if not, the dosage schedule should be adjusted accordingly. If testosterone deficiency results from testicular disease, as indicated by an elevated serum LH concentration, adequacy of testosterone treatment also can be judged indirectly by the normalization of LH within 2 months of treatment initiation (Snyder and Lawrence, 1980).

Normalization of the serum testosterone concentration induces normal virilization in prepubertal boys and restores virilization in adult men who became hypogonadal as adults. Within a few months, and often sooner, libido, energy, and hemoglobin return to normal. Within 6 months, muscle mass increases and fat mass decreases. Bone density and trabecular connectivity, however, continue to increase for 2 years (Al Mukaddam et al., 2014; Snyder et al., 2000).

Monitoring for Deleterious Effects

Testosterone administered by itself as a transdermal preparation has no “side effects” (i.e., no effects that endogenously secreted testosterone does not have), as long as the dose is not excessive. Some of these undesirable effects occur shortly after testosterone administration is initiated, whereas others usually do not occur until administration has been continued for many years. Raising the serum testosterone concentration can result in undesirable effects similar to those that occur during puberty, including acne, gynecomastia, and more aggressive sexual behavior. Physiological amounts of testosterone do not appear to affect serum lipids or apolipoproteins.

Replacement of physiological levels of testosterone occasionally has undesirable effects in the presence of concomitant illnesses. If the testosterone dose is excessive, erythrocytosis and, uncommonly, salt and water retention and peripheral edema occur even in men who have no predisposition to these conditions.

When a man is more than 40 years of age, he is subject to certain testosterone-dependent diseases, including benign prostatic hyperplasia and prostate cancer. Modified testosterones, such as the 17α-alkylated androgens, do have side effects even when the doses are targeted to be physiologic. The principal side effects are hepatic, including cholestasis and, uncommonly, peliosis hepatitis, blood-filled hepatic cysts. Hepatocellular cancer has been reported rarely. Additionally, they may lower serum high-density lipoprotein cholesterol, especially at large doses.

Monitoring at the Anticipated Time of Puberty

Testosterone accelerates epiphyseal maturation, leading initially to a growth spurt but then to epiphyseal closure and permanent cessation of linear growth. Consequently, the height and growth hormone status of the boy being treated must be considered. Boys who are short because of growth hormone deficiency should be treated with growth hormone before their hypogonadism is treated with testosterone.

Male Senescence

Serum testosterone levels decrease as men age, and the parallels between the consequences of aging and those of hypogonadism due to pituitary or testicular disease, such as decreases in sexual function, bone density, and hemoglobin, suggest the possibility that the decrease in testosterone with aging may contribute to these changes of aging. A study of 788 men 65 years or older with low testosterone concentrations demonstrated that testosterone treatment for 1 year, compared to placebo, improved sexual function, mood, depressive symptoms, anemia, and bone density and strength (Roy et al., 2017; Snyder et al., 2016, 2017). Testosterone replacement, however, did not improve cognitive function or glucose or lipid metabolism.

No studies to date have been large enough or long enough to determine whether testosterone treatment of older men will increase the risk of prostate cancer, urinary tract symptoms, or heart disease. The FDA, however, has become sufficiently concerned about the possible risk of cardiovascular disease, based on epidemiological studies and small clinical trials, that it has required a label change for testosterone preparations to indicate that they are approved only for men with “classical hypogonadism,” meaning hypogonadism due to discernible pituitary or testicular disease, and not for idiopathic or age-related hypogonadism (Ngyuen et al., 2015).

Female Hypogonadism

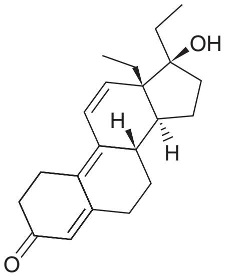
Few data exist regarding whether increasing the serum testosterone concentrations of women whose serum testosterone concentrations are below normal will improve their libido, energy, muscle mass and strength, or bone mineral density. In a study of women with low serum testosterone concentrations due to panhypopituitarism, increasing the testosterone concentration to high-normal was associated with small increases in bone mineral density, fat-free mass, and sexual function compared to placebo (Miller et al., 2006).

Enhancement of Athletic Performance

Some athletes take drugs, including androgens, in an attempt to improve their performance. Because androgens for this purpose usually are taken surreptitiously, information about their possible effects is not as complete as that for androgens taken for treatment of male hypogonadism. Citing potentially serious health risks, the FDA has recommended against the use of body-building products that are marketed as containing steroids or steroid-like substances (FDA, 2017a, 2017b).

Kinds of Androgens Used

Virtually all androgens produced for human or veterinary purposes have been taken by athletes. When such use by athletes began more than 35 years ago, 17α-alkylated androgens and other compounds (the so-called anabolic steroids) were thought to have greater anabolic effects than androgen effects relative to testosterone and were used most commonly. Because these compounds can be detected readily by organizations that govern athletic competitions, other agents that increase the serum concentration of testosterone itself, such as the testosterone esters or hCG, have increased in popularity. Testosterone precursors, such as androstenedione and dehydroepiandrosterone, are also popular because they are treated as nutritional supplements and thus are not regulated by athletic organizations. Selective androgen receptor modulators, although not approved for clinical use, are available on the Internet. *THG*, a potent androgen, appears to have been designed and synthesized to avoid detection by antidoping laboratories on the basis of its novel structure and rapid catabolism.



Tetrahydrogestrinone (THG)

Efficacy

The few controlled studies of the effects of pharmacological doses of androgens suggest a dose-dependent effect of testosterone on muscle strength that acts synergistically with exercise. In one controlled study, 43 normal young men were randomized to one of four groups: strength training with or without 600 mg of testosterone enanthate once a week (more than six times the replacement dose) or no exercise with or without testosterone. The men who received testosterone experienced an increase in muscle strength compared to those who received placebo, and the men who exercised simultaneously experienced even greater increases (Bhasin et al., 1996). In another study, normal young men were treated with a GnRH analogue to reduce endogenous testosterone secretion severely and, in a random blinded fashion, weekly doses of testosterone enanthate from 25 to 600 mg. There was a dose-dependent effect of testosterone on muscle strength (Bhasin et al., 2001). In contrast, in a double-blind study of androstenedione, men who took 100 mg three times a day for 8 weeks did not experience an increase in muscle strength compared to men who took placebo. The treatment also did not increase the mean serum testosterone concentration (King et al., 1999).

Side Effects

All androgens suppress gonadotropin secretion when taken in high doses and thereby suppress endogenous testicular function. This decreases endogenous testosterone and sperm production, resulting in diminished fertility. If administration continues for many years, testicular size may diminish. Testosterone and sperm production usually return to normal within 4 months of discontinuation but may take more than a year. High doses of androgens also cause erythrocytosis.

When administered in high doses, androgens that can be converted to estrogens, such as testosterone itself, cause gynecomastia. Androgens whose A ring has been modified so that it cannot be aromatized, such as dihydrotestosterone, do not cause gynecomastia even in high doses.

The 17α-alkylated androgens are the only androgens that cause hepatotoxicity. These androgens, when administered in high doses, affect serum lipid concentrations, specifically to decrease high-density lipoprotein cholesterol and increase low-density lipoprotein cholesterol. Women and children experience virilization, including facial and body hirsutism, temporal hair recession in a male pattern, and acne. Boys experience phallic enlargement, and women experience clitoral enlargement. Boys and girls whose epiphyses have not yet closed experience premature closure and stunting of linear growth.

Detection

An androgen other than testosterone can be detected by gas chromatography and mass spectroscopy if the athlete is still taking it when tested. Exogenous testosterone itself can be detected by one of two methods. One is the T/E ratio, the ratio of testosterone glucuronide to its endogenous epimer, epitestosterone glucuronide, in urine. Administration of exogenous testosterone suppresses secretion of both testosterone and epitestosterone and replaces them with only testosterone, so the T/E ratio is higher than normal. This technique is limited, however, by heterozygosity in the UDP-glucuronosyl transferase that converts testosterone to testosterone glucuronide. An athlete who has a deletion of one or both copies of the gene coding for this enzyme and who takes exogenous testosterone will have a much lower T/E ratio than one who has both copies (Schulze et al., 2008).

A second technique for detecting administration of exogenous testosterone employs gas chromatography-combustion-isotope ratio mass spectrometry to detect the presence of ^{13}C and ^{12}C compounds. Urinary steroids with a low $^{13}C/^{12}C$ ratio are likely to have originated from pharmaceutical sources as opposed to endogenous physiological sources (Aguilera et al., 2001).

Catabolic and Wasting States

Testosterone, because of its anabolic effects, has been used in attempts to ameliorate catabolic and muscle-wasting states, but this has not been generally effective. One exception is in the treatment of muscle wasting associated with AIDS, which often is accompanied by hypogonadism. Treatment of men with AIDS-related muscle wasting and subnormal serum testosterone concentrations increases their muscle mass and strength (Bhasin et al., 2000).

Angioedema

Chronic androgen treatment of patients with angioedema effectively prevents attacks. The disease is caused by hereditary impairment of C1-esterase inhibitor or acquired development of antibodies against it. The 17α-alkylated androgens (e.g., *stanozolol*, *danazol*) stimulate hepatic synthesis of the esterase inhibitor. In women, virilization is a potential side effect. In children, virilization and premature epiphyseal closure prevent chronic use of androgens for prophylaxis, although they are used occasionally to treat acute episodes. Alternatively, concentrated C1-esterase inhibitor derived from human plasma may be used for protection in patients with hereditary angioedema.

Blood Dyscrasias

Androgens once were employed to attempt to stimulate erythropoiesis in patients with anemias of various etiologies, but the availability of erythropoietin has supplanted that use. Androgens, such as *danazol*, still are used occasionally as adjunctive treatment of hemolytic anemia and idiopathic thrombocytopenic purpura that are refractory to first-line agents.

Antiandrogens

Because some effects of androgens are undesirable, at least under certain circumstances, agents have been developed specifically to inhibit androgen synthesis or effects. Other drugs, originally developed for different purposes, have been accidentally found to be antiandrogens and now are used intentionally for this indication. See Chapter 73 for a more detailed discussion of androgen deprivation therapy for prostate cancer.

Inhibitors of Testosterone Secretion

Analogues of GnRH effectively inhibit testosterone secretion by inhibiting LH secretion. GnRH analogues, given repeatedly, downregulate the GnRH receptor and are available for treatment of prostate cancer.

Some antifungal drugs of the imidazole family, such as *ketoconazole* (see Chapter 61), inhibit CYPs and thereby block the synthesis of steroid hormones, including testosterone and cortisol. Because they may induce adrenal insufficiency and are associated with hepatotoxicity, these drugs generally are not used to inhibit androgen synthesis but sometimes are employed in cases of glucocorticoid excess (see Chapter 50).

Inhibitors of Androgen Action

These drugs inhibit the binding of androgens to the AR or inhibit 5α-reductase.

Androgen Receptor Antagonists

Flutamide, Bicalutamide, Nilutamide, Enzalutamide, Apalutamide and Darolutamide. Although relatively potent, these AR antagonists have limited efficacy when used alone because the increased LH secretion stimulates higher serum testosterone concentrations. They are used primarily in conjunction with a GnRH analogue in the treatment of metastatic prostate cancer (see Chapter 73). In this situation, they block the action of adrenal androgens, which are not inhibited by GnRH analogues. *Flutamide* also has been used to treat hirsutism in women; however, its association with hepatotoxicity warrants caution against its use for this cosmetic purpose.

Spironolactone. *Spironolactone* (see Chapter 29) is an inhibitor of aldosterone that also is a weak inhibitor of the AR and a weak inhibitor of testosterone synthesis. When the agent is used to treat fluid retention or hypertension in men, gynecomastia is a common side effect. In part because of this adverse effect, the selective mineralocorticoid receptor antagonist *eplerenone* was developed. *Spironolactone* can be used in women to treat hirsutism.

Cyproterone Acetate. *Cyproterone acetate* is a progestin and a weak antiandrogen by virtue of binding to the AR. It is moderately effective in reducing hirsutism alone or in combination with an oral contraceptive but is not approved for use in the U.S.

5α-Reductase Inhibitors

Finasteride and *dutasteride* are antagonists of 5α-reductase. They block the conversion of testosterone to dihydrotestosterone, especially in the male

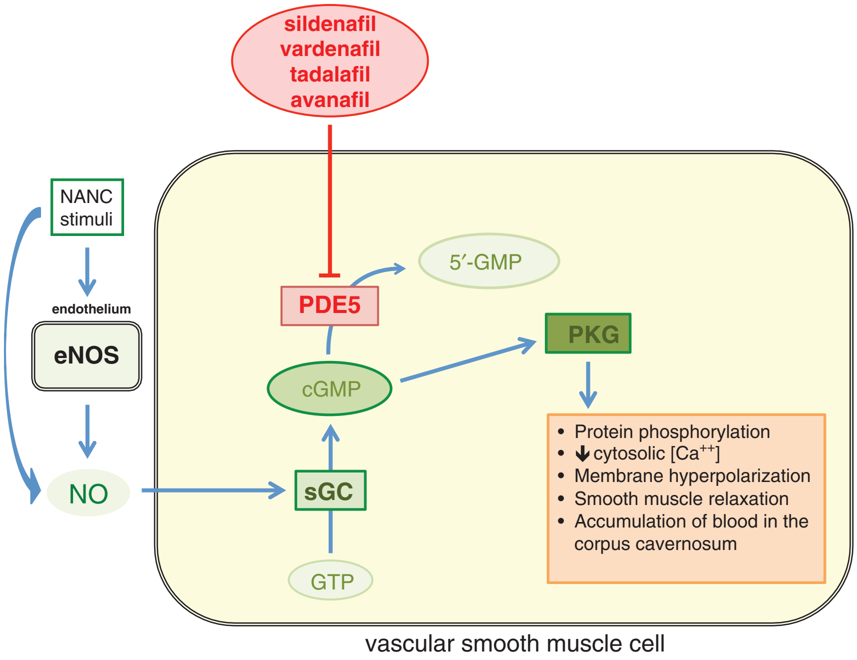

sildenafil
vardenafil
tadalafil
avanafil
NANC stimuli
endothelium
eNOS
NO
PDE5
5′-GMP
cGMP
PKG
sGC
GTP
• Protein phosphorylation
• ↓cytosolic [Ca++]
• Membrane hyperpolarization
• Smooth muscle relaxation
• Accumulation of blood in the corpus cavernosum
vascular smooth muscle cell

Figure 49–6 *Mechanism of action of PDE5 inhibitors in the corpus cavernosum.* Physiologically, penile erection is initiated by nonadrenergic/noncholinergic (NANC) neural stimulation that results in NO release from neurons and endothelial cells. PDE5 inhibitors enhance signaling through the NO–guanylyl cyclase–cGMP–PKG pathway by inhibiting the degradation of cGMP, thereby enhancing the activation of PKG. PKG activation leads to relaxation of cavernosal smooth muscle, which permits engorgement of the corpus cavernosum with blood, resulting in penile erection. eNOS, endothelial nitric oxide synthase.

external genitalia. These drugs were developed to treat benign prostatic hyperplasia and are approved in the U.S. and many other countries for this purpose. When they are administered to men with moderate-to-severe symptoms due to obstruction of urinary tract outflow, serum and prostatic concentrations of dihydrotestosterone decrease, prostatic volume decreases, and urine flow rate increases (McConnell et al., 1998). Impotence is a documented, albeit infrequent, side effect of this use. Gynecomastia is a rare side effect. *Finasteride* also is approved for use in the treatment of male pattern baldness and is effective in the treatment of hirsutism.

Pharmacological Treatment of Erectile Dysfunction

Normal erectile function depends on a combination of many factors, including visual, psychological, hormonal, and neurological factors, that act via the common mechanism of increasing the synthesis of NO by vascular endothelium in the arterioles supplying the corpora cavernosa and in the corpora cavernosa. NO diffuses to adjacent smooth muscle cells and causes vasodilation of arterioles and increased compliance of the cavernosal space, permitting its engorgement with blood. This accumulation of blood also restricts the outflow by compressing the veins against the surrounding sheath (*tunica albuginea*). The overall result is penile erection.

Erectile dysfunction can result from psychological, hormonal, and vascular causes, including damage to endothelium and from side effects of various drugs, including some that are used in the therapy of hypertension; it is associated with a variety of disease states, including diabetes (Dean and Lue, 2005).

Erectile Signaling and Erectile Dysfunction

Nitric oxide acts by binding and activating sGC, which catalyzes the production of cyclic GMP from cellular GTP. Cyclic GMP is a second messenger that activates *protein kinase G* (PKG), leading to phosphorylation of contractile proteins and ion channels to decrease the concentration of intracellular Ca^{2+}, resulting in smooth muscle relaxation and increased blood flow to corpora cavernosa. Phosphodiesterase type 5 (PDE5) degrades cyclic GMP; thus, erectile dysfunction can be improved by drugs that retard the degradation of cyclic GMP by inhibiting PDE5 (Goldstein et al., 1998) (Figure 49–6).

PDE5 Inhibitors

Available inhibitors of PDE5 include *sildenafil*, *vardenafil*, *tadalafil*, and *avanafil*. All these agents compete for cyclic GMP binding at the site of cyclic GMP hydrolysis on PDE5. PDE5 inhibitors are also used in treating pulmonary arterial hypertension (see Chapter 35).

ADME

Table 49–2 summarizes pharmacokinetic properties of the available PDE5 inhibitors. These agents are adequately absorbed orally, widely distributed, and act fairly quickly (within ~30 min). Their affinities, time to onset, and half-lives differ somewhat (see Table 49–2), giving patients options for onset and duration of effect. The drugs are metabolized by hepatic CYP3A4, with minor contributions by CYP2C9 (20% for *sildenafil*). Excretion of metabolites is largely via the feces, with urinary excretion playing a secondary role in excretion of *tadalafil* (36%) (Mehrotra et al., 2007).

Clinical Use

All these agents produce satisfactory results in most patients. The starting dose recommendations vary, and patients should start at the lowest recommended dose. This is especially important in patients over 65 years.

Adverse Effects, Precautions

Adverse effects are similar but not identical across this drug class owing to their similar mechanism of action but their differing specificities toward PDE5 compared to other PDE isoforms. Common complaints are headache, flushing, dyspepsia, nasal congestion, dizziness, and back pain. Some patients using *sildenafil* or *vardenafil* may notice blurred vision and a blue-green tinting of vision, referable to inhibition of retinal PDE6, which is involved in phototransduction (see Chapter 74).

TABLE 49–2 ■ PHARMACOKINETIC PROPERTIES OF PDE5 INHIBITORS

	SILDENAFIL	VARDENAFIL	TADALAFIL	AVANAFIL
K_i (nM)	4	0.1	2	4
Plasma $t_{1/2}$ (h)	4	4	17.5	1.3–2
Oral bioavailability (%)	40	15	40	70
Onset of action (min)	30–60	30–60	30–120	15–30
Time to C_{Pmax} (min)	60	60	120	30
Maximum duration of action (h)[a]	12	10	36	6
Optic effects/PDE6	+	+	–	–
Food[b] alters AUC, C_{Pmax}?	+	+	–	±

[a] Duration will vary with dose and rate of clearance.
[b] High-fat meal; generally reduces AUC and C_{Pmax} but, for avanafil, prolongs absorption period and time to C_{Pmax} (by 1 h), decreases C_{Pmax} (−24%), and increases AUC (+14%).
For pharmacokinetic data on PDE5 inhibitors, see FDA, 2012, and Mehrotra et al., 2007.

Concomitant administration of potent CYP3A inducers (e.g., *bosentan*) will generally cause substantial decreases in plasma levels of drugs in this class. CYP3A inhibitors (e.g., protease inhibitors used in human immunodeficiency virus therapy, *erythromycin*, and *cimetidine*) inhibit metabolism of PDE5 inhibitors, thereby prolonging the half-lives and elevating blood levels of these agents. Consistent with their mechanism of action, potentiation of cyclic GMP signaling, PDE5 inhibitors potentiate the hypotensive effects of nitrate vasodilators, producing dangerously low blood pressures. Thus, the administration of PDE5 inhibitors to patients receiving organic nitrates is contraindicated. The patient's underlying cardiovascular status and concurrent use of hypotensive agents (e.g., nitrates, α adrenergic antagonists) must be considered prior to use of this class of drugs. Priapism (erection lasting longer than 4 h) induced by PDE5 inhibitors runs the risk of ischemic damage to the cavernosal smooth muscle and sinusoidal epithelium and requires medical attention.

Drug Facts for Your Personal Formulary: *Androgens; Antiandrogens; PDE5 Inhibitors*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Testosterone Esters • Effective for weeks to months. Wide fluctuations in serum concentrations		
Testosterone enanthate, testosterone cypionate	• Treatment of male hypogonadism	• Formulated in oil for injection • Administer as a deep IM injection every 1–2 weeks • Effective in causing and maintaining virilization • Fluctuations in serum concentrations may result in fluctuations in energy, mood, and libido
Testosterone undecanoate	• Treatment of male hypogonadism	• Formulated in oil for injection • Administer as a deep IM gluteal injection. Observe for 30 min after injection for anaphylaxis or pulmonary microembolism • Administer every 10 weeks
Testosterone undecanoate for oral administration	• Treatment of male hypogonadism	• Taken 2–3 times a day with food • Absorbed into lymphatics
Testosterone Transdermal Patch		
One FDA-approved product	• Treatment of male hypogonadism	• Worn without interruption and changed once a day • High rate of skin rash
Transdermal Testosterone Gels		
Several FDA-approved products	• Treatment of male hypogonadism	• Applied once a day • Relatively steady serum testosterone concentration • Effective in causing and maintaining virilization
17α-Alkylated Androgens		
Danazol Methyltestosterone Oxandrolone	• Treatment of angioedema • Treatment of hemolytic anemia • Angioedema prophylaxis • Endometriosis • Fibrocystic breast disease	• Oral administration • Risk of hepatoxicity
GnRH Analogues		
Leuprolide Goserelin Triptorelin Histrelin Buserelin (not available in the U.S.)	• Treatment of metastatic prostate cancer • Leuprolide also approved for endometriosis, precocious puberty, prostate cancer, and uterine leiomyomata • Goserelin also approved for breast cancer, dysfunctional uterine bleeding, and endometriosis • Histrelin also approved for precocious puberty and prostate cancer	• Parenteral administration • Suppresses LH and FSH secretion and thereby causes profound hypogonadism
Androgen Receptor Antagonists		
Flutamide Bicalutamide Nilutamide Enzalutamide	• Adjuvant treatment of metastatic prostate cancer	• Used in conjunction with GnRH agonists
5α-Reductase inhibitors		
Finasteride Dutasteride	• Treatment of lower urinary tract symptoms due to benign prostatic hyperplasia • Finasteride also approved for alopecia	• Shrinks the size of the prostate by decreasing the production of dihydrotestosterone in the prostate • Dutasteride also marketed as fixed-dose combination with tamsulosin
PDE5 Inhibitors		
Sildenafil, vardenafil, tadalafil, avanafil	• Male erectile dysfunction • Pulmonary arterial hypertension	• Contraindicated in patients using nitrate vasodilators (can cause dangerously low blood pressure) • Side effects: headache, flushing, blue-green tinted vision • Erection lasting >4 h requires medical attention

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Chapter 50

Adrenocorticotropic Hormone, Adrenal Steroids, and the Adrenal Cortex

Christopher J. Hupfeld and Jorge A. Iñiguez-Lluhí

CORTICOTROPIN (ACTH)

- Actions on the Adrenal Cortex
- Mechanism of Action
- Regulation of ACTH Secretion
- Therapeutic Uses and Diagnostic Applications of ACTH and CRH

ADRENOCORTICAL STEROIDS

- Synthesis, Circulating Levels, and Interconversion
- Physiological Functions and Pharmacological Effects
- Pharmacokinetics
- Toxicity of Adrenocortical Steroids
- Therapeutic Uses and Diagnostic Applications in Endocrine Diseases
- Therapeutic Uses in Nonendocrine Diseases

INHIBITORS OF ACTH SECRETION AND THE BIOSYNTHESIS AND ACTIONS OF ADRENOCORTICAL STEROIDS

- Inhibitors of ACTH Secretion and Function
- Inhibitors of Steroidogenesis and Adrenolytic Agents
- Glucocorticoid Receptor Antagonists
- Mineralocorticoid Receptor Antagonists

Corticotropin, also known as adrenocorticotropic hormone (ACTH), is secreted by specialized cells in the anterior pituitary gland known as the *corticotrophs.* They constitute about 20% of the anterior pituitary cells. Besides ACTH, corticotrophs also release melanocyte-stimulating hormone (MSH) and lipotropin. While synthetic derivatives of ACTH are used commonly in the diagnostic assessment of adrenal cortex function, synthetic corticosteroids, rather than ACTH, are used therapeutically.

The adrenal cortex synthesizes and secretes steroid hormones essential for adaptive responses to stress (glucocorticoid) and mineral balance (mineralocorticoid) and for some direct and indirect androgenic functions, particularly in women (adrenal androgens). Glucocorticoids and mineralocorticoids are collectively referred as corticosteroids, with cortisol and aldosterone being the main physiologic forms. Corticosteroids and their biologically active synthetic derivatives may differ individually in their glucocorticoid (metabolic/anti-inflammatory) and mineralocorticoid (electrolyte-regulating) actions. These agents are used as physiological replacement therapy when endogenous production is impaired, as in adrenal insufficiency.

The broad anti-inflammatory and immunosuppressive properties of glucocorticoids are of great therapeutic value in numerous conditions where suppression of inflammation is needed (such as autoimmune diseases and allergic reactions), making them among the most frequently prescribed classes of drugs. Shortly after synthetic *cortisone* became available, Hench and colleagues demonstrated its dramatic effect in the treatment of rheumatoid arthritis, setting the stage for the clinical use of corticosteroids in a wide variety of diseases, as discussed in this chapter. Because glucocorticoids exert effects on almost every organ system, their administration and withdrawal may be complicated by severe side effects. Therefore, the decision to institute therapy with systemic glucocorticoids always requires careful consideration of the relative risks and benefits in each patient.

Corticotropin (ACTH)

Human ACTH, a peptide containing 39 amino acids, is synthesized as part of a larger precursor protein, pro-opiomelanocortin (POMC). In the pituitary, POMC undergoes proteolytic cleavage at dibasic residues by two serine endoproteases, proprotein convertase subtilisin/kexin 1/3 and 2 (PCSK1/3 and 2; Figure 50–1). This process produces ACTH as well as

HISTORICAL PERSPECTIVE

Addison described fatal outcomes in patients with adrenal destruction in 1849. A few years later, Brown-Séquard demonstrated that bilateral adrenalectomy was fatal in laboratory animals. It became clear that the adrenal cortex, rather than the medulla, was essential for survival in these ablation experiments and that the cortex regulated carbohydrate metabolism and fluid and electrolyte balance. The isolation and identification of the adrenal steroids by Reichstein and Kendall and the effects of these compounds on carbohydrate metabolism (hence the term glucocorticoids) culminated with the synthesis of *cortisone*, the readily available, pharmacologically effective glucocorticoid. Subsequently, Tait and colleagues isolated and characterized aldosterone, which potently affected fluid and electrolyte balance and was termed a mineralocorticoid. That distinct corticosteroids regulated carbohydrate metabolism and fluid/electrolyte balance led to the concept that the adrenal cortex comprises two largely independent units: an outer zone that produces mineralocorticoids and an inner region that synthesizes glucocorticoids and androgen precursors. Kendall, Reichstein, and Hench shared the 1950 Nobel Prize in Physiology/Medicine "for their discoveries relating to the hormones of the adrenal cortex, their structure and biological effects."

Studies of adrenocortical steroids also were key in delineating the role of the anterior pituitary. As early as 1912, Cushing described patients with hypercorticism; he later recognized that pituitary basophilism caused the adrenal overactivity, thus establishing the link between the anterior pituitary and adrenal function. These studies led to the purification of ACTH and the determination of its chemical structure. ACTH was shown to be essential for maintaining the structural integrity and steroidogenic capacity of the adrenal's inner cortical zones. Harris established the role of the hypothalamus in pituitary control and postulated that a soluble factor produced by the hypothalamus activated ACTH release. These investigations culminated with the determination of the structure of *corticotropin-releasing hormone* (CRH), a hypothalamic peptide that, together with *arginine vasopressin* (AVP), regulates secretion of ACTH from the pituitary (Miller, 2013).

Abbreviations

ACTH: corticotropin (or adrenocorticotropic hormone)
AngII: angiotensin II
AVP: arginine vasopressin
CAH: congenital adrenal hyperplasia
CBG: corticosteroid-binding globulin
COX: cyclooxygenase
CRH: corticotropin-releasing hormone
CYP: cytochrome P450
CYP11A1: cholesterol side-chain cleavage enzyme
CYP11B1: 11β-hydroxylase
CYP11B2: aldosterone synthase
CYP17A1: 17α-hydroxylase
CYP19A1: aromatase
CYP21A2: steroid 21-hydroxylase
DHEA: dehydroepiandrosterone
DHEAS: dehydroepiandrosterone sulfate
GR: glucocorticoid receptor
HPA: hypothalamic-pituitary-adrenal
3β-HSD: 3β-hydroxysteroid dehydrogenase
11β-HSD1: 11β-hydroxysteroid dehydrogenase (type 1)
11β-HSD2: 11β-hydroxysteroid dehydrogenase (type 2)
5HT: serotonin
IL: interleukin
MCR: melanocortin receptor (5 subtypes; # = 1 to 5)
MR: mineralocorticoid receptor
MSH: melanocyte-stimulating hormone
NF-κB: nuclear factor kappa B
PK: protein kinase
PLC: phospholipase C
POMC: pro-opiomelanocortin
TNF: tumor necrosis factor

other biologically active peptides including endorphins, lipotropins, and MSHs (see also Table 23–1 and Harno et al., 2018).

The actions of the POMC-derived melanocortins (ACTH and MSHs) are mediated by their specific interactions with five melanocortin receptor (MCR) subtypes (MC1R–MC5R) comprising a subfamily of GPCRs (Cone, 2006; Novoselova et al., 2018). The effect of MSH on pigmentation results from interactions with MC1R on melanocytes. ACTH, which is identical to α-MSH in its first 13 amino acids, exerts its effects at the adrenal cortex through MC2R. The affinity of ACTH for MC1R is much lower than for MC2R; however, under pathological conditions in which ACTH levels are persistently elevated (such as primary adrenal insufficiency), ACTH itself may lead to hyperpigmentation through MC1R. In the hypothalamus, activation of MC4R and MC3R by MSH peptides as well as antagonism by agouti signaling protein (ASIP) participate in the regulation of body weight and appetite. The function of MC5R is less well defined, but studies in rodents suggest roles in exocrine secretion, particularly sebogenesis, lacrimal secretion and release of sex pheromones (Morgan and Cone, 2006). Recent translational efforts have focused on developing novel therapies for seborrhea and acne vulgaris based on antagonizing MC5R (Xu et al., 2020).

Actions on the Adrenal Cortex

ACTH, acting via the MC2R, stimulates enzyme activity in the adrenal cortex, regulating production of the main hormones cortisol, aldosterone, and the androgen precursor *dehydroepiandrosterone* (DHEA). The adrenal cortex, both histologically and functionally, can be separated into three zones (Figure 50–2) that produce different steroids under different regulatory influences:

- The outer zona glomerulosa produces aldosterone, the main mineralocorticoid.
- The middle zona fasciculata produces cortisol, the main glucocorticoid.
- The inner zona reticularis produces the androgen precursor DHEA and the much more abundant sulfated derivative DHEAS.

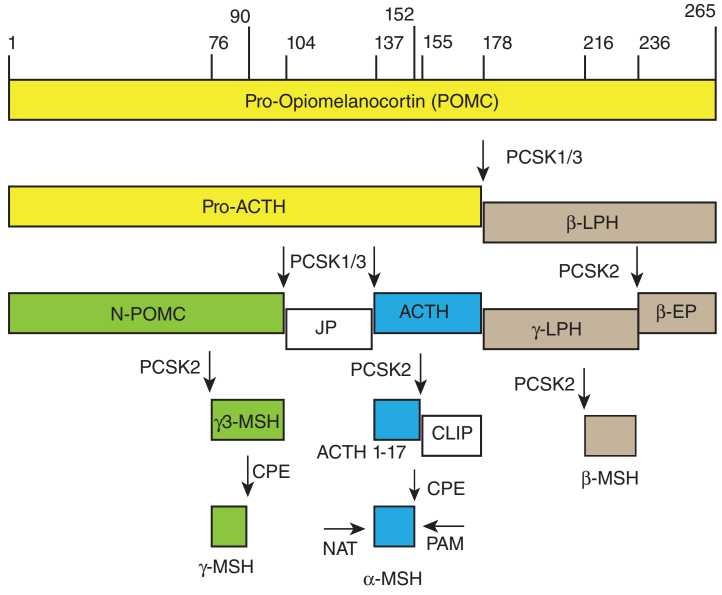

Figure 50–1 *Processing of POMC to ACTH.* POMC is converted to ACTH and other peptides in anterior pituitary, pars intermedia, hypothalamus, and skin. Human peptide cleavage sites are listed above. Anterior pituitary expresses PCSK1/3 only, whereas at other sites, all enzymes are expressed. CLIP, corticotropin-like intermediate peptide; CPE, carboxypeptidase E; JP, joining peptide; MSH, melanocyte-stimulating hormone; NAT, *N*-acetyltransferase; PAM, peptidyl-glycine alpha-amidating monooxygenase; PCSK, prohormone convertase subtilisin/kexin.

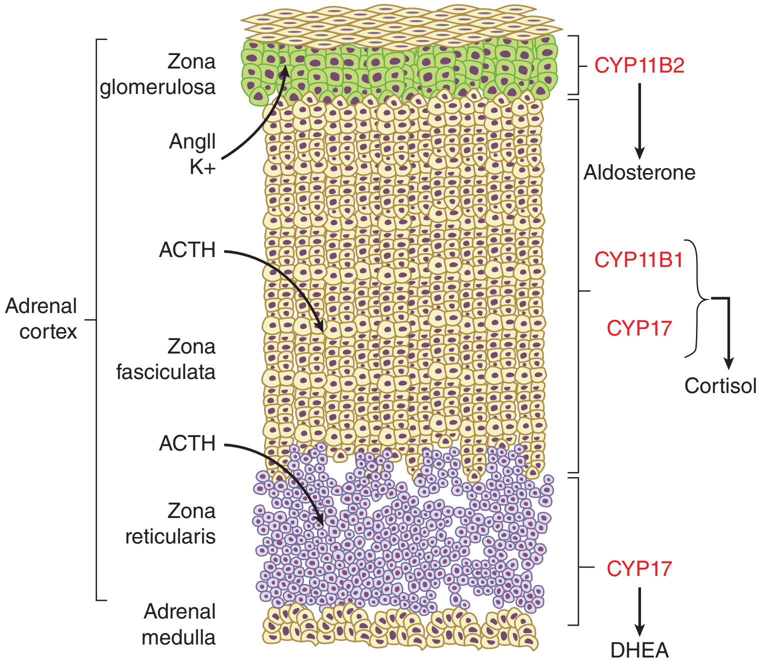

Figure 50–2 *The three anatomically and functionally distinct compartments of the adrenal cortex.* The major functional compartments of the adrenal cortex are shown, along with the steroidogenic enzymes that determine the unique profiles of corticosteroid products. Also shown are the predominant physiological regulators of steroid production: AngII and K^+ for the zona glomerulosa and ACTH for the zona fasciculata. Although ACTH acutely increases DHEA biosynthesis, the physiological regulators of DHEA production by the zona reticularis are not completely known.

Adrenal steroid hormones are produced from the same precursor, cholesterol, by a set of cytochrome P450 enzymes (CYP11A1, CYP11B1 and CYP11B2, and CYP17A1 and CYP21A2) and the steroid dehydrogenase 3β-HSD. These enzymes are differentially expressed in the three zones of the adrenal cortex giving rise to zone-specific hormone production. Some of the enzymes encoded by these genes can catalyze more than one reaction.

Cells in the zona glomerulosa have receptors for both ACTH and angiotensin II (AngII) and express aldosterone synthase (CYP11B2), the enzyme that catalyzes the terminal reactions in mineralocorticoid biosynthesis. Although ACTH acutely stimulates mineralocorticoid production, this zone is regulated primarily by AngII and extracellular K^+ (see Chapter 30) and remains functional in the absence of pituitary function. With persistently elevated ACTH, mineralocorticoid levels initially increase but then return to normal (a phenomenon termed ACTH escape).

Cells of the zona fasciculata have receptors for ACTH and express 17α-hydroxylase (CYP17A1) and 11β-hydroxylase (CYP11B1). These enzymes catalyze the production of glucocorticoids under the control of ACTH. In the zona reticularis, 17α-hydroxylase carries out an additional C17-20 lyase reaction that converts C21 corticosteroids into the C19 androgen precursors.

In the absence of ACTH stimulation (as may occur in hypopituitarism), the zona fasciculata and reticularis undergo atrophy, and production of glucocorticoids and adrenal androgens is severely impaired. Conversely, persistently elevated levels of ACTH induce hypertrophy and hyperplasia of these inner zones, with concomitant overproduction of glucocorticoids and adrenal androgens. Adrenal hyperplasia is most marked in congenital disorders of steroidogenesis, in which ACTH levels are continually elevated as a secondary response to impaired cortisol biosynthesis.

Mechanism of Action

ACTH stimulates the synthesis and release of the adrenocortical hormones by increasing *de novo* biosynthesis (steroid hormones are hydrophobic and thus cannot be stored). ACTH binding to MC2R activates the G_s–adenylyl cyclase–cyclic AMP–PKA pathway. Cyclic AMP is the second messenger for most effects of ACTH on steroidogenesis.

Temporally, the response of adrenocortical cells to ACTH has two phases. The acute phase, which occurs within seconds to minutes, largely reflects an increased supply of cholesterol substrate to the steroidogenic enzymes. The chronic phase, which occurs over hours to days, results from increased transcription of the steroidogenic enzymes.

A number of transcriptional regulators participate in the induction of the steroidogenic enzymes by ACTH. Among these is the nuclear receptor NRSA1 (steroidogenic factor 1), a transcription factor required for the development of the adrenal cortex and for the expression of most of the steroidogenic enzymes (Schimmer and White, 2010). Pathways of adrenal steroid biosynthesis and the structures of the major steroid intermediates and products of the human adrenal cortex are shown in Figure 50–3. The rate-limiting step in steroid hormone production is the translocation of cholesterol across mitochondrial membranes by the steroid acute regulatory (StAR) protein. Cholesterol is then converted to pregnenolone by the side-chain cleavage enzyme CYP11A1, which represents the first enzymatic step in steroid hormone biosynthesis (Miller and Auchus, 2011). Most of the enzymes required for steroid hormone biosynthesis, including CYP11A1, are members of the cytochrome P450 superfamily (see Chapter 5). To ensure an adequate supply of substrate, the adrenal cortex uses multiple sources of cholesterol, including circulating cholesterol and cholesterol esters taken up via the low-density lipoprotein and high-density lipoprotein receptor pathways; endogenous cholesterol liberated from cholesterol ester stores via activation of cholesterol esterase; and endogenous cholesterol from *de novo* biosynthesis.

Regulation of ACTH Secretion

Hypothalamic-Pituitary-Adrenal (HPA) Axis

The rate of glucocorticoid secretion is determined by fluctuations in the release of ACTH by the pituitary corticotrophs. These corticotrophs are regulated by CRH and AVP, peptide hormones made by specialized neurons of the hypothalamus and released into the network of portal veins bathing the anterior pituitary (Sheng et al., 2021). This HPA axis forms an integrated system that maintains appropriate levels of glucocorticoids (Figure 50–4). The three characteristic modes of physiological regulation of the HPA axis are:

- Diurnal rhythm in basal steroidogenesis
- Negative-feedback regulation by adrenal corticosteroids
- Marked increases in steroidogenesis in response to stress

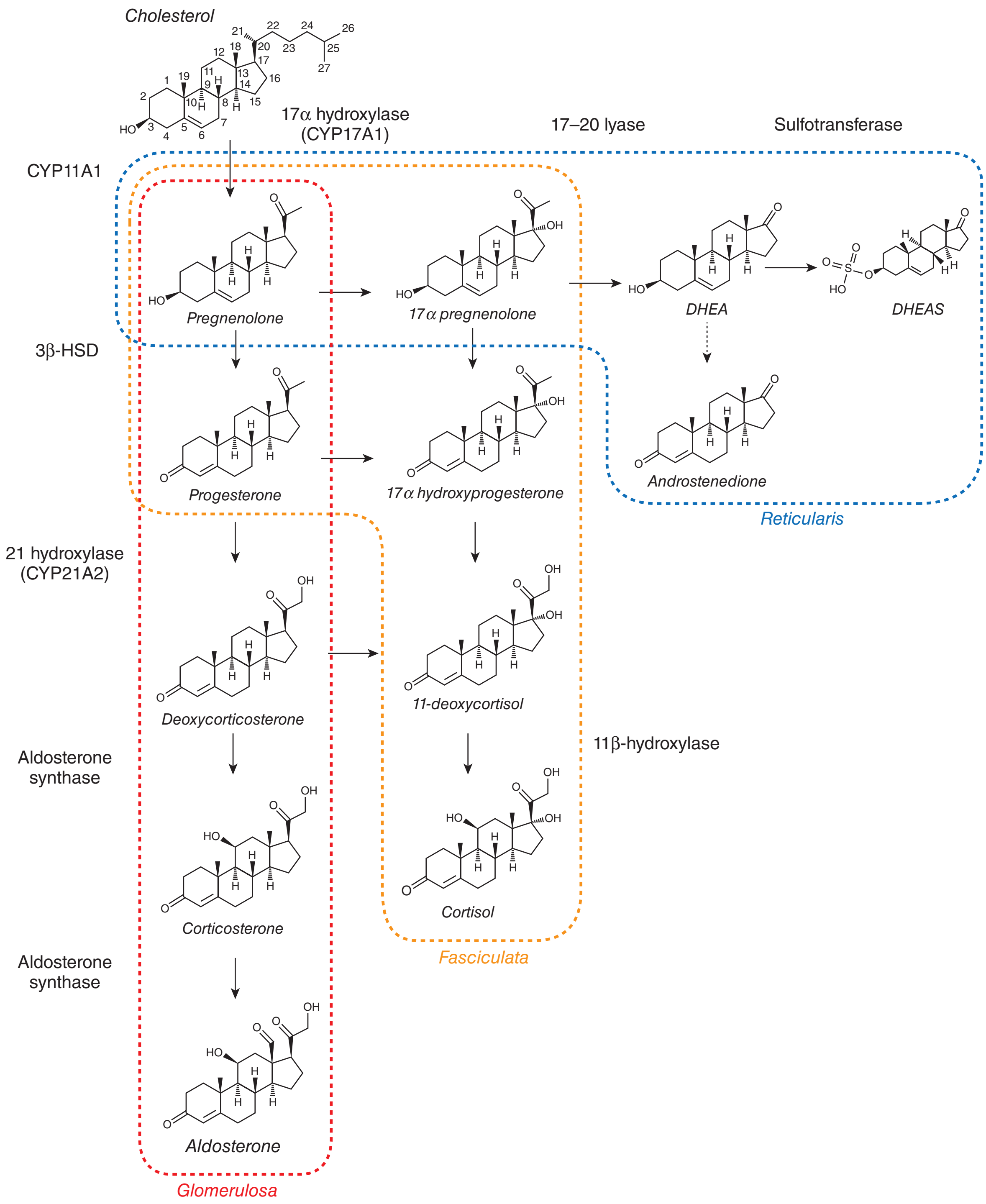
Cholesterol
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
HO
H
17α hydroxylase (CYP17A1)
17–20 lyase
Sulfotransferase
CYP11A1
Pregnenolone
17α pregnenolone
DHEA
DHEAS
3β-HSD
Progesterone
17α hydroxyprogesterone
Androstenedione
Reticularis
21 hydroxylase (CYP21A2)
Deoxycorticosterone
11-deoxycortisol
11β-hydroxylase
Aldosterone synthase
Corticosterone
Cortisol
Fasciculata
Aldosterone synthase
Aldosterone
Glomerulosa
O
OH

Figure 50–3 *Pathways of corticosteroid synthesis.* The steroidogenic pathways used in the biosynthesis of the corticosteroids are shown, along with the structures of the intermediates and products. The reactions in each of the adrenal zones are grouped by dashed lines. The numbering is indicated in the cholesterol structure.

Pathological elevation of steroidogenesis is seen in Cushing disease, in the setting of ectopic secretion of CRH or ACTH (often part of a paraneoplastic syndrome), and in rare conditions as a result of defects in corticosteroid receptor–mediated feedback mechanisms.

The diurnal rhythm is determined by circadian clocks in the hypothalamic suprachiasmatic nucleus and in the adrenal gland itself and is entrained by higher neuronal centers in response to sleep-wake cycles (Oster et al., 2017). Levels of ACTH peak in the early morning hours, causing the circulating glucocorticoid levels to peak at about 8 AM (Leliavski et al., 2015). Negative-feedback regulation occurs at multiple levels of the HPA axis and is the major mechanism that maintains circulating glucocorticoid levels in the appropriate range. Stress can override the normal negative-feedback control mechanisms, leading to marked increases in plasma concentrations of glucocorticoids.

Corticotropin-Releasing Hormone

Following release into the hypophyseal plexus, CRH is transported via this portal system to the anterior pituitary, where it binds to specific membrane receptors on corticotrophs. Upon CRH binding, the CRH receptor activates the G_s–adenylyl cyclase–cyclic AMP pathway within corticotrophs, ultimately stimulating both ACTH biosynthesis and secretion.

Arginine Vasopressin

Arginine vasopressin acts as a weak secretagogue for corticotrophs on its own but significantly potentiates the effects of CRH. AVP is produced

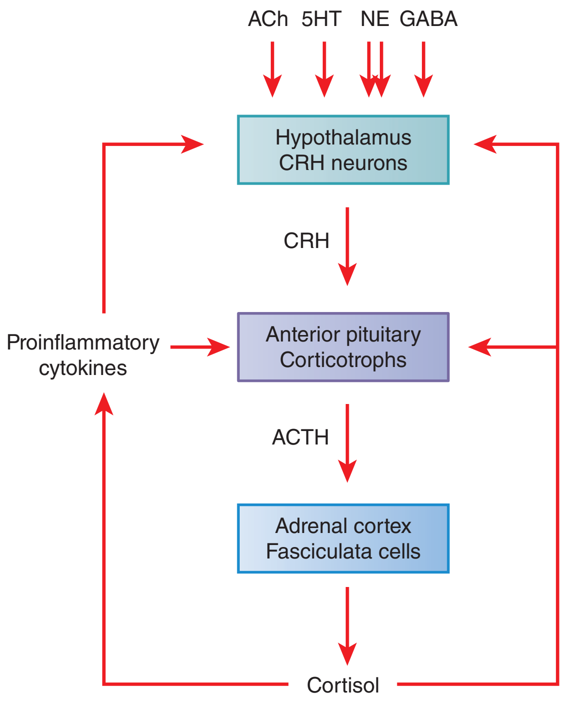

Figure 50–4 *The HPA axis and the immune inflammatory network.* Positive effects are in green, and negative effects are in red. Inputs from higher neuronal centers regulate CRH secretion. In addition, AVP stimulates release of ACTH from corticotrophs. ACh, acetylcholine; GABA, γ-aminobutyric acid; NE, norepinephrine.

in the paraventricular nucleus of the hypothalamus and secreted into the pituitary portal veins from the median eminence. AVP binds to V1b receptors and activates the G_q-PLC-IP_3-Ca^{2+} pathway to enhance the release of ACTH. In contrast to CRH, AVP by itself does not increase *de novo* ACTH synthesis.

Negative Feedback of Glucocorticoids

Glucocorticoid feedback of the HPA axis occurs at both the hypothalamus and the pituitary, and the effects are both rapid (seconds to minutes) and delayed (requiring hours and involving changes in gene transcription) (Keller-Wood, 2015). Glucocorticoids inhibit hypothalamic CRH secretion via direct effects on hypothalamic CRH neurons by decreasing CRH mRNA levels and CRH release. Indirect effects of cortisol on hypothalamic CRH secretion are mediated by both the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) acting on separate CRH neurons in the hippocampus. In the pituitary, glucocorticoids inhibit ACTH secretion through GR by inhibiting corticotroph responsiveness to CRH (rapid response) as well as suppressing POMC expression (delayed response).

The involvement of MR in cortisol feedback mechanisms in the CNS is based on the ability of MR to bind and respond to cortisol in cells lacking 11β-HSD2. Because of the higher intrinsic affinity of MR (relative to GR) for cortisol, the major CNS receptor species occupied during periods of low cortisol levels is MR. At higher blood cortisol levels, MR becomes saturated, and GR occupancy increases. Both MR and GR control the basal activity of the HPA axis, whereas feedback inhibition by glucocorticoids predominately occurs via GR.

The Stress Response

Stress overcomes negative feedback regulation of the HPA axis, leading to a marked rise in corticosteroid levels. Examples of stress signals include trauma, hemorrhage, severe infection, major surgery, hypoglycemia, cold, pain, and fear. Different brain regions are involved in the processing of these stimuli. Although the precise mechanisms that underlie this stress response, and the essential functions of corticosteroids during this process, are not fully defined, increased corticosteroid secretion is vital to maintain homeostasis in these settings. As discussed further in the chapter, complex interactions between the HPA axis and the immune system may be a fundamental physiological component of this stress response.

Therapeutic Uses and Diagnostic Applications of ACTH and CRH

Except for the treatment of infantile spasms, most proven therapeutic effects of ACTH can be achieved with appropriate doses of corticosteroids. Moreover, therapy with ACTH is less predictable and less convenient than therapy with corticosteroids. ACTH acutely stimulates mineralocorticoid and adrenal androgen secretion and may therefore cause acute retention of salt and water, as well as virilization. ACTH and CRH, however, have important diagnostic applications.

Infantile Spasms

The mainstay treatment for this rare epileptic disorder of infancy and early childhood is hormonal therapy with ACTH. The mechanism of action is not known but may involve effects independent of adrenal corticosteroid release since ACTH can control spasms in patients with adrenal suppression. Antiepileptic effects involving suppression of CRH in the CNS have been proposed. The formulation used most commonly in the U.S. is measured in units as an injectable gel delivered intramuscularly or subcutaneously. In other countries, a long-acting depot formulation of synthetic ACTH is available (*tetracosactide depot*).

Diagnostic Use

Cosyntropin, a synthetic peptide that corresponds to residues 1–24 of human ACTH, is used in testing the integrity of the HPA axis. At the supraphysiological dose of 0.25 mg, *cosyntropin* maximally stimulates adrenocortical steroidogenesis. An increase in the circulating cortisol level to a level greater than 18 to 20 μg/dL using many of the standard assays indicates a normal response. Newer cortisol assay platforms have higher specificity for cortisol and have corresponding lower normal cutoffs. *Cosyntropin* may also be used diagnostically during adrenal venous sampling, a procedure used to distinguish between unilateral and bilateral aldosterone secretion in primary aldosteronism.

Ovine CRH (*corticorelin*) and human CRH (not available in the U.S.) are used rarely for diagnostic testing of the HPA axis. When evaluating patients with documented ACTH-dependent hypercortisolism, CRH stimulation testing is sometimes used to differentiate pituitary from ectopic sources of ACTH, either alone or more commonly during inferior petrosal sinus sampling. In both cases, pituitary ACTH production is increased by CRH, whereas ectopic ACTH production is not. CRH stimulation testing, after *dexamethasone* suppression, is also occasionally used to assist differentiation of pseudo-Cushing states (as occurs with alcoholism and some neuropsychiatric disorders) from true Cushing syndrome.

Assays for ACTH

Immunoassays that use two separate antibodies directed at distinct epitopes on the ACTH molecule are now widely available. These assays allow differentiation of patients with primary adrenal insufficiency due to intrinsic adrenal disease, who have high ACTH levels due to the loss of normal glucocorticoid feedback inhibition, from patients with secondary adrenal insufficiency due to low ACTH levels from hypothalamic or pituitary disorders. ACTH immunoassays are also useful during the evaluation of patients with hypercortisolism (Cushing syndrome): normal or high ACTH levels are seen in patients with hypercortisolism from either a pituitary (Cushing disease) or an ectopic (nonpituitary tumor) source, whereas low ACTH levels are invariably seen in patients with hypercortisolism due to an adrenal source. One problem with the immunoassays for ACTH is that their specificity for intact ACTH can lead to falsely low values in patients with ectopic ACTH secretion; these tumors can rarely secrete aberrantly processed forms of ACTH that have biologic activity but do not react in the immunoassays.

Absorption, Fate, and Toxicity

ACTH is readily absorbed from parenteral sites. The hormone rapidly disappears from the circulation after intravenous administration; in humans, the $t_{1/2}$ in plasma is about 15 min, primarily due to rapid enzymatic hydrolysis. Aside from rare hypersensitivity reactions, any toxicity is primarily attributable to the increased secretion of corticosteroids. *Cosyntropin* is generally less antigenic than native ACTH.

Adrenocortical Steroids

Synthesis, Circulating Levels, and Interconversion

The adrenal cortex synthesizes two classes of steroids: the *corticosteroids* (glucocorticoids and mineralocorticoids, which have a 21-carbon pregnane structure) and *androgens*, which have a 19-carbon androstane structure (see Figure 50–3). The actions of corticosteroids historically were described as *glucocorticoid* (reflecting their activity regulating carbohydrate metabolism) and *mineralocorticoid* (reflecting their activity regulating electrolyte balance). In humans, *cortisol* is the main physiological glucocorticoid, and aldosterone is the main physiological mineralocorticoid. Although biosynthetic intermediates such as *corticosterone* and *deoxycorticosterone* have significant glucocorticoid and mineralocorticoid activities, their influence is limited due to their low circulating levels under normal conditions. They can, however, exert important effects when elevated, mainly as the result of rare genetic defects in biosynthetic enzymes or their pharmacological inhibition.

Cortisol is relatively abundant in circulation and is extensively (>95%) bound, primarily to *corticosteroid-binding globulin* (CBG). In contrast, aldosterone circulates mostly in free form at approximately 1000-fold lower levels. Both cortisol and aldosterone are secreted episodically and vary in a similar diurnal manner, with the highest levels reached in the early morning hours and the lowest levels reached several hours after the onset of sleep (Figure 50–5).

In the periphery, cortisol is reversibly converted to the inactive 11-keto derivative cortisone. Quantitatively, the forward inactivating reaction is carried out mainly in the kidney by the enzyme 11β-HSD2. The reverse reactivating reaction is carried out by a different isozyme, 11β-HSD1, mainly but not exclusively in the liver. Therefore, cortisone can be viewed as an inactive circulating reserve for cortisol. Under normal conditions, the circulating levels of cortisone and cortisol are found at an approximately 1:4 ratio. The local availability of cortisol varies greatly and is influenced by the relative activities of the interconverting enzymes, which have distinct tissue expression patterns. The extensive conversion of inactive 11-keto steroids to the active 11-hydroxy forms in the liver forms the basis for the clinical activity of 11 keto prodrug forms of corticosteroid drugs such as *prednisone* (Figure 50–6).

Adrenal Androgens

The adrenal cortex also produces the C19 steroids DHEA and, to a lesser extent, androstenedione. DHEA has limited androgenic potency but serves as the major precursor for most sex steroids. The bulk of DHEA is secreted as its sulfated derivative DHEAS. Although both forms are albumin bound, the interaction of DHEAS is much tighter. Thus, the circulating levels of DHEAS are more than 100-fold higher than those of DHEA.

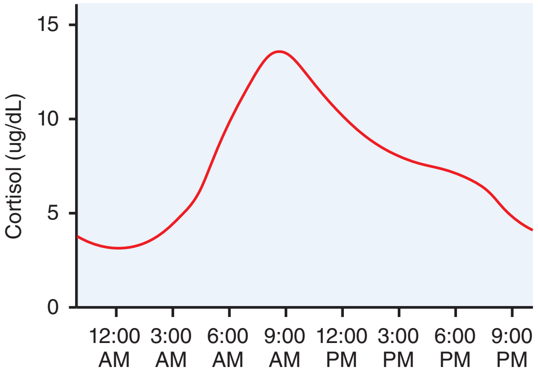

Figure 50–5 *Approximate diurnal pattern of cortisol and aldosterone levels.*

In gonads and other peripheral tissues, steroid sulfatases can convert DHEAS back to DHEA to serve as precursor to more potent androgens and estrogens. Levels of adrenal androgens in circulation rise gradually in childhood, peak in the third decade of life, and decline progressively thereafter in a pattern that reflects the size of the adrenal zona reticularis. Functionally, adrenal androgens drive the first appearance of pubic hair or pubarche and are the precursor source for more than half of the testosterone in women. Patients with adrenal insufficiency do not require adrenal androgen replacement, indicating that these hormones are not essential for survival. Elevated circulating DHEAS levels are associated with hyperandrogenic states, particularly in women. Furthermore, for a number of chronic diseases, affected patients can have very low DHEA levels. Based on this, some have proposed that DHEA treatment might be beneficial in these diseases and could partly alleviate the loss of libido, the decline in cognitive function, the decreased sense of well-being, and other adverse physiological consequences of aging. However, studies on the benefits of addition of DHEA to the standard replacement regimen in women with adrenal insufficiency have been inconclusive. Despite the absence of definitive data, DHEA is widely used as an over-the-counter nutritional supplement for its alleged health benefits.

Physiological Functions and Pharmacological Effects

Corticosteroids have numerous effects, which include alterations in carbohydrate, protein, and lipid metabolism; maintenance of fluid and electrolyte balance; and preservation of normal function of the cardiovascular system, the immune system, the kidney, skeletal muscle, the endocrine system, and the nervous system. In addition, corticosteroids endow the organism with the capacity to resist stressful and noxious stimuli and environmental changes. In the absence of adequate secretion of corticosteroids from the adrenal cortex, stresses such as infection, trauma, and extremes in temperature can be fatal.

The actions of corticosteroids are related to those of other hormones. For example, in the absence of lipolytic hormones, cortisol has virtually no effect on the rate of lipolysis by adipocytes. Conversely, in the absence of glucocorticoids, epinephrine and norepinephrine have only minor effects on lipolysis. Administration of a small dose of glucocorticoid, however, markedly potentiates the lipolytic action of these catecholamines. Those effects of corticosteroids that involve concerted actions with other hormonal regulators are termed permissive and most likely reflect steroid-induced changes in protein synthesis, which, in turn, modify tissue responsiveness to other hormones.

Corticosteroids are termed either mineralocorticoids or glucocorticoids, according to their relative effects in Na^+ retention and effects on carbohydrate metabolism (i.e., hepatic deposition of glycogen and gluconeogenesis). In general, the potencies of steroids on glucose metabolism closely parallel their potencies as anti-inflammatory agents. The effects on Na^+ retention and the carbohydrate/anti-inflammatory actions are not closely related and reflect selective actions at distinct receptors. As noted in further discussion (see structure-activity relationships), some steroid derivatives provide relative selectivity as stimulants of Na^+ retention or anti-inflammatory effects.

General Mechanisms for Corticosteroid Effects

Corticosteroids exert their effects principally by causing both positive and negative changes in the transcription of corticosteroid-responsive genes leading to changes in the protein composition and thus function of target cells and tissues. These effects are mediated by GR and MR, two closely related members of the nuclear receptor superfamily of ligand regulated transcription factors. Although some genes are regulated in most target cells, the array of responsive genes and the extent and pattern of regulation are tissue and cell type specific. The effects are governed by the expression and intrinsic function of the receptors, their relative affinities, local availability of the ligands, and the array of other transcription factors present in a particular cell. It is also becoming recognized that in addition to protein-coding genes, the receptors regulate the expression of noncoding RNAs, the physiological function

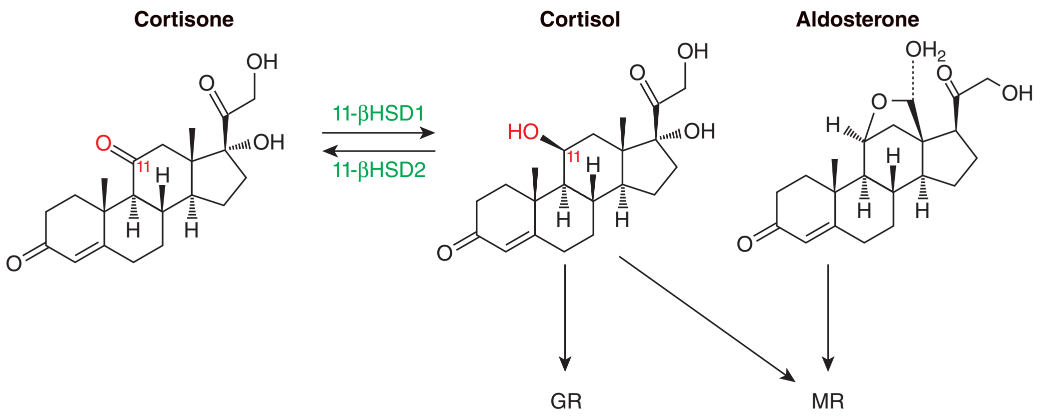

Figure 50–6 *Corticosteroid conversion and receptor specificity.* Cortisol and aldosterone bind to the MR with equally high affinity; cortisol binds with more modest affinity to the GR, which binds aldosterone only marginally. Cortisol is converted to the inactive cortisone by 11β-HSD2, and cortisone is activated to cortisol by 11β-HSD1.

of which remains largely unexplored. Given the multistep process of transcription and translation leading to net changes in protein levels, many effects of corticosteroids are not immediate but become apparent after several hours. In many cases, but not invariably, the therapeutic effects of corticosteroids become clinically apparent only after a time delay. Corticosteroids also have more rapid effects that are unlikely to be dependent on gene regulation. The exact mechanisms are not fully understood, although in some circumstances, GR and MR have been implicated.

Glucocorticoid and Mineralocorticoid Receptors. GR (gene name *NR3C1*, nuclear receptor subfamily 3, group C, member 1) and MR (*NR3C2*) share with other members of the nuclear receptor family of transcription factors a common architecture composed of a central DNA binding domain (DBD), a C-terminal ligand binding domain (LBD), and an N-terminal region harboring transcriptional regulatory functions (AF-1) (Figure 50–7A). GR and MR reside predominately in the cytoplasm in an inactive form complexed with molecular chaperones that maintain the receptor in a ligand binding competent state (Kirschke et al., 2014). Steroid binding to the LBD results in receptor activation and translocation to the nucleus where the receptors exert both positive and negative effects on transcription (see Figure 50–7B for GR).

Determinants of Specificity and Action. The receptor responsible for the effects of a given corticosteroid depend not only on receptor affinity but also on local availability of the ligand such that different combinations of receptors and ligands occur in distinct tissues and conditions. In terms of affinities, the mineralocorticoid receptor binds comparably and with very high affinity to cortisol, aldosterone, precursors such as deoxycorticosterone (which act as agonists), and progesterone and 17α-progesterone (which act as antagonists). In contrast, GR binds with more modest affinity to both cortisol and the precursors corticosterone and deoxycorticosterone as agonists (and to a lesser extent to progesterone as antagonist) while having very low affinity for aldosterone. Neither receptor binds appreciably to 11-keto forms such as cortisone.

In epithelial cells of the kidney, colon, and salivary glands, aldosterone specifically activates MR in the face of much higher circulating levels of glucocorticoids due to the co-expression of 11β-HSD2. As mentioned above, this enzyme metabolizes glucocorticoids such as cortisol to inactive 11-keto derivatives such as cortisone. Aldosterone escapes this inactivation and maintains mineralocorticoid activity because it exists predominantly in the hemiacetal form that is resistant to 11β-HSD action. In the absence of 11β-HSD2, as occurs in the inherited disease *syndrome of apparent mineralocorticoid excess*, MR is inappropriately activated by cortisol, leading to severe hypokalemia and hypertension. A similar state is elicited acutely when 11β-HSD2 is inhibited by *glycyrrhizic acid*, a component of licorice implicated in licorice-induced hypertension.

MR is also expressed in cells not involved in Na^+ reabsorption, including endothelial cells, vascular smooth muscle cells, cardiomyocytes, certain neuronal populations, and inflammatory cells. When examined, many cells lack 11β-HSD2, and thus, MR likely responds physiologically to cortisol.

Similarly, in cells that express 11β-HSD1 such as hepatocytes and adipocytes, GR is exposed to higher levels of cortisol relative to the circulation due to local activation of cortisone to cortisol (see Figure 50–6).

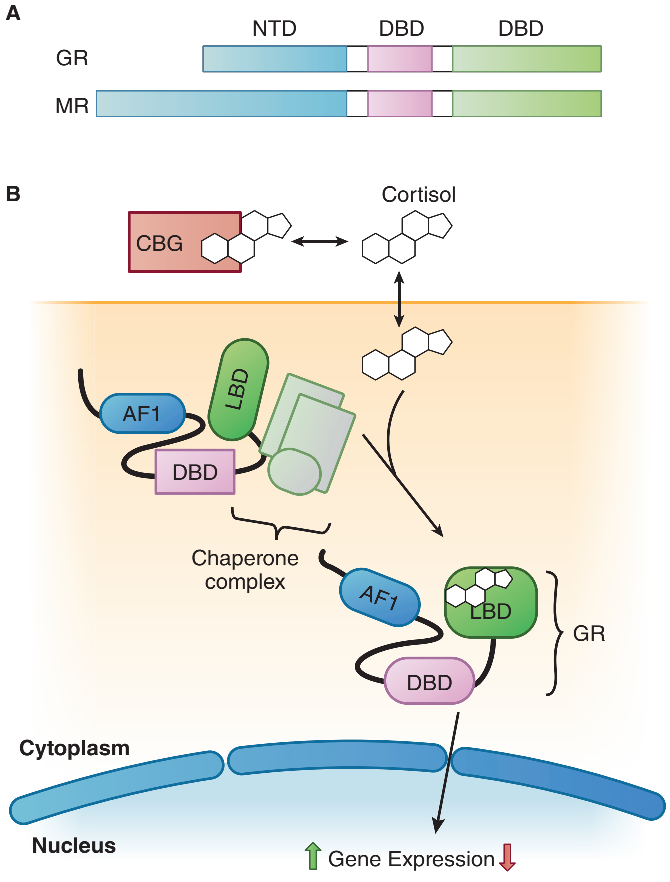

Figure 50–7 *Domain organization of GR and MR and mechanism of action of GR.* **A.** GR and MR share a common organization composed of a central DNA binding domain (DBD), a C-terminal ligand binding domain (LBD), and a more variable N-terminal domain (NTD). **B.** Cortisol circulates mostly bound to CBG. Free cortisol enters cells and interacts with GR, inducing a conformational change that activates the receptor and allows for translocation to the nucleus where it causes both positive and negative changes in gene expression.

Regulation of Gene Expression by the Glucocorticoid Receptor. After ligand binding and nuclear translocation, GR is recruited to specific sites on the genome. The location and extent of recruitment vary extensively between cell types (John et al., 2011). At these sites, GR nucleates or modifies the assembly of transcriptional regulatory complexes through interactions with both sequence-specific factors and coregulator complexes and exerts positive or negative effects on transcription (Sacta et al., 2016). At most positive sites, GR homodimers bind directly to specific DNA sequences termed glucocorticoid-response elements and recruit coregulators that enhance transcription by altering chromatin structure or contacting the basal RNA polymerase II transcription apparatus (Figure 50–8A). Genes regulated in this manner include metabolism genes such as phosphoenolpyruvate carboxykinase (*PEPCK*) and tyrosine aminotransferase as well as anti-inflammatory genes such as the inhibitory subunit of NF-κB. GR repression at negative sites can occur through multiple mechanisms. A frequent pattern involves recruitment of GR monomers to DNA-bound positively acting transcription factors such as AP-1 or NF-κB. The resulting complexes interfere with successful transcriptional initiation or elongation (Figure 50–8B). This type of mechanism mediates GR repression of genes for multiple proinflammatory cytokines such as IL-6 and IL-2, as well as for collagenase and stromelysin. GR inhibition of POMC gene transcription illustrates a different mechanism of repression. In this case, direct binding of GR to DNA sequences that overlap and occlude the binding of basal transcriptional machinery components interferes with transcription, thereby contributing to the negative-feedback regulation of the HPA axis. Other genes negatively regulated by glucocorticoids include genes for cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase.

Several GR isoforms result from alternative RNA splicing and from translation initiation at alternative sites. Of these, GRα is the prototypical glucocorticoid-responsive isoform. GRγ is a splice variant with a single amino acid insertion in the DNA binding domain that has altered DNA binding specificity. GRβ has a truncated C terminus that is unable to bind ligands and has been implicated in decreased responsiveness to glucocorticoids. Multiple polymorphisms in the human GR are associated with differences in GR function, but they appear to account for only a small fraction of clinical variability to glucocorticoid responsiveness.

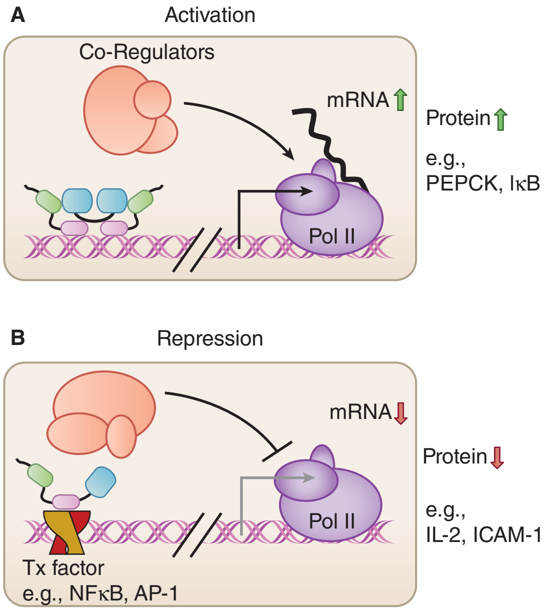

Figure 50–8 *Mechanisms of transcriptional regulation by GR.* **A.** At activated genes, GR binds directly to specific sequences (glucocorticoid-response element [GRE]) as a dimer and regulates the assembly of coregulator complexes that enhance mRNA transcription by RNA polymerase II (Pol II). **B.** Gene repression often involves GR monomers binding to other transcription (Tx) factors and recruiting repressive coregulator complexes that inhibit Pol II.

Regulation of Gene Expression by the Mineralocorticoid Receptor. Like GR, the MR is also a ligand-activated transcription factor and binds to similar specific DNA sequences. MRs also associate with molecular chaperone proteins and regulate the transcription of discrete sets of genes in target tissues. MR and GR also differ in their ability to interact functionally with other transcription factors and this may in part underly the contrasting pro-inflammatory effects of MR relative to the anti-inflammatory effects of GR. In contrast to the essentially ubiquitous expression of GR, MR is expressed in a much more restricted manner in epithelial tissues involved in electrolyte transport (i.e., the kidney, colon, salivary glands, and sweat glands) and in some nonepithelial tissues (e.g., hippocampus, heart, vasculature, and adipose tissue).

Aldosterone exerts its effects on Na$^+$ and K$^+$ homeostasis via MR primarily through its actions on the principal cells of the distal renal tubules and collecting ducts, whereas effects on H$^+$ secretion largely are exerted in the intercalated cells. The binding of aldosterone to MR in the kidney initiates a sequence of events that includes the prompt induction of serum and glucocorticoid-regulated kinase, which in turn phosphorylates and activates amiloride-sensitive epithelial Na$^+$ channels (also called ENaC) in the apical membrane. Thereafter, increased Na$^+$ influx stimulates the Na$^+$/K$^+$-ATPase in the basolateral membrane (see Chapter 29). In addition to these rapid genomic effects, aldosterone increases the synthesis of these membrane proteins as part of a more delayed effect.

Carbohydrate and Protein Metabolism

Glucocorticoids markedly effect carbohydrate and protein metabolism. Glucocorticoids stimulate the liver to form glucose from amino acids (gluconeogenesis) while also promoting glucose storage as glycogen. In the periphery, glucocorticoids diminish glucose utilization, increase protein breakdown and the synthesis of glutamine, and activate lipolysis, thereby providing amino acids and glycerol for gluconeogenesis. The net result is to increase blood glucose levels. Through their effects on glucose metabolism, glucocorticoids can worsen glycemic control in patient with diabetes mellitus and can precipitate the new onset of hyperglycemia in susceptible patients.

Lipid Metabolism

Two effects of glucocorticoids on lipid metabolism are firmly established. The first is the dramatic redistribution of body fat that occurs in hypercortisolism, as in patients with Cushing syndrome. In this setting, there is increased fat in the back of the neck ("buffalo hump"), face ("moon facies"), and supraclavicular area, coupled with a loss of fat in the extremities. The molecular mechanisms underlying these depot-specific effects remain incompletely understood. The other effect of glucocorticoids on lipid metabolism is the permissive facilitation of the lipolytic effect of other agents, such as growth hormone and β adrenergic receptor agonists, resulting in an increase in free fatty acids after glucocorticoid administration.

Electrolyte and Water Balance

Aldosterone is by far the most potent endogenous corticosteroid with respect to fluid and electrolyte balance. Mineralocorticoids act on the distal tubules and collecting ducts of the kidney to enhance reabsorption of Na$^+$ from the tubular fluid; they also increase the urinary excretion of K$^+$ and H$^+$. These actions on electrolyte transport, in the kidney and in other tissues (e.g., colon, salivary glands, and sweat glands), appear to account for the physiological and pharmacological activities that are characteristic of mineralocorticoids and are the result of selective activation of MR by aldosterone in tissues expressing 11β-HSD2. Thus, the primary features of hyperaldosteronism are positive Na$^+$ balance with consequent expansion of extracellular fluid volume, normal or slight increases in plasma Na concentration, normal or low K$^+$, and alkalosis. Mineralocorticoid deficiency, in contrast, leads to Na$^+$ wasting and contraction of extracellular fluid volume, hyponatremia, hyperkalemia, and acidosis. Chronically, hyperaldosteronism causes hypertension, whereas aldosterone deficiency can lead to hypotension and vascular collapse.

Glucocorticoids also exert effects on fluid and electrolyte balance, largely due to permissive effects on tubular function and actions that maintain the glomerular filtration rate. Glucocorticoids play a permissive role in the renal excretion of free water. In part, the inability of patients with glucocorticoid deficiency to excrete free water results from the increased secretion of AVP, which stimulates water reabsorption in the kidney. In addition to their effects on monovalent cations and water, glucocorticoids exert multiple effects on Ca^{2+} metabolism, lowering Ca^{2+} uptake from the gut, increasing mobilization from bone, and increasing Ca^{2+} excretion by the kidney, collectively leading to decreased total body Ca^{2+} stores.

Cardiovascular System

The most striking effects of corticosteroids on the cardiovascular system result from MR-mediated changes in renal Na^+ retention, as evident in primary hyperaldosteronism. MR activation also has direct effects on the heart and vessel walls; aldosterone induces hypertension and interstitial cardiac fibrosis in animal models. The clinical efficacy of MR antagonists in heart failure at doses that do not alter blood pressure argue for a direct effect on the heart. These effects are thought to reflect at least in part MR occupancy by cortisol since cardiomyocytes are devoid of 11β-HSD2 (Richardson et al., 2016). A third major action of corticosteroids on the cardiovascular system is to enhance vascular reactivity to other vasoactive substances. Hypoadrenalism is associated with reduced responsiveness to vasoconstrictors such as norepinephrine and AngII, perhaps due to decreased expression of receptors in the vascular wall. Conversely, hypertension is seen in patients with excessive glucocorticoid secretion, occurring in most patients with Cushing syndrome and in a subset of patients treated with synthetic glucocorticoids (even those lacking any significant mineralocorticoid action).

Skeletal Muscle

Permissive concentrations of corticosteroids are required for the normal function of skeletal muscle, and diminished work capacity is a prominent sign of adrenocortical insufficiency. In patients with Addison disease, weakness and fatigue are frequent symptoms. Excessive amounts of either glucocorticoids or mineralocorticoids also impair muscle function. In primary hyperaldosteronism, muscle weakness results primarily from hypokalemia rather than from a direct effect of mineralocorticoids on skeletal muscle. In contrast, glucocorticoid excess over prolonged periods causes skeletal muscle wasting, likely reflecting the need for precursor mobilization to sustain glucocorticoid-induced gluconeogenesis (see Carbohydrate and Protein Metabolism above). Glucocorticoid-induced myopathy accounts in part for weakness and fatigue in patients with glucocorticoid excess.

CNS

Corticosteroids exert a number of indirect effects on the CNS, through maintenance of blood pressure, plasma glucose, and electrolyte concentrations. Corticosteroids also have direct effects on the CNS that involve both GR and MR with effects on the latter likely due to cortisol occupancy in regions devoid of 11β-HSD2 such as the hippocampus. Corticosteroids affect mood, behavior, cognition, memory, and brain excitability. Patients with adrenal insufficiency exhibit a diverse array of neurological manifestations, including apathy, depression, irritability, and even psychosis. Appropriate replacement therapy corrects these abnormalities. Conversely, glucocorticoid administration can induce multiple CNS reactions. Most patients respond with mood elevation, which may impart a sense of well-being despite the persistence of underlying disease. Some patients exhibit more pronounced behavioral changes, such as mania, insomnia, restlessness, and increased motor activity. A smaller but significant percentage of patients treated with glucocorticoids become anxious, depressed, or overtly psychotic. A high incidence of neuroses and psychoses is seen in patients with Cushing syndrome. These abnormalities usually, but not always, improve or disappear after cessation of glucocorticoid therapy or treatment of endogenous Cushing syndrome.

Formed Elements of Blood

Glucocorticoids exert minor effects on hemoglobin and the erythrocyte content of blood, as evidenced by the frequent occurrence of polycythemia in Cushing syndrome and of normochromic, normocytic anemia in adrenal insufficiency. Corticosteroids also affect circulating white blood cells. Addison disease is associated with an increased mass of lymphoid tissue and lymphocytosis; in contrast, Cushing syndrome is characterized by lymphocytopenia and a decreased mass of lymphoid tissue. The administration of glucocorticoids leads to a decreased number of circulating lymphocytes, eosinophils, monocytes, and basophils. A single dose of *hydrocortisone* leads to a decline of these circulating cells within 4 to 6 h; this effect persists for 24 h and results from the redistribution of cells rather than from increased destruction. In contrast, glucocorticoids increase circulating polymorphonuclear leukocytes as a result of increased release from the marrow, diminished rate of removal from the circulation, and decreased adherence to vascular walls. Finally, glucocorticoids are effective in the treatment of certain lymphoid malignancies, in large part due to the capacity of glucocorticoids to induce lymphocyte apoptosis.

Anti-inflammatory and Immunosuppressive Actions

In addition to their effects on lymphocyte number, glucocorticoids profoundly alter the immune responses of lymphocytes. These effects are important facets of the anti-inflammatory and immunosuppressive actions of the glucocorticoids. Although the use of glucocorticoids as anti-inflammatory agents for the most part does not address the underlying cause of the disease, the suppression of inflammation is of vital clinical utility and has made these drugs among the most frequently prescribed agents. Similarly, glucocorticoids are of great value in treating diseases that result from undesirable immune reactions. These diseases range from conditions that predominately result from humoral immunity, such as urticaria (see Chapter 75), to those that are mediated by cellular immune mechanisms, such as transplantation rejection (see Chapter 39). The immunosuppressive and anti-inflammatory actions of glucocorticoids are inextricably linked, perhaps because they both involve inhibition of leukocyte function.

Multiple mechanisms are involved in the suppression of inflammation by glucocorticoids. Glucocorticoids induce the expression of lipocortin proteins, which inhibit phospholipase A2 and thus arachidonic acid–derived leukotriene mediators (see Chapter 41). In numerous cell types, glucocorticoids inhibit the production of factors that are critical in generating the inflammatory response. As a result, there is decreased release of vasoactive and chemoattractant factors, diminished secretion of lipolytic and proteolytic enzymes, decreased extravasation of leukocytes to areas of injury, and ultimately, decreased fibrosis. Glucocorticoids can also reduce expression of proinflammatory cytokines, COX-2, and inducible nitric oxide synthase. Some of the cell types and mediators that are inhibited by glucocorticoids are summarized in Table 50–1.

Among the proinflammatory cytokines, IL-1, IL-6, and TNF-α stimulate the HPA axis, with IL-1 having the broadest range of actions. IL-1 stimulates the release of CRH by hypothalamic neurons, interacts directly with the pituitary to increase the release of ACTH, and may directly stimulate the adrenal gland to produce glucocorticoids. The increased production of glucocorticoids, in turn, profoundly inhibits the immune system at multiple sites as discussed previously. Thus, the HPA axis and the immune system are capable of bidirectional interactions in response to stress, and these interactions appear to be important for homeostasis (Turnbull and Rivier, 1999).

Pharmacokinetics

Absorption

Hydrocortisone (cortisol) and numerous congeners, including the synthetic analogues, are orally effective. Certain water-soluble esters of *hydrocortisone* and its synthetic congeners are administered intravenously to achieve high concentrations of drug rapidly in systemic or targeted body fluids. More prolonged effects are obtained by intramuscular injection of suspensions of *hydrocortisone*, its esters, and congeners. Minor changes in chemical structure markedly alter the rate of absorption, time of onset of effect, and duration of action. Glucocorticoids are also absorbed systemically from sites of local administration, such as synovial spaces, the conjunctival sac, skin, and respiratory tract. When administration is

TABLE 50–1 ■ INHIBITORY EFFECTS OF GLUCOCORTICOIDS ON INFLAMMATORY/IMMUNE RESPONSES

CELL TYPE	FACTOR INHIBITED	COMMENTS
Macrophages and monocytes	Arachidonic acid, PGs, and LTs Cytokines: IL-l, IL-6, and TNF-α Acute-phase reactants	Mediated by glucocorticoid inhibition of COX-2 and PLA_2 Production and release are blocked; cytokines exert multiple effects on inflammation (e.g., ↑ T cells, ↑ fibroblast proliferation) Including the third component of complement
Endothelial cells	ELAM-1 and ICAM-1 Acute-phase reactants Cytokines (e.g., IL-1) Arachidonic acid derivatives	ELAM-1 and ICAM-1 are critical for leukocyte localization Same as above for macrophages and monocytes
Basophils	Histamine, LTC_4	IgE-dependent release ↓ by glucocorticoids
Fibroblasts	Arachidonic acid metabolites	Same as above for macrophages and monocytes Glucocorticoids ↓ growth factor–induced DNA synthesis and fibroblast proliferation
Lymphocytes	Cytokines (IL-1, IL-2, IL-3, IL-6, TNF-α, GM-CSF, interferon γ)	Same as above for macrophages and monocytes

ELAM-1, endothelial-leukocyte adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; LT, leukotriene; PG, prostaglandin; PL, phospholipase.

prolonged, when the site of application is covered with an occlusive dressing, or when large areas of skin are involved, absorption may be sufficient to cause systemic effects, including suppression of the HPA axis.

Distribution, Metabolism, and Excretion

After absorption of cortisol into plasma, 90% or more is reversibly bound to protein under normal circumstances. In most tissues, only the fraction of corticosteroid that is unbound is active and can enter cells. Two plasma proteins account for almost all the corticosteroid-binding capacity: CBG and albumin. CBG is an α globulin secreted by the liver that has relatively high affinity for glucocorticoids (dissociation constant of ~1 nM) but relatively low total binding capacity, whereas albumin, also produced by the liver, has a relatively large binding capacity but low affinity (estimated dissociation constant of 1 mM). In tissues with prolonged capillary transit time (e.g., liver, spleen), corticosteroid released from albumin is available for delivery. At high corticosteroid concentrations, the capacity of CBG binding is exceeded, and a slightly greater fraction of the corticosteroid exists in the free state. CBG has relatively high affinity for cortisol and some of its synthetic congeners and low affinity for aldosterone and glucuronide-conjugated steroid metabolites; thus, greater percentages of these last steroids are found in the free form. A special state of physiological hypercortisolism occurs during pregnancy. The elevated circulating estrogen levels induce CBG production, and CBG and total plasma cortisol levels increase several-fold, with only small increases in free cortisol. The physiological significance of these changes remains to be established. Similar effects on CBG and total cortisol levels are seen in women taking estrogen-containing contraceptives. The aldosterone levels also rise 3- to 10-fold during pregnancy, in part reflecting the marked elevations of progesterone and 17α-progesterone, which are effective MR and, to a lesser extent, GR antagonists. GR antagonism may also contribute to the mild elevation in free cortisol.

As a general rule, the metabolism of steroid hormones involves sequential additions of O or H atoms, followed by conjugation to form water-soluble derivatives. Reduction of the 4,5 double bond (see Figure 50–6) occurs at both hepatic and extrahepatic sites, yielding inactive compounds. Subsequent reduction of the 3-ketone substituent to the 3-hydroxyl derivative, forming tetrahydrocortisol, occurs only in the liver. Most of these A ring-reduced compounds are conjugated through the 3-hydroxyl group with sulfate or glucuronide by enzymatic reactions that take place in the liver and, to a lesser extent, in the kidney. The resultant sulfate esters and glucuronides are water soluble and are excreted in urine. Neither biliary nor fecal excretion is of quantitative importance in humans.

Synthetic steroids with an 11-keto group, such as *cortisone* and *prednisone*, must be enzymatically reduced to the corresponding 11β-hydroxy derivative before they are biologically active. The type 1 isoenzyme of 11β-HSD (11β-HSD1) catalyzes this reduction, predominately in the liver, but also in specialized sites such as adipocytes, bone, eye, and skin (see Figure 50–6). In settings in which this enzymatic activity is impaired, it is prudent to use steroids that do not require enzymatic activation (e.g., *hydrocortisone* or *prednisolone* rather than *cortisone* or *prednisone*). Such settings include individuals with severe hepatic failure and patients with rare mutations causing cortisone reductase deficiency.

Structure-Activity Relationships

Chemical modifications of the cortisol molecule have generated derivatives with greater separation of glucocorticoid and mineralocorticoid activity (Table 50–2); for several synthetic glucocorticoids, the effects on electrolytes are minimal even at the highest doses used. In addition, these modifications have led to derivatives with greater potencies and with longer durations of action. A vast array of steroid preparations is available for oral, parenteral, and topical use. Some of these are summarized in Table 50–3. None of these currently available derivatives effectively separates anti-inflammatory effects from effects on carbohydrate, protein, and fat metabolism or from suppressive effects on the HPA axis.

Estimates of Na$^+$-retaining and anti-inflammatory potencies of representative steroids are listed in Table 50–2. Some steroids that are classified predominately as glucocorticoids (e.g., cortisol) also possess modest but significant mineralocorticoid activity and thus may affect fluid and electrolyte handling in the clinical setting. At doses used for replacement therapy in patients with primary adrenal insufficiency, the mineralocorticoid effects of these "glucocorticoids" are insufficient to replace that of aldosterone, and concurrent therapy with a more potent mineralocorticoid is needed. In contrast, aldosterone is exceedingly potent with respect to Na$^+$ retention but has only minimal effects on carbohydrate metabolism. Even at levels that maximally affect electrolyte balance, aldosterone has no significant glucocorticoid activity and thus acts as a pure mineralocorticoid.

Toxicity of Adrenocortical Steroids

Two categories of toxic effects result from the therapeutic use of glucocorticoids: those resulting from withdrawal of steroid therapy and those resulting from continued use at supraphysiological doses. The side effects from both categories are potentially life threatening and require a careful assessment of the risks and benefits in each patient.

Withdrawal of Therapy

The most frequent problem with steroid withdrawal is flare-up of the underlying disease for which steroids were prescribed. Several other complications are associated with steroid withdrawal. The most severe,

TABLE 50–2 ■ RELATIVE POTENCIES AND EQUIVALENT DOSES OF REPRESENTATIVE CORTICOSTEROIDS

COMPOUND	ANTI-INFLAMMATORY POTENCY	NA$^+$-RETAINING POTENCY	DURATION OF ACTION[a]	EQUIVALENT DOSE (mg)[b]
Hydrocortisone[c]	1	1	S	20
Cortisone	0.8	0.8	S	25
Fludrocortisone	10	125	I	—[d]
Prednisone	4	0.8	I	5
Prednisolone	4	0.8	I	5
Methylprednisolone	5	0.5	I	4
Triamcinolone	5	0	I	4
Betamethasone	25	0	L	0.75
Dexamethasone	25	0	L	0.75

[a] Biological $t_{1/2}$: S, short (8–12 h); I, intermediate (12–36 h); L, long (36–72 h).

[b] Dose relationships apply only to oral or intravenous administration; potencies may differ greatly following intramuscular or intra-articular administration.

[c] The name for cortisol when used as a drug.

[d] This agent is used for its mineralocorticoid effects, not for glucocorticoid effects.

acute adrenal insufficiency, results from overly rapid withdrawal of corticosteroids after prolonged therapy has suppressed the HPA axis. Many patients recover from glucocorticoid-induced HPA suppression within several weeks to months; however, in some individuals, the time to full recovery can be a year or longer.

Most protocols for discontinuing glucocorticoid therapy in patients receiving long-term treatment involve gradual reduction in daily dosage or transition to an alternate-day regimen before gradual taper. If signs of acute adrenal insufficiency develop, immediate return to a higher dose is warranted. Patients who have received supraphysiological doses of glucocorticoids for a period of 2 to 4 weeks within the preceding year or with cushingoid appearance should be considered to have some degree of HPA impairment until proven otherwise. A characteristic glucocorticoid withdrawal syndrome consists of fever, myalgia, arthralgias, and malaise, which may be difficult to differentiate from some of the underlying diseases for which steroid therapy was instituted. Finally, pseudotumor cerebri, a clinical syndrome that includes increased intracranial pressure with papilledema, is a rare condition that sometimes is associated with reduction or withdrawal of corticosteroid therapy.

Chronic Use of Supraphysiologic Glucocorticoid Doses

Besides the consequences that result from the suppression of the HPA axis, a number of other complications result from prolonged therapy with glucocorticoids. These include fluid and electrolyte abnormalities, hypertension, hyperglycemia, increased susceptibility to infection, peptic ulcers, osteoporosis, myopathy, behavioral disturbances, cataracts, growth arrest, and the characteristic habitus of steroid overdose, including fat redistribution, striae, and ecchymoses.

Fluid and Electrolyte Handling. Alterations in fluid and electrolyte handling can cause hypokalemic alkalosis and hypertension, particularly in patients treated concomitantly with mineralocorticoids. Similarly, hypertension is a relatively common manifestation of exogenous glucocorticoid administration, even in patients treated with glucocorticoids lacking appreciable mineralocorticoid activity.

Metabolic Changes. The effects of glucocorticoids on intermediary metabolism were described previously. Hyperglycemia usually can be managed with diet and antidiabetes medications, including *insulin*. With proper management, this effect should not be a major factor in the decision to initiate or continue glucocorticoid therapy in diabetic patients.

Immune Responses. Because of their multiple effects to inhibit the immune system and the inflammatory response, glucocorticoid use is associated with an increased susceptibility to infection and a risk for reactivation of latent tuberculosis. In the presence of known infections, glucocorticoids should be administered only if necessary and concomitantly with appropriate and effective antimicrobial or antifungal therapy. Whenever possible, it is generally advised to provide appropriate vaccination before initiation of long courses of glucocorticoids, especially in vulnerable populations. The efficacy of some vaccines is reduced in patients already on glucocorticoids.

Gastrointestinal Effects. Glucocorticoids alone mildly increase the risk of gastritis, ulcer formation, and gastrointestinal bleeding; however, combination with nonsteroidal anti-inflammatory drugs leads to a synergistic increase in incidence. Glucocorticoids can also mask the symptoms of serious gastrointestinal disease, which may account for the associated increased risk of perforated sigmoid diverticular abscesses.

Myopathy. Myopathy, characterized by weakness of proximal limb muscle, can occur in patients taking large doses of corticosteroids and is also part of the clinical picture in patients with endogenous Cushing syndrome. It can be of sufficient severity to impair ambulation and is an indication for withdrawal of therapy. Attention also has focused on steroid myopathy of the respiratory muscles in patients with asthma or chronic obstructive pulmonary disease (see Chapter 44); this complication can diminish respiratory function. Recovery from the steroid myopathies may be slow and incomplete.

Behavioral Changes. Behavioral disturbances are common after administration of high-dose corticosteroids and in patients with endogenous Cushing syndrome; these disturbances may take many forms, including anxiety, insomnia, depression, and overt psychosis.

Cataracts. Cataracts are a well-established complication of glucocorticoid therapy and are related to dosage and duration of therapy. Children appear to be particularly at risk. Cessation of therapy may not lead to complete resolution of cataracts, which may progress despite reduction or cessation of therapy. Patients on long-term glucocorticoid therapy at *prednisone* doses of 10 to 15 mg/day or greater should receive periodic slit-lamp examinations to detect glucocorticoid-induced posterior subcapsular cataracts.

Osteoporosis. Osteoporosis, a frequent serious complication of glucocorticoid therapy, occurs in patients of both genders and all ages and is related to dosage and duration of therapy. About 30% to 50% of all patients who receive chronic glucocorticoid therapy ultimately develop osteoporotic fractures. Glucocorticoids preferentially affect trabecular bone and cortical rim of the vertebral bodies; the ribs and vertebrae are the most frequent sites of fracture. Glucocorticoids decrease bone density by multiple mechanisms, including inhibition of gonadal steroid hormone production via lowering gonadotropin release, diminished GI absorption of Ca^{2+} both via inhibition of vitamin D action and via decreased intestinal calcium channel expression, and inhibition of bone formation due to suppressive effects on osteoblasts and stimulation of resorption by osteoclasts via changes in the production of osteoprotegerin and receptor

TABLE 50–3 ■ SOME AVAILABLE PREPARATIONS OF ADRENOCORTICAL STEROIDS AND THEIR SYNTHETIC ANALOGUES

NONPROPRIETARY NAME	TYPE OF PREPARATION
Alclometasone dipropionate	Topical
Amcinonide	Topical
Beclomethasone dipropionate	Inhaled, nasal, topical
Betamethasone acetate	Injectable
Betamethasone sodium phosphate	Oral, injectable
Betamethasone valerate	Topical
Budesonide	Oral, inhaled, nasal, rectal
Ciclesonide	Inhaled, nasal
Clobetasol propionate	Topical, shampoo
Clocortolone pivalate	Topical
Desonide	Topical
Desoximetasone	Topical
Dexamethasone	Oral, ophthalmic, intravitreal implant, ophthalmic insert, intraocular
Dexamethasone sodium phosphate	Ophthalmic, otic, injectable
Diflorasone diacetate	Topical
Fludrocortisone acetate[a]	Oral
Flunisolide	Inhaled, nasal
Fluocinolone acetonide	Topical, shampoo, otic, intravitreal implant
Fluocinonide	Topical, otic
Fluorometholone	Ophthalmic
Fluorometholone acetate	Ophthalmic
Flurandrenolide	Impregnated dressing, topical
Halcinonide	Topical
Hydrocortisone	Topical, oral, rectal
Hydroxycortisone acetate	Topical, rectal
Hydroxycortisone butyrate	Topical
Hydrocortisone probutate	Topical
Hydrocortisone sodium succinate	Injectable
Hydrocortisone valerate	Topical
Methylprednisolone	Oral
Methylprednisolone acetate	Injectable
Methylprednisolone sodium succinate	Injectable
Mometasone furoate	Inhaled, nasal, topical
Prednisolone	Oral
Prednisolone acetate	Oral, ophthalmic
Prednisolone sodium phosphate	Oral, ophthalmic
Prednisone	Oral
Triamcinolone acetonide	Nasal, topical, injectable, dental
Triamcinolone hexacetonide	Injectable

Note: *Topical* preparations include agents for application to skin or mucous membranes in creams, foams, solutions, ointments, gels, pastes (for oral lesions), oils, tape, and aerosols; *ophthalmic* preparations include solutions, suspensions, and ointments; *inhalation* preparations include agents for nasal or oral inhalation.
[a]Fludrocortisone acetate is intended for use as a mineralocorticoid.

for activating NF-κB ligand (see Chapter 52). In addition, glucocorticoid inhibition of intestinal Ca^{2+} uptake and increased renal calcium excretion may lead to secondary increases in parathyroid hormone, thereby increasing bone resorption.

The initiation of glucocorticoid therapy at 5 mg/day or more of *prednisone* (or its equivalent) for 3 months or longer is an indication for bone densitometry to detect abnormalities in trabecular bone. Importantly, bone loss associated with glucocorticoids predominantly occurs within the first 6 months of therapy, and preventive treatment, if indicated, should be promptly initiated. Calcium (to provide 1200 mg/day from dietary or supplemental sources) and vitamin D (800 IU/day) are typically provided to all patients. In patients clinically determined to be at higher risk, bisphosphonate treatment has been shown to decrease the decline in bone density and the incidence of fractures in patients receiving glucocorticoid therapy. Additional discussion of these issues is found in Chapters 48 and 52.

Osteonecrosis. Osteonecrosis (also known as avascular or aseptic necrosis) is a relatively common complication of glucocorticoid therapy, particularly with prolonged therapy using higher doses. The femoral head is affected most frequently, but this process also may affect the humeral head and distal femur. Joint pain and stiffness usually are the earliest symptoms, and this diagnosis should be considered in patients receiving glucocorticoids who abruptly develop hip, shoulder, or knee pain. Although the risk increases with the duration and dose of glucocorticoid therapy, osteonecrosis also can occur when high doses of glucocorticoids are given for short periods of times. Osteonecrosis generally progresses, and most affected patients ultimately require joint replacement.

Regulation of Growth and Development. Growth retardation in children can result from administration of relatively small doses of glucocorticoids. Although the precise mechanism is unknown, there are reports that collagen synthesis and linear growth in these children can be restored by (off-label) treatment with growth hormone; further studies are needed to define the role of concurrent treatment with growth hormone in this setting. In experimental animals, antenatal exposure to glucocorticoids is clearly linked to cleft palate and altered neuronal development, ultimately resulting in complex behavioral abnormalities. The actions of glucocorticoids to promote cellular differentiation play important physiological roles in human development in late gestation and in the neonatal period (e.g., production of pulmonary surfactant and induction of hepatic gluconeogenic enzymes); those actions notwithstanding, antenatal steroids may lead to subtle abnormalities in fetal development. Babies born to women receiving large doses of corticosteroids during pregnancy should be monitored for signs of adrenal insufficiency and appropriate therapy initiated, if necessary.

Therapeutic Uses and Diagnostic Applications in Endocrine Diseases

With the exception of replacement therapy in deficiency states, the use of glucocorticoids largely is empirical. Given the number and severity of potential side effects, the decision to institute therapy with glucocorticoids always requires careful consideration of the relative risks and benefits in each patient. For any disease and in any patient, the optimal dose to achieve a therapeutic effect must be determined by trial and error and periodic reevaluation as the activity of the underlying disease changes or as complications of therapy arise. A single dose of glucocorticoid, even a large one, is virtually without harmful effects, and a short course of therapy (up to 1 week), in the absence of specific contraindications, is unlikely to be harmful. As the duration of glucocorticoid therapy is increased beyond 1 week, there are time- and dose-related increases in the incidence of disabling and potentially lethal effects. Except in patients receiving replacement therapy, glucocorticoids are neither specific nor curative; rather, they are palliative by virtue of their anti-inflammatory and immunosuppressive actions. Finally, abrupt cessation of glucocorticoids after prolonged therapy is associated with the risk of adrenal insufficiency, which may be fatal.

When glucocorticoids are to be given over long periods, the dose, determined empirically, must be the lowest that will achieve the desired effect. When the therapeutic goal is relief of painful or distressing symptoms not associated with an immediately life-threatening disease, complete relief is not sought, and the steroid dose is reduced gradually until worsening symptoms indicate that the minimal acceptable dose has been found. Where possible, the substitution of other medications, such as nonsteroidal anti-inflammatory drugs, may facilitate tapering the glucocorticoid dose once the initial benefit of therapy has been achieved. When therapy is directed at a life-threatening disease (e.g., pemphigus or lupus cerebritis), the initial dose should be a large one aimed at achieving rapid control of the crisis. If some benefit is not observed quickly, then the dose should be doubled or tripled. After initial control a potentially life threatening condition, dose reduction should be carried out under conditions that permit frequent accurate observations of the patient.

The lack of demonstrated deleterious effects of a single dose of glucocorticoids within the conventional therapeutic range justifies empiric administration to critically ill patients suspected of having adrenal insufficiency. This is lifesaving in cases of previously undiagnosed adrenal insufficiency. If the treated patient turns out not to have adrenal insufficiency, the single dose will not cause harm. Long courses of therapy at high doses are typically reserved for life-threatening conditions.

To diminish HPA axis suppression with longer-term treatment of an underlying condition responsive to glucocorticoids, the intermediate-acting steroid preparations (e.g., *prednisone* or *prednisolone*) should be given in the morning as a single dose. Alternate-day therapy with these glucocorticoids can often be used in patients who have achieved adequate therapeutic responses on the daily regimen. Pulse therapy with higher glucocorticoid doses (e.g., doses as high as 1–1.5 g/day IV of *methylprednisolone* for 3 days) can be used as initial therapy in patients with fulminant, immunologically related disorders and with acute exacerbations of conditions such as multiple sclerosis, acute transplant rejection, necrotizing glomerulonephritis, and lupus nephritis.

Replacement Therapy for Adrenal Insufficiency

Adrenal insufficiency can result from structural or functional lesions of the adrenal cortex (primary adrenal insufficiency, or Addison disease) or from structural or functional lesions of the anterior pituitary of hypothalamus (secondary adrenal insufficiency). In resource rich regions, primary adrenal insufficiency is most commonly caused by autoimmune adrenal disease, whereas tuberculous adrenalitis is the most frequent etiology in resource limited regions. Other causes of primary adrenal insufficiency include adrenalectomy, bilateral adrenal hemorrhage, neoplastic infiltration of the adrenal glands, AIDS, inherited disorders of steroidogenic enzymes, and X-linked adrenoleukodystrophy. Secondary adrenal insufficiency resulting from pituitary or hypothalamic dysfunction generally presents in a more insidious manner than does the primary disorder, probably because mineralocorticoid biosynthesis is preserved.

Acute Adrenal Insufficiency. This life-threatening condition is characterized by GI symptoms (nausea, vomiting, and abdominal pain), dehydration, hyponatremia, hyperkalemia, weakness, lethargy, and hypotension. It is more commonly associated with disorders of the adrenal rather than with disorders of the pituitary or hypothalamus, although it can also occur following abrupt withdrawal of glucocorticoids used at high doses or for prolonged periods.

The immediate management of patients with acute adrenal insufficiency includes intravenous therapy with isotonic NaCl solution supplemented with 5% glucose and corticosteroids and appropriate therapy for precipitating causes such as infection, trauma, or hemorrhage. Because cardiac function often is reduced in the setting of adrenocortical insufficiency, the patient should be monitored for evidence of volume overload, such as rising central venous pressure or pulmonary edema. After an initial intravenous bolus of 100 mg, *hydrocortisone* should be given by continuous infusion at a rate of 50 to 100 mg every 8 h, a dose that confers sufficient mineralocorticoid activity to meet all requirements. As the patient stabilizes, the *hydrocortisone* dose may be decreased to 25 mg every 6 to 8 h. Thereafter, patients are treated in the same fashion as those with chronic adrenal insufficiency. For the initial management of unconfirmed acute adrenal insufficiency, 4 mg *dexamethasone sodium phosphate* can be substituted for *hydrocortisone*; *dexamethasone* does not cross-react in the cortisol assay and will not interfere with the measurement of cortisol (either basally or in response to the *cosyntropin* [ACTH] stimulation test). Failure to respond to *cosyntropin* in this setting is diagnostic of adrenal insufficiency.

Chronic Adrenal Insufficiency. Patients with chronic adrenal insufficiency present with many of the same manifestations seen in adrenal crisis but with lesser severity. These patients require daily treatment with corticosteroids. The adequacy of corticosteroid replacement therapy is judged by clinical criteria and biochemical measurements. The subjective well-being of the patient is an important clinical parameter of glucocorticoid dosing in primary and secondary disease. Overtreatment with glucocorticoid may cause manifestations of Cushing syndrome in adults and decreased linear growth in children. The plasma ACTH levels are not typically used to monitor therapy in patients with primary adrenal insufficiency; due to an altered feedback relationship between cortisol and ACTH in this condition, persistent ACTH elevation should be expected at usual treatment doses, and suppression of ACTH can often indicate overtreatment.

Traditional replacement regimens have used *hydrocortisone* in doses of 20 to 30 mg/day; however, most current guidelines recommend lower doses of 15 to 20 mg/day based on estimates of normal daily rates of endogenous cortisol production. *Cortisone acetate*, which is inactive until converted to cortisol by 11β-HSD1, has been used in doses ranging from 25 to 37.5 mg/day. In an effort to mimic the normal diurnal rhythm of cortisol secretion, these glucocorticoids have been given in divided doses, with two-thirds of the dose given in the morning and one-third given in the afternoon. *Dexamethasone* and *prednisone* have also been used as chronic replacement; however, careful monitoring for iatrogenic development of cushingoid features is required due to their longer duration of action.

Although some patients with primary adrenal insufficiency can be maintained on *hydrocortisone* and liberal salt intake, most of these patients also require mineralocorticoid replacement; *fludrocortisone acetate* is used in doses of 0.05 to 0.2 mg/day. In primary adrenal insufficiency, mineralocorticoid dosing is based on resolution of electrolyte abnormalities and postural hypotension; reduction of salt craving is another valuable indicator of adequate mineralocorticoid replacement. Mineralocorticoid overtreatment may cause hypertension, hypokalemia, and edema. In secondary adrenal insufficiency, the administration of a glucocorticoid alone is generally adequate because mineralocorticoid synthesis in the zona glomerulosa usually remains intact.

When initiating treatment in patients with adrenal insufficiency associated with panhypopituitarism, glucocorticoids need to be administered before initiating treatment with thyroid hormone. If used in isolation, thyroid hormone (and correction of hypothyroidism) will accelerate metabolism of any remaining endogenous cortisol present and thus may precipitate adrenal crisis.

Standard doses of glucocorticoids often must be adjusted upward in patients who are also taking drugs that increase their metabolic clearance (i.e., *phenytoin*, barbiturates, or *rifampin*) or who suffer the stress of intercurrent illness. All patients with adrenal insufficiency should wear a medical alert bracelet or tag that lists their diagnosis and carries information about their steroid regimen. During minor illness, the glucocorticoid dose should be doubled. The patient and family members should also be trained to administer parenteral *dexamethasone* (4 mg intramuscularly) in the event that severe nausea or vomiting precludes the oral administration of medications; they then should seek medical attention immediately. Glucocorticoid doses are also adjusted when patients with adrenal insufficiency undergo surgery. In this setting, the doses are designed to approximate or exceed the maximal cortisol secretory rate of 200 mg/day; a standard regimen is *hydrocortisone* 100 mg parenterally every 8 h. Following surgery, the dose is halved each day until it is reduced to routine maintenance levels.

Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) encompasses a group of genetic disorders in which there is a deficiency in the activity of one of several enzymes required for the biosynthesis of glucocorticoids. The impaired production of cortisol and the consequent lack of negative-feedback inhibition lead to the increased release of ACTH. As a result, other hormonally active steroids that are proximal to the enzymatic block in the steroidogenic pathway are produced in excess. CAH includes a spectrum for which precise clinical presentation, laboratory findings, and treatment depend on which of the steroidogenic enzymes is deficient.

In about 95% of patients, CAH results from mutations in CYP21, the enzyme that carries out the 21-hydroxylation reaction (see Figure 50–3). Clinically, patients with 21-hydroxylase deficiency are divided into those with classic CAH, who have severe defects in enzymatic activity and first present during childhood, and those with nonclassic CAH, who present after puberty with signs and symptoms of mild androgen excess, such as hirsutism, amenorrhea, infertility, and acne. Female patients with classic CAH frequently are born with virilized external genitalia that result from elevated production of adrenal androgen precursors at critical stages of sexual differentiation in utero and often require reconstructive genital surgery. Some medical centers have successfully experimented with *dexamethasone* administration in utero with a goal of reducing female genital virilization; however, this approach is controversial because of concerns regarding abnormal behavioral development after prenatal exposure to glucocorticoids (Miller and Witchel, 2013). Males appear normal at birth and later may have precocious development of secondary sexual characteristics (isosexual precocious puberty). In both sexes, linear growth is accelerated in childhood, but the adult height is reduced by premature closure of the epiphyses. About 75% of patients with classic CAH have inadequate aldosterone production associated with salt wasting, failure to thrive, and potentially fatal hypovolemia and shock.

All patients with classic CAH require replacement therapy with *hydrocortisone* or a suitable congener, and those with salt wasting also require mineralocorticoid replacement. The goals of therapy are to restore physiological levels of steroid hormones and to suppress ACTH and thereby abrogate the effects of overproduction of adrenal androgens. The typical oral dose of *hydrocortisone* is about 0.6 mg/kg daily in two or three divided doses. The mineralocorticoid used is *fludrocortisone acetate* (0.05–2.0 mg/day). Many experts also administer tablet salt to infants (one-fifth of a teaspoon dissolved in formula daily) until the child is eating solid food. Therapy is guided by gain in weight and height, by plasma levels of 17-hydroxyprogesterone and androgens, and by blood pressure. Elevated plasma renin activity suggests that the patient is receiving an inadequate dose of mineralocorticoid. Sudden spurts in linear growth often indicate inadequate ACTH suppression and excessive androgen secretion, whereas growth failure suggests glucocorticoid overtreatment.

Miscellaneous Endocrine Conditions

Prednisone rapidly reduces the neck pain associated with subacute thyroiditis in patients not responding to more conservative measures, and this treatment may be associated with shortened disease duration. High-dose glucocorticoids are also used in severe hyperthyroidism, or thyroid storm, due to their ability to downregulate the deiodinase involved in the conversion of T_4 to its active form T_3. *Prednisone* is the primary therapy for hypercalcemia related to vitamin D intoxication, as it reduces the renal 1α-hydroxylase necessary for converting 25-hydroxy vitamin D to its active 1,25-dihydroxy vitamin D form.

Diagnostic Applications of Dexamethasone

In addition to its therapeutic uses, *dexamethasone* is used as a diagnostic aid in patients suspected of having Cushing syndrome. To determine if patients with clinical manifestations suggestive of hypercortisolism have biochemical evidence of increase cortisol biosynthesis, an overnight *dexamethasone* suppression test has been devised. Patients are given 1 mg of *dexamethasone* orally at 11 PM, and cortisol is measured at 8 AM the following morning. Suppression of cortisol to less than 1.8 μg/dL suggests strongly that the patient does not have Cushing syndrome. Drugs such as barbiturates that enhance *dexamethasone* metabolism or drugs (estrogens) or conditions (pregnancy) that increase the concentration of CBG can interfere with suppression and compromise the test.

A high-dose *dexamethasone* suppression test can be used as part of an evaluation to determine the etiology of biochemically documented Cushing syndrome. Following determination of baseline cortisol levels for 48 h, *dexamethasone* (2 mg every 6 h for 48 h, or 8 mg overnight) is administered orally. In many, but not all, patients with a pituitary source of ACTH excess (i.e., Cushing disease), cortisol will be suppressed. Conversely, in patients with most ectopic ACTH sources or with adrenocortical tumors, cortisol levels fail to be suppressed under these conditions. Since certain ectopic sources of ACTH, such as bronchial carcinoids are nevertheless suppressed by *dexamethasone*, the gold standard for establishing a pituitary source of ACTH is inferior petrosal sinus sampling after CRH administration.

Therapeutic Uses in Nonendocrine Diseases

There are important uses of glucocorticoids in diseases that do not directly involve the HPA axis. The disorders discussed next illustrate the principles governing glucocorticoid use in selected diseases. The dosage of glucocorticoids varies considerably depending on the nature and severity of the underlying disorder. Approximate doses of a representative glucocorticoid (e.g., *prednisone*) are provided.

Rheumatic Diseases

Glucocorticoids are used widely in the treatment of rheumatic disorders and are a mainstay in the treatment of the more serious inflammatory rheumatic diseases, such as systemic lupus erythematosus, and a variety of vasculitic disorders, such as polyarteritis nodosa, granulomatosis with polyangiitis, Churg-Strauss syndrome, and giant cell arteritis. For these more serious disorders, the starting dose of glucocorticoids should be sufficient to suppress the disease rapidly and minimize resultant tissue damage. Initially, *prednisone* (1 mg/kg per day in divided doses) often is used, generally followed by consolidation to a single daily dose, with subsequent tapering to a minimal effective dose as determined by the clinical picture.

Glucocorticoids are often used in conjunction with other immunosuppressive agents such as *cyclophosphamide* and *methotrexate*, which offer better long-term control than steroids alone. The exception is giant cell arteritis, for which glucocorticoids remain superior to other agents. Caution should be exercised in the use of glucocorticoids in some forms of vasculitis (e.g., polyarteritis nodosa), for which underlying infections with hepatitis viruses may play a pathogenetic role. Intermediate-acting glucocorticoids, such as *prednisone* and *prednisolone*, are generally preferred over longer-acting steroids such as *dexamethasone*.

In rheumatoid arthritis, because of the serious and debilitating side effects associated with their chronic use, glucocorticoids are used as stabilizing agents for progressive disease that fails to respond to first-line treatments such as physiotherapy and nonsteroidal anti-inflammatory drugs. In this case, glucocorticoids provide relief until other, slower-acting antirheumatic drugs (e.g., *methotrexate* or agents targeted at TNF) take effect. A typical starting dose is 5 to 10 mg of *prednisone* per day. In the setting of an acute exacerbation, higher doses of glucocorticoids may be employed (typically 20–40 mg/day of *prednisone* or equivalent), with rapid taper thereafter. Alternatively, patients with major symptomatology confined to one or a few joints may be treated with intra-articular steroid injections. Depending on joint size, typical doses are 5 to 20 mg of the very long-lasting *triamcinolone acetonide* or its equivalent.

In noninflammatory degenerative joint diseases (e.g., osteoarthritis) or in a variety of regional pain syndromes (e.g., tendinitis or bursitis), glucocorticoids may be administered by local injection for the treatment of episodic disease flare-up. It is important to use a glucocorticoid that does not require bioactivation (e.g., *prednisolone* rather than *prednisone*) and to minimize the frequency of local steroid administration whenever possible. In the case of repeated intra-articular injection of steroids, there is a significant incidence of painless joint destruction, resembling

Charcot arthropathy. It is recommended that intra-articular injections be performed with intervals of at least 3 months to minimize complications.

Renal Diseases

Patients with nephrotic syndrome secondary to minimal change disease generally respond well to steroid therapy, and glucocorticoids are the first-line treatment in both adults and children. Initial daily doses of *prednisone* are 1 to 2 mg/kg for 6 weeks, followed by a gradual tapering of the dose over 6 to 8 weeks, although some nephrologists advocate alternate-day therapy. Objective evidence of response, such as diminished proteinuria, is seen within 2 to 3 weeks in 85% of patients, and more than 95% of patients enter remission within 3 months. Patients with renal disease secondary to systemic lupus erythematosus also are generally given a therapeutic trial of glucocorticoids. In the case of membranous glomerulonephritis, many nephrologists recommend a trial of alternate-day glucocorticoids for 8 to 10 weeks (e.g., *prednisone* 120 mg every other day), followed by a 1- to 2-month period of tapering.

Allergic Diseases

The onset of action of glucocorticoids in allergic diseases is delayed, and patients with severe allergic reactions such as anaphylaxis require immediate therapy with *epinephrine*. The manifestations of allergic diseases of limited duration—such as hay fever, serum sickness, urticaria, contact dermatitis, drug reactions, bee stings, and angioedema—can be suppressed by adequate doses of glucocorticoids given as supplements to the primary therapy. In severe disease, intravenous glucocorticoids (*methylprednisolone* 125 mg IV every 6 h or equivalent) are appropriate. For allergic rhinitis, many experts recommend intranasal steroids.

Pulmonary Diseases

The use of glucocorticoids in bronchial asthma and other pulmonary diseases is discussed in Chapter 44. Antenatal glucocorticoids are used frequently in the setting of premature labor, decreasing the incidence of respiratory distress syndrome, intraventricular hemorrhage, and death in infants delivered prematurely. *Betamethasone* (12 mg IM every 24 h for two doses) or *dexamethasone* (6 mg IM every 12 h for four doses) is administered to women with definitive signs of premature labor between 26 and 34 weeks of gestation. For women still at risk of preterm birth 7 or more days after receiving the initial glucocorticoid dose, a meta-analysis of 10 randomized clinical trials involving over 4730 women and 5700 infants showed that a second course of treatment reduced the risk of respiratory distress syndrome and serious neonatal morbidity without adverse effects in infants followed for 2 to 3 years after (McKinlay et al., 2012). Beyond 34 weeks of gestation, the lower risks of respiratory problems make the short-term value of antenatal glucocorticoids questionable given the potential for adverse long-term neuropsychiatric outcomes.

Infectious Diseases

Although the use of immunosuppressive glucocorticoids in infectious diseases may seem paradoxical, there are a limited number of settings in which they are indicated in the therapy of specific infectious pathogens. A recent and impactful example is the significant survival benefit of *dexamethasone* treatment in hospitalized COVID-19 patients requiring mechanical ventilation, which was established early during the pandemic (Horby et al., 2021). Similarly, glucocorticoids clearly decrease the incidence of long-term neurological impairment associated with *Haemophilus influenzae* type b meningitis in infants and children 2 months of age or older, and in patients with HIV and *Pneumocystis carinii* pneumonia, glucocorticoids in combination with antimicrobial therapy can decrease the incidence of respiratory failure and mortality.

Ocular Diseases

Glucocorticoids frequently are used to suppress inflammation in the eye and can preserve sight when used properly. They are administered topically for diseases of the outer eye and anterior segment and attain therapeutic concentrations in the aqueous humor after instillation into the conjunctival sac. For diseases of the posterior segment, intraocular injection or systemic administration is required. These uses of glucocorticoids are discussed in Chapter 74.

Skin Diseases

Glucocorticoids are remarkably efficacious in the treatment of a wide variety of inflammatory dermatoses. A typical regimen for an eczematous eruption is 1% *hydrocortisone* ointment applied locally twice daily. Effectiveness is enhanced by application of the topical steroid under an occlusive film, such as plastic wrap; unfortunately, the risk of systemic absorption also is increased by occlusive dressings, and this can be a significant problem when the more potent glucocorticoids are applied to inflamed skin. Glucocorticoids are administered systemically for severe episodes of acute dermatological disorders and for exacerbations of chronic disorders. The dose in these settings is usually 40 mg/day of *prednisone*. Systemic steroid administration can be lifesaving in pemphigus, which may require daily doses of up to 120 mg of *prednisone*. Chapter 75 presents the dermatologic uses of glucocorticoids.

Gastrointestinal Diseases

Patients with inflammatory bowel disease (chronic ulcerative colitis and Crohn's disease) who fail to respond to more conservative management (i.e., rest, diet, and *sulfasalazine*) may benefit from glucocorticoids, particularly for acute exacerbations. For Crohn's disease, enteric-coated *budesonide* preparations are available that provide controlled ileal release while minimizing systemic exposure due to high first-pass metabolism (see Chapter 55).

Hepatic Diseases

Glucocorticoids are of benefit in autoimmune hepatitis both alone and in combination with immunosuppressants such as *azathioprine* or *6-mercaptopurine* as induction therapy. As many as 80% of patients show histological remission when treated with *prednisone* (40–60 mg daily initially, with tapering to a maintenance dose of 7.5–10 mg daily after serum transaminase levels fall). The role of corticosteroids in alcoholic liver disease is not fully defined; the most recent meta-analyses did not support a beneficial role of corticosteroids. In the setting of severe hepatic disease, *prednisolone* should be used instead of *prednisone*, which requires hepatic conversion to be active.

Malignancies

Glucocorticoids are potent inducers of lymphocyte apoptosis, and this forms the basis for their use in the chemotherapy of many lymphoid malignancies, most commonly as a component of combination therapy (see Chapter 70–73). The therapeutic role in nonhematological malignancies is limited. Glucocorticoids are also widely used as adjuvants to mitigate side effects of chemotherapy or radiotherapy of many cancers. Glucocorticoids increase appetite, decrease weight loss, reduce fatigue, and are used to manage chemotherapy-induced nausea and vomiting. They also reduce hypersensitivity reactions and mitigate fluid retention associated with taxane chemotherapy (see Chapter 70). In the palliative setting, short-term glucocorticoids can provide moderate control for pain and radiotherapy pain flare for patients with bone metastases.

Cerebral Edema

Corticosteroids at very high doses (e.g., *dexamethasone* 4–16 mg every 6 h) are commonly used in the reduction or prevention of cerebral edema associated with parasites and neoplasms, especially those that are metastatic.

Sarcoidosis

Corticosteroids are indicated therapy for patients with debilitating symptoms or life-threatening forms of sarcoidosis. Patients with severe pulmonary involvement are treated with 20 to 40 mg/day of *prednisone*, or an equivalent dose of alternative steroids, to induce remission. Higher doses may be required for other forms of this disease. Maintenance doses may be as low as 5 mg/day of *prednisone*.

Thrombocytopenia

In thrombocytopenia, *prednisone* (0.5 mg/kg) is used to decrease the bleeding tendency. In more severe cases and for initiation of treatment of idiopathic thrombocytopenia, daily doses of *prednisone* (1–1.5 mg/kg) are employed. Patients with refractory idiopathic thrombocytopenia may respond to pulsed high-dose glucocorticoid therapy.

Autoimmune Destruction of Erythrocytes

Patients with autoimmune destruction of erythrocytes (i.e., hemolytic anemia with a positive Coombs test) are treated with *prednisone* (1 mg/kg per day). In the setting of severe hemolysis, higher doses may be used, with tapering as the anemia improves. Small maintenance doses may be required for several months in patients who respond.

Organ Transplantation

Glucocorticoids remain key components of immunosuppressive regimens in organ transplantation and are the standard therapy for graft-versus-host disease. High doses of *prednisone* (50–100 mg) are given at the time of transplant surgery, in conjunction with other immunosuppressive agents, and most patients are kept on a maintenance regimen that includes lower doses of glucocorticoids (see Chapter 39). For some solid-organ transplants (e.g., pancreas), protocols that either withdraw corticosteroids early after transplantation or that avoid them completely have become more common (Niederhaus et al., 2013).

Spinal Cord Injury

Large doses of *methylprednisolone sodium succinate* (30 mg/kg initially followed by an infusion of 5.4 mg/kg/h for 23 h) are a treatment option for patients with acute spinal cord injury. Although, multicenter controlled trials have demonstrated decreases in neurological defects in patients with acute spinal cord injury treated within 8 h of injury (Bracken, 2012), concerns regarding statistical analysis, reproducibility of data, and potential side effects of treatment have caused some experts to advocate against use of *methylprednisolone* in this setting (Evaniew and Dvorak, 2016).

Inhibitors of ACTH Secretion and the Biosynthesis and Actions of Adrenocortical Steroids

Hypercortisolism (Cushing syndrome), with its attendant morbidity and mortality, is most frequently caused by corticotroph adenomas that overproduce ACTH (Cushing disease) or by adrenocortical tumors or bilateral hyperplasia that overproduces cortisol. Less frequently, hypercortisolism may result from adrenocortical carcinomas or from ectopic ACTH- or CRH-producing tumors. Although surgery is the treatment of choice for any endogenous cause of Cushing syndrome, it is not always effective, and adjuvant therapy with pharmacological inhibitors becomes necessary. In these settings, inhibitors of ACTH secretion and of adrenal steroidogenesis are clinically useful. Inhibition of cortisol production by direct inhibition of steroidogenic enzymes leads to a compensatory increase in ACTH, which can partially override inhibition and often lead to accumulation of precursor steroids that can elicit undesirable responses. All of these agents pose the common risk of precipitating adrenal insufficiency; thus, they must be used in appropriate doses, and the status of the patient's HPA axis must be carefully monitored (Hinojosa-Amaya et al., 2019). Most of the inhibitors discussed here are considered in detail in other chapters; mineralocorticoid receptor antagonists are not considered here but are discussed in Chapter 29.

Inhibitors of ACTH Secretion and Function

Pasireotide

Pasireotide is a somatostatin analogue that is an agonist at four of the five subtypes of somatostatin receptors, with especially high affinity for type 5. Through these interactions, *pasireotide* effectively inhibits growth hormone secretion and is used in the treatment of acromegaly (see Chapter 46). *Pasireotide* also inhibits ACTH secretion and reduces circulating levels of cortisol in patients with ACTH-producing pituitary tumors; the agent is FDA-approved for use in patients with Cushing disease who are not candidates for surgery or who have recurrent disease. At subcutaneous doses of 0.6 or 0.9 mg twice daily, urine cortisol concentrations normalized in 15% and 26% of patients, respectively. A long-acting preparation is now commonly used currently, in doses of 10 to 40 mg every 4 weeks. Treatment improves signs and symptoms of hypercortisolism, including hypertension, elevated low-density lipoprotein cholesterol, and elevated body mass index. Common adverse effects include hyperglycemia, gallstones, and transient GI discomfort (Colao et al., 2012).

Cabergoline

Cabergoline is a potent long-acting dopamine (D_2) receptor agonist used primarily to treat hyperprolactinemia (see Chapter 46). *Cabergoline* also inhibits ACTH secretion from corticotroph tumors, which are often D_2 receptor positive, and is occasionally used off-label for this purpose. In a nonrandomized retrospective study of 30 patients with Cushing disease treated with *cabergoline* monotherapy, 30% achieved normalization of urinary free cortisol for at least 1 year (mean 37 months; average dose 2.1 mg weekly) (Godbout et al., 2010).

Inhibitors of Steroidogenesis and Adrenolytic Agents

Ketoconazole

Ketoconazole is a CYP inhibitor and antifungal agent (see Chapter 61). At doses higher than those employed in antifungal therapy, it is an effective inhibitor of adrenal and gonadal steroidogenesis because it inhibits multiple steroidogenic P450 enzymes, including CYP11A1, the common initial step (see Figure 50–3). At sufficiently high doses, it effectively blocks steroidogenesis in all primary steroidogenic tissues. *Ketoconazole* is an effective inhibitor of steroid hormone biosynthesis in patients with hypercortisolism. In most cases, a dosage regimen of 600 to 800 mg/day (in two divided doses) is required, and some patients may require up to 1200 mg/day (in two or three doses). Side effects include hepatic dysfunction with the possibility of severe hepatic injury. The potential of *ketoconazole* to alter drug transport and metabolism by inhibiting P-glycoprotein and CYP3A4 can lead to serious drug interactions (see Chapters 4 and 5). *Levoketoconazole* is the 2S,4R enantiomer of *ketoconazole* and responsible for most of its steroidogenesis inhibitory activity. It is currently in development for treatment of Cushing syndrome and has received orphan drug status in the U.S. and Europe. A recent clinical trial demonstrated successful control in 36% of enrolled patients at a median dose of 300 mg twice daily (Fleseriu et al., 2019).

Metyrapone

Metyrapone is a relatively selective inhibitor of CYP11B1 and thus inhibits the conversion of 11-deoxycortisol to cortisol, thereby reducing cortisol production and leading to elevation of its precursors (11-deoxycortisol, 11-deoxycorticosterone). Although biosynthesis of aldosterone is also impaired, the mineralocorticoid activity of 11-deoxycortisol and 11-deoxycorticosterone compensates for this and can lead to excessive mineralocorticoid effects, accounting for the hypertension and hypokalemia side effects of chronic *metyrapone* use. Off-label use in the treatment of endogenous hypercortisolism usually requires doses of 4 g/day. Because incomplete responses are common, it is often combined at lower doses (500–750 mg three to four times daily) with other agents that inhibit steroidogenesis. Side effects of chronic use, in addition to hypertension and hypokalemia, include hirsutism (due to upstream overproduction of adrenal androgens), nausea, headache, sedation, and rash.

The FDA-approved indication for *metyrapone* is for diagnostic assessment of HPA axis function, commonly in patients with suspected secondary adrenal insufficiency. *Metyrapone* (30 mg/kg, maximum dose of 3 g) is administered orally with a snack at midnight, and plasma cortisol and 11-deoxycortisol are measured at 8 AM the next morning. A plasma cortisol less than 8 μg/dL validates adequate inhibition of CYP11B1; in this setting, an 11-deoxycortisol level less than 7 μg/dL is highly suggestive of impaired HPA function.

Osilodrostat

Osilodrostat is a nonsteroidal, orally available, selective inhibitor of CYP11B1 and CYP11B2; it is FDA-approved for treatment of Cushing disease in patients who are not surgical candidates or who have recurrent disease. Initial dosing is 2 mg twice daily, and it is titrated by 1- to 2-mg twice-daily increments every 2 weeks until satisfactory control is achieved. In a recent trial of patients with Cushing disease, initial control was achieved with an average dose of 5 mg twice daily. In the following discontinuation phase, 86% of those randomly assigned to continue on *osilodrostat* for 12 weeks stayed in control compared to 34% assigned to placebo. Adrenal insufficiency and adverse effects due to adrenal hormone precursors were common (Pivonello et al., 2020).

Etomidate

Etomidate, a substituted imidazole used primarily as an anesthetic agent and sedative, inhibits cortisol secretion at subhypnotic doses, primarily by inhibiting CYP11B1 activity. *Etomidate* has been used off-label to treat hypercortisolism when rapid control is required in the emergency setting ("Cushing crisis"). *Etomidate* is administered as a bolus of 0.03 mg/kg intravenously, followed by an infusion of 0.1 mg/kg/h to a maximum of 0.3 mg/kg/h (Biller et al., 2008).

Abiraterone Acetate

Abiraterone acetate, a CYP17A1 inhibitor approved for the treatment of prostate cancer (see Chapter 73), is under evaluation for treatment of endogenous Cushing syndrome and for reduction of adrenal androgen production in CAH (Auchus et al., 2014).

Mitotane

Mitotane is an adrenocorticolytic agent used to treat inoperable adrenocortical carcinoma. Its cytolytic action is due to its metabolic conversion to a reactive acyl chloride by adrenal mitochondrial CYPs and subsequent reactivity with cellular proteins. It also inhibits CYP11A1, thereby reducing steroid synthesis. Initial doses range from 2 to 6 g/day administered orally in three to four divided doses. The maximal dose can be as high as 16 g/day, if tolerated. Its onset of action takes weeks to months, and GI disturbances and ataxia are its major toxicities. Primary adrenal insufficiency is an expected consequence, and glucocorticoid replacement is required, typically in higher than usual doses. See Chapter 70 for the structure of *mitotane* and additional details on its use.

Glucocorticoid Receptor Antagonists

Mifepristone

Mifepristone, a progesterone receptor antagonist used to terminate early pregnancy (see Chapter 48), is also a high-affinity GR antagonist. This latter activity reduces cortisol effects, including loss of negative feedback, which causes increases in serum levels of ACTH, cortisol, 11-deoxycortisol, and adrenal androgens. *Mifepristone* is approved for control of hyperglycemia in adults secondary to endogenous Cushing syndrome who have type 2 diabetes. The usual dose is 300 mg once daily. It is used off-label as treatment of intractable Cushing syndrome. Side effects include hypertension and hypokalemia due to action of deoxycortisol at the MR. Dose adjustments are made based on improvement in general symptoms such as fatigue and depression and reduction of cortisol-induced hyperglycemia. The expected paradoxical rise in cortisol level, if coupled with symptomatic response, can be an indication of drug activity.

GR Antagonists/Modulators in Development

Relacorilant is an orally available, high-affinity, selective antagonist for the glucocorticoid receptor currently undergoing clinical trials as a treatment for endogenous Cushing syndrome and adrenocortical carcinoma. Unlike *mifepristone*, it does not have appreciable affinity at the progesterone receptor and is thus expected to be devoid of antiprogesterone effects. *Miricorilant* is a high-affinity GR antagonist/modulator with significant MR antagonist activity. It is in development for nonalcoholic steatohepatitis and for antipsychotic-induced weight gain.

Mineralocorticoid Receptor Antagonists

MR antagonists have multiple therapeutic applications, and third-generation, highly selective, nonsteroidal agents are in advanced clinical development or approved in other countries. These drugs are covered in detail in Chapter 29.

Drug Facts for Your Personal Formulary: *Adrenal Related*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Replacement Therapy		
Hydrocortisone/cortisone	• Primary and secondary chronic adrenal insufficiency	• Hydrocortisone is the synthetic equivalent of cortisol. • Daily oral dose of hydrocortisone is 20–30 mg, preferably as divided doses. • Although nonphysiological glucocorticoids are sometimes used, hydrocortisone or cortisone is preferred for replacement therapy. • Tip: Two-thirds of dose in the morning, one-third of dose in the evening.
Hydrocortisone, other glucocorticoids	• Acute adrenal insufficiency • Critical illness-related cortisol insufficiency (CIRCI)	• CIRCI reflects inadequate cortisol production or may occur with abrupt cessation of administered glucocorticoids. • High-dose intravenous hydrocortisone (50–100 mg/6 h) or a constant infusion of 10 mg/h is needed. • An alternative is prednisone at 1 mg/kg per day.
Fludrocortisone (9α-fluorocortisol)	• Mineralocorticoid replacement	• Doses of 0.05–0.2 mg/day. • Lower dose is used initially and is titrated upward as required by blood pressure, plasma renin levels, and response to upright posture. • Fludrocortisone has a $t_{1/2}$ ≥24 h so divided doses are not necessary.
Anti-inflammatory Agents: Systemic		
Prednisolone, methylprednisolone Dexamethasone, budesonide Others	• Across the spectrum of inflammatory disease • Preterm (24–34 weeks) delivery	• Initial high-dose tapering to low dose in short-course therapy. • In early therapy: insomnia, weight gain, emotional lability. • With high-dose/long-term therapy: psychosis, increased susceptibility to infection, osteoporosis, osteonecrosis, myopathy, HPA axis suppression. • On cessation of therapy: acute hypocortisolism. • Tip: Constant vigilance.

Drug Facts for Your Personal Formulary: *Adrenal Related* (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Anti-inflammatory Agents: Topical		
Betamethasone Hydrocortisone Beclomethasone Dexamethasone Triamcinolone acetonide	• Dermatitis, pemphigus, atopic dermatitis, vitiligo, psoriasis, etc.	• Fluorinated steroids have better skin penetration than hydrocortisone. • Effects are magnified by occlusive dressings. • Local adverse events: atrophy, striae, and exacerbation of skin infection. • Tip: Skin-lightening cosmetics include corticosteroids and may produce serious systemic adverse events.
Anti-inflammatory Agents: Ophthalmic		
Dexamethasone Triamcinolone acetonide Fluocinolone acetate (implant)	• Macular disease (degeneration, edema, retinal vein occlusion) • Postoperative inflammation • Corneal injury • Uveitis	• Commonly repeated at 3-month intervals. • Adverse effects: glaucoma, cataract formation. • Contraindications: glaucoma, eye infections.
Anti-inflammatory Agents: Inhaled		
Beclomethasone, budesonide, ciclesonide, flunisolide, fluticasone, mometasone, triamcinolone acetonide	• Asthma, chronic obstructive pulmonary disease	• Rapid metabolism postabsorption into blood is the key for lung selectivity and lower incidence of adverse events. • Chronic use in children may slow growth velocity without compromising final height. • Tip: Ciclesonide, a prodrug converted to active des-ciclesonide in the lung, has low oral bioavailability and less HPA suppression.
Anti-inflammatory Agents: Intranasal		
Mometasone furoate Fluticasone furoate Fluticasone propionate	• Allergic rhinitis, rhinosinusitis, rhinoconjunctivitis, nasal polyposis, postoperatively for sinus ostia stenosis surgery	• Potent localized activity, minimal systemic risk. • Tip: Avoid frequent use.
Anti-inflammatory Steroids: Intra-articular		
Hydrocortisone	• Relief of joint pain	• Local and systemic adverse events rare. • Success varies with difficulty (e.g., vertebral facet joints versus knees).
Chemotherapy		
Dexamethasone Prednisolone Methylprednisolone Prednisone	• Acute lymphatic leukemia • Chronic lymphatic leukemia • Thymoma • Non-Hodgkin lymphoma • Multiple myeloma, breast cancer	• Used in combination with a variety of chemotherapeutic agents. • Used for primary cytotoxic effects, plus relief of pain and nausea and appetite stimulation. • Tip: No place in acute or chronic myelogenous leukemia.
Diagnostics		
Dexamethasone	• Cushing disease	• ↓ ACTH secretion from pituitary corticotrophs but not from ectopic sources.
Metyrapone	Integrity of entire HPA axis	• Inhibits CYP11B1, thereby reducing cortisol and ↑ levels of precursor steroids. • Failure to adequately ↑ precursor levels indicates impaired HPA function.
Cosyntropin (synthetic ACTH)	• Ectopic ACTH secretion • Adrenal insufficiency • Lateralization of aldosterone overproduction	• Cosyntropin is a truncated synthetic form of ACTH used to test adrenal reserve. • Tip: Cosyntropin is commonly used as either a bolus before or a continuous infusion during adrenal venous sampling to distinguish between unilateral and bilateral aldosterone oversecretion in primary aldosteronism.
Stimulant of ACTH Secretion		
Corticorelin	• Peritumoral brain edema postsurgery (off-label use); diagnostic testing	• A synthetic CRH, preferred to high-dose dexamethasone in relieving peritumoral brain edema. • Used diagnostically to distinguish Cushing disease from ectopic ACTH syndrome.
Inhibitors of ACTH Secretion		
Pasireotide	• ACTH oversecretion (Cushing disease)	• Targets $SSTR_5$ (abundant on corticotrophs), ↓ ACTH secretion; used for recurrent or nonresectable ACTH-secreting adenomas.
Cabergoline	• ACTH oversecretion and hyperprolactinemia	• D_2 receptor agonist; ↓ ACTH secretion, ↓ prolactin secretion; useful but not FDA-approved for Cushing disease.
Inhibitors of Corticosteroid Production		
Ketoconazole	• Hypercortisolism (off-label use) • (Used at lower doses as antifungal agent; see Chapter 61)	• ↓ CYP17A1 (17α-hydroxylase) and CYP11A1 (cholesterol side chain cleavage), ↓ adrenal and gonadal steroidogenesis. • Adverse effects: hepatic toxicity; drug interactions due to inhibition of CYP3A4 and P-glycoprotein.

Drug Facts for Your Personal Formulary: *Adrenal Related* (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Metyrapone	• Hypercortisolism; adjunctive therapy after pituitary irradiation	• Inhibits CYP11B1 (11-deoxy cortisol → cortisol). • ↓ cortisol; 4 g/day to maximally ↓ steroidogenesis. • Chronic use may cause hirsutism and hypertension.
Etomidate	• Rapid control of hypercorticolism (off-label use) • (Also a short-acting anesthetic; see Chapter 25)	• Inhibits CYP11B1 (11-deoxy cortisol → cortisol). • ↓ cortisol production at subanesthetic doses. • Administer as IV bolus, 0.03 mg/kg.
Mitotane	• Treating inoperable adrenocortical carcinoma (See also Chapter 70)	• Activated by adrenal cortical CYPs to an acyl chloride with cytolytic effects. • Inhibits CYP11A1 (cholesterol side chain cleavage), ↓ steroidogenesis.
Glucocorticoid Antagonist		
Mifepristone (RU486)	• Hypercortisolism • (Used at lower doses as antiprogesterone for termination of early pregnancy; see Chapter 48)	• GR antagonist, IC_{50} ~2.2 nM (IC_{50} for antiprogesterone effect, ~0.025 nM). • Used at 300–1200 mg/day to treat inoperable hypercortisolism that is resistant to other agents.

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Chapter 51

Endocrine Pancreas and Pharmacotherapy of Diabetes Mellitus and Hypoglycemia

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PHYSIOLOGY OF GLUCOSE HOMEOSTASIS

- Regulation of Blood Glucose
- Pancreatic Islet Physiology and Insulin Secretion
- Insulin Action
- The Insulin Receptor

PATHOPHYSIOLOGY AND DIAGNOSIS OF DIABETES MELLITUS

- Glucose Homeostasis and the Diagnosis of Diabetes
- Screening for Diabetes and Categories of Increased Risk of Diabetes
- Pathogenesis of Type 1 Diabetes
- Pathogenesis of Type 2 Diabetes
- Pathogenesis of Other Forms of Diabetes
- Diabetes-Related Complications

THERAPY OF DIABETES

- Goals of Therapy
- Nonpharmacological Aspects of Diabetes Therapy
- Insulin Therapy
- Management of Diabetes in Hospitalized Patients
- Insulin Secretagogues and Glucose-Lowering Agents
- Combined Pharmacological Approaches to Type 2 Diabetes
- Emerging Therapies for Diabetes

HYPOGLYCEMIA

- Agents Used to Treat Hypoglycemia

OTHER PANCREATIC ISLET–RELATED HORMONES OR DRUGS

- Diazoxide
- Somatostatin

Diabetes mellitus is a heterogeneous spectrum of metabolic disorders, likely arising from disparate genetic and environmental factors, with a common outcome of impaired glucose homeostasis and hyperglycemia. The pathogenesis, for most persons, involves some combination of insufficient insulin secretion, reduced responsiveness to endogenous or exogenous insulin, increased glucose production, and abnormalities in fat and protein metabolism. The resulting hyperglycemia may lead to both acute symptoms and metabolic abnormalities. A major source of the morbidity of diabetes is chronic end-organ damage that arises from prolonged hyperglycemia and includes retinopathy, neuropathy, nephropathy, and cardiovascular disease. These chronic complications can be mitigated in many patients by sustained control of the blood glucose and treatment of comorbidities such as hypertension and dyslipidemia (Nathan and DCCT/EDIC Research Group, 2013). A wide variety of treatment options for hyperglycemia that target different processes involved in glucose regulation or dysregulation are available (ADA, 2022c).

Physiology of Glucose Homeostasis

Regulation of Blood Glucose

The maintenance of glucose homeostasis is a highly developed systemic process involving the integration of several major organs (Figure 51–1). *Glucose tolerance* refers specifically to tests of this system using standardized oral or intravenous glucose challenges. The actions of insulin are of central importance for glucose homeostasis with webs of interorgan communication via other hormones, nerves, local factors, and substrates also playing vital roles. The pancreatic β cell is essential for normal glucose tolerance, adjusting the amount of insulin secreted very precisely to promote glucose uptake after meals and regulating glucose output from the liver during fasting.

In the *fasting state* (Figure 51–1A), most of the fuel demands of the body are met by the oxidation of fatty acids. The brain does not effectively use fatty acids to meet energy needs and in the fasting state requires glucose for normal function; glucose requirements are about 2 mg/kg/min in adult humans, largely to supply the CNS with an energy source. *Fasting glucose requirements are primarily provided by the liver.* Liver glycogen stores provide some of this glucose; conversion of lactate, alanine, and glycerol into glucose accounts for the remainder. The dominant regulation of hepatic *glycogenolysis* and *gluconeogenesis* is controlled by the pancreatic islet hormones insulin and *glucagon*. Insulin inhibits hepatic glucose production, and the decline of the circulating insulin concentration in the postabsorptive state (fasting) is permissive for higher rates of hepatic glucose output. Glucagon maintains blood glucose concentrations at physiological levels in the absence of exogenous carbohydrate (overnight and in between meals) by stimulating hepatic gluconeogenesis and glycogenolysis. Insulin secretion is stimulated by *food ingestion*, nutrient absorption, and elevated blood glucose, and activation of the insulin receptor promotes glucose, lipid, and protein anabolism (Figure 51–1B). The centrality of insulin in glucose metabolism is emphasized by the fact that all the forms of human diabetes have as a root cause some abnormality of insulin secretion or action.

Pancreatic *β cell* function is primarily controlled by plasma glucose concentrations. Elevations of blood glucose are necessary for insulin release above basal levels, and other stimuli are relatively ineffective when plasma glucose is in the fasting range (4.4–5.5 mM or 80–100 mg/dL). These other stimuli include nutrient substrates such as amino acids, *insulinotropic hormones* released from the GI tract and islet α cells, and autonomic neural pathways. Neural stimuli cause some increase of insulin secretion prior to food consumption and throughout the meal. Arrival of nutrient chyme to the intestine leads to the release of insulinotropic peptides from specialized endocrine cells in the intestinal mucosa. *Glucose-dependent insulinotropic polypeptide (GIP)* and *glucagon-like peptide 1 (GLP-1)*, together termed *incretins,* are the essential gut hormones contributing to *glucose tolerance*. They are secreted in proportion to the ingested nutrient load and relay this information to the islet as part of a feed-forward mechanism that allows an insulin response appropriate to meal size. Insulin secretion rates in healthy humans are highest in the early digestive phase of meals, preceding and limiting the peak in blood glucose. This pattern of anticipatory, rapid insulin secretion is an

Abbreviations

A1c: hemoglobin A_{1c}
ADA: American Diabetes Association
CGM: continuous glucose monitoring
CSII: continuous subcutaneous insulin infusion
CVD: cardiovascular disease
DPP-4: dipeptidyl peptidase 4
GFR: glomerular filtration rate
GIP: glucose-dependent insulinotropic polypeptide
GK: glucokinase (hexokinase IV)
GLP-1: glucagon-like peptide 1
GLP-1RA: GLP-1 receptor agonist
GLUT: glucose transporter
G6P: glucose-6-phosphate
GPCR: G protein-coupled receptor
Hb: hemoglobin
HDL: high-density lipoprotein
HGP: hepatic glucose production
IAPP: islet amyloid polypeptide
IFG: impaired fasting glucose
IGT: impaired glucose tolerance
IRS: insulin receptor substrate
Kir: inward rectifying K^+ channel
LDL: low-density lipoprotein
MODY: maturity-onset diabetes of the young
mTOR: mammalian target of rapamycin
NPH: neutral protamine Hagedorn
PI3K: phosphatidylinositol-3-kinase
PPAR: peroxisome proliferator-activated receptor
SGLT2: sodium-glucose cotransporter 2
SST: somatostatin
SUR: sulfonylurea receptor

essential feature of normal glucose tolerance, and mimicking this pattern is one of the key challenges for successful insulin therapy in individuals with diabetes.

Elevated circulating insulin concentrations lower glucose in blood by inhibiting hepatic glucose production (HGP) and stimulating the uptake and metabolism of glucose by liver, muscle, and adipose cells. Production of glucose is inhibited half-maximally by an insulin concentration of about 120 pmol/L, whereas glucose utilization is stimulated half-maximally at about 300 pmol/L. Some of the effects of insulin on the liver occur rapidly, within the first 20 min of meal ingestion, whereas stimulation of peripheral glucose uptake may require up to an hour to reach significant rates. Insulin has potent effects to reduce lipolysis from adipocytes, primarily through the inhibition of hormone-sensitive lipase; insulin also increases lipid storage by promoting lipoprotein-lipase synthesis and adipocyte glucose uptake. In muscle and other tissues, insulin stimulates amino acid uptake and protein synthesis and inhibits protein degradation. The extracellular matrix, e.g., that is between the intravascular space and skeletal muscle cells or adipocytes, is also important in insulin action and insulin resistance.

The limited glycogen stores in skeletal muscle are mobilized at the onset of physical activity, but most of the glucose support for exercise comes from hepatic gluconeogenesis. The dominant regulation of hepatic glucose production during exercise comes from epinephrine and norepinephrine. The catecholamines stimulate glycogenolysis and gluconeogenesis, inhibit insulin secretion, and enhance release of glucagon, all contributing to increased hepatic glucose output. In addition, catecholamines promote lipolysis, freeing fatty acids for oxidation in exercising muscle and glycerol for hepatic gluconeogenesis.

Pancreatic Islet Physiology and Insulin Secretion

The pancreatic islets compose 1% to 2% of the pancreatic volume. The pancreatic islet is a vascularized, innervated miniorgan containing five endocrine cell types: *α cells* that secrete *glucagon, β cells* that secrete insulin, *δ cells* that secrete *somatostatin (SST), PP* cells that secrete *pancreatic polypeptide*, and *ε cells* that secrete *ghrelin* (Walker et al., 2021).

Insulin is initially synthesized as a single polypeptide chain, *preproinsulin* (110 amino acids), which is processed first to *proinsulin* and then to *insulin* and *C-peptide* (Figure 51–2). This complex and highly regulated process involves the Golgi complex, the endoplasmic reticulum, and the secretory granules of the β cell. The chemical properties of secretory granules are critical in the cleavage and processing of the prohormone to the final secretion products, insulin and C-peptide, and their regulated intracellular transport carries insulin to the cell membrane for exocytosis. Equimolar quantities of insulin and C-peptide are co-secreted. Insulin has a $t_{1/2}$ of 5 to 6 min due to extensive hepatic clearance and renal filtration. C-peptide, in contrast, with no known physiological function or receptor, has a $t_{1/2}$ of about 30 min. Because of minimal hepatic clearance, measurements of peripheral C-peptide concentrations are useful in assessment of β cell secretion and to distinguish endogenous and exogenous hyperinsulinemia (e.g., in the evaluation of insulin-induced hypoglycemia). The β cell also synthesizes and secretes *islet amyloid polypeptide* (IAPP) or *amylin*, a 37–amino acid peptide. IAPP influences GI motility and the speed of glucose absorption. *Pramlintide* is an agent used in the treatment of diabetes that mimics the action of IAPP.

Insulin secretion is tightly regulated to provide stable concentrations of glucose in blood during both fasting and feeding. This regulation is achieved by the coordinated interplay of circulating nutrients, GI hormones, pancreatic hormones, and autonomic neurotransmitters. Glucose, amino acids, fatty acids, and ketone bodies promote the secretion of insulin. Glucose is the primary insulin secretagogue, and insulin secretion is tightly coupled to the extracellular glucose concentration. Insulin secretion is much greater when the same amount of glucose is delivered orally compared to intravenously, a response termed the *incretin effect* and attributed to the insulinotropic GI peptides GIP and GLP-1. Somatostatin release from islet δ cells acts on SST receptors on α and β cells to inhibit glucagon and insulin release through local, paracrine regulation. Recent studies have demonstrated that glucagon and GLP-1 produced in α cells also have local effects to promote insulin secretion. Islets are richly innervated by both adrenergic and cholinergic nerves. Stimulation of α_2 adrenergic receptors inhibits insulin secretion, whereas β_2 adrenergic receptor agonists and vagal nerve stimulation enhance release. In general, any condition that activates the sympathetic branch of the autonomic nervous system (e.g., hypoxia, hypoglycemia, exercise, hypothermia, surgery, or severe burns) suppresses the secretion of insulin by stimulation of α_2 adrenergic receptors.

The molecular events controlling glucose-stimulated insulin secretion begin with the transport of glucose into the β cell via a facilitative glucose transporter, primarily glucose transporter type 1 (GLUT1) in human β cells (Figure 51–3) (Campbell and Newgard, 2021). On entry into the β cell, glucose is quickly phosphorylated by *glucokinase* (GK; also known as hexokinase IV); *this phosphorylation is the rate-limiting step in glucose metabolism in the β cell.* β Cell GK phosphorylates glucose most actively when blood glucose concentrations are 5 to 10 mM, accounting for high rates of intracellular glucose metabolism across the physiological range from fasting to prandial glycemia when insulin secretion is greatest. The *glucose-6-phosphate* (G6P) produced by GK activity enters the glycolytic pathway, producing changes in NADPH and the ratio of ADP/ATP. Elevated ATP inhibits an ATP-sensitive K^+ channel (K_{ATP} channel), leading to cell membrane depolarization. This heteromeric K_{ATP} channel consists of an inward rectifying K^+ channel (Kir6.2) and a closely associated protein known as the sulfonylurea receptor (SUR). Mutations in the K_{ATP} channel are responsible for specific types of neonatal diabetes and hyperinsulinemic hypoglycemia. Membrane depolarization following K_{ATP} closure leads to opening of a voltage-dependent Ca^{2+} channel and increased intracellular Ca^{2+}, resulting in exocytotic release of insulin from storage vesicles. These intracellular events are modulated by changes in

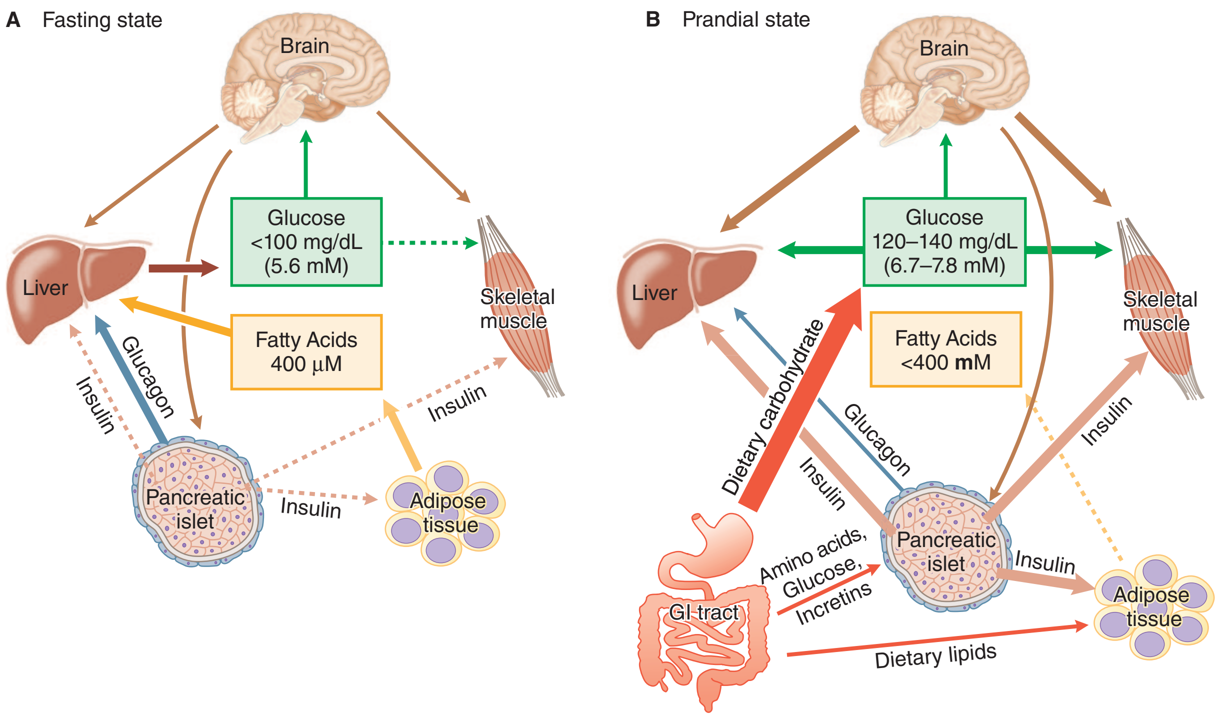
A Fasting state
Brain
Glucose <100 mg/dL (5.6 mM)
Liver
Skeletal muscle
Fatty Acids 400 µM
Glucagon
Insulin
Insulin
Pancreatic islet
Insulin
Adipose tissue
B Prandial state
Brain
Glucose 120–140 mg/dL (6.7–7.8 mM)
Liver
Skeletal muscle
Fatty Acids <400 mM
Dietary carbohydrate
Glucagon
Insulin
Insulin
Pancreatic islet
Insulin
Amino acids, Glucose, Incretins
GI tract
Adipose tissue
Dietary lipids

Figure 51–1 *Insulin, glucagon, and glucose homeostasis.* **A.** *Fasting State*—In healthy humans, plasma glucose is maintained in a range from 4.4 to 5 mM and fatty acids near 400 µM. In the absence of nutrient absorption from the GI tract, glucose is supplied primarily from the liver and fatty acids from adipose tissue. During overnight fasting, plasma insulin concentrations decrease, and plasma glucagon rises modestly, contributing to increased hepatic glycogenolysis and gluconeogenesis; low insulin also releases adipocytes from inhibition, permitting increased release of fatty acids into the circulation. Most tissues oxidize primarily fatty acids during fasting, sparing glucose for use by the CNS. **B.** *Prandial State*—During feeding, nutrient absorption causes an increase in plasma glucose and release of incretins from the gut and neural stimuli from the CNS. This combination of factors stimulates insulin secretion to rise in proportion to meal size. Protein-containing meals also cause a modest increase in glucagon secretion. Under the control of these changes in islet hormones, glucose is distributed primarily to the liver, skeletal muscle, and adipose tissue, which each take up glucose under the influence of insulin. Hepatic glucose production is diminished, and lipolysis inhibited, while total body glucose oxidation increases. The brain senses plasma glucose concentrations and provides regulatory inputs contributing to fuel homeostasis. The boldness of the *arrows* reflects relative intensity of action; a *dashed line* indicates little or no activity.

cAMP production and amino acid metabolism. G protein-coupled receptors (GPCRs) for glucagon, GIP, and GLP-1 and other regulatory peptides couple to G_s to stimulate adenylyl cyclase and insulin secretion; receptors for SST and α_2 adrenergic agonists couple to G_i to reduce cellular cAMP production and secretion.

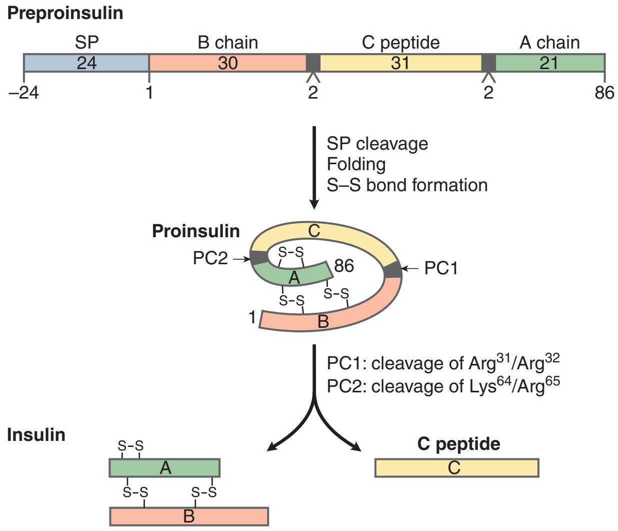
Preproinsulin
SP
24
B chain
30
C peptide
31
A chain
21
–24
1
2
2
86
SP cleavage
Folding
S–S bond formation
Proinsulin
C
PC2
S–S
A
86
PC1
S–S
S–S
1
B
PC1: cleavage of Arg^{31}/Arg^{32}
PC2: cleavage of Lys^{64}/Arg^{65}
Insulin
S–S
A
S–S
S–S
B
C peptide
C

Figure 51–2 *Synthesis and processing of insulin.* The initial peptide, preproinsulin (110 amino acids) consists of a signal peptide (SP), B chain, C-peptide, and A chain. The SP is cleaved and S–S bonds form as the proinsulin folds. Two prohormone convertases, PC1 and PC2, cleave proinsulin into insulin, C-peptide, and two dipeptides. Insulin and C-peptide are stored in granules and cosecreted in equimolar quantities.

The pancreatic α cell secretes *glucagon*, most dramatically in response to hypoglycemia, but also after short periods of fasting or after protein-containing meals. Glucagon biosynthesis begins with *preproglucagon*, which is processed in a cell-specific fashion to several biologically active peptides, such as glucagon, GLP-1, and GLP-2 (see Figure 51–9). *In general, glucagon and insulin secretion are regulated in a reciprocal fashion by the blood glucose; that is, hyperglycemia stimulates insulin secretion and inhibits glucagon secretion. However, pharmacological administration of arginine and other amino acids stimulates both islet hormones, and SST inhibits the secretion of both insulin and glucagon.*

Insulin Action

The insulin receptor is expressed on virtually all mammalian cell types. Tissues that are critical for regulation of blood glucose are liver, skeletal muscle, fat (see Figure 51–1), specific regions of the brain, and the pancreatic islet. The actions of insulin are generally anabolic, and insulin signaling is critical for promoting the uptake, use, and storage of the major nutrients: glucose, lipids, and amino acids. Insulin stimulates glycogenesis, lipogenesis, and protein synthesis; it also inhibits the catabolism of these compounds. On a cellular level, insulin stimulates transport of substrates and ions into cells, promotes translocation of proteins between cellular compartments, regulates the action of specific enzymes, and controls gene transcription and mRNA translation. Some effects of insulin (e.g., activation of glucose and ion transport systems, phosphorylation or dephosphorylation of specific enzymes) occur within seconds or minutes; other effects (e.g., those promoting protein synthesis and regulating gene transcription and cell proliferation) manifest over minutes to hours to days. The effects of insulin on cell proliferation and differentiation occur over an even longer period of time.

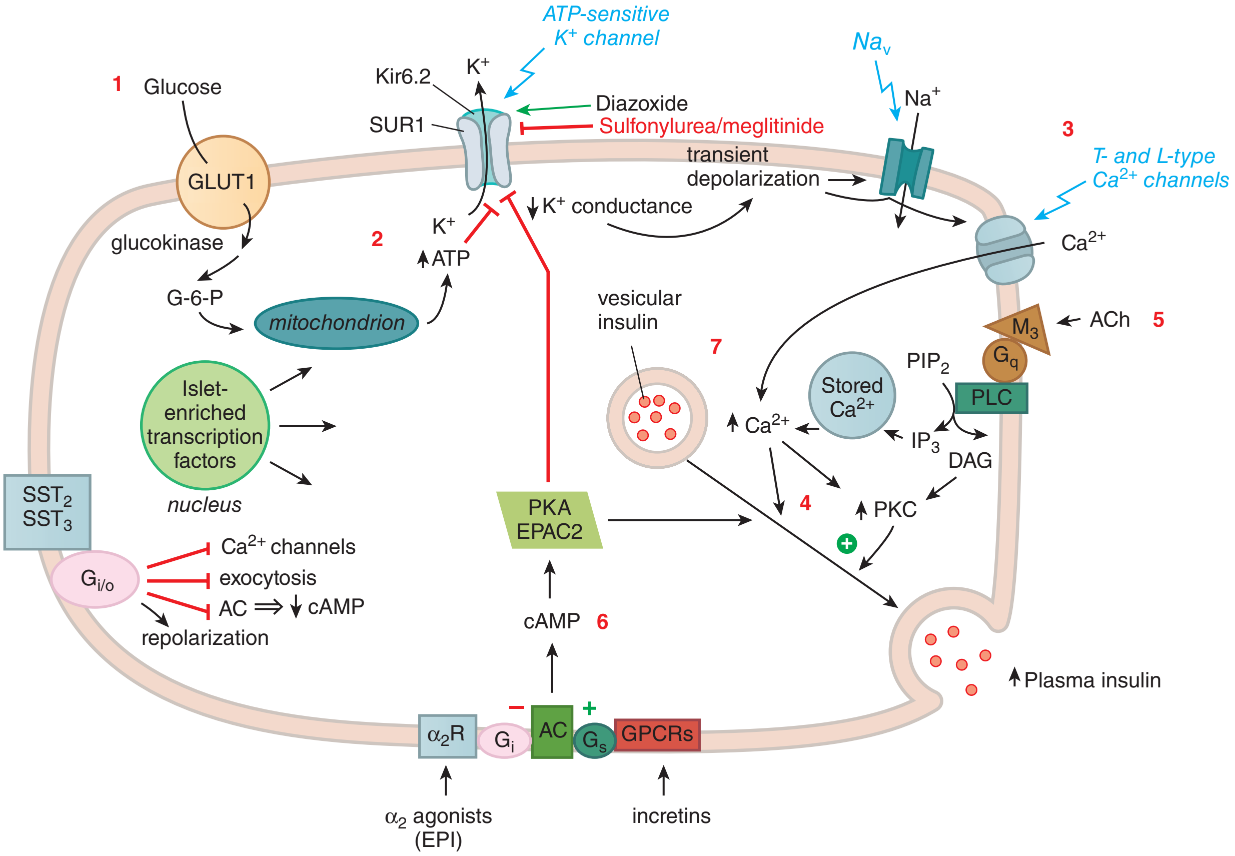

Figure 51–3 *Regulation of insulin secretion from a pancreatic β cell.* In the fasting state of basal glycemia, pancreatic β cells are hyperpolarized. With increases in glycemia, e.g., after meals, glucose transport into the β cells increases, primarily via GLUT1 in humans (1). Increased intracellular glucose leads to more glucose metabolism and elevated levels of ATP, which reduces K^+ conductance through the K_{ATP} channel (2); the decreased K^+ conductance causes local membrane depolarization and activation of Ca^{2+} and Na^+ channels (3), leading to a rise of intracellular calcium and stimulation of release of stored insulin (4), using the basic mechanisms described for exocytosis of neurotransmitters (see Chapter 10, Figures 10–4 to 10–6). ACh, acting via M_3 receptors, can activate the G_q-PLC-IP_3-Ca^{2+}-PKC pathway, potentiating insulin exocytosis (5); incretins, also acting via GPCRs, can activate the G_s-AC-cAMP-PKA/EPAC2 pathways, both of which enhance exocytosis (6). Elevated cAMP also leads to inhibition of the K_{ATP} channel, enhancing depolarization and furthering exocytosis (7). The depolarization/exocytosis period is limited by closure of voltage-sensitive ion channels, by export of Ca^{2+} and Na^+, and by sequestration of Ca^{2+} within the sarcoplasmic reticulum (SR) by the SERCA transporter. SST, acting via SST2 and SST3 that couple to $G_{i/o}$, can aid in restoring the hyperpolarized state of the cell, as can α_2 agonists. The K_{ATP} channel has SUR1 and Kir6.2 subunits; ATP binds to and inhibits Kir6.2; sulfonylureas and meglitinides bind to and inhibit SUR1; all three agents thereby promote insulin secretion. *Diazoxide* and ADP-Mg^{2+} (low ATP) bind to and activate SUR1, thereby inhibiting insulin secretion. Mitochondrial mutations and islet-enriched transcription factors can contribute to the development of diabetes. This schematic is a simplification; see Campbell and Newgard (2021) for greater detail. G, G protein, with subtype indicated by subscript; AC, adenylyl cyclase; EPAC, exchange protein activated by cAMP; GLUT, GLUT1 glucose transporter; GPCR, G protein-coupled receptor; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; SST2/3, somatostatin receptors.

The Insulin Receptor

Insulin action is transmitted through a receptor tyrosine kinase that bears functional similarity to the *insulin-like growth factor 1 receptor* (Haeusler et al., 2018; Saltiel, 2021). The insulin receptor is composed of linked α/β subunit dimers that are products of a single gene; dimers linked by disulfide bonds form a transmembrane heterotetramer glycoprotein composed of two extracellular α subunits and two membrane-spanning β subunits (Figure 51–4). The number of receptors varies from 40/cell on erythrocytes to 300,000/cell on adipocytes and hepatocytes.

The α subunits inhibit the inherent tyrosine kinase activity of the β subunits. Insulin binding to the α subunits releases this inhibition and allows transphosphorylation of one β subunit by the other, and autophosphorylation at specific sites from the juxtamembrane region to the intracellular tail of the receptor. Activation of the insulin receptor initiates signaling by phosphorylating a set of intracellular mediators, including the *insulin receptor substrate* (IRS) and Src-homology-2-containing protein. These proteins interact with effectors that amplify and extend the signaling cascade.

Insulin action on glucose transport depends on the activation of *phosphatidylinositol-3-kinase* (PI3K). PI3K is activated by interaction with IRS proteins and generates phosphatidylinositol 3,4,5-trisphosphate, which regulates the localization and activity of mTOR. The isoform Akt2 appears to control the downstream steps that are important for glucose uptake in skeletal muscle and adipose tissue and to regulate glucose production in the liver. Substrates of Akt2 coordinate the translocation of GLUT4 to the plasma membrane through processes involving actin remodeling and other membrane trafficking systems.

GLUT4

GLUT4 is expressed in insulin-responsive tissues such as skeletal muscle and adipose tissue. In the basal state, most GLUT4 resides in the intracellular space; following activation of insulin receptors, GLUT4 is shifted rapidly and in abundance to the plasma membrane (Saltiel, 2021), where it facilitates inward transport of glucose from the circulation. Insulin signaling also reduces GLUT4 endocytosis, increasing the residence time of the protein in the plasma membrane. Following the facilitated diffusion into cells along a concentration gradient, glucose is phosphorylated to G6P by hexokinases. Hexokinase II is found in association with GLUT4 in skeletal and cardiac muscle and in adipose tissue. Like GLUT4, hexokinase II is regulated transcriptionally by insulin. G6P can be isomerized to G1P and stored as glycogen (insulin enhances the activity of glycogen

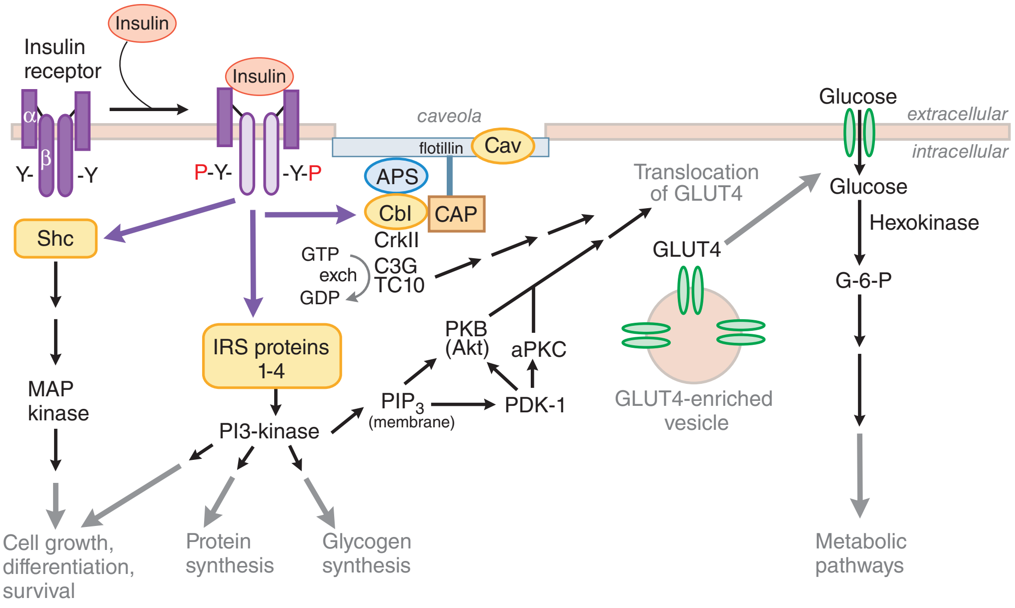

Figure 51–4 *Pathways of insulin signaling.* The binding of insulin to its plasma membrane receptor activates a cascade of downstream signaling events. Insulin binding activates the intrinsic tyrosine kinase activity of the receptor dimer, resulting in the tyrosine phosphorylation (Y-P indicates the phosphorylated tyrosine residue, Y) of the receptor's β subunits and a small number of specific substrates (yellow shapes): the IRS proteins and Src-homology-2-containing protein; within the membrane, a caveolar pool of insulin receptor phosphorylates Cav, APS, and Cbl. These tyrosine-phosphorylated proteins interact with signaling cascades via SH2 and SH3 domains to mediate the effects of insulin, with specific effects resulting from each pathway. In the target tissues such as skeletal muscle and adipocytes, a key event is the translocation of GLUT4 from intracellular vesicles to the plasma membrane; this translocation is stimulated by both the caveolar and noncaveolar pathways. In the noncaveolar pathway, the activation of PI3K is crucial, and PKB/Akt (anchored at the membrane by phosphatidylinositol 3,4,5-trisphosphate) or an atypical form of PKC is involved. In the caveolar pathway, caveolar protein flotillin localizes the signaling complex to the caveola; the signaling pathway involves a series of SH2 domain interactions that add the adaptor protein CrkII, the guanine nucleotide exchange protein C3G, and small GTP-binding protein TC10. The pathways are inactivated by specific phosphoprotein phosphatases (e.g., PTB1B). In addition to the actions shown, insulin stimulates the plasma membrane Na^+/K^+-ATPase by a mechanism that is still being elucidated; the result is an increase in pump activity and a net accumulation of K^+ in the cell. APS, adaptor protein with PH and SH2 domains; CAP, Cbl associated protein; CAV, caveolin; CrkII, chicken tumor virus regulator of kinase II; GLUT4, glucose transporter 4; PDK, phosphoinositide-dependent kinase; Y-P, phosphorylated tyrosine residue.

synthase); G6P can also enter the glycolytic pathway (for ATP production) or the pentose phosphate pathway.

Pathophysiology and Diagnosis of Diabetes Mellitus

Glucose Homeostasis and the Diagnosis of Diabetes

Broad categories of glucose homeostasis are defined by the blood glucose in the fasting or fed state or the glucose following an oral glucose challenge. These include the following:

- Normal glucose homeostasis: fasting plasma glucose less than 5.6 mmol/L (100 mg/dL)
- Impaired fasting glucose (IFG): 5.6–6.9 mmol/L (100–125 mg/dL)
- Impaired glucose tolerance (IGT): glucose level between 7.8 and 11.1 mmol/L (140 and 199 mg/dL) 120 min after ingestion of 75 g liquid glucose solution
- Hyperglycemia diagnostic for diabetes mellitus (Table 51–1)

The American Diabetes Association (ADA) and the World Health Organization (WHO) have adopted criteria for the diagnosis of diabetes based on the fasting blood glucose, the glucose value following an oral glucose challenge, or the level of hemoglobin (Hb) type A_{1c} (A1c); exposure of proteins to elevated glucose produces nonenzymatic glycation of proteins, including Hb, so the level of A1c represents a measure of the average glucose concentration to which the Hb has been exposed (see Table 51–1). IFG and IGT portend a markedly increased risk of progressing to type 2 diabetes and are associated with increased risk of cardiovascular disease.

The four categories of diabetes include type 1 diabetes, type 2 diabetes, other forms of diabetes, and gestational diabetes mellitus (Table 51–2). Although hyperglycemia is common to all forms of diabetes, the pathogenic mechanisms leading to diabetes are quite diverse.

Screening for Diabetes and Categories of Increased Risk of Diabetes

Many individuals with type 2 diabetes are asymptomatic at the time of diagnosis, and diabetes is often found on routine blood testing for

TABLE 51–1 ■ CRITERIA FOR THE DIAGNOSIS OF DIABETES

- Symptoms of diabetes plus random blood glucose concentration ≥11.1 mM (200 mg/dL)[a] or
- Fasting plasma glucose ≥7.0 mM (126 mg/dL)[b] or
- Two-hour plasma glucose ≥11.1 mM (200 mg/dL) during an oral glucose tolerance test[c] or
- HbA_{1c} ≥6.5%

Note: In the absence of unequivocal hyperglycemia and acute metabolic decompensation, these criteria should be confirmed by repeat testing on a different day.

[a] *Random* is defined as without regard to time since the last meal.

[b] *Fasting* is defined as no caloric intake for at least 8 h.

[c] The test should be performed using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water; this test is not recommended for routine clinical use.

Adapted from American Diabetes Association (2022a).

TABLE 51–2 ■ DIFFERENT FORMS OF DIABETES MELLITUS BY ETIOLOGY

I. **Type 1 diabetes β-cell destruction**, usually leading to absolute insulin deficiency

II. **Type 2 diabetes** (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect)

III. **Other specific types of diabetes**

- A. Monogenic disorders of β-cell function
    1. HNF-4α (MODY 1)
    2. Glucokinase (MODY 2)
    3. HNF-1α (MODY 3)
    4. Other forms of MODY: insulin promoter factor 1, HNF-1β, HNF-1α, NeuroD1, and other regulators of islet cells
    5. Permanent neonatal diabetes *KCNJ11* gene encoding Kir6.2 subunit of β cell K_{ATP} channel, insulin gene
    6. Mitochondrial DNA
- B. Genetic defects in insulin action, including type A insulin resistance, leprechaunism, Rabson-Mendenhall syndrome, lipodystrophy syndromes
- C. Diseases of the exocrine pancreas—pancreatitis, pancreatectomy, neoplasia, cystic fibrosis, hemochromatosis, fibrocalculous pancreatopathy, mutations in carboxyl ester lipase
- D. Endocrinopathies—acromegaly, Cushing syndrome, glucagonoma, pheochromocytoma, hyperthyroidism, somatostatinoma, aldosteronoma
- E. Drug or chemical induced—glucocorticoid, calcineurin and mTOR inhibitors, antipsychotics (atypical, others), protease inhibitors, β adrenergic agonists such as epinephrine, pyrinuron (a rodenticide no longer sold in the U.S.)
- F. Infections—congenital rubella, cytomegalovirus
- G. Uncommon forms of immune-mediated diabetes—“stiff-person” syndrome, anti-insulin receptor antibodies
- H. Other genetic syndromes sometimes associated with diabetes—Wolfram, Down, Klinefelter, Laurence-Moon-Biedl, Prader-Willi, and Turner syndromes; Friedreich ataxia; Huntington’s disease; myotonic dystrophy; porphyria

IV. **Gestational diabetes mellitus**

MODY, maturity-onset diabetes of the young.
Adapted from American Diabetes Association (2017, 2022c).

non–glucose-related reasons. The ADA recommends widespread screening for type 2 diabetes of adults with the following features:

- Age more than 45 years
- Body mass index greater than 25 kg/m^2 (or >23 kg/m^2 in persons of Asian descent) with one of these additional risk factors: first-degree relative with diabetes, physical inactivity, hypertension, low HDL value, high-risk ethnic group (African American, Latino, Native American, Asian American, and Pacific Islander), history of abnormal glucose testing (IFG, IGT, A1c of 5.7%–6.4%), cardiovascular disease, features of insulin resistance, or women with polycystic ovary syndrome
- Women who have previously had gestational diabetes mellitus

In screening for diabetes, fasting plasma glucose, A1c, and plasma glucose after an oral glucose tolerance test are equally valid, but the fasting glucose and A1c are used most commonly. Early diagnosis and treatment of type 2 diabetes should delay diabetes-related complications and reduce the burden of the disease. A number of interventions, including lifestyle modification and pharmacological agents, are effective. Screening for type 1 diabetes is not currently recommended outside a clinical trial.

Pathogenesis of Type 1 Diabetes

Type 1 diabetes accounts for 5% to 10% of diabetes and results from autoimmune-mediated destruction of the β cells of the islet, leading to total or near-total insulin deficiency (Powers, 2021). Type 1 diabetes can occur at any age, with up to 40% of individuals developing type 1 diabetes after the age of 30. Individuals with type 1 diabetes and their families have an increased prevalence of autoimmune diseases such as autoimmune adrenal insufficiency, Graves or Hashimoto disease, pernicious anemia, vitiligo, and celiac sprue. The concordance of type 1 diabetes in genetically identical twins is 60% to 70%, indicating a significant genetic component. The major genetic risk (40%–50%) is conferred by HLA class II genes encoding HLA-DR and HLA-DQ. However, there likely is a critical interaction of genetics and an environmental or infectious agent. Most individuals with type 1 diabetes (>75%) do not have a family member with type 1 diabetes, and the genes conferring genetic susceptibility are found in a significant fraction of the nondiabetic population.

Genetically susceptible individuals are thought to have a normal β cell number or mass until β cell–directed autoimmunity develops and β cell loss begins. The initiating or triggering stimulus for the autoimmune process is not known, but most favor exposure to viruses (enterovirus, etc.) or other ubiquitous environmental agents. The β cell destruction is likely cell mediated, and there is also evidence that infiltrating cells produce local inflammatory agents such as tumor necrosis factor-α, interferon-γ, and interleukin-1, all of which can lead to β cell death. The β cell destruction occurs over a period of months to years, and when the majority of β cells are destroyed, hyperglycemia ensues and the clinical diagnosis of type 1 diabetes is made. The ADA and others now recognize three stages of type 1 diabetes: stage 1: autoimmunity as reflected by two autoantibodies with normoglycemia; stage 2: autoimmunity with dysglycemia; and stage 3: autoimmunity with hyperglycemia (usually symptomatic). Most patients report several weeks of polyuria and polydipsia, fatigue, and often abrupt and significant weight loss. Some adults with the phenotypic appearance of type 2 diabetes (obese, not insulin-requiring initially) have islet cell autoantibodies suggesting autoimmune-mediated β cell destruction and are diagnosed as having latent-autoimmune diabetes of adults (Mishra et al., 2018).

Pathogenesis of Type 2 Diabetes

Type 2 diabetes is best thought of as a heterogeneous syndrome of dysregulated glucose homeostasis associated with impaired insulin secretion and action (Gloyn and Drucker, 2018). Recent analyses of large databases of persons with type 2 diabetes have identified discrete clusters of patients with common features based on demographic and clinical factors (Ahlqvist et al., 2018). These clusters are reproducible in different patient cohorts and have clear associations with specific genetic loci and diabetic complications, supporting a model whereby several distinct pathogenic mechanisms lead to type 2 diabetes. Overweight or obesity is a common correlate of type 2 diabetes, occurring in approximately 80% of affected individuals. For the vast majority of individuals developing type 2 diabetes, there is no clear inciting incident; rather, the condition develops gradually over years, often with progression through an identifiable prediabetic stage.

In general, type 2 diabetes results when there is insufficient insulin action to maintain plasma glucose levels in the normal range. Insulin action is the composite effect of plasma insulin concentrations (determined by islet β cell function) and insulin sensitivity of key target tissues (liver, skeletal muscle, and adipose tissue). These sites of regulation are all impaired to variable extents in patients with type 2 diabetes (Figure 51–5). The etiology of type 2 diabetes has a strong genetic component. It is a heritable condition, and persons with a diabetic parent or sibling have a relative 4-fold increased risk of disease, increasing to 6-fold if both parents have type 2 diabetes. Although more than 400 genetic loci with clear associations to type 2 diabetes have been identified through genome-wide association studies, the contribution of each is relatively small (Cole and Florez, 2020).

Impaired β Cell Function

In type 2 diabetes, the sensitivity of the β cell to glucose is impaired, with associated loss of responsiveness to other stimuli, such as insulinotropic GI hormones and neural signaling (Kahn et al., 2021). This results in delayed or insufficient insulin secretion, allowing the blood glucose to

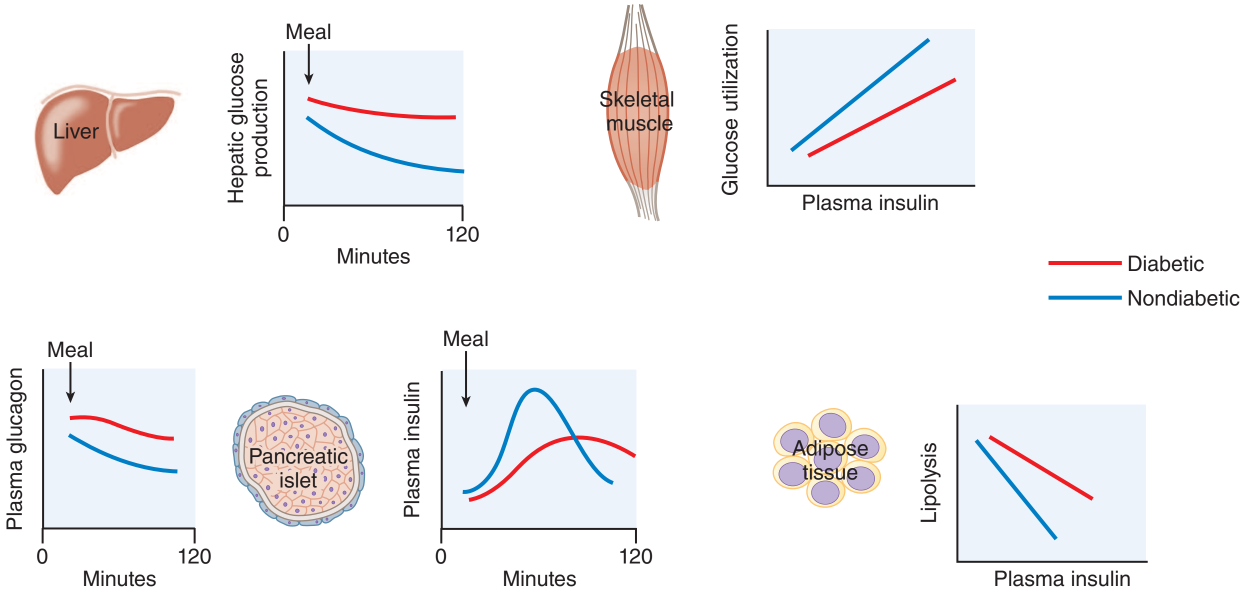

Figure 51–5 *Pathophysiology of type 2 diabetes mellitus.* Graphs show data from nondiabetic individuals (blue lines) and from individuals with diabetes (red lines), comparing postprandial insulin and glucagon secretion and hepatic glucose production, and comparing the sensitivities of muscle glucose use and adipocyte lipolysis to insulin.

rise dramatically after meals, and failure to restrain liver glucose release during fasting. The absolute mass of β cells may also be reduced in patients with type 2 diabetes, but studies are not definitive. Progressive reduction of functional β cell mass explains the natural history of type 2 diabetes in most patients who require steadily increasing therapy to maintain glucose control.

Type 2 diabetic patients sometimes have elevated levels of fasting insulin, a result of their higher fasting glucose level and insulin resistance. Another factor contributing to apparently high insulin levels early in the course of the disease is the presence of increased amounts of proinsulin. Proinsulin, the precursor to insulin, is inefficiently processed in the type 2 diabetic islet. Whereas healthy subjects have only 2% to 4% of total circulating insulin as proinsulin, patients with type 2 diabetes can have 10% to 20% of the measurable plasma insulin in this form. Proinsulin has a considerably attenuated effect for lowering blood glucose compared to insulin. Many diabetic patients have elevated fasting and prandial glucagon secretion (Sandoval and D'Alessio, 2015).

Insulin Resistance

Insulin sensitivity is measured as the amount of glucose cleared from the blood in response to a fixed dose, or plasma concentration, of insulin. The failure of normal amounts of insulin to elicit the expected response is referred to as *insulin resistance.* There is considerable variability of insulin sensitivity among cells, tissues, and individuals, with insulin sensitivity being affected by many factors, including age, body weight, physical activity levels, illness, and medications. Persons with type 2 diabetes or glucose intolerance have reduced responses to insulin and can be distinguished from groups with normal glucose tolerance.

The major insulin-responsive tissues are skeletal muscle, adipose tissue, and liver. Insulin resistance in muscle and fat is generally marked by a decrease in transport of glucose from the circulation into these tissues. Insulin resistance in adipocytes also causes increased rates of lipolysis and release of fatty acids into the circulation, which can contribute to insulin resistance in other tissues, as well as hepatic steatosis and dyslipidemia. Hepatic insulin resistance generally refers to a blunted ability of insulin to suppress glucose production, but insulin-mediated glucose uptake into hepatocytes can also be impaired. The sensitivity of humans to the effects of insulin administration is inversely related to the amount of fat stored in the abdominal cavity; more visceral adiposity leads to more insulin resistance. Intracellular lipid or its by-products may have direct effects to impede insulin signaling. Enlarged collections of adipose tissue, visceral or otherwise, are often infiltrated with macrophages and can become sites of chronic inflammation. Adipocytokines, secreted from adipocytes and immune cells, including tumor necrosis factor-α, interleukin-6, resistin, and retinol-binding protein 4, can also cause systemic insulin resistance.

Sedentary persons are more insulin resistant than active ones, and physical training can improve insulin sensitivity. Physical activity can decrease the risk of developing diabetes and improve glycemic control in persons who have diabetes. Insulin resistance is more common in the elderly as insulin sensitivity decreases with age. At the cellular level, insulin resistance involves blunted steps in the cascade from the insulin receptor tyrosine kinase to translocation of GLUT4 transporters, but the molecular mechanisms are incompletely understood. While many different mutations have been identified in the insulin receptor, including those that affect insulin receptor number, ligand binding, receptor phosphorylation, and trafficking, it does not appear that mutations in specific components of the insulin signaling cascade are responsible for clinical insulin resistance in most individuals (Saltiel, 2021). Very rare mutations involving the insulin binding domains of the extracellular α-chain can cause very severe syndromes and sometimes are associated with lipodystrophy (Angelidi et al., 2021). Insulin resistance is a major risk factor for the development of type 2 diabetes.

Dysregulated Hepatic Glucose Metabolism

In type 2 diabetes, hepatic glucose output is excessive in the fasting state and inadequately suppressed after meals. Abnormal secretion of the islet hormones, insufficient insulin and excessive glucagon, likely accounts for a significant portion of dysregulated hepatic glucose metabolism in type 2 diabetes. Increased concentrations of glucagon, especially in conjunction with hepatic insulin resistance, can lead to excessive hepatic gluconeogenesis and glycogenolysis and abnormally high fasting glucose concentrations. The liver is resistant to insulin action in type 2 diabetes, and the capacity of insulin to suppress HGP and promote hepatic glucose uptake and glycogen synthesis after meals is reduced. Despite ineffective insulin effects on hepatic glucose metabolism, the lipogenic effects of insulin in the liver are relatively intact and may be accentuated by fasting hyperinsulinemia. This contributes to hepatic steatosis and further worsening of insulin resistance.

Pathogenesis of Other Forms of Diabetes

Mutations in key genes involved in glucose homeostasis cause monogenic diabetes, which is inherited in an autosomal dominant fashion (Hattersley and Patel, 2017). These fall in two broad categories: diabetes onset in the neonatal period (<12 months of age) and monogenic diabetes in children or adults. Some forms of neonatal diabetes are caused by mutations in SUR or its accompanying inward rectifying K^+ channel or by mutations in the insulin gene. Monogenic diabetes beyond the first year of life may appear clinically similar to type 1 or type 2 diabetes. In other instances, children, adolescents, and young adults may present with monogenic forms of diabetes known as MODY (maturity-onset diabetes of the young). Phenotypically, these individuals are often not obese or insulin resistant and may initially have only modest hyperglycemia. The most common causes are mutations in key islet-enriched hepatocyte nuclear transcription factors (HNF1A and HNF4A) or GK (see Table 51–2). Most individuals with MODY are treated similarly to those with type 2 diabetes, but patients with MODY3, the most common of the monogenic forms of diabetes, can be effectively treated with sulfonylureas.

Chronic diseases of the pancreas, such as pancreatitis or cystic fibrosis, impair insulin secretion, and endocrinopathies such as acromegaly and Cushing disease cause insulin resistance (see Table 51–2) and are secondary causes of diabetes. In addition, a variety of commonly used medications, such as glucocorticoids, atypical antipsychotics, *calcineurin* and mTOR inhibitors after organ transplantation, or protease inhibitors, can raise the blood glucose or lead to diabetes over time (see Table 51–2) (Fève, 2022).

Diabetes-Related Complications

Untreated diabetes can lead to severe metabolic disturbances that can be acutely life threatening, such as diabetic ketoacidosis and a hyperglycemic hyperosmolar state. These require hospitalization for insulin administration, rehydration with intravenous fluids, and careful monitoring of electrolytes and metabolic parameters. Chronic end-organ effects of diabetes are commonly divided into microvascular and macrovascular complications. The microvascular complications are specific to people with diabetes and include retinopathy, nephropathy, and neuropathy. Macrovascular complications related to atherosclerosis, such as myocardial infarction and stroke, occur more frequently in individuals with diabetes but are not specific to diabetes like the microvascular complications. In the U.S., diabetes is the leading etiology of blindness in adults, the major reason for renal failure requiring dialysis or renal transplantation, and the most common cause of nontraumatic lower extremity amputations. The results of clinical trials indicate that microvascular complications can be prevented, delayed, or reduced by effective glucose lowering and macrovascular complications with use of specific diabetes medications.

Hyperglycemia duration and severity of hyperglycemia are major determinants of diabetes-related complications with the precise molecular mechanisms appearing to involve epigenetic changes from hyperglycemia, oxidative stress, dyslipidemia, advanced glycation end products, disordered sphingolipid metabolism (neuropathy), and increased growth factors such as VEGF-A in retinopathy (Jampol et al., 2020; Kato and Natarajan, 2019; Ruiz et al., 2019). The pathogenic processes may be different in each affected organ and influenced by genetic susceptibility.

Therapy of Diabetes

Goals of Therapy

The goals of therapy for diabetes are to alleviate the symptoms related to hyperglycemia (fatigue, polyuria, weight loss), to prevent or reduce the acute metabolic decompensation and chronic end-organ complications, and to allow the individual with diabetes to conduct the normal activities of life (e.g., exercise).

Glycemic control is assessed using both short-term (blood glucose self-monitoring; continuous glucose monitoring) and long-term (A1c, fructosamine) metrics. A1c reflects glycemic control over the prior 3 months, whereas measures of glycosylated serum proteins or albumin (fructosamine) reflect glycemic control over the preceding 2 weeks.

Continuous glucose monitoring (CGM) is a rapidly evolving technology that allows near real-time tracking of interstitial glucose as a reflection of blood glucose levels. Current CGM technology uses a sensor or electrode and detects a reaction between interstitial glucose and a glucose oxidase. It is being used very frequently in the management of type 1 diabetes and in some individuals with type 2 diabetes (Boughton and Hovorka, 2021). CGM provides glycemic data sets that translate into glycemic metrics such as the ambulatory glucose profile and time in a defined glycemic range (target range and above or below target range). The calculated glucose management indicator based on the mean glucose correlates with the A1c. Using capillary blood glucose measurements or CGM, patients can measure their glucose throughout their usual fasting and feeding periods and report these values to the diabetes management team and integrate with diet and exercise.

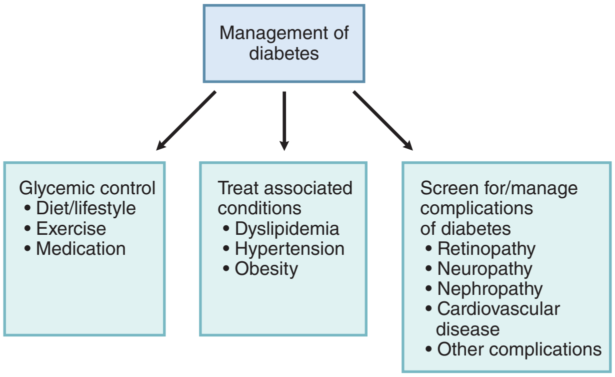

Figure 51–6 *Components of comprehensive diabetes care.*

The term *comprehensive diabetes care* describes optimal therapy for individuals with diabetes that is patient-centered and individualized and includes glucose management, treatment of abnormalities in blood pressure and lipids, and detection and management of diabetes-related complications (Figure 51–6). Table 51–3 shows the ADA-recommended treatment goals for comprehensive diabetes care for adults for glucose, blood pressure, and lipids (see Chapters 32 and 37). The treatment goals should be individualized to the patient and take into account factors such as risk of hypoglycemia, life expectancy, age, other medical conditions, duration of diabetes, and advanced macrovascular/microvascular complications of diabetes (see Table 51–3). Also, the patient's attitude toward diabetes, expectations, resources, and support systems should be considered.

Nonpharmacological Aspects of Diabetes Therapy

Patients with diabetes should be given education on glucose lowering through diet, exercise, and medications (ADA, 2022c). In type 1 diabetes, matching caloric intake and insulin dosing is critical for glucose control. In type 2 diabetes, lifestyle measures are directed at weight loss and reduction of blood pressure and atherosclerotic risk. There is now compelling evidence that metabolic surgery or marked caloric restriction can prevent or even reverse type 2 diabetes, with clinical trials of metabolic surgery showing greater efficacy than medical management (Douros et al., 2019).

Insulin Therapy

Insulin is the mainstay for treatment of virtually all patients with type 1 and many with type 2 diabetes (Hirsch et al., 2020). Although there are specific preparations of insulin that may be administered intramuscularly, intravenously, or nasally, long-term treatment relies predominantly on subcutaneous injection. Subcutaneous administration of insulin delivered into the peripheral circulation can lead to near-normal glycemia but differs from physiological secretion of insulin in two major ways:

- The absorption kinetics do not reproduce the rapid rise and decline of endogenous insulin in response to changes in blood glucose.
- Injected insulin is delivered into the peripheral circulation instead of being released into the portal circulation. Thus, the portal/peripheral insulin concentration is not physiological, and this may alter the influence of insulin on hepatic metabolism.

TABLE 51–3 ■ GOALS OF THERAPY FOR DIABETES IN NONPREGNANT ADULTS

INDEX	GOAL[a]
Glycemic control[b]	
A1c	<7.0%
Preprandial capillary blood glucose	4.4–7.2 mmol/L (80–130 mg/dL)
Peak postprandial capillary blood glucose	<10.0 mmol/L (<180 mg/dL)[c]
Time in range (as defined by CGM) 3.9–10.0 mmol/L (70–180 mg/dL)[d]	>70%
Blood pressure	<140/90[d]
Intensity of statin therapy for lipids (in addition to lifestyle therapy)[e]	
Age <40 years	
-No ASCVD risk factors	No statin
-ASCVD risk factors	Consider moderate dose
-ASCVD	High dose
Age 40–75 years	
-No ASCVD risk factors	Moderate dose
-ASCVD risk factors	High dose
-ASCVD	High dose
Age >75 years	
-No ASCVD risk factors	Consider moderate dose
-ASCVD risk factors	Moderate or high dose
-ASCVD	High dose

ASCVD, arteriosclerotic cardiovascular disease; CGM, continuous glucose monitoring.

[a] Goals should be individualized for each patient and may be different for certain patient populations (lower or higher). According to the ADA, "Goals should be individualized based on duration of diabetes, age/life expectancy, comorbid conditions, known CVD or advanced microvascular complications, hypoglycemia unawareness, and individual patient considerations."

[b] Achieving A1c value is an important goal, but a higher A1c value may be appropriate for older individuals or those with complex illnesses or moderate to severe cognitive impairment.

[c] At 1–2 h after beginning of a meal.

[d] Optimal from CGM assessment: <5% below 3.9 mmol/L (70 mg/dL) and <25% above 10.0 mmol/L (180 mg/dL).

[e] Lower blood pressure targets (<130/80 mmHg) may be appropriate for certain individuals with diabetes.

[e] For individuals with diabetes, very high risk for ASCVD, and LDL >70 mg/dL (1.3 mmol/L) or who cannot tolerate high-dose statins, see Chapter 37 for alternative approaches.

Adapted from American Diabetes Association (2022b, 2022c, 2022e).

Insulin Preparation and Chemistry

Human *insulin*, produced by recombinant DNA technology, is soluble in aqueous solution (Hirsch et al., 2020). Doses and concentrations of clinically used *insulin* preparations are expressed in international units. One international unit of *insulin* is defined as the bioequivalent of 34.7 µg of crystalline *insulin*; this is equivalent to the older working definition of a U.S. Pharmacopeia unit as the amount required to reduce the blood glucose concentration to 45 mg/dL (2.5 mM) in 2.2-kg rabbit fasted for 24 h. Most preparations of *insulin* are supplied in solution or suspension at a concentration of 100 units/mL, which is about 3.6 mg *insulin* per milliliter (0.6 mM) and termed U-100. *Insulin* also is available in more concentrated preparations (200 [*degludec* and *lispro insulins*], 300 [*glargine insulin*], or 500 [*regular* insulin] units/mL) for patients who are resistant to the hormone and require higher doses.

Insulin Formulations

Preparations of *insulin* are classified according to their duration of action into *short acting* and *long acting* (Table 51–4). Within the short-acting category, some distinguish the *very rapid-acting insulins* (*aspart*, *glulisine*, *lispro*) from regular *insulin*. Likewise, some distinguish formulations with a *longer duration of action* (*degludec, detemir, glargine*) from *NPH insulin*. Two approaches are used to modify the absorption and pharmacokinetic profile of *insulin*. The first approach is based on formulations that slow the absorption following subcutaneous injection. The other approach is to alter the amino acid sequence or protein structure of human *insulin* so that it retains its ability to bind to the insulin receptor, but its behavior in solution or following injection is either accelerated or prolonged in comparison to native or regular *insulin* (Figure 51–7). There is wide variability in the kinetics of *insulin* action among individuals and even with repeated doses in the same individual. The time to peak hypoglycemic effect and insulin levels can vary by 50%, due in part by large variations in the rate of subcutaneous absorption.

Short-Acting Regular Insulin. Native or regular *insulin* molecules associate as hexamers in aqueous solution at a neutral pH, and this aggregation slows absorption following subcutaneous injection. Regular *insulin* should be injected 30 to 45 min before a meal. Regular, unbuffered, *100-units/mL insulin* also may be given intravenously or intramuscularly. However, unbuffered, regular *insulin* (*500 units/mL*) is for subcutaneous injection only and should not be given by intravenous or intramuscular injection.

Short-Acting Insulin Analogues. The short-acting *insulin* analogues are absorbed more rapidly from subcutaneous sites than regular *insulin* (see Figures 51–7 and 51–8; see Table 51–4) (Hirsch et al., 2020). *Insulin* analogues should be injected 15 min or less before a meal. When used to treat glycemia at meals, the more rapid acting analogues have lower rates of hypoglycemia and modestly improved A1c levels compared to regular *insulin*.

Insulin lispro is identical to human insulin except at positions B28 and B29. Unlike regular *insulin*, *lispro* dissociates into monomers almost instantaneously following subcutaneous injection. This property results in more rapid absorption and shorter duration of action compared with regular *insulin*.

Insulin aspart is formed by the replacement of proline at B28 with aspartic acid, reducing self-association. Like *lispro*, *insulin aspart* dissociates rapidly into monomers following injection.

Insulin glulisine is formed when glutamic acid replaces lysine at B29 and lysine replaces asparagine at B3; these substitutions also result in a reduction in self-association and rapid dissociation into active monomers.

Long-Acting Insulins. *NPH insulin* (*insulin isophane*) is a suspension of native *insulin* complexed with zinc and protamine in a phosphate buffer. This produces a cloudy or whitish solution in contrast to the clear appearance of other *insulin* solutions. *NPH insulin* dissolves more gradually when injected subcutaneously; thus, its duration of action is prolonged. *NPH insulin* is usually given either once a day (at bedtime) or twice a day in combination with short-acting *insulin*.

Insulin glargine is a long-acting analogue of human insulin. Two arginine residues are added to the C terminus of the B chain, and an asparagine molecule in position 21 on the A chain is replaced with glycine. *Insulin glargine* is a clear solution with a pH of 4.0, which stabilizes the *insulin* hexamer. When injected into the neutral pH of the subcutaneous space, aggregation occurs, resulting in prolonged, predictable absorption from the injection site. Owing to *insulin glargine*'s acidic pH, it cannot be mixed with short-acting *insulin* preparations that are formulated at a neutral pH. *Glargine* has a minimal peak absorption profile and provides more predictable 24-h insulin coverage than *NPH insulin* when injected once daily. Clinical trial data suggest that *glargine* has a lower risk of hypoglycemia, particularly overnight, compared to *NPH insulin*. *Glargine* may be administered at any time during the day with equivalent efficacy and does not accumulate after several injections. Most commonly, *glargine* formulation of 100 units/mL is used. It is also available in a formulation of 300 units/mL. *Glargine* biosimilar formulations are now available.

Insulin detemir is an *insulin* analogue modified by the addition of a saturated fatty acid to the ε amino group of Lys^{B29}, yielding a myristoylated *insulin*. When *insulin detemir* is injected subcutaneously, it binds to albumin via its fatty acid chain. In patients with type 1 diabetes, *insulin*

TABLE 51–4 ■ TIME-ACTION PROFILES OF INSULIN PREPARATIONS[a]

		TIMES		
TYPE	PREPARATION	ONSET (h)	PEAK (h)	EFFECTIVE DURATION (h)
Short acting				
	Aspart[b] Glulisine Lispro[c]	<0.25	0.5–1.5	3–5
	Regular	0.5–1.0	2–3	4–8
Long acting				
	Detemir	1–4	0[d]	12–24
	Glargine	2–4	0[d]	20–24
	Degludec	1–9	0[d]	42
	NPH	3–4	6–10	10–16
Inhaled insulin		<0.5	1–2	3
Insulin combinations				
	Mixture: short acting (25%–50%) and long acting (50%–76%)	<0.25–1.0	1.5[e]	Up to 10–16

[a] Insulin preparations available in the U.S.; see text for additional information about preparations.
[b] Aspart formulation with niacinamide (vitamin B$_3$) has more rapid onset of action.
[c] Lispro-aabc formulation has more rapid onset of action.
[d] Glargine, degludec, and detemir have little peak activity at steady state.
[e] Some mixtures will have dual peaks, one at 2–3 h and the second one several hours later.

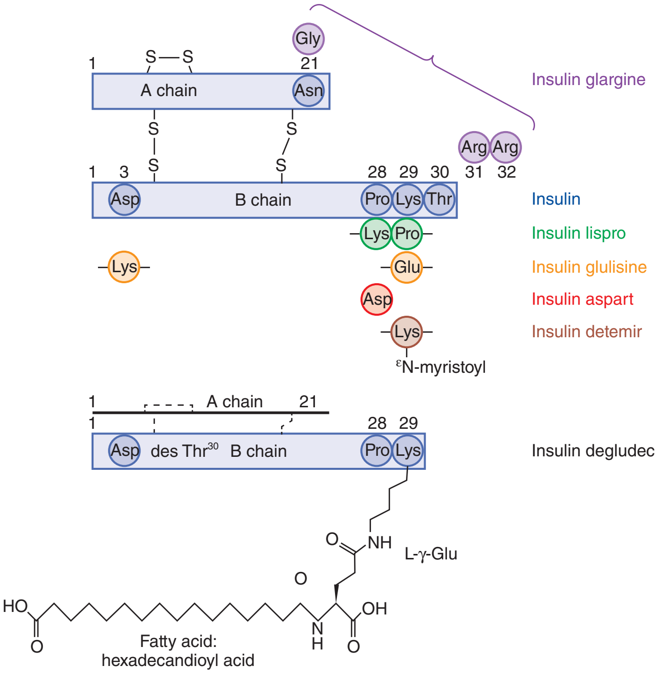

Figure 51–7 *Insulin analogues.* Modifications of native insulin can alter its pharmacokinetic profile. Reversing amino acids 28 and 29 in the B chain (*lispro*) or substituting Asp for Pro^{28B} (*aspart*) gives analogues with reduced tendencies for molecular self-association that are faster acting. Altering Asp^{3B} to Lys and Lys^{29B} to Glu produces an insulin (*glulisine*) with a more rapid onset and a shorter duration of action. Substituting Gly for Asn^{21A} and lengthening the B chain by adding Arg^{31} and Arg^{32} produces a derivative (*glargine*) with reduced solubility at pH 7.4 that is, consequently, absorbed more slowly and acts over a longer period of time. Deleting Thr^{30B} and adding a myristoyl group to the ε-amino group of Lys^{29B} (*detemir*) enhances reversible binding to albumin, thereby slowing transport across vascular endothelium to tissues and providing prolonged action. *Insulin degludec* is Lys^{29B} (Nε-hexadecandioyl-γ-Glu) des^{B30} human insulin. When *degludec* is injected subcutaneously, it forms multihexameric complexes that slow absorption; *degludec* also binds well to albumin; these two characteristics contribute to the prolonged effect of degludec (>24 h at steady state).

detemir, administered twice a day, has a smoother time-action profile and produces a reduced prevalence of hypoglycemia than *NPH insulin*. The absorption profiles of *glargine* and *detemir insulin* are similar, but *detemir* often requires twice-daily administration.

Insulin degludec is a modified *insulin* with one amino acid deleted (threonine at position B30) and is conjugated to hexadecanedioic acid via γ-L-glutamyl spacer at the amino acid lysine at position B29. *Degludec*, which is active at a physiological pH, forms multihexamers after injection subcutaneously. It is associated with less severe hypoglycemia than *glargine*.

Other Insulin Formulations. Stable combinations of short-acting and long-acting insulins provide convenience by reducing the number of daily injections.

Inhaled insulin is formulated for inhalation using a manufacturer-specific device (Heinemann and Parkin, 2018). This formulation should be used in combination with a long-acting *insulin* and has a more rapid onset and shorter duration than injected *insulin* analogues. It is not widely used. Adverse events include cough and throat irritation. It should not be used in individuals who smoke.

Insulin Delivery

Most *insulin* is injected subcutaneously. Pen devices containing prefilled *insulin* are popular. "Smart" pens record and report *insulin* injection and connect to CGM devices. Intravenous infusions of *insulin* are useful in patients with ketoacidosis or when requirements for *insulin* may change rapidly, such as during the perioperative period, during labor and delivery, and in intensive care situations. Long-acting *insulin* should not be given intravenously or intramuscularly or in an infusion device.

Continuous Subcutaneous Insulin Infusion. Short-acting insulins are the only form of the hormone used in subcutaneous *insulin* infusion devices. Several pumps are available for continuous subcutaneous *insulin* infusion (CSII) therapy; this technology is rapidly evolving with improvements in hardware and software (Boughton and Hovorka, 2021). *Insulin* infusion devices provide a constant basal infusion of *insulin* and have the option of different infusion rates during the day and night to help avoid the rise in blood glucose that occurs just prior to awakening from sleep (the dawn phenomenon) and bolus injections that are programmed according to the size and nature of a meal. *Insulin* infusion devices allow one to deliver a more physiological profile of *insulin* replacement during exercise (where insulin production is decreased) and thus less hypoglycemia than traditional subcutaneous *insulin* injections provide. The integration of an *insulin* infusion device and CGM (open and closed loop systems) is rapidly evolving with algorithms and communication that alter the infusion rate for *insulin* delivery based on CGM data.

Factors That Affect Insulin Absorption

Factors that determine the rate of absorption of insulin after subcutaneous administration include the site of injection, the type of insulin, subcutaneous blood flow, smoking, regional muscular activity at the site of the injection, the volume and concentration of the injected insulin, and depth of injection (*insulin* has a more rapid onset of action if delivered intramuscularly rather than subcutaneously). Increased subcutaneous blood flow (brought about by massage, hot baths, or exercise) increases the rate of absorption. The abdomen currently is the preferred site of injection in the morning because *insulin* is absorbed 20% to 30% faster from that site than from the arm. Rotation of *insulin* injection sites is recommended to avoid or limit subcutaneous scarring, lipohypertrophy, or lipoatrophy.

Insulin Dosing and Regimens

Several commonly used dosage regimens that include mixtures of *insulin* given in two or more daily injections are depicted in Figure 51–8. For most patients, *insulin*-replacement therapy includes long-acting *insulin* (basal) and a short-acting *insulin* to provide postprandial needs. In a mixed population of patients with type 1 diabetes, the dose of *insulin* is usually 0.4 to 0.7 units/kg body weight per day. Obese patients, those with type 2 diabetes, and pubertal adolescents may require more (about 1–2 units/kg per day) because of the accompanying *insulin* resistance. The basal dose is usually 40% to 50% of the total daily dose, with the remainder as prandial or premeal *insulin*. The *insulin* dose at mealtime should reflect the anticipated carbohydrate intake. A correction dose of short-acting *insulin* is added to the prandial *insulin* dose to allow correction of the blood glucose. *Administration of a single daily dose of long-acting insulin is not sufficient to achieve optimal glycemic control. More complex regimens that include multiple injections of long-acting or short-acting insulin are needed to reach this goal. In all patients, careful monitoring of therapeutic end points determines the insulin dose. This approach is facilitated by self-monitoring of glucose (capillary or CGM), measurements of A1c, and individualization of the patient's therapeutic regimen (see Tables 51–3 and 51–4).* In patients who have gastroparesis or loss of appetite, injection of a short-acting analogue postprandially, based on the amount of food actually consumed, may improve glycemic control.

Adverse Events

Hypoglycemia is the major risk that must be weighed against benefits of efforts to normalize glucose control. *Insulin* treatment of both type 1 and type 2 diabetes is associated with modest weight gain. Although uncommon, allergic reactions to recombinant human *insulin* may occur as a result of reaction to the small amounts of aggregated or denatured *insulin* in preparations, to minor contaminants, or because of sensitivity to a component added to *insulin* in its formulation (protamine, Zn^{2+}, etc.). Atrophy of subcutaneous fat at the site of *insulin* injection (lipoatrophy) was a rare side effect of older *insulin* preparations. Lipohypertrophy (enlargement of subcutaneous fat depots) has been ascribed to the lipogenic action of high local concentrations of *insulin*.

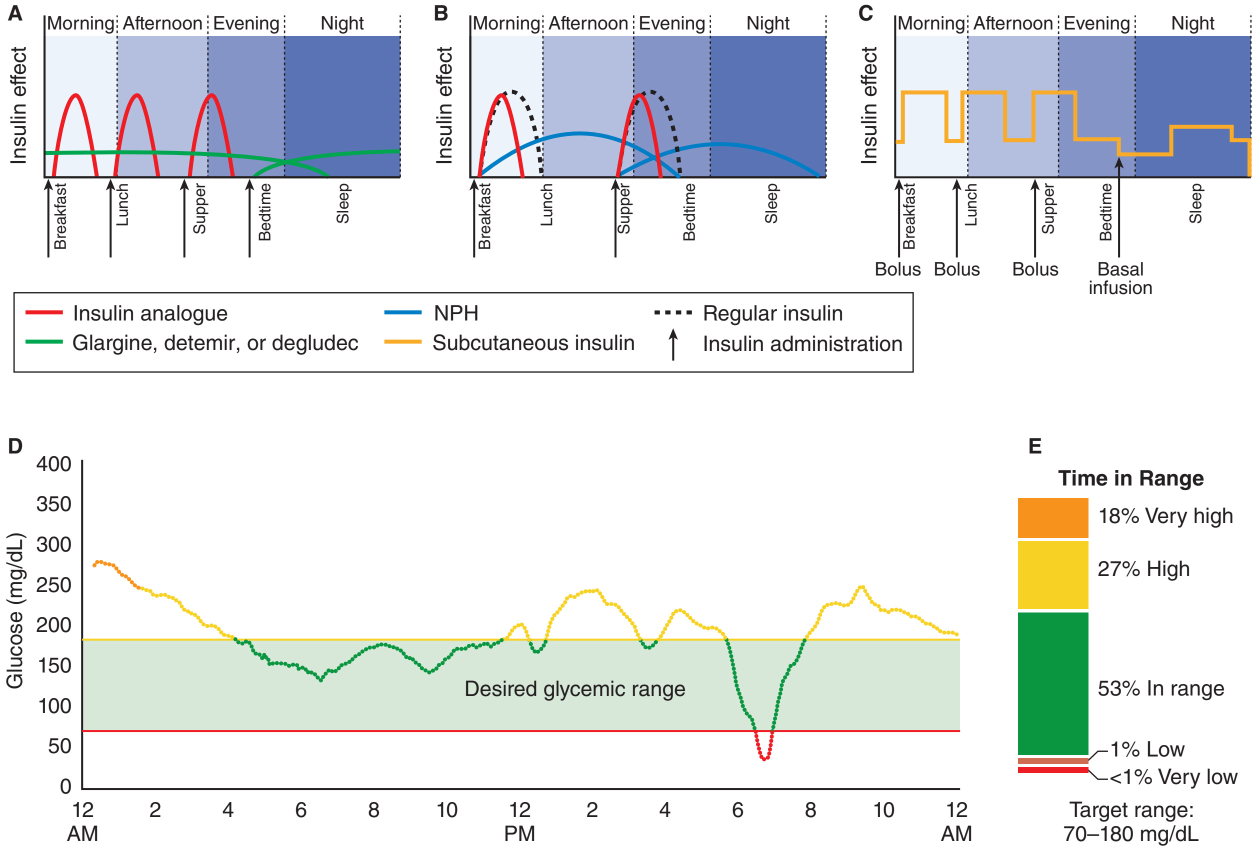

Figure 51–8 *Commonly used insulin regimens.* Panel **A** shows administration of a long-acting *insulin* like *glargine* (*detemir* or *degludec* could also be used; *detemir* may require twice-daily administration; *degludec* is used once daily; see text for details) to provide basal *insulin* and a premeal short-acting *insulin* analogue (see Table 51–4). Panel **B** shows a less-intensive *insulin* regimen with twice-daily injection of *NPH insulin* providing basal *insulin* and regular *insulin* or an *insulin* analogue providing mealtime *insulin* coverage. Only one type of short-acting *insulin* would be used. Panel **C** shows the insulin level attained following subcutaneous *insulin* (short-acting *insulin* analogue) by an *insulin* pump programmed to deliver different basal rates. At each meal, an *insulin* bolus is delivered. (Adapted from Kaufman FR, ed. *Medical Management of Type 1 Diabetes*, 6th ed. American Diabetes Association, Alexandria, VA, **2012**.) Panel **D** shows a daily glucose profile in an individuals with type 1 diabetes with the desired glycemic range shown in light green. The color of the glucose line corresponds to the glucose value category shown in Panel E. Panel E shows the time in range of glucose values over one month in an individual with type 1 diabetes.

Insulin Treatment of Ketoacidosis and Other Special Situations

Intravenous administration of *insulin* may be most appropriate in patients with ketoacidosis or severe hyperglycemia with a hyperosmolar state (Umpierrez and Korytkowski, 2016). Subcutaneous *insulin* regimens are also effective. *Insulin* inhibits lipolysis and gluconeogenesis completely and produces near-maximal stimulation of glucose uptake. In most patients with diabetic ketoacidosis, blood glucose concentrations will fall by about 10% per hour; the acidosis is corrected more slowly. As treatment proceeds, it often is necessary to administer glucose along with the *insulin* not only to prevent hypoglycemia but also to allow clearance of all ketones. Patients with a nonketotic hyperglycemic hyperosmolar state may be more sensitive to *insulin* than are those with ketoacidosis. Appropriate replacement of fluid and electrolytes, particularly K^+, is an integral part of the therapy because of the major K^+ deficit. A long-acting *insulin* should be administered subcutaneously before the *insulin* infusion is discontinued.

Treatment of Diabetes in Children or Adolescents

Diabetes is one of the most common chronic diseases of childhood in the U.S., with the rate gradually increasing over the past decade. An unfortunate corollary of the growing rates of obesity over the past three decades is an increase in the numbers of children and adolescents with non-autoimmune, or type 2, diabetes. Current estimates are that 15% to 20% of new cases of pediatric diabetes may be type 2 diabetes; rates vary by ethnicity, with disproportionately high rates in Native Americans, African Americans, and Latinos. Current therapy for type 1 diabetes in children and adolescents is intensive, physiologically based *insulin* replacement using combinations of basal and prandial *insulin* replacement or CSII with a goal of near-normal glucose control while avoiding hypoglycemia (see Figure 51–8). The primary limiting factor of aggressive *insulin* therapy is hypoglycemia. In children and adolescents with type 1 diabetes, the recommended A1c goal is less than 7%, with a slightly higher goal (7.5%) in individuals who cannot recognize hypoglycemia or have hypoglycemia unawareness. *Insulin* infusion devices and CGM are being used with increasing frequency in the pediatric diabetic population and in older children and adolescents.

Because of the association of type 2 diabetes with obesity in the pediatric age group, lifestyle management is the recommended first step in therapy. Goals of reducing body weight and increasing physical activity are broadly recommended. The only medication currently approved by the FDA specifically for medical treatment of type 2 diabetes is *metformin*. *Metformin* is approved for children as young as 10 years of age and is available in a liquid formulation (100 mg/mL). *Insulin* is the typical second line of therapy after *metformin*; basal *insulin* can be added to oral agent therapy or multiple daily injections can be used when simpler regimens are not successful. The response to treatment is different in adolescents with type 2 diabetes, suggesting a different underlying pathophysiology. Weight gain is a more significant problem than hypoglycemia with *insulin* treatment in pediatric type 2 diabetes.

Management of Diabetes in Hospitalized Patients

Hyperglycemia is common in hospitalized patients and predisposes to worse outcomes and greater complications. The prevalence of elevated blood glucose levels during hospitalization is not well established but may exceed 30% of inpatients at tertiary care medical centers, with one-third of cases occurring in people without a prior diagnosis of diabetes. Rates of hyperglycemia are particularly high in persons with critical illness cared for in intensive care units where blood glucose levels correlate with severity of illness. Depending on the patient population, a significant number of patients with a first detection of hyperglycemia during hospitalization will have persistent hyperglycemia after recovery and meet diagnostic criteria for diabetes. Stress of illness has been associated with insulin resistance, often attributed to counterregulatory hormone secretion, cytokines, and other inflammatory mediators, although the role of specific mediators has not been proven. Medications used in the hospital, such as glucocorticoids or dextrose-containing intravenous solutions, can exacerbate tendencies toward hyperglycemia. Finally, fluid balance and tissue perfusion can affect the absorbance of subcutaneous *insulin* and the clearance of glucose. Therapy of hyperglycemia in hospitalized patients should be adjusted for these variables.

Insulin is the cornerstone of treatment of hyperglycemia in hospitalized patients. Oral agents have a limited place in treatment of hyperglycemic patients in the hospital because of slow onset of action, insufficient potency, variable absorption from the gut, and side effects. In noncritically ill hospitalized patients, a basal plus bolus correction *insulin* regimen, adjusted for oral intake, is optimal. There is evidence from randomized trials that using only short-acting *insulin* in response to hyperglycemia (i.e., sliding-scale regimens) is less effective than scheduled *insulin* regimens. For the critically ill and those with variable blood pressure, edema, and diminished tissue perfusion, intravenous *insulin* is the treatment of choice. Intravenous administration of *insulin* also is well suited to the treatment of diabetic patients during the perioperative period and during childbirth. Although optimal blood glucose targets in hospitalized patients are incompletely defined, in general, levels of 140 to 180 m/dL (7.8–10.0 mM) in most hospitalized patients, with more stringent goals, such as 110 to 140 mg/dL (6.1–7.8 mmol/L), in some critically ill patients, are recommended.

Insulin Secretagogues and Glucose-Lowering Agents

A variety of *sulfonylureas, meglitinides, GLP-1 agonists, and inhibitors of dipeptidyl peptidase 4* (DPP-4) are used as secretagogues to stimulate insulin release (Table 51–5).

K_{ATP} Channel Modulators: Sulfonylureas

Sulfonylureas in current use includes *glyburide* (*glibenclamide*), *glipizide*, and *glimepiride* (Khunti et al., 2018). Some are available in an extended-release (*glipizide*) or a micronized (*glyburide*) formulation. *Glyburide* is generally not recommended because of increased risk of hypoglycemia and putative adverse effects during cardiac ischemia.

Mechanism of Action. Sulfonylureas stimulate insulin release by binding to a specific site on the β cell K_{ATP} channel complex (SUR) and inhibiting its activity. K_{ATP} channel inhibition causes cell membrane depolarization and the cascade of events leading to insulin secretion (see Figure 51–3). The acute administration of sulfonylureas to patients with type 2 diabetes increases insulin release from the pancreas and shifts the insulin-glucose dose-response relationship to the left.

ADME. Sulfonylureas are effectively absorbed from the GI tract, but food and hyperglycemia can reduce absorption. Sulfonylureas in plasma are largely (90%–99%) bound to protein, especially albumin. The volumes of distribution of most of the sulfonylureas are about 0.2 L/kg. Although their half-lives are short (3–5 h), their hypoglycemic effects are evident for 12 to 24 h, and they often can be administered once daily. The liver metabolizes all sulfonylureas, and the metabolites are excreted in the urine. Thus, sulfonylureas should be administered with caution to patients with either renal or hepatic insufficiency.

Therapeutic Uses. Sulfonylureas are used to treat hyperglycemia in type 2 diabetes. Of properly selected patients, 50% to 80% respond to this class of agents. All members of the class appear to be equally efficacious. A significant number of patients who respond initially later cease to respond to the sulfonylurea and develop progressive hyperglycemia (so-called secondary failure). This may occur as a result of a change in drug metabolism but is more likely the result of progressive β cell failure. Some individuals with neonatal diabetes or MODY-3 respond to these agents with long-lasting therapeutic efficacy. Contraindications to the use of these drugs include type 1 diabetes, pregnancy, lactation, and significant hepatic or renal insufficiency.

Adverse Effects; Drug Interactions. Sulfonylureas may cause hypoglycemic reactions, including coma. Weight gain of 1 to 3 kg is a common side effect of improving glycemic control with sulfonylurea treatment.

TABLE 51–5 ■ PROPERTIES OF INSULIN SECRETAGOGUES

CLASS AND GENERIC NAME	DOSAGE[a] (mg)	DURATION OF ACTION (HOURS OR DOSING FREQUENCY)
Sulfonylureas[b]		
Glimepiride	1–8	24
Glipizide	5–40	12–18
Glipizide (extended release)	5–20	24
Glyburide[c]	1.25–20	12–24
Glyburide (micronized)[c]	0.75–12	12–24
Nonsulfonylureas (Meglitinides)[c]		
Nateglinide	180–360	2–4
Repaglinide	0.5–16	2–6
GLP-1 Agonists		
Dulaglutide	0.75–4.5	Weekly
Exenatide	0.005–0.010	Daily
Exenatide, extended release	2	Weekly
Liraglutide[d]	1.2–1.8	Daily
Lixisenatide	0.010–0.020	Daily
Semaglutide[d]	0.5–1.0	Weekly
Semaglutide, oral	7–14	Daily
Dipeptidyl Peptidase-4 Inhibitors		
Alogliptin	25	Daily
Linagliptin	5	Daily
Saxagliptin	2.5–5	Daily
Sitagliptin	25–100	12–16
Vildagliptin	50–100	Twice daily

[a] Dose should be lower in some patients.

[b] Glyburide no longer recommended because of hypoglycemia profile.

[c] Labeled for administration 3–4 times daily.

[d] Liraglutide 3.0 mg and semaglutide 2.4 mg are approved for weight loss independent of diabetes status.

Less frequent side effects include nausea and vomiting, cholestatic jaundice, agranulocytosis, aplastic and hemolytic anemias, generalized hypersensitivity reactions, and dermatological reactions. Rarely, patients treated with these drugs develop an alcohol-induced flush similar to that caused by *disulfiram* or hyponatremia. Although there has been longstanding controversy over the cardiovascular safety of sulfonylureas, recent comparative clinical trials indicate that this class of drugs has no more risk of cardiovascular events than other commonly used glucose-lowering agents (Rosenstock et al., 2019; Vaccaro et al., 2017).

The hypoglycemic effect of sulfonylureas may be enhanced by various mechanisms (decreased hepatic metabolism or renal excretion, displacement from protein-binding sites). Some drugs (sulfonamides, *clofibrate*, and salicylates) displace the sulfonylureas from binding proteins, thereby transiently increasing the concentration of free drug. Ethanol may enhance the action of sulfonylureas and cause hypoglycemia. Hypoglycemia may be more frequent in patients taking a sulfonylurea in combination with one or more of the following agents: androgens, anticoagulants, azole antifungals, *fenfluramine*, *gemfibrozil*, H_2 antagonists, magnesium salts, *methyldopa*, *probenecid*, sulfonamides, tricyclic antidepressants, and urinary acidifiers. Other drugs may decrease the glucose-lowering effect of sulfonylureas by increasing hepatic metabolism, increasing renal excretion, or inhibiting insulin secretion (β blockers, Ca^{2+} channel blockers, *cholestyramine*, *diazoxide*, estrogens, hydantoins, *isoniazid*, *nicotinic acid*, phenothiazines, *rifampin*, sympathomimetics, thiazide diuretics, and urinary alkalinizers).

Dosage Forms Available. Treatment is initiated at the lower end of the dose range and titrated upward based on the patient's glycemic response. Some have a longer duration of action and can be prescribed in a single daily dose (*glimepiride*), whereas others are formulated as extended-release or micronized formulations to extend their duration of action (see Table 51–5). Sulfonylureas such as *glipizide* or *glimepiride* appear safer than longer-acting sulfonylureas in elderly individuals with type 2 diabetes, but even the short-duration agents should be used with caution in elderly patients.

K_{ATP} Channel Modulators: Nonsulfonylureas

Repaglinide. *Repaglinide* is an oral insulin secretagogue that also stimulates insulin release by closing K_{ATP} channels in pancreatic β cells, although not by direct binding to the SUR (see Table 51–5) (Chen et al., 2015). The drug is absorbed rapidly from the GI tract, and peak blood levels are obtained within 1 h with a $t_{1/2}$ of approximately 1 h. These features allow for a rapid on-off effect suitable for selected preprandial use. *Repaglinide* is metabolized primarily by the liver (CYP3A4) to inactive derivatives. Because a small proportion (~10%) is metabolized by the kidney, dosing of the drug in patients with renal insufficiency also should be performed cautiously.

The major side effect of *repaglinide* is hypoglycemia. *Repaglinide* also is associated with a decline in efficacy (secondary failure) after initially improving glycemic control. Certain drugs may potentiate the action of *repaglinide* by displacing it from plasma protein-binding sites (β blockers, *chloramphenicol*, *warfarin*, monoamine oxidase inhibitors, nonsteroidal anti-inflammatory drugs, *probenecid*, salicylates, and *sulfonamide*) or altering its metabolism (*gemfibrozil*, *itraconazole*, *trimethoprim*, *cyclosporine*, *simvastatin*, *clarithromycin*).

Nateglinide. *Nateglinide* is an orally effective insulin secretagogue. *Nateglinide* also stimulates insulin secretion by blocking K_{ATP} channels in pancreatic β cells. *Nateglinide* promotes a more rapid but less-sustained secretion of insulin than other available oral antidiabetic agents. The drug's major therapeutic effect is reducing postprandial glycemic elevations in patients with type 2 diabetes.

Nateglinide is most effective when administered at a dose of 120 mg, three times daily, 1 to 10 min before a meal. It is metabolized primarily by hepatic CYPs (2C9, 70%; 3A4, 30%) and should be used cautiously in patients with hepatic insufficiency. About 15% of an administered dose is excreted by the kidney as unchanged drug. Some drugs reduce the glucose-lowering effect of *nateglinide* (corticosteroids, rifamycins, sympathomimetics, thiazide diuretics, thyroid products); others (alcohol, nonsteroidal anti-inflammatory drugs, salicylates, monoamine oxidase inhibitors, and nonselective β blockers) may increase the risk of hypoglycemia with *nateglinide*. *Nateglinide* therapy may produce fewer episodes of hypoglycemia than other currently available oral insulin secretagogues, including *repaglinide*. As with sulfonylureas and *repaglinide*, secondary failure occurs.

Biguanides

Metformin is the only member of the biguanide class of oral hypoglycemic drugs available for use today. Previously available biguanides, *phenformin* and *buformin*, were removed from the market in the 1970s due to unacceptable rates of associated lactic acidosis.

Mechanism of Action. Several mechanisms have been proposed to explain the central pharmacological action of *metformin*, reduction of HGP primarily by limiting gluconeogenesis (LaMoia and Shulman, 2020). *Metformin* has specific actions on mitochondrial respiration that reduce intracellular ATP and increase AMP. Experimental evidence supports activation of AMP-dependent protein kinase by *metformin*, leading to stimulation of hepatic fatty acid oxidation, glucose uptake, and nonoxidative glucose metabolism and reduction of lipogenesis, and gluconeogenesis. *Metformin* also inhibits the mitochondrial glycerol phosphate dehydrogenase, thereby changing the redox state of the cell. Other evidence implicates other mechanisms, including blunting the effects of glucagon, inhibiting conversion of lactate and glycerol to glucose, and shifting the liver toward negative lipid balance. *Metformin* also appears to act in the gut.

Most of the pharmacological effects of *metformin* are mediated in the liver with minimal action on glucose metabolism or insulin sensitivity in skeletal muscle. *Metformin* has little effect on blood glucose in normoglycemic states, does not stimulate the release of insulin or other islet hormones, and rarely causes hypoglycemia. However, even in persons with only mild hyperglycemia, *metformin* lowers blood glucose by reducing HGP. There is little information to support a direct effect of *metformin* on hepatic insulin signaling, but there are at least complementary effects of the drug to improve the dose-response relationship between insulin and hepatic glucose production.

ADME. Based on the pharmacokinetics of the common immediate-release form of *metformin*, it is recommended for twice-daily administration at doses of 0.5 to 1.0 g. The maximum dose is 2550 mg, but therapeutic benefit starts to plateau at 2000 mg. A sustained-release preparation is available for once-daily dosing starting at 500 mg daily, with titration up to 2000 mg as necessary. Fixed-dose combinations of *metformin* with *glipizide*, *glyburide*, *pioglitazone*, *linagliptin*, *saxagliptin*, *sitagliptin*, *alogliptin*, *canagliflozin*, *dapagliflozin*, and *empagliflozin* are available.

Metformin is absorbed primarily from the small intestine with a bioavailability of 70% to 80%. Peak concentrations after an oral dose occur at about 2 h; the drug's plasma $t_{1/2}$ is 4 to 5 h. *Metformin* does not bind to plasma proteins and is excreted unchanged in the urine. The transport of *metformin* into hepatocytes is mediated primarily by organic cation transporter type 1; renal uptake is mediated by organic cation transporter type 2. Export into the urine is by MATE1/2 (multidrug and toxin extrusion proteins).

Therapeutic Uses. *Metformin* is generally accepted as the first-line treatment of type 2 diabetes and is the most commonly used oral agent for this condition. *Metformin* is effective as monotherapy and in combination with other glucose-lowering medications. *Metformin* has been shown to be safe and effective to treat gestational diabetes, although it has not yet been given FDA approval for this indication.

Metformin has superior or equivalent efficacy of glucose lowering compared to other oral agents used to treat diabetes and reduces microvascular complications in patients with type 2 diabetes; more limited data support a beneficial effect to reduce macrovascular disease as well. *Metformin* does not typically cause weight gain and, in some cases, causes mild weight reduction. In persons with IGT, treatment with *metformin* delays the progression to diabetes. *Metformin* has been used as a treatment of infertility in women with polycystic ovarian syndrome. Although not formally approved for this purpose, *metformin* demonstrably improves ovulation and menstrual cyclicity and reduces circulating androgens and hirsutism.

Adverse Effects; Drug Interactions. The most common side effects (10%–25%) of *metformin* are GI: nausea, indigestion, abdominal cramps or bloating, diarrhea, or some combination of these. *Metformin* has direct effects on GI function, including interference with the absorption of glucose and bile salts. Use of *metformin* is also associated with 20% to 30% lower blood levels of vitamin B_{12}, and these levels should be monitored. Most adverse GI effects of *metformin* abate over time with continued use and can be minimized by starting at a low dose and gradually titrating to a target dose over several weeks, as well as by having patients take the drug with meals. There is evidence suggesting that extended-release *metformin* has decreased GI side effects and can be substituted for immediate-release *metformin* in patients who are having difficulty tolerating the drug.

Because the previously available biguanides *phenformin* and *buformin* caused lactic acidosis, *metformin* has been carefully scrutinized for this side effect. Lactic acidosis associated with *metformin* has been rarely reported in patients with concurrent conditions that can cause poor tissue perfusion (e.g., sepsis, myocardial infarction, and congestive heart failure). However, recent analyses have raised doubts regarding whether the association of *metformin* with lactic acidosis is causal. Renal failure is a common comorbidity in patients with lactic acidosis associated with *metformin* use, and plasma *metformin* levels are inversely related to glomerular filtration rate (GFR) due to reduced clearance of drug from the circulation (e.g., levels rise above the usual therapeutic range when creatinine clearance drops below 40–50 mL/min). However, in recent studies of patients with severe renal failure, including some requiring dialysis, rates of lactic acidosis were not increased in those taking *metformin*. Current guidelines suggest that *metformin* may be used safely when the GFR is greater than 30 mL/min/1.73 m^2.

It is important to assess renal function before starting *metformin* and to monitor function at least annually. *Metformin* should be discontinued preemptively if renal function could decline precipitously, such as before radiographic procedures that use contrast dyes and during admission to the hospital for severe illness. Metformin should not be used in patients with severe pulmonary disease, decompensated heart failure, severe liver disease, or chronic alcohol abuse. Cationic drugs that are eliminated by renal tubular secretion have the potential for interaction with *metformin* by competing for common renal tubular transport systems. Adjustment of *metformin* is recommended in patients who are taking cationic medications such as *cimetidine*, *furosemide*, and *nifedipine*.

Thiazolidinediones

Thiazolidinediones are ligands for the *peroxisome proliferator-activated receptor γ* (PPARγ) receptor, a nuclear hormone receptor that has two isoforms and is involved in the regulation of genes related to glucose and lipid metabolism. Two thiazolidinediones are currently available to treat patients with type 2 diabetes, *rosiglitazone* and *pioglitazone*; a third, *troglitazone*, was removed from the market in 2000 due to hepatotoxicity (Lebovitz, 2019).

Mechanism of Action; Pharmacological Effects. Thiazolidinediones activate PPARγ receptors, which are expressed primarily in adipose tissue with lesser expression in cardiac, skeletal, and smooth muscle cells; islet β cells; macrophages; and vascular endothelial cells. The endogenous ligands for PPARγ include small lipophilic molecules such as oxidized linoleic acid, arachidonic acid, and the prostaglandin metabolite 15d-PGJ_2. Ligand binding to PPARγ causes heterodimer formation with the retinoid X receptor and interaction with PPAR response elements on specific genes (see Chapter 3). The principal response to PPARγ activation is adipocyte differentiation. PPARγ activity also promotes uptake of circulating fatty acids into fat cells and shifts of lipid stores from extra-adipose sites to adipose tissue.

One consequence of the cellular responses to PPARγ activation is increased tissue sensitivity to insulin. *Pioglitazone* and *rosiglitazone* are insulin sensitizers and increase insulin-mediated glucose uptake by 30% to 50% in patients with type 2 diabetes. Although adipose tissue seems to be the primary target for PPARγ agonists, both clinical and preclinical models support a role for skeletal muscle, the major site for insulin-mediated glucose disposal, in the response to thiazolidinediones. In addition to promoting glucose uptake into muscle and adipose tissue, the thiazolidinediones reduce HGP and increase hepatic glucose uptake. It is not clear whether thiazolidinedione-induced improvement of insulin resistance is due to direct effects on key target tissues (skeletal muscle and liver), indirect effects mediated by secreted products of adipocytes (e.g., adiponectin), or some combination of these.

Thiazolidinediones also affect lipid metabolism. Treatment with *rosiglitazone* or *pioglitazone* reduces plasma levels of fatty acids by increasing clearance and reducing lipolysis. These drugs also cause a shift of triglyceride stores from nonadipose to adipose tissues and from visceral to subcutaneous fat depots. *Pioglitazone* reduces plasma triglycerides by 10% to 15% and raises HDL cholesterol levels. In contrast, *rosiglitazone* has minimal effects on plasma triglycerides, and the only consistent effect on circulating lipids is an increase of LDL cholesterol.

ADME. *Rosiglitazone* and *pioglitazone* are dosed once daily. The starting dose of *rosiglitazone* is 4 mg, and the maximum dose should not exceed

8 mg daily. The starting dose of *pioglitazone* is 15 to 30 mg, increased up to a maximum of 45 mg daily. Both agents are absorbed within 2 to 3 h, and bioavailability is unaffected by food. The thiazolidinediones are metabolized by the liver and may be administered to patients with renal insufficiency but should not be used if there is active hepatic disease. *Rifampin* induces hepatic CYPs and causes a significant decrease in plasma concentrations of *rosiglitazone* and *pioglitazone*; *gemfibrozil* impedes metabolism of the thiazolidinediones and can increase plasma levels by about 2-fold; a dose reduction is suggested with this combination. The onset of action of thiazolidinediones is relatively slow; maximal effects on glucose homeostasis develop gradually over the course of 1 to 3 months.

Therapeutic Uses. Thiazolidinediones enhance insulin action on liver, adipose tissue, and skeletal muscle; confer improvements in glycemic control in persons with type 2 diabetes; and cause average reductions in A1c of 0.5% to 1.4%. A key feature of their pharmacology is that they reduce hyperglycemia, but do not cause hypoglycemia. Thiazolidinediones require the presence of endogenous insulin for pharmacological activity and are not used in type 1 diabetes. Both *pioglitazone* and *rosiglitazone* are effective as monotherapy and when added to *metformin*, sulfonylureas, or *insulin*. *Pioglitazone* is marketed in a fixed-dose combination with *alogliptin*.

Adverse Effects; Drug Interactions. The most common adverse effects of the thiazolidinediones are weight gain and edema. Thiazolidinediones cause an increase in body adiposity and an average weight gain of 2 to 4 kg over the first year of treatment. The use of insulin with thiazolidinedione treatment roughly doubles the incidence of edema and amount of weight gain compared with either drug alone. Macular edema has been reported in patients using either *rosiglitazone* or *pioglitazone*.

As with other side effects of thiazolidinediones, fluid retention is dose related. Use of thiazolidinediones is associated with a mild reduction of hematocrit, which may be an effect of fluid retention, although a primary effect on hematopoiesis has not been excluded.

Exposure to these drugs over several years in clinical trials has been associated with an increased incidence of heart failure of up to 2-fold. This has generally been attributed to the effect of the drugs to expand plasma volume in patients with type 2 diabetes who have an increased risk for heart failure. There does not appear to be an acute effect of *pioglitazone* or *rosiglitazone* to reduce myocardial contractility or ejection fraction. Thiazolidinediones can be used in diabetic patients without a history of heart failure or with compensated heart failure, but careful monitoring is important, especially when *insulin* is also used. Thiazolidinediones should not be used in patients with moderate-to-severe heart failure.

In the past, evidence suggested that *rosiglitazone* increased the risk of cardiovascular events (myocardial infarction, stroke). For this reason, the FDA restricted its use for several years, but this regulation has now been lifted as trial results suggested a neutral effect. Most evidence supports a mild beneficial effect of *pioglitazone* on overall cardiovascular events. In a trial of nondiabetic, insulin-resistant individuals who had a recent history of ischemic stroke or transient ischemic attack, *pioglitazone* reduced the risk of subsequent stroke or myocardial infarction and diabetes.

Treatment with thiazolidinediones has been associated with an increased risk of bone fracture in women, with some studies also showing effects in men. Therefore, the presence of osteoporosis and other risks for fracture should be considered before starting thiazolidinediones.

Pioglitazone and *rosiglitazone* are associated with a lowering of transaminases, probably reflective of reductions in hepatic steatosis. In clinical trials of patients with nonalcoholic steatohepatitis, *pioglitazone* reduced both hepatic lipid accumulation and inflammation (Gastaldelli and Cusi, 2019) and is the pharmacological agent with the strongest evidence for efficacy in this syndrome. Although *troglitazone* was removed from the market because of hepatotoxicity, case reports of acute liver injury with *pioglitazone* and *rosiglitazone* are rare. Nonetheless, thiazolidinediones should be withheld from patients with clinically apparent liver disease.

GLP-1–Based Agents

Incretins are GI hormones that are released after meals and stimulate insulin secretion. The currently established incretins are GLP-1 and GIP, and both act through specific receptors on β cells to stimulate insulin secretion. The GLP-1 receptor has become an effective drug target, with numerous glucose-lowering drugs developed that work through this mechanism. The GLP-1 receptor is also expressed in the brain, and GLP-1 receptor agonists activate these to reduce food intake and possibly augment the glucose-lowering actions that occur through enhanced β cell function. Efforts to incorporate the GIP receptor into diabetes therapeutics are under development.

Both GLP-1 and glucagon are derived from preproglucagon, a 180–amino acid precursor with five separately processed domains (Figure 51–9). An amino-terminal signal peptide is followed by glicentin-related pancreatic peptide, glucagon, GLP-1, and GLP-2. Processing of the protein is sequential and occurs in a tissue-specific fashion. Pancreatic α cells cleave proglucagon primarily into glucagon and a large C-terminal peptide that includes both of the GLPs. Intestinal L cells and hindbrain neurons process proglucagon mostly into a large N-terminal peptide that includes glucagon and GLP-1 and GLP-2. GLP-2 affects the proliferation of epithelial cells lining the GI tract. *Teduglutide*, a GLP-2 analogue, is approved for treatment of short-bowel syndrome (see Chapter 54).

Given intravenously to diabetic subjects in supraphysiological amounts, GLP-1 stimulates insulin secretion, inhibits glucagon release, delays gastric emptying, reduces food intake, and normalizes fasting and postprandial insulin secretion. The insulinotropic effect of GLP-1 is glucose dependent in that insulin secretion at fasting glucose concentrations, even with high levels of circulating GLP-1, is minimal. Native GLP-1 is rapidly inactivated by the enzyme DPP-4, yielding a plasma $t_{1/2}$ of 1 to 2 min; thus, the endogenous peptide is not a useful therapeutic agent.

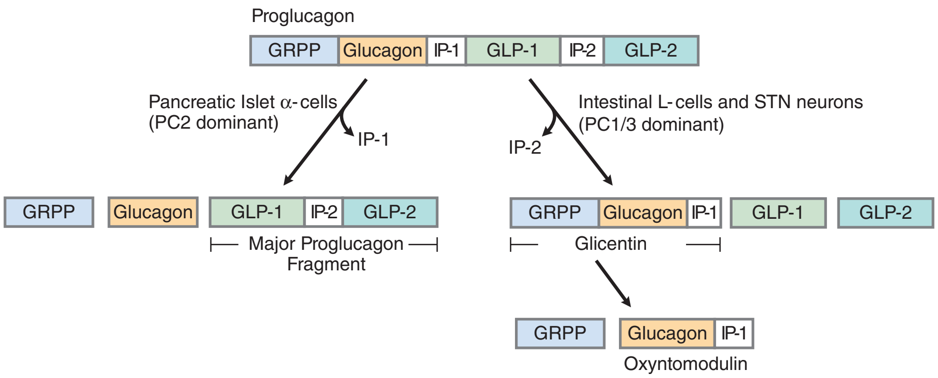

Figure 51–9 *Processing of proglucagon to glucagon, GLP-1, GLP-2, and glicentin-related pancreatic polypeptide (GRPP).* Proglucagon is synthesized in islet α cells, intestinal enteroendocrine cells (L cells), and a subset of neurons in the hindbrain. In α cells, prohormone processing is primarily by proconvertase 2, releasing glucagon, GRPP, and a major proglucagon fragment containing the two GLPs. In L cells and neurons, proglucagon cleavage is mostly through proconvertase 1/3, giving glicentin, oxyntomodulin, GLP-1, and GLP-2. IP-1, intervening peptide-1; STN, solitary tract nucleus.

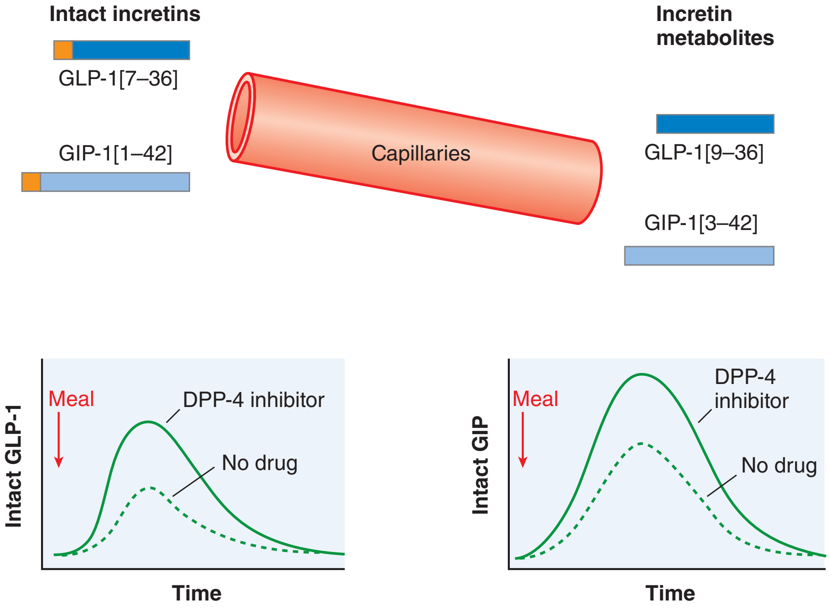

Figure 51–10 *Pharmacological effects of DDP-4 inhibition.* DPP-4, an ectoenzyme located on the luminal side of capillary endothelial cells, metabolizes the incretins, GLP-1, and GIP by removing the two N-terminal amino acids. The target for DPP-4 cleavage is a proline or alanine residue in the second position of the primary peptide sequence. The truncated metabolites GLP-1[9–36] and GIP[3–42] are the major forms of the incretins in plasma and are inactive as insulin secretagogues. Treatment with a DPP-4 inhibitor increases the concentrations of intact GLP-1 and GIP.

Two broad strategies have been taken to applying GLP-1 to therapeutics: the development of injectable, DPP-4-resistant peptide agonists of the GLP-1 receptor and the creation of small-molecule inhibitors of DPP-4 (Figure 51–10; see Table 51–5).

GLP-1 Receptor Agonists

Six *GLP-1 receptor agonists* (GLP-1RAs; *albiglutide, dulaglutide, exenatide, liraglutide*, *lixisenatide*, *semaglutide*) have been FDA-approved for treatment of diabetic patients, although *albiglutide* is no longer marketed in the U.S. (Table 51–5) (Nauck et al., 2022c).

Exenatide. Exendin-4 is a naturally occurring 39–amino acid reptilian peptide with 53% sequence homology to GLP-1. This peptide is a potent GLP-1RA that shares many of the physiological and pharmacological effects of GLP-1. It is not metabolized by DPP-4 and so has a longer circulating $t_{1/2}$ following injection than GLP-1. Synthetic exendin-4, *exenatide*, is approved for use as monotherapy and as adjunctive therapy for patients with type 2 diabetes not achieving glycemic targets with other drugs.

In clinical trials, *exenatide*, alone or in combination with *metformin*, *sulfonylurea*, or *thiazolidinedione*, was associated with improved glycemic control, as reflected in an approximately 1% decrease in A1c and weight loss that averaged 2.5 to 4 kg. Evidence from clinical trials indicated that *exenatide* can also be used in conjunction with basal *insulin*. An extended-release form of *exenatide* that is embedded in a biodegradable polymer to delay absorption into the circulation is administered by subcutaneous injection once a week with greater A1c lowering effectiveness than twice-daily *exenatide*.

Liraglutide. This is long-acting, DPP-4-resistant form of GLP-1 with a $\text{Lys}^{34}\text{Arg}$ substitution and addition of an α-glutamic acid spacer coupled to a C16 fatty acyl group at Lys^{26}. The fatty acid side chain delays absorption from the subcutaneous space and permits binding to albumin and other plasma proteins and accounts for a $t_{1/2}$ of approximately 13 h that permits once-daily administration; the fatty acid also seems to confer some protection from cleavage of the N-terminus by DPP-4. The pharmacodynamic profile of *liraglutide* mimics GLP-1 and *exenatide*. In clinical trials, *liraglutide* improved glycemic control and caused weight loss. *Liraglutide* can be added to oral agents or basal insulin. It is usually started at a subclinical dose and titrated over several weeks to treatment amounts to mitigate side effects (see Table 51–5). *Liraglutide* is available in a fixed-dose combination with *insulin degludec* that delivers a dose of 50 units of *insulin* with 1.8 mg, the top diabetes treatment dose, of *liraglutide*.

Dulaglutide. This is a fusion protein consisting of two molecules of a modified version of GLP-1 linked to the Fc portion of human immunoglobulin; the GLP-1 sequences are modified to protect against the action of DPP-4. *Dulaglutide* has a $t_{1/2}$ of approximately 5 days and is injected once weekly; like other GLP-1RAs, it is started at a low dose and escalated to treatment doses (see Table 51–5). Pharmacodynamics are comparable to other GLP-1RAs, and the drug can be used with other antidiabetic agents.

Lixisenatide. This is a modified form of exendin-4 with a C-terminal polylysine extension that has comparable pharmacodynamics to *exenatide*. *Lixisenatide* is rapidly absorbed to peak levels within 2 h and has a plasma $t_{1/2}$ of 2 h. *Lixisenatide* is available in fixed-dose combinations with *insulin glargine* that deliver doses of 15 to 60 units of *insulin* (in increments of 1 unit) with 5 to 20 μg of *liraglutide* (in increments of 0.33 μg).

Semaglutide. *Semaglutide* is a long-acting, DPP-4-resistant drug identical to *liraglutide* with the exception of an aminobutyric acid substitution at position 2, an α-glutamic acid–oligoethylene glycol linker at position 26, and coupling to a C-18 diacid (Knudsen and Lau, 2019). *Semaglutide* has a $t_{1/2}$ of 7 days that is due to tighter binding with albumin, and the injectable formulation is dosed once weekly. A separate formulation that includes the short-chain fatty acid sodium *N*-amino caprylate permits passage of sufficient *semaglutide* across the gastric mucosa to allow oral daily dosing.

While all of the GLP-1RAs have demonstrated efficacy as monotherapy, they are recommended as first-line agents. Although there are no systematic comparisons of all the drugs in this class, several clinical trials have directly compared two or three GLP-1RAs (Trujillo et al., 2021). While the differences in efficacy are modest relative to the overall effect of the drugs, *semaglutide* appears to be the most potent and *exenatide* for daily use and *lixisenatide* the weakest.

Weight Loss. All long-acting GLP-1RAs were reported to reduce body weight by 3 to 5 kg over 4 to 6 months in clinical trials of persons with type 2 diabetes. *Liraglutide* and *semaglutide* have been studied at higher doses (3.0 and 2.4 mg, respectively) in nondiabetic subjects with a body mass index greater than 30 and demonstrated to cause significant weight loss. In these trials, *liraglutide* reduced body weight by 6 kg compared to a placebo-treated group and *semaglutide* by 13 kg compared to controls. Both drugs are now approved in the U.S. for this use.

Effects on Cardiovascular Risk. All of the approved GLP-1RAs currently available have been tested in placebo-controlled trials for safety in persons with established cardiovascular conditions or at high risk for cardiovascular disease (CVD). *Exenatide* and *lixisenatide* were both noninferior to placebo and are considered to confer no risk for CVD events. *Liraglutide* and *dulaglutide* both demonstrated superiority to placebo and significantly reduced risk of a composite endpoint including death from CVD, stroke, or myocardial infarction. Both oral and injectable *semaglutide* have demonstrated safety in diabetic persons with high risk for CVD; both drugs also conferred protection from events, although these were not prespecified endpoints in the studies, and trials that are testing superiority are ongoing. The findings from cardiovascular trials have led to current expert guidelines recommending a GLP-1RA for patients at high risk for CVD if access to these medications is available.

Mechanism of Action. All GLP-1RAs share a common mechanism, activation of the GLP-1 receptor, a member of glucagon receptor family of GPCRs (class B GPCRs). GLP-1 receptors are expressed by β cells, cells in the peripheral and central nervous systems, the heart and vasculature, kidney, lung, and GI mucosa. Binding of agonists to the GLP-1 receptor activates the cAMP-PKA pathway and several guanine nucleotide exchange factors. GLP-1 receptor activation also initiates signaling via PKC, PI3K, and β-arrestin and alters the activity of several ion channels (McLean et al., 2020). In β cells, the end result of these actions is increased insulin biosynthesis and exocytosis in a glucose-dependent manner (see Figure 51–3). Activation of GLP-1 receptors in the CNS accounts for the effects of GLP-1RAs on food intake and gastric emptying and for side effects such as nausea.

ADME. *Exenatide* is given as a subcutaneous injection twice daily, typically before meals. It is rapidly absorbed, reaches peak concentrations in about 2 h, and has a plasma $t_{1/2}$ of 2 to 3 h. Clearance of the drug occurs primarily by glomerular filtration, with tubular proteolysis and minimal reabsorption. *Exenatide* is marketed as a pen that delivers 5 or 10 μg; dosing is typically started at the lower amount and increased as needed. There is a weekly preparation based on the embedding of *exenatide* in a polymeric microsphere that releases drug slowly after injection. Weekly *exenatide* is given as a suspension of 2 mg that is prepared from lyophilized material and diluent immediately prior to injection. Once in the circulation, the drug is metabolized similarly to short-acting *exenatide*; however, based on the extended rate of delivery, 5 to 6 weekly doses are required to reach therapeutic steady state. *Lixisenatide*, the other GLP-1RA based on exendin-4, has an elimination $t_{1/2}$ of approximately 3 h that involves a significant degree of renal clearance.

Liraglutide is given as a subcutaneous injection once daily. Peak levels occur in 8 to 12 h; the elimination $t_{1/2}$ is 12 to 14 h. There is little renal or intestinal excretion of *liraglutide*; clearance is primarily through the metabolic pathways of large plasma proteins. *Liraglutide* is supplied in a pen injector that delivers 0.6, 1.2, 1.8, 2.4, or 3 mg of drug; the lowest dose is for treatment initiation, with elevation to the higher doses based on clinical response.

Dulaglutide and *semaglutide* have extended plasma $t_{1/2}$, are dosed weekly, and require 1 to 2 months to reach steady state. No effects of hepatic or renal impairment on clearance of these compounds has been demonstrated.

Adverse Effects; Drug Interactions. Intravenous or subcutaneous administration of GLP-1 causes nausea and vomiting; this is thought to be mediated through neural activation of specific CNS neurons that are activated following peripheral dosing of peptide. The doses above which GLP-1 causes GI side effects are higher than those needed to regulate blood glucose. Nonetheless, up to 30% to 50% of subjects report nausea at the initiation of therapy with any of the GLP-1RAs, although the GI side effects of these drugs wane over time. Activation of GLP-1 receptors in the CNS mediates the typical delay of gastric emptying, and GLP-1 agonists may alter the pharmacokinetics of drugs that require rapid GI absorption, such as oral contraceptives and antibiotics. In the absence of other diabetes drugs that cause low blood glucose, hypoglycemia associated with GLP-1 agonist treatment is rare, but the combination of GLP-1 agonist with sulfonylurea drugs causes an increased rate of hypoglycemia compared to sulfonylurea treatment alone. Because of the reliance on renal clearance, *exenatide*, and probably *lixisenatide*, should not be given to persons with moderate-to-severe renal failure (creatinine clearance <30 mL/min). In clinical trials, patients treated with *liraglutide* and *semaglutide* had increased risk of gallbladder disease. Based on surveillance data, there is a possible association of GLP-1RA and pancreatitis, although this risk has not been observed in the analyses of clinical trials. Nonetheless, GLP-1RA should be used with caution in persons with a history of pancreatitis. The GLP-1 receptor is expressed by thyroid C cells. Although there is not an established clinical association with medullary carcinoma of the thyroid, GLP-1 agonists should not be given to these patients.

DPP-4 Inhibitors

Dipeptidyl peptidase 4 is a serine protease that is widely distributed throughout the body, expressed as an ectoenzyme on vascular endothelial cells, on the surface of T lymphocytes, and in a circulating form. DPP-4 cleaves the two N-terminal amino acids from peptides with a proline or alanine in the second position and seems to be especially critical for the inactivation of GLP-1 and GIP (Deacon and Lebovitz, 2016). DPP-4 inhibitors increase the area under the curve (AUC) of intact, bioactive GLP-1 and GIP after meal consumption (see Figure 51–10). A variety of small-molecule, orally available agents provide nearly complete and long-lasting inhibition of DPP-4, thereby increasing the proportion of active GLP-1 from 10% to 20% of total circulating GLP-1 immunoreactivity to nearly 100%. *Sitagliptin*, *saxagliptin, linagliptin*, and *alogliptin* are available in the U.S.; *vildagliptin* is available in the E.U.

Mechanism of Action; Pharmacological Effects. *Alogliptin*, *linagliptin*, and *sitagliptin* are competitive inhibitors of DPP-4; *vildagliptin* and *saxagliptin* bind the enzyme covalently. All five drugs can be given in doses that lower measurable activity of DPP-4 by more than 95% for 12 h. This causes a greater than 2-fold elevation of plasma concentrations of active GIP and GLP-1 and is associated with increased insulin secretion, reduced glucagon levels, and improvements in both fasting and postprandial hyperglycemia. Inhibition of DPP-4 does not appear to have direct effects on insulin sensitivity, gastric motility, or satiety; chronic treatment with a DPP-4 inhibitor also does not affect body weight. DPP-4 inhibitors, used as monotherapy in type 2 diabetic patients, reduce A1c levels by an average of 0.4–0.8%. These compounds are also effective for chronic glucose control when added to *metformin*, thiazolidinediones, and sulfonylureas. The effects of DPP-4 inhibitors in combination regimens appear to be additive.

ADME. The recommended doses of the DPP4 inhibitors are *alogliptin*, 25 mg daily; *linagliptin*, 5 mg daily; *saxagliptin*, 5 mg daily; *sitagliptin*, 100 mg daily; and *vildagliptin*, 50 mg one or two times daily. DPP-4 inhibitors are absorbed effectively from the small intestine. *Alogliptin*, *saxagliptin*, *sitagliptin*, and *vildagliptin* circulate primarily in unbound form and are excreted largely unchanged in the urine; lower doses should be given to patients with reduced renal function. *Linagliptin* binds extensively to plasma proteins and is cleared primarily by the hepatobiliary system, with little renal clearance. Only *saxagliptin* is metabolized by hepatic microsomal enzymes, and its dose should be lowered to 2.5 mg daily when coadministered with strong CYP3A4 inhibitors (e.g., *ketoconazole, atazanavir, clarithromycin, indinavir, itraconazole, nefazodone, nelfinavir, ritonavir, saquinavir*, and *telithromycin*).

Adverse Effects. There are no consistent adverse effects that have been noted in clinical trials with any of the DPP-4 inhibitors. Large cardiovascular safety studies have been completed for *alogliptin*, *saxagliptin*, and *sitagliptin*. There was no impact of these drugs on the incidence of cardiovascular events in diabetic patients, although patients treated with *saxagliptin* had an increase in hospitalization for heart failure. The FDA has issued a warning that this class of drugs is rarely associated with severe joint pain. DPP-4 is expressed on lymphocytes and is also referred to as CD26.

α-Glucosidase Inhibitors

α-Glucosidase inhibitors reduce intestinal absorption of starch, dextrin, and disaccharides by inhibiting the action of α-glucosidase in the intestinal brush border (Hedrington and Davis, 2019). These drugs also increase the release of the glucoregulatory hormone GLP-1 into the circulation, which may contribute to their glucose-lowering effects. The drugs in this class are *acarbose*, *miglitol*, and *voglibose* (not available in the U.S.).

ADME. Dosing of *acarbose* and *miglitol* are similar. Both are provided as 25-, 50-, or 100-mg tablets that are taken before meals. Treatment should start with lower doses and be titrated as indicated by balancing postprandial glucose, A1c, and GI symptoms. *Acarbose* is minimally absorbed; the small amount of drug reaching the systemic circulation is cleared by the kidney. *Miglitol* absorption is saturable, with 50% to 100% of any dose entering the circulation. *Miglitol* is cleared almost entirely by the kidney, and dose reductions are recommended for patients with creatinine clearance less than 30 mL/min.

Adverse Effects; Drug Interactions. The most prominent adverse effects are malabsorption, flatulence, diarrhea, and abdominal bloating. Mild-to-moderate elevations of hepatic transaminases are reported with *acarbose*, but symptomatic liver disease is very rare. Cutaneous hypersensitivity has been described but is also rare. Hypoglycemia has been described when α-glucosidase inhibitors are added to *insulin* or an insulin secretagogue. *Acarbose* can decrease the absorption of *digoxin*; *miglitol* can decrease the absorption of *propranolol* and *ranitidine*. The α-glucosidase inhibitors are contraindicated in patients with stage 4 renal disease.

Therapeutic Use. α-Glucosidase inhibitors are indicated as adjuncts to diet and exercise in type 2 diabetic patients not reaching glycemic targets. They can also be used in combination with other oral antidiabetic agents or *insulin*. In clinical studies, α-glucosidase inhibitors reduced A1c by 0.5% to 0.8%, fasting glucose by about 1 mM, and postprandial glucose by 2.0 to 2.5 mM. These agents do not cause weight gain or have significant effects on plasma lipids.

Na$^+$-Glucose Transporter 2 Inhibitors

The sodium-glucose cotransporter 2 (SGLT2) is a Na$^+$-glucose cotransporter located almost exclusively in the proximal portion of the renal tubule. SGLT2 is a high-affinity, low-capacity transporter that moves glucose against a concentration gradient from the tubular lumen using energy generated from Na$^+$ flux through the epithelial cells. Renal retention of glucose is nearly complete in nondiabetic persons, and SGLT2 accounts for 80% to 90% of this reclamation; the remainder is recovered by SGLT1 more distally in the tubule. Early studies in diabetic animals demonstrated that hyperglycemia could be nearly ameliorated by the naturally occurring compound *phlorizin*, an SGLT inhibitor. Based on this proof of principle, drugs that are specific inhibitors of SGLT2 have been developed to treat diabetes (Thomas and Cherney, 2018). These agents block glucose transport in the proximal tubule and lower blood glucose by promoting urinary loss.

Mechanism of Action; Pharmacological Effects. SGLT2 inhibitors reduce the rate of glucose reclamation in the proximal tubule and lower the renal threshold for glucose excretion from about 180 to 50 mg/dL (10 to 2.8 mM). In monotherapy, they reduce A1c by 0.7% to 1.0%, cause weight loss of 2 to 4 kg, and decrease blood pressure by 2 to 4 mmHg. There are currently four SGLT2 inhibitors available for clinical use—*canagliflozin, dapagliflozin, empagliflozin,* and *ertugliflozin*. These agents are indicated for use in combination with other oral agents and *insulin*; such use leads to an additional decrease of A1c of 0.5% to 0.8%. SGLT2 inhibitors are available in combination with *metformin* and DPP-4 inhibitors.

Effects on Cardiovascular Risk (See Also Chapter 33 for Details). All four of the currently available SGLT2 inhibitors have been tested in placebo-controlled trials to assess their impact on cardiovascular risk. *Empagliflozin* and *canagliflozin* decreased specified cardiovascular events in persons with type 2 diabetes and established CVD or at high risk for CVD. *Dapagliflozin* and *ertugliflozin* had effects on cardiac events that did not differ from placebo-treated patients; that is, they conferred no added risk. All the SGLT2 inhibitors decreased the risk of hospitalization for heart failure compared to placebo. *Dapagliflozin* had a significant effect on reducing hospitalization for heart failure and cardiovascular death in diabetic and nondiabetic subjects with established heart failure and reduced ejection fraction. *Canagliflozin* decreased admissions for heart failure in subjects with type 2 diabetes and diabetic nephropathy.

Effects on Diabetic Nephropathy. *Canagliflozin*, *dapagliflozin*, and *empagliflozin* each decreased the progression of renal disease compared to placebo in randomized trials of patients with reduced glomerular filtration and albuminuria. Based on the results of a series of clinical trials, current recommendations emphasize the use of SGLT2 inhibitors early in the management of individuals with diabetes and CVD, heart failure, or nephropathy.

ADME. Available SGLT2 inhibitors share favorable pharmacokinetic properties. They have good oral bioavailability (60%–80%) that is not affected by food and reach peak levels 1 to 2 h after ingestion. They are approximately 90% bound to circulating proteins with plasma $t_{1/2}$ of 12 h, making them suitable for once-daily dosing. The compounds are metabolized by glucuronidation, and the inactive metabolites are renally excreted; there is virtually no renal excretion of the parent drugs. All four drugs are available in tablets with two doses, *dapagliflozin* 5 and 10 mg, *canagliflozin* 100 and 300 mg, *empagliflozin* 10 and 25 mg, and *ertugliflozin* 5 and 15 mg; the higher-dose tablet is the maximum recommended amount of each drug.

Adverse Effects; Drug Interactions. The side effects of SGLT2 inhibitors are predictable from their mechanism of action. There is increase in lower urinary tract infections of approximately 2% and a 3% to 5% increase in genital mycotic infections. Urine glucose losses cause mild diuresis, which can lead to hypotension and associated symptoms in a small percentage of, usually older, patients. Importantly, because SGLT2 inhibitors ultimately depend on the rate of glucose filtration to be effective, potency decreases by 40% to 80% across the spectrum of stage 3 kidney disease (GFR 60–30 mL/min). SGLT2 inhibitors do not cause hypoglycemia alone but can potentiate the effects of drugs that do.

Early clinical trials with *canagliflozin* suggested that treated patients might have an increased risk of fractures. This finding has not been born out in subsequent studies of *canagliflozin* or reported for other SGLT2 inhibitors. Similarly, results from two trials suggested that use of *canagliflozin* doubled the risk for lower extremity amputation. While these results triggered a warning for the drug, this has been removed based on evidence from further clinical studies. Diabetic ketoacidosis (often with near-normal blood glucose) can occur in patients treated with SGLT2 inhibitors, especially during a concurrent illness. Patients starting SGLT2 inhibitors may have mild, transient worsening of renal function that resolves once the initial phase of diuresis has been compensated.

Other Glucose-Lowering Agents

Pramlintide. Islet amyloid polypeptide (amylin) is a 37–amino acid peptide produced in the pancreatic β cell and secreted with insulin. A synthetic form of amylin with several amino acid modifications to improve bioavailability, *pramlintide,* has been developed as a drug for the treatment of diabetes. *Pramlintide* likely acts through the amylin receptor in specific regions of the hindbrain. Activation of the amylin receptor reduces glucagon secretion, delays gastric emptying, and fosters a feeling of satiety.

ADME. *Pramlintide* is administered as a subcutaneous injection prior to meals. The drug is not extensively bound by plasma proteins and has a $t_{1/2}$ of 50 min. Metabolism and clearance are primarily renal. The doses in patients with type 1 diabetes start at 15 μg and are titrated upward to a maximum of 60 μg; in type 2 diabetes, the starting dose is 60 μg, and the maximum is 120 μg. Because of differences in the pH of the solutions, *pramlintide* should not be administered in the same syringe as insulin.

Adverse Effects; Drug Interactions. The most common adverse effects are nausea and hypoglycemia. Although *pramlintide* alone does not lower blood glucose, addition to insulin at mealtimes can cause increased rates of hypoglycemia, occasionally severe. It is currently recommended that prandial *insulin* doses be reduced 30% to 50% at the time of *pramlintide* initiation and then retitrated. Because of its effects on GI motility, *pramlintide* is contraindicated in patients with gastroparesis or other disorders of motility. *Pramlintide* is a pregnancy category C drug. *Pramlintide* can be used in persons with moderate renal disease (creatinine clearance >20 mL/min).

Therapeutic Uses. *Pramlintide* is approved for treatment of types 1 and 2 diabetes as an adjunct in patients who take *insulin* with meals.

Bile Acid–Binding Resins. The only bile acid sequestrant specifically approved for the treatment of type 2 diabetes is *colesevelam*.

Mechanism of Action. Bile acid metabolism is abnormal in patients with type 2 diabetes, and there have been intermittent reports that bile acid–binding resins lower plasma glucose in diabetic patients. The mechanism by which bile acid binding and removal from enterohepatic circulation lowers blood glucose has not been established. Bile acid sequestrants could reduce intestinal glucose absorption, although there is no direct evidence of this. Bile acids also act as signaling molecules through nuclear receptors, some of which may act as glucose sensors.

ADME. *Colesevelam* is provided as a powder for oral solution and as 625-mg tablets; typical usage is 3 tablets twice daily before lunch and dinner or 6 tablets prior to the patient's largest meal. The drug is absorbed from the intestinal tract only in trace amounts, so its distribution is limited to the GI tract.

Adverse Effects; Drug Interactions. Common side effects of *colesevelam* are GI, with constipation, dyspepsia, abdominal pain, and nausea affecting up to 10% of treated patients. Like other bile acid–binding resins, *colesevelam* can increase plasma triglycerides in persons with an inherent tendency to hypertriglyceridemia and should be used cautiously in patients with plasma triglycerides greater than 200 mg/dL. *Colesevelam* can interfere with the absorption of commonly used agents (e.g., *phenytoin*, *warfarin*, *verapamil*, *glyburide*, L-*thyroxine*, and *ethinyl estradiol* and fat-soluble vitamins). *Colesevelam* is a pregnancy category B drug that has no contraindications in patients with renal or liver disease.

Therapeutic Uses. *Colesevelam* is approved for treatment of hypercholesterolemia and may be used for treatment of type 2 diabetes as an adjunct to diet and exercise. In clinical trials, *colesevelam* reduced A1c by 0.5% when added to *metformin*, sulfonylurea, or *insulin* treatment in type 2 diabetic patients.

Bromocriptine. A low-dose (0.8-mg) tablet formulation of *bromocriptine*, a dopamine receptor agonist, is approved for the treatment of type 2 diabetes. *Bromocriptine* is an established treatment of Parkinson's disease and hyperprolactinemia (see Chapters 15, 21, and 46). Effects of *bromocriptine* on blood glucose are modest and may reflect an action in the CNS. The dose range for *bromocriptine* is 1.6 to 4.8 mg, taken with food in the morning within 3 h of awakening. Side effects include nausea, fatigue, dizziness, orthostatic hypotension, vomiting, and headache.

Combined Pharmacological Approaches to Type 2 Diabetes

Managing the Progression of Type 2 Diabetes

For most patients, the pathological changes causing hyperglycemia in type 2 diabetes progress over time. Thus, most patients require stepwise intensification of therapy to maintain glycemic goals. Several academic societies and health organizations have issued guidelines, algorithms, or flowcharts for the treatment of type 2 diabetes. Figure 51–11 presents a simplified version; more details can be found in guidelines from the ADA, European Society for Study of Diabetes, American Association of

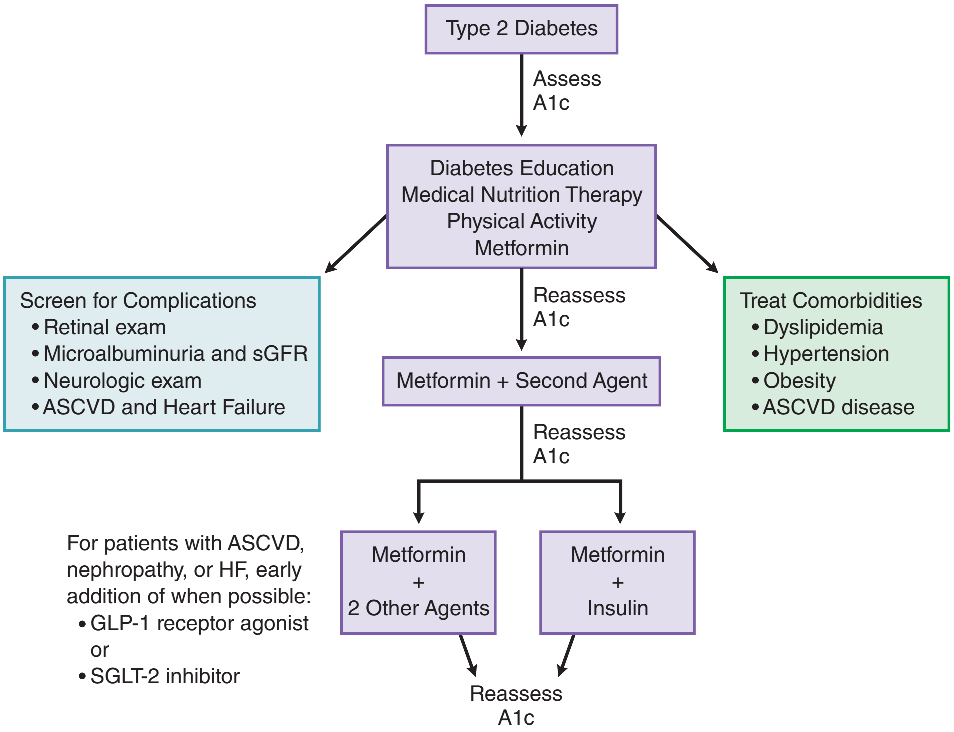

Figure 51–11 *Treatment algorithm for management of type 2 diabetes mellitus.* Patients diagnosed with type 2 diabetes, by fasting glucose, oral glucose tolerance testing, or A1c measurement, should have diabetes education that includes instruction on medical nutrition therapy and physical activity. *Metformin* remains the consensus first line of therapy and should be started at the time of diagnosis. Failure to reach the glycemic target, generally A1c of 7% or less within 2 to 3 months, should prompt the addition of a second agent (*insulin*, sulfonylurea, thiazolidinedione, DPP-IV inhibitor, GLP-1 agonist, or SGLT-2 inhibitor). Reinforce lifestyle interventions at every visit, and check A1c every 3 months. Treatment may escalate to *metformin* plus *insulin* or *metformin* plus two other agents from the list given. For individuals with established clinical atherosclerotic cardiovascular disease, a GLP-1 receptor agonist or SGLT-2 inhibitor should be added early in the course of treatment; for patients with heart failure or diabetic nephropathy, SGLT-2 inhibitors are favored as second agents (see text for details). ASCVD, atherosclerotic cardiovascular disease; eGFR, estimated glomerular filtration rate; HF, heart failure.

Clinical Endocrinology, and National Institute for Health and Care Excellence (United Kingdom) (ADA, 2022c). Table 51–6 summarizes available pharmacological agents for the treatment of diabetes. While there is no consensus as to preferred combinations or the order in which they are used, in general, selection involves consideration of comorbidities, side effect profile, cost, and A1c goal. Recent evidence suggests that individuals with type 2 diabetes who are at high risk for cardiovascular or renal disease benefit from SGLT2 inhibitors or GLP-1 agonists. The National Institutes of Health–funded Glycemia Reduction Approaches in Diabetes: A Comparative Effectiveness Study (GRADE) found that addition of *liraglutide* or basal *insulin* to metformin leads to slightly better glycemic control than *glimepiride* or *sitagliptin*. SLGT2 inhibitors were not tested in this study.

There is consensus that *metformin* and lifestyle changes should be the first interventions. After that, a number of pathways or combination of drugs can be used for treatment of type 2 diabetes if the glucose control does not reach the therapeutic target (ADA, 2022c; Davies et al., 2018). For example, the addition of a second oral agent may provide good therapeutic results. Combinations of fixed doses of most of the oral agents are now available; while most oral agents have additive effects, no specific combinations have been demonstrated to have particular efficacy that can be predicted for most patients. Another approach is to introduce basal long-acting *insulin* (at bedtime) in combination with an oral glucose-lowering agent. This combination allows the oral agent to provide postprandial glycemic control, while the basal *insulin* provides the foundation for normalizing fasting or basal glucose levels. Long-acting *insulin* can be combined with almost all of the oral antihyperglycemic agents in Table 51–6. The combination of therapies can be guided by an estimation of the β cell secretory *reserve* in the patient (i.e., a measurement of C-peptide level) and individualized patient glycemic goals. The progressive insulin deficiency in type 2 diabetes often makes it increasingly difficult to achieve the glycemic goal solely with oral antihyperglycemic agents; thus, *insulin* is often required.

Costs of Diabetes Drugs. Treatment of diabetes can be very expensive, especially because most patients use multiple agents, as well as drugs for associated conditions such as hypertension, dyslipidemia, and cardiovascular disease. Thus, cost has become a key issue in the management of diabetic patients since it has been documented to affect adherence to and choice of treatment plans (Table 51–7). Newer agents are costlier, while older drug classes like sulfonylureas and biguanides are inexpensive and available in generic formulations. In recent years, the price of all *insulin* formulations has increased steadily despite the addition of numerous new products to the market, including insulin biosimilars (Cefalu et al., 2018). The balance of cost-benefit ratio, especially of the new agents, remains a topic under debate but is important for clinical management. Shared decision making with patients, incorporating their circumstances and wishes, can be effective in diabetes treatment.

Emerging Therapies for Diabetes

A number of immunomodulatory approaches are being investigated to prevent or block the autoimmune process central to type 1 diabetes. Early in the disease course (stage 2 type 1 diabetes), suppression of the autoimmune process with anti-CD3 monoclonal antibodies that target T lymphocytes has been shown to delay the onset of type 1 diabetes. Recently, targeting the thioredoxin-interacting protein (TXNIP) with Ca^{2+} channel blockers has had a positive impact on recent-onset type 1 diabetes. The integration of an *insulin* infusion device and a glucose sensor is progressing rapidly so that the *insulin* infusion is being automated based on sensor results (closed loop).

Advances in protein chemistry have allowed the development of peptides that activate more than one receptor to improve glucose regulation. Most of these incorporate GLP-1 receptor agonism with the capacity to activate receptors for glucagon or the GIP receptor. Other new therapies being evaluated for type 2 diabetes include glucokinase activators, inhibitors of 11β-hydroxysteroid dehydrogenase-1, GPR40 agonists, and combined SLGT1 and SLGT2 inhibitors.

Metabolic or bariatric surgery is quite effective for obese individuals with type 2 diabetes. Whole pancreas and islet transplantation can normalize glucose control in type 1 diabetes.

Hypoglycemia

In the absence of prolonged fasting, healthy humans almost never have blood glucose levels below 3.5 mM. This is due to a highly adapted neuroendocrine counterregulatory system that prevents acute hypoglycemia, a hazardous and potentially lethal situation. The three most common clinical scenarios for hypoglycemia are as follows:

- Treatment of diabetes with *insulin* or oral agents that promote insulin secretion (sulfonylureas)
- Inappropriate production of endogenous insulin by a pancreatic islet tumor (insulinoma) or insulin-like growth factors by non-islet tumors such as hepatomas or sarcomas
- Use (purposeful or inadvertent) of a glucose-lowering agent in an individual without diabetes

Hypoglycemia in the first and third scenarios can occur in either the fasting or the fed state, whereas hypoglycemia secondary to neoplasms occurs primarily in the fasting or postabsorptive state.

Hypoglycemia is an adverse reaction to a number of oral therapies and is most pronounced and serious with *insulin* therapy. Hypoglycemia may result from an inappropriately large dose of *insulin*, from a mismatch between the time of peak delivery of *insulin* and food intake, or from superposition of additional factors that increase sensitivity to *insulin* (e.g., adrenal or pituitary insufficiency) or that increase insulin-independent glucose uptake (e.g., exercise). Hypoglycemia is the major risk that always must be weighed against benefits of pharmacological interventions to normalize glucose control. Hypoglycemia is especially problematic in the elderly and should be given strong consideration when individualizing glycemic goals.

The first physiological response to hypoglycemia is a reduction of endogenous insulin secretion, which occurs at a plasma glucose level of about 70 mg/dL (3.9 mM); thereafter, the counterregulatory hormones (glucagon, epinephrine, norepinephrine, growth hormone, and cortisol) are released. Symptoms of hypoglycemia typically become manifest at a plasma glucose level of 60 to 70 mg/dL (3.3–3.9 mM). Sweating, hunger, paresthesias, palpitations, tremor, and anxiety, principally of autonomic origin, usually are seen first. Difficulty in concentrating, confusion, weakness, drowsiness, a feeling of warmth, dizziness, blurred vision, and loss of consciousness (i.e., most important neuroglycopenic symptoms) usually occur at lower plasma glucose levels than do autonomic symptoms. Severe hypoglycemia can lead to seizure and coma.

In patients with type 1 and type 2 diabetes of longer duration, the glucagon secretory response to hypoglycemia becomes deficient. These individuals with diabetes thus become dependent on epinephrine for counter-regulation, and if this mechanism becomes deficient, the incidence of severe hypoglycemia increases; this phenomenon is central to hypoglycemia unawareness and autonomic neuropathy. With the ready availability of glucose monitoring, hypoglycemia can be documented in most patients who experience suggestive symptoms. Hypoglycemia that occurs during sleep may be difficult to detect, but affected patients often give a history of morning headaches, night sweats, or symptoms of hypothermia. Mild-to-moderate hypoglycemia may be treated simply by ingestion of oral glucose (15–20 g of carbohydrate). When hypoglycemia is severe, it should be treated with intravenous glucose or an injection of *glucagon*.

Agents Used to Treat Hypoglycemia

Glucagon, a single-chain polypeptide of 29 amino acids interacts with the glucagon GPCR on the plasma membrane of target cells, most importantly the hepatocyte, activating the G_s-cAMP-PKA pathway with an acute rise in glucose production, primarily from glycogenolysis. *Glucagon* should be prescribed for individuals at risk for severe hypoglycemia,

TABLE 51–6 ■ COMPARISON OF AGENTS USED FOR TREATMENT OF DIABETES

TYPE AND AGENT	MECHANISM OF ACTION	HBA$_{1c}$ REDUCTION (%)[a]	AGENT-SPECIFIC ADVANTAGES	AGENT-SPECIFIC DISADVANTAGES	CONTRAINDICATIONS AND PRECAUTIONS
Oral					
Biguanides[c]	↓ Hepatic glucose production, ↑ insulin sensitivity, influence gut function	1–2	Weight neutral, do not cause hypoglycemia, inexpensive	Diarrhea, nausea, vitamin B_{12} deficiency, lactic acidosis	GFR <30 mL/min, CHF, radiographic contrast studies, seriously ill patients, acidosis
Dipeptidyl peptidase 4 inhibitors[c]	Prolong endogenous GLP-1 action	0.4–0.8	Do not cause hypoglycemia		↓ Dose with renal disease
α-Glucosidase inhibitors[c]	↓ GI glucose absorption	0.5–0.8	↓ Postprandial glycemia	GI flatulence, elevated liver function tests	Renal/liver insufficiency
Insulin secretagogues—sulfonylureas[c]	↑ Insulin secretion	1–2	Inexpensive	Hypoglycemia, weight gain	Renal/liver insufficiency
Insulin secretagogues—nonsulfonylureas[c]	↑ Insulin secretion	1–2	Rapid onset of action, lower postprandial glucose	Hypoglycemia, precautions for elderly and renal impairment	Renal/liver insufficiency
SGLT2 inhibitors[c] (*the gliflozins*)	↑ Renal glucose excretion	0.5–0.8	Mild weight loss and BP reduction; do not cause hypoglycemia; CV and heart failure benefit	↑ Rate of lower urinary tract and genital mycotic infections; exacerbate tendency to hyperkalemia and DKA; see text for canagliflozin	Renal insufficiency
Thiazolidinediones[c] (*the glitazones*)	↓ Insulin resistance, ↑ glucose utilization	0.5–1.2	Lower insulin requirements	Peripheral edema, CHF, weight gain, fractures in females, macular edema	CHF, liver or renal insufficiency
Parenteral					
Insulin	↑ Glucose utilization, ↓ hepatic glucose production, and other anabolic actions	Not limited	Well-known safety/adverse effect profile from much clinical experience	Injection, weight gain, hypoglycemia	Hypoglycemia
GLP-1 receptor agonists[c,d]	↑ Insulin, ↓ glucagon, slow gastric emptying, satiety	0.5–1.5	Weight loss, do not cause hypoglycemia, ↓ CV events	Injection, nausea, pancreatitis	Renal disease, agents that also slow GI motility, pancreatic disease, medullary carcinoma of thyroid
Amylin agonists[b,c]	Slow gastric emptying, ↓ glucagon	0.25–0.5	Reduce postprandial glycemia; weight loss	Injection, nausea, ↑ risk of hypoglycemia with insulin	Agents that also slow GI motility
Other					
Medical nutrition therapy and physical activity[c]	↓ Insulin resistance, ↑ insulin secretion	1–3	Weight loss, improved CV health	Compliance difficult, long-term success low	
Inhaled insulin[c]	↑ Glucose utilization, ↓ hepatic glucose production, other anabolic actions	0.25–0.5	Rapid onset of action	Limited clinical experience	Pulmonary disease, smoking

BP, blood pressure; CHF, congestive heart failure; CV, cardiovascular; DKA, diabetic ketoacidosis.

[a] A1c reduction (absolute) depends partly on starting A1c value.

[b] Used in conjunction with insulin for treatment of type 1 diabetes.

[c] Used for treatment of type 2 diabetes.

[d] Semaglutide is available in an oral formulation.

TABLE 51–7 ■ RELATIVE COSTS OF THERAPEUTIC AGENTS FOR DIABETES

DRUG CLASS	AGENT	RELATIVE COST
α-Glucosidase inhibitors	Acarbose	+
	Miglitol	+++
Amylin analogue	Pramlintide	++++
Biguanides	Metformin, metformin ER	+
Dipeptidyl peptidase 4 inhibitors	Alogliptin, linagliptin, saxagliptin, sitagliptin, vildagliptin	++++
GLP-1 receptor agonists	Dulaglutide, exenatide, liraglutide, lixisenatide, semaglutide	++++
Meglitinides	Nateglinide, repaglinide	++
SGLT2 inhibitors	Canagliflozin, dapagliflozin, empagliflozin, ertugliflozin	++++
Sulfonylureas	Glimepiride, glipizide, glyburide	+
Thiazolidinediones	Pioglitazone	+
	Rosiglitazone	+++
Recombinant human insulin	Humulin regular, NPH and U-500, Novolin regular and NPH	+++
Basal insulin analogues	Degludec, detemir, glargine	++++
Prandial insulin analogues	Aspart, glulisine, lispro	++++
Inhaled insulin	Afrezza	+++

ER, extended release. Cost scale is based on average 2021 retail prices in the U.S. +, <\$10/month; ++, <\$100/month; +++, \$100–\$300/month; ++++, >\$300/month.

and the patient's family or friends should be trained to inject this in an emergency. *Glucagon* is used to treat severe hypoglycemia when the diabetic patient cannot safely consume oral glucose and intravenous glucose is not available.

Until recently, lyophilized preparations of *glucagon*, produced by recombinant DNA technology, were the only available formulation for clinical use. Because native *glucagon* forms fibrils and is unstable in solution for more than a short period, these kits include a diluent for dissolving the peptide before treatment, a feature that delays and confounds use in emergencies. For hypoglycemic reactions, 1 mg is administered intravenously, intramuscularly, or subcutaneously, although in most circumstances, the intramuscular route is most applicable to emergency use. The onset of hyperglycemic action after *glucagon* is 5 to 10 min after injection and is maximal after 15 to 20 min. However, the effect is relatively short-lived and may be attenuated if hepatic stores of glycogen are depleted. After the initial response to glucagon, patients should be given oral glucose or urged to eat to prevent recurrent hypoglycemia. Nausea and vomiting are the most frequent adverse effects.

Recently, three new *glucagon* preparations have become available to treat hypoglycemia (Diana et al., 2021). A nasal formulation is available as single-use, 3-mg delivery devices, and while peak levels are lower and several minutes delayed compared to IM glucagon, in clinical trials, the efficacy in correcting hypoglycemia was comparable. Moreover, in tests of successful use in simulated emergency settings, nasal *glucagon* was superior. In addition, a liquid formulation of native *glucagon* is also in use. *Glucagon* dissolved in dimethyl sulfoxide is provided as an autoinjector or a prefilled syringe. The kinetics and clinical response are identical to older *glucagon* kits, but ease of use, time for successful administration, and proportion of rescued hypoglycemic events were better with the liquid stable *glucagon*. Finally, a *glucagon* analogue, *dasiglucagon* has 7 amino acid substitutions to the native sequence that improve solubility and allow a stable liquid formulation. Also available as a prefilled syringe or autoinjector, *dasiglucagon* has similar pharmacodynamics to native *glucagon*. All forms of *glucagon*, given in the recommended amounts to treat hypoglycemia, have GI side effects, with nausea occurring in 30% to 40% of patients and vomiting in 10% to 20%.

Other Pancreatic Islet–Related Hormones or Drugs

Diazoxide

Diazoxide (see Chapter 32) is an antihypertensive, antidiuretic benzothiadiazine derivative with potent hyperglycemic actions when given orally. *Diazoxide* interacts with the K_{ATP} channel on the β cell membrane and either prevents its closing or prolongs the open time. This effect, opposite to that of the sulfonylureas (see Figure 51–3), inhibits insulin secretion.

The usual oral dose is 3 to 8 mg/kg per day in adults and children and 8 to 15 mg/kg per day in infants and neonates. The drug can cause nausea and vomiting and thus usually is given in divided doses with meals. *Diazoxide* circulates largely bound to plasma proteins and has a $t_{1/2}$ of about 48 h. *Diazoxide* has a number of adverse effects, including retention of Na^+ and fluid, hyperuricemia, hypertrichosis, thrombocytopenia, and leukopenia, which sometimes limit its use. Despite these side effects, the drug may be useful in patients with inoperable insulinomas and in children with neonatal hyperinsulinism.

Somatostatin

Somatostatin is produced by δ cells of the pancreatic islet, by cells of the GI tract, and in the CNS. SST, which circulates primarily as 14– or a 28–amino acid forms, acts through a family of five GPCRs, $SSTR_{1-5}$. SST inhibits a wide variety of endocrine and exocrine secretions, including thyroid-stimulating hormone and growth hormone from the pituitary, and gastrin, motilin, VIP (vasoactive intestinal peptide), glicentin, insulin, glucagon, and pancreatic polypeptide from the GI tract/pancreatic islet. The physiological role of SST has not been defined precisely, but its short $t_{1/2}$ (3–6 min) prevents its use therapeutically. Longer-acting analogues such as *octreotide*, *lanreotide*, or *pasireotide* are useful for treatment of severe secretory diarrhea (see Chapter 54) and carcinoid tumors, glucagonomas, VIPomas, acromegaly, and Cushing disease (see Chapter 50). Gallbladder abnormalities (stones and biliary sludge) occur frequently with chronic use of the SST analogues, as do GI symptoms.

Drug Facts for Your Personal Formulary: *Agents for Diabetes and Hypoglycemia*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Insulin Formulations		
Insulin—short acting (regular)	• Type 1 and type 2 diabetes • Control prandial rise in blood glucose • Acute correction of hyperglycemia • Intravenous infusion for DKA and hyperglycemia in hospitalized setting	• Injected SC, IM, or IV • Onset of action 30–45 min after subcutaneous injection • Duration of action of 4–6 h after subcutaneous injection • Major adverse event: hypoglycemia
Insulin analogues—short acting (lispro, aspart, glulisine)	• Type 1 and type 2 diabetes • Control prandial rise in blood glucose • Used in insulin pump for treatment of diabetes	• Genetically modified to accelerate insulin absorption profile • Injected SC or IM • Onset of action 5–15 min after SC injection • Duration of action of 3–5 h after SC injection • Major adverse event: hypoglycemia
Insulin—long acting (NPH)	• Provide basal insulin in type 1 and type 2 diabetes • Reduce fasting hyperglycemia in type 2 diabetes	• Formulated to prolong insulin absorption • Usually requires twice-daily subcutaneous injection to provide 24-h basal insulin coverage • Combined with short-acting insulin in basal/bolus regimen • Given at bedtime in type 2 diabetes to reduce hepatic glucose production • Duration of action of 8–12 h • Major adverse event: hypoglycemia
Insulin analogues—long acting (glargine, detemir, degludec)	• Provide basal insulin in type 1 and type 2 diabetes	• Genetically modified to prolong absorption • Once-a-day subcutaneous injection → 24-h basal insulin coverage • Combined with shorting-acting insulin in basal/bolus regimen • Duration of action of 18–42 h • Major adverse event: hypoglycemia
Oral Glucose-Lowering Agents		
Biguanides Metformin	• Therapy of type 2 diabetes • Usually initial agent in type 2 diabetes	• Reduce hepatic glucose production • Weight neutral • Do not cause hypoglycemia • Adverse events include diarrhea, nausea, lactic acidosis (black-box warning) • Use cautiously in renal insufficiency, hospitalized patients; temporarily discontinue therapy prior to potential renal insults (e.g., radiocontrast media) • Avoid use in patients with hepatic dysfunction • Can be combined with other agents • Inexpensive
α-Glucosidase inhibitors Acarbose, miglitol, voglibose	• Therapy of type 2 diabetes	• Reduce carbohydrate breakdown in GI tract • Adverse effects: GI flatulence, elevated liver function tests • Can be combined with other agents • Relatively modest glucose lowering
Dipeptidyl peptidase 4 inhibitors Sitagliptin, saxagliptin, linagliptin, alogliptin, vildagliptin	• Therapy of type 2 diabetes	• Prolong action of GLP-1; promotes insulin secretion • Can be combined with other agents • Relatively modest glucose lowering
Insulin secretagogues—sulfonylureas Second generation: glibenclamide, glipizide, and others	• Therapy of type 2 diabetes	• Stimulate insulin secretion • Major adverse event is hypoglycemia • Adjustments needed in renal/liver disease • Can be combined with other agents • Modest weight gain • Inexpensive
Insulin secretagogues—nonsulfonylureas Repaglinide, nateglinide	• Therapy of type 2 diabetes	• ↑ Insulin secretion; quicker onset and shorter duration than sulfonylureas • Major adverse event: hypoglycemia • Adjustments needed in renal/liver disease • Can be combined with other agents
SLGT2 inhibitors Canagliflozin, dapagliflozin, empagliflozin, ertugliflozin	• Therapy of type 2 diabetes	• Prevent glucose reabsorption and promote renal glucose excretion • Mild weight loss and blood pressure reduction • Improve cardiovascular outcomes, reduce heart failure admissions, and improve heart failure outcomes • Slow diabetic nephropathy • Do not cause hypoglycemia • May ↑ rate of lower urinary tract and genital mycotic infections, hypotension, and DKA • Can be combined with other agents

Drug Facts for Your Personal Formulary: *Agents for Diabetes and Hypoglycemia* (continued)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Oral Glucose-Lowering Agents (cont.)		
Thiazolidinediones Rosiglitazone, pioglitazone	• Therapy of type 2 diabetes	• Increase insulin sensitivity • Adverse effects: peripheral edema, CHF, weight gain, fractures, macular edema • Use with caution in CHF, liver disease • Can be combined with other agents
Other Glucose-Lowering Agents		
GLP-1 agonists Dulaglutide, exenatide, liraglutide, lixisenatide, semaglutide	• Therapy of type 2 diabetes	• ↑ Insulin secretion, ↓ gastric emptying, ↓ glucagon • Injected subcutaneously (one oral formulation available) • Often associated with weight loss • Improve cardiovascular outcomes • Adverse events include nausea • Do not use with agents that ↓ GI motility • Risk of hypoglycemia with insulin
Amylin analogue Pramlintide	• Adjunctive therapy with insulin in type 1 and type 2 diabetes	• Slows gastric emptying, decreases glucagon • Injected subcutaneously • ↓ Postprandial glycemia • Often associated with weight loss • Adverse events include nausea • Do not use with agents that ↓ GI motility • Risk of hypoglycemia with insulin
Drugs to Reverse Hypoglycemia		
Glucagon	• Emergency treatment of severe hypoglycemia	• Injected SC, IM, IV, or intranasal • Quickly raises blood glucose • Relaxes smooth muscles of the GI tract • Positive inotropism and chronotropism on heart
Other Pancreatic Islet-Related Hormones or Drugs		
Diazoxide	• Treatment of hypertensive crisis • Treatment of pathologic hyperinsulinemia	• Inhibits insulin secretion • Adverse events include nausea, vomiting, fluid retention, hyperuricemia, hypertrichosis, thrombocytopenia, and leukopenia
Somatostatin analogues Octreotide, lanreotide, pasireotide	• Treatment of carcinoid tumors, glucagonomas, VIPomas, acromegaly, and Cushing disease	• Injected intramuscularly • Inhibits hormone release • Adverse events include gallbladder abnormalities

Abbreviations: CHF, congestive heart failure; DKA, diabetic ketoacidosis.

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Chapter 52

Agents Affecting Mineral Ion Homeostasis and Bone Turnover

Thomas D. Nolin and Peter A. Friedman

PHYSIOLOGY OF MINERAL ION HOMEOSTASIS
- Calcium
- Phosphate

HORMONAL REGULATION OF CALCIUM AND PHOSPHATE HOMEOSTASIS
- Parathyroid Hormone
- Fibroblast Growth Factor 23
- Vitamin D
- Calcitonin

BONE PHYSIOLOGY
- Bone Mass
- Bone Remodeling

DISORDERS OF MINERAL HOMEOSTASIS AND BONE
- Abnormal Calcium Metabolism
- Disturbed Phosphate Metabolism
- Disorders of Vitamin D
- Osteoporosis
- Paget Disease
- Chronic Kidney Disease–Mineral Bone Disease

PHARMACOLOGICAL TREATMENT OF DISORDERS OF MINERAL ION HOMEOSTASIS AND BONE METABOLISM
- Hypercalcemia
- Hypocalcemia and Other Therapeutic Uses of Calcium
- Vitamin D
- Calcitonin
- Bisphosphonates
- Parathyroid Hormone
- Calcium-Sensing Receptor Mimetics
- Fluoride

INTEGRATED APPROACH TO PREVENTION AND TREATMENT OF OSTEOPOROSIS
- Antiresorptive Agents
- Anabolic Agents
- Combination Therapies

This chapter presents a primer on mineral ion homeostasis and the endocrinology of Ca^{2+} and phosphate metabolism, then some relevant pathophysiology, and finally pharmacotherapeutic options in treating disorders of mineral ion homeostasis.

PHYSIOLOGY OF MINERAL ION HOMEOSTASIS

Calcium

Elemental calcium is essential for many biological functions, ranging from muscle contraction and intracellular signaling (see Chapter 3) to blood coagulation (Chapter 36) supporting the formation and continuous remodeling of the skeleton.

Extracellular Ca^{2+} is in the millimolar range, whereas intracellular free Ca^{2+} is maintained at submicromolar levels. Different mechanisms evolved that regulate Ca^{2+} over this 10,000-fold concentration span. Changes in cytosolic Ca^{2+} (whether released from intracellular stores or entering via membrane Ca^{2+} channels) can modulate effector targets, often by interacting with the ubiquitous Ca^{2+}-binding protein *calmodulin*. The rapid association-dissociation kinetics of Ca^{2+} permit effective regulation of cytosolic Ca^{2+} over the range of 100 nM to 1 μM.

The body content of calcium in healthy adult men and women, respectively, is about 1300 and 1000 g, of which more than 99% is in bone and teeth. Ca^{2+} in extracellular fluids is stringently regulated within narrow limits. In adult humans, the normal serum Ca^{2+} concentration ranges from 8.5 to 10.4 mg/dL (4.25–5.2 mEq/L, 2.1–2.6 mM) and includes three distinct chemical forms: *ionized* (50%), *protein bound* (40%), and *complexed* (10%). Thus, whereas total plasma Ca^{2+} concentration is about 2.5 mM, the concentration of ionized Ca^{2+} in plasma is about 1.2 mM. The various pools of Ca^{2+} are illustrated schematically in Figure 52–1.

Albumin accounts for some 90% of the serum Ca^{2+} bound to plasma proteins; a change of plasma albumin concentration of 1.0 g/dL from the normal value of 4.0 g/dL can be expected to alter total Ca^{2+} concentration by about 0.8 mg/dL. The remaining 10% of the serum Ca^{2+} is complexed with small polyvalent anions, primarily phosphate and citrate. Only diffusible Ca^{2+} (i.e., ionized plus complexed) crosses cell membranes. The degree of complex formation depends on the ambient pH and the concentrations of ionized Ca^{2+} and complexing anions. Ionized Ca^{2+} is the physiologically relevant component, mediating calcium's biological effects, and, when perturbed, produces the characteristic signs and symptoms of hypo- or hypercalcemia. Hormones that affect intestinal calcium absorption and renal calcium excretion tightly control the extracellular Ca^{2+} concentration; when needed, these same hormones regulate withdrawal from the large skeletal reservoir.

Calcium Stores

The skeleton contains 99% of total body calcium in a crystalline form resembling the mineral hydroxyapatite; other ions, including Na^+, K^+, Mg^{2+}, and F^-, also are present in the crystal lattice. The steady-state content of Ca^{2+} in bone reflects the net effect of bone resorption and bone formation. Although the bulk of skeletal calcium is not readily available for meeting short-term needs, a rapidly exchangeable calcium pool at the endosteal surface can be both mobilized and serve to sequester acute increases of extracellular calcium.

Calcium Absorption and Excretion

In the U.S., about 75% of dietary Ca^{2+} is obtained from milk and dairy products. Guidelines for daily vitamin D and calcium supplementation (Institute of Medicine, 2011) are shown in Table 52–1. Figure 52–2 illustrates the components of whole-body daily Ca^{2+} turnover. Ca^{2+} enters the body only through the intestine. *Vitamin D–dependent Ca^{2+} transport* occurs in the proximal duodenum, whereas most Ca^{2+} uptake is mediated by *passive absorption* throughout the small intestine. When calcium intake is adequate or high, passive calcium absorption in the jejunum and ileum is the major absorptive process. Conversely, when intake is

Abbreviations

BMD: bone mineral density
CaSR: calcium-sensing receptor
CGRP: calcitonin gene–related peptide
CKD-MBD: chronic kidney disease–mineral bone disease
CTR: calcitonin receptor
CYP: cytochrome P450
DHT: dihydrotachysterol
ERK: extracellular signal–regulated kinase
FGF: fibroblast growth factor
FGF23: fibroblast growth factor 23
FGFR: FGF receptor
FGFR/KL: FGF receptor/klotho
FRS2α: FGFR substrate 2α
GPCR: G protein-coupled receptor
HRT: hormone replacement therapy
HVDDR: hereditary 1,25-dihydroxyvitamin D resistance
Ig: immunoglobulin
IL: interleukin
IP$_3$: inositol triphosphate
KL: klotho
MTC: medullary thyroid carcinoma
NF-κB: nuclear factor kappa B
25-OHD$_3$: 25-OH-cholecalciferol
OPG: osteoprotegerin
NPT2: sodium-dependent phosphate transport protein 2
PDDR: pseudovitamin D–deficiency rickets
P$_i$: inorganic phosphate
PKC: protein kinase C
PLC: phospholipase C
PTH: parathyroid hormone
PTHR: PTH receptor
PTHrP: PTH-related protein
RANK: receptor for activating NF-κB
RANKL: RANK ligand
RDA: recommended daily allowance
REMS: Risk Evaluation and Mitigation Strategy
SERM: selective estrogen receptor modulator
SGK1: serum and glucocorticoid–regulated kinase 1
TK: tyrosine kinase
TRPV6: transient receptor potential cation channel V6
VDDR-1: vitamin D–dependent rickets type I
VDR: vitamin D receptor
XLH: X-linked hypophosphatemia

low, vitamin D–dependent active calcium absorption is upregulated in the duodenum and accounts for the larger proportion of calcium that is absorbed.

This uptake, whether active or passive, is counterbalanced by an obligatory daily intestinal Ca^{2+} loss of about 150 mg/day that reflects the Ca^{2+} content of mucosal and biliary secretions and in sloughed intestinal cells. The efficiency of intestinal Ca^{2+} absorption is inversely related to calcium intake. Thus, a diet low in calcium leads to a compensatory increase in fractional absorption owing partly to activation of vitamin D. In older persons, this response is considerably less robust. Disease states associated with steatorrhea, chronic diarrhea, or malabsorption promote fecal loss of Ca^{2+}. Drugs such as glucocorticoids and phenytoin depress intestinal Ca^{2+} transport.

Urinary Ca^{2+} excretion is the difference between the amount filtered at the glomerulus and the quantity reabsorbed. About 9 g of calcium are filtered each day, of which more than 98% is reabsorbed by the tubules. The efficiency of reabsorption is highly regulated by PTH and is influenced by filtered Na$^+$, the presence of nonreabsorbed anions, and diuretic agents (see Chapter 25). Sodium intake, and therefore Na$^+$ excretion, is directly related to urinary Ca^{2+} excretion. Diuretics that act on the ascending limb of the loop of Henle (e.g., *furosemide*) increase Ca^{2+} excretion. By contrast, thiazide diuretics uncouple the relationship between Na$^+$ and Ca^{2+} excretion, increasing sodium excretion but diminishing calcium excretion. Urinary Ca^{2+} excretion is a direct function of dietary protein intake, presumably owing to the effect of sulfur-containing amino acids on renal tubular function.

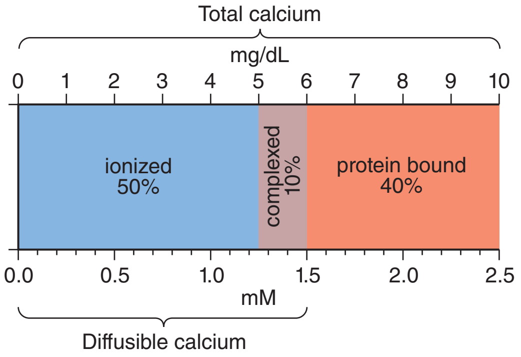


Figure 52–1 *Pools of calcium in serum.* Concentrations are expressed as milligrams per deciliter (top axis) and as millimoles per liter (bottom axis). The total serum calcium concentration is 10 mg/dL or 2.5 mM, divided into three pools: protein bound (40%), complexed with small anions (10%), and ionized calcium (50%). The complexed and ionized pools represent the diffusable forms of calcium that can enter cells.

Phosphate

Phosphate is present in plasma, extracellular fluid, cell membrane phospholipids, intracellular fluid, collagen, and bone tissue. More than 80% of total body phosphorus is found in bone; about 15% is in soft tissue. In addition, phosphate is a dynamic constituent of intermediary and energy metabolism and acts as a key regulator of enzyme activity when

TABLE 52–1 ■ RECOMMENDED DAILY ALLOWANCE OF CALCIUM AND VITAMIN D

LIFE STAGE GROUP	CALCIUM (mg/day)[a]	VITAMIN D (IU/day)[a,b]
Infants 0–6 months	200[c]	400[d]
Infants 6–12 months	260[c]	400[d]
1–3 years old	700	600
4–8 years old	1000	600
9–13 years old	1300	600
14–18 years old	1300	600
19–30 years old	1000	600
31–50 years old	1000	600
51–70 years old	1000	600
51- to 70-year-old females	1200	600
>70 years old	1200	600
14–18 years old, pregnant/lactating	1300	600
19–50 years old, pregnant/lactating	1000	600

[a] Intake covering needs of ≥97.5% of population.
[b] Covers all forms of vitamin D. For details, see Institute of Medicine, 2011.
[c] For infants 0 to 6 months of age, adequate intake is 200 mg/day; it is 260 mg/day for infants 6 to 12 months of age. RDAs have not been established for infants.
[d] For infants 0 to 6 months of age, adequate intake is 400 IU/day; it is 400 IU/day for infants 6 to 12 months of age. RDAs have not been established for infants.

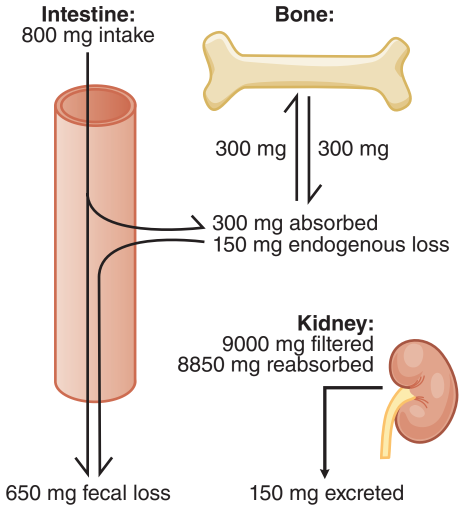


Figure 52–2 *Whole-body daily turnover of calcium.* In healthy adults, calcium intake is equal to calcium excretion, and no net gain or loss of skeletal calcium occurs. Daily dietary calcium intake averages 800 mg. Net intestinal absorption amounts to 150 mg and is balanced by an equivalent amount of calcium excretion by the kidneys. Fecal calcium excretion amounts to 650 mg. In the absence of a challenge to calcium homeostasis such as lactation, the kidneys are the primary site of calcium metabolism. (Adapted with permission from Yanagawa N, Lee DBN. Renal handling of calcium and phosphorus. In: Coe FL, Favus MJ, eds. *Disorders of Bone and Mineral Metabolism*. Raven Press, New York, **1992**, 3–40.)

transferred by protein kinases from ATP to phosphorylatable serine, threonine, and tyrosine residues.

Biologically, phosphorus exists in both organic and inorganic (P_i) forms. Organic forms include phospholipids and various organic esters. In extracellular fluid, the bulk of phosphorus is present as inorganic phosphate in the form of NaH_2PO_4 and Na_2HPO_4. At pH 7.4, the ratio of disodium to monosodium phosphate is 4:1, so plasma phosphate has an intermediate valence of 1.8. Owing to its relatively low concentration in extracellular fluid, phosphate contributes little to buffering capacity. The aggregate level of P_i modifies tissue concentrations of Ca^{2+} and plays a major role in renal H^+ excretion. Within bone, phosphate is complexed with Ca^{2+} as hydroxyapatites and as calcium phosphate.

Absorption, Distribution, and Excretion

Phosphate is an abundant dietary component; even an inadequate diet rarely causes phosphate depletion. Phosphate is extensively absorbed from the GI tract primarily by passive movement (proportional to the concentration in the intestinal lumen), with a smaller fraction mediated by active vitamin D–dependent transport. The fact that most intestinal phosphate absorption is passive may explain why it continues in the presence of hyperphosphatemia, whereas renal phosphate transport is downregulated by elevated phosphate concentrations. The NPT2B Na-phosphate cotransporter mediates active GI phosphate transport, which proceeds through a classic feedback mechanism: Decreases of serum phosphate enhance the biogenesis of vitamin D, which in turn upregulates NPT2B expression.

In adults, about two-thirds of ingested phosphate is absorbed and is excreted almost entirely into the urine. Small amounts of phosphate are secreted into the intestine. In growing children, phosphate balance is positive, and plasma concentrations of phosphate are higher than in adults.

Renal phosphate excretion is the difference between the amount filtered and that reabsorbed. More than 90% of plasma phosphate is freely filtered at the glomerulus, and 80% is reabsorbed, predominantly by proximal tubules. Renal phosphate absorption is regulated by PTH and FGF23 and by other factors, primarily dietary phosphate. Additional hormonal regulators of intestinal phosphate absorption include glucocorticoids, estradiol, and epidermal growth factor. Nonhormonal factors contributing to phosphate homeostasis include extracellular volume and acid-base status.

Dietary phosphate deficiency upregulates renal phosphate transporters and decreases excretion, whereas a high-phosphate diet increases phosphate excretion; these changes are independent of effects on plasma P_i, Ca^{2+}, PTH, or FGF23 (Bourgeois et al., 2013). PTH and FGF23 increase urinary phosphate excretion by blocking phosphate reabsorption. Expansion of plasma volume increases urinary phosphate excretion.

Role of Phosphate in Urine Acidification

Phosphate is concentrated progressively as it traverses the renal tubule and becomes the most abundant buffer system in the distal tubule and terminal nephron. The exchange of H^+ and Na^+ in the tubular urine converts Na_2HPO_4 to NaH_2PO_4, permitting the excretion of large amounts of acid without lowering the urine pH to a degree that would block H^+ transport.

Pharmacological Actions of Phosphate

Phosphate salts are employed as mild laxatives (see Chapter 54) and to acidify the urine and treat hypophosphatemia.

Hormonal Regulation of Calcium and Phosphate Homeostasis

A number of hormones interact to control extracellular Ca^{2+} and phosphate balance. The most important are PTH, FGF23, and *1,25-dihydroxyvitamin* D_3 (*calcitriol*), which regulate mineral homeostasis by effects on the kidney, intestine, and bone (Figure 52–3).

Parathyroid Hormone

Parathyroid hormone is a polypeptide that helps to regulate plasma Ca^{2+} by affecting bone resorption/formation, renal Ca^{2+} excretion/reabsorption, and calcitriol synthesis (thus, GI Ca^{2+} absorption).

HISTORY

Sir Richard Owen, the curator of the British Museum of Natural History, discovered the parathyroid glands in 1852 while dissecting a rhinoceros that had died in the London Zoo. Credit for discovery of the human parathyroid glands usually is given to Sandstrom, a Swedish medical student who published an anatomical report in 1890. In 1891, von Recklinghausen reported a new bone disease, which he termed *osteitis fibrosa cystica*, which Askanazy subsequently described in a patient with a parathyroid tumor in 1904. The glands were rediscovered a decade later by Gley, who determined the effects of their extirpation with the thyroid. Vassale and Generali then successfully removed only the parathyroids and noted that tetany, convulsions, and death quickly followed unless calcium was given postoperatively.

MacCallum and Voegtlin first noted the effect of parathyroidectomy on plasma Ca^{2+}. The relation of low plasma Ca^{2+} concentration to symptoms was quickly appreciated, and a comprehensive picture of parathyroid function began to form. Active glandular extracts alleviated hypocalcemic tetany in parathyroidectomized animals and raised the level of plasma Ca^{2+} in normal animals. For the first time, the relation of clinical abnormalities to parathyroid hyperfunction was appreciated.

Chemistry

Parathyroid hormone is a single polypeptide chain of 84 amino acids with a molecular mass of about 9500 Da. Biological activity is associated with the N-terminal portion of the peptide; residues 1 to 27 are required for optimal binding to the PTHR and hormone activity. Derivatives lacking the first and second residues bind to PTHRs but do not activate the cyclic AMP or IP_3-Ca^{2+} signaling pathways. The PTH fragment lacking the first six amino acids inhibits PTH action.

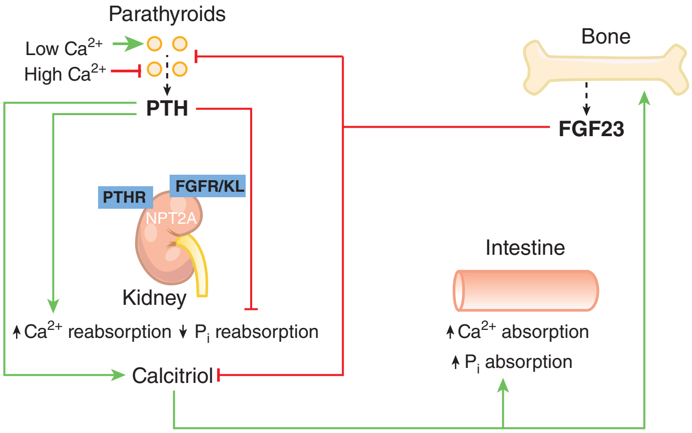


Figure 52–3 *Calcium homeostasis and its regulation by PTH, FGF23, and 1,25-dihydroxyvitamin D.* PTH, released from parathyroids (dotted line), has stimulatory effects on bone and kidney, increasing calcium mobilization and reabsorption, decreasing phosphate reabsorption, and stimulating 1α-hydroxylase activity in kidney mitochondria, leading to the production of 1,25-dihydroxyvitamin D (calcitriol) from 25-hydroxycholecalciferol (Figure 52–6). FGF23, released from bone (dotted line), likewise dampens renal phosphate reabsorption and augments calcium recovery but decreases the production of 1,25-dihydroxyvitamin D by inhibiting 25(OH)1-α-hydroxylase (*CYP27B1*) and increasing metabolism by inducing 1,25(OH)$_2$vitamin D 24-hydroxylase (*CYP24A1*). FGF23 also suppresses PTH release by the parathyroid glands. Calcitriol, the biologically active metabolite of vitamin D, increases intestinal calcium and phosphate absorption and regulates FGF23 synthesis and release and calcium mobilization in bone.

Synthesis and Secretion

Parathyroid hormone is synthesized as a 115–amino acid peptide called *preproparathyroid hormone*, which is converted to *proparathyroid hormone* by cleavage of 25 amino-terminal residues in the endoplasmic reticulum. Proparathyroid hormone is converted in the Golgi complex to PTH by cleavage of six amino acids. PTH(1–84) resides within secretory granules until it is discharged into the circulation. PTH(1–84) has a $t_{1/2}$ in plasma of about 4 min; removal by the liver and kidney accounts for about 90% of its clearance. Proteolysis of PTH generates smaller fragments [e.g., a 33– to 36–amino acid N-terminal fragment that is fully active, a larger C-terminal peptide, and PTH(7–84)]. PTH(7–84) and other amino-truncated PTH fragments are normally cleared from the circulation predominantly by the kidneys, whereas intact PTH is also removed by extrarenal mechanisms. PTH is commonly measured by a two-site immunoassay encompassing the PTH amino terminus and a downstream location. Such "whole" or "intact" PTH assays avoid fragments lacking residues 1 to 6 that circulate normally and accumulate in kidney failure (Smit et al., 2019; Suva and Friedman, 2020).

Physiological Functions and Mechanism of Action

The primary function of PTH is to maintain a constant concentration of Ca^{2+} and P$_i$ in the extracellular fluid. The principal regulated processes are P$_i$ excretion and Ca^{2+} absorption in kidney and mobilization of Ca^{2+} from bone (see Figure 52–3). Renal PTH activity on P$_i$ and Ca^{2+} are spatially separated along the nephron. The actions of PTH on its target tissues are mediated by at least two GPCRs that can couple with G$_s$, G$_q$, and G$_{12/13}$ in cell type–specific manners (Garrido et al., 2009). The type I PTHR, which also binds PTH-related protein (PTHrP) mediates mineral ion homeostasis and the skeletal actions of PTH. A second PTHR expressed in arterial and cardiac endothelium, brain, pancreas, placenta, and elsewhere binds PTH but not PTHrP. A third putative PTHR, designated the cPTH receptor, interacts with carboxy-terminal PTH fragments that are truncated in the amino-terminal region, contain most of the carboxy terminus, and are inactive at the PTH$_1$ receptor; CPTH receptors are expressed on osteocytes (Scillitani et al., 2011).

Regulation of Secretion. *Plasma Ca^{2+} is the major factor regulating PTH secretion.* As the concentration of Ca^{2+} diminishes, PTH secretion increases; hypocalcemia induces parathyroid hypertrophy and hyperplasia. Conversely, if the concentration of Ca^{2+} is high, PTH secretion decreases. Changes in plasma Ca^{2+} regulate PTH secretion by the plasma membrane–associated calcium-sensiing receptor on parathyroid cells. The CaSR is a GPCR that couples with G$_q$ and G$_i$. Occupancy of the CaSR by Ca^{2+} stimulates the G$_q$-PLC-IP$_3$-Ca^{2+}, pathway leading to activation of PKC; this results in inhibition of PTH secretion, an unusual case in which elevation of cellular Ca^{2+} inhibits rather than stimulates secretion (another being the granular cells in the juxtaglomerular complex of the kidney, where elevation of cellular Ca^{2+} inhibits renin secretion). Simultaneous activation of the CaSR-G$_i$ pathway by Ca^{2+} reduces cyclic AMP synthesis and lowers the activity of PKA, also a negative signal for PTH secretion. Conversely, reduced occupancy of CaSR by Ca^{2+} reduces signaling through G$_i$ and G$_q$, thereby promoting PTH secretion. Other agents that increase parathyroid cell cyclic AMP levels, such as β adrenergic receptor agonists and dopamine, also increase PTH secretion, but much less than does hypocalcemia. The active vitamin D metabolite 1,25-dihydroxyvitamin D (*calcitriol*) directly suppresses PTH gene expression. Severe hypermagnesemia or hypomagnesemia can inhibit PTH secretion.

Effects on Bone. Parathyroid hormone exerts both catabolic and anabolic effects on bone. Chronically elevated PTH enhances bone resorption and thereby increases Ca^{2+} delivery to the extracellular fluid, whereas intermittent exposure to PTH promotes anabolic actions. The primary skeletal target cell for PTH is the osteoblast.

Effects on Kidney. In the kidney, PTH enhances the efficiency of Ca^{2+} reabsorption, inhibits tubular reabsorption of phosphate, and stimulates conversion of vitamin D to its biologically active form, 1,25-dihydroxy vitamin D$_3$ (calcitriol; see Figure 52–3). As a result, filtered Ca^{2+} is avidly retained, and its concentration increases in plasma, whereas phosphate is excreted, and its plasma concentration falls. Newly synthesized 1,25-dihydroxy vitamin D$_3$ interacts with specific high-affinity receptors in the intestine to increase the efficiency of intestinal Ca^{2+} absorption, thereby contributing to the increase in plasma Ca^{2+}.

Calcitriol Synthesis. The final step in the activation of vitamin D to calcitriol occurs in kidney proximal tubule cells. Three primary regulators govern the enzymatic activity of the 25-hydroxyvitamin D$_3$–1α-hydroxylase that catalyzes this step: P$_i$, PTH, and Ca^{2+} (see discussion later in this chapter). Reduced circulating or tissue phosphate content rapidly increases calcitriol production, whereas hyperphosphatemia or hypercalcemia suppresses it. PTH powerfully stimulates calcitriol synthesis. Thus, when hypocalcemia causes a rise in PTH concentration, both the PTH-dependent lowering of circulating P$_i$ and a more direct effect of the hormone on the 1α-hydroxylase lead to increased circulating concentrations of calcitriol.

Integrated Regulation of Extracellular Ca^{2+} and Phosphate by PTH. Even modest reductions of serum Ca^{2+} stimulate PTH secretion. For minute-to-minute regulation of Ca^{2+}, adjustments in renal Ca^{2+}

handling suffice to maintain plasma calcium homeostasis. With prolonged hypocalcemia, the renal 1α-hydroxylase is induced, enhancing the synthesis and release of calcitriol that directly stimulates intestinal Ca^{2+} absorption (see Figure 52–3), and delivery of Ca^{2+} from bone into the extracellular fluid is augmented. With prolonged and severe hypocalcemia, new bone-remodeling units are activated to restore circulating Ca^{2+} concentrations, albeit at the expense of skeletal integrity.

When plasma Ca^{2+} activity rises, PTH secretion is suppressed, and tubular Ca^{2+} reabsorption decreases. The reduction in circulating PTH promotes renal phosphate conservation, and both the decreased PTH and the increased phosphate depress calcitriol production and thereby decrease intestinal Ca^{2+} absorption. Finally, bone remodeling is suppressed. These integrated physiological events ensure a coherent response to positive or negative excursions of plasma Ca^{2+} concentrations.

Fibroblast Growth Factor 23

Fibroblast growth factor 23 is a member of the FGF19 family of endocrine FGFs. Osteocytes and other bone cells, including osteoblasts, and lining cells are the primary source of FGF23. FGF23 is secreted in response to dietary phosphorus load and to changes in serum phosphate and 1,25-dihydroxyvitamin D. *Its main function is the promotion of urinary phosphate excretion and the suppression of active vitamin D production by the kidney*. FGF23, unlike PTH, also suppresses intestinal phosphate absorption.

Synthesis and Secretion

Fibroblast growth factor 23 is synthesized as a 251–amino acid peptide. Cleavage of the amino-terminal signal sequence and glycosylation produce the active protein consisting of an amino-terminal core sequence and a shorter carboxy-terminal fragment (Figure 52–4). FGF23 is extensively O-glycosylated, which shields it from proteolysis and enhances secretion. FGF23 possesses a furin cleavage site, ^{176}RXXR^{179}. Hydrolysis produces FGF23 fragments that are biologically inactive or act as antagonists. Phosphorylation at Ser^{180} inhibits O-glycosylation, thereby making FGF23 more susceptible to protease degradation.

Regulated FGF23 proteolysis may contribute to maintaining serum phosphate levels by generating more of a carboxy-terminal cFGF23 peptide (cFGF23) relative to the amount of iFGF23 (intact FGF23). cFGF23 is now thought to inhibit FGF23 action by competitively blocking binding to FGFRs and klotho.

Unlike paracrine FGFs that require heparan sulfate for FGFR activation, FGF23 (and FGF19) binds heparan sulfate with very low affinity, and it is not necessary for signal transduction. FGF23 inhibits renal phosphate transport by activating FGFRs through a mechanism that entails the transmembrane form of α-klotho. *Klotho is as an essential coreceptor for FGFR activation and transduction of FGF23 signaling*. Cleaved klotho increases FGF23 formation, while FGF23 decreases expression of transmembrane klotho (Smith et al., 2012). FGFRs are receptor TKs consisting of an extracellular domain with immunoglobulin-like loops and an intracellular region that harbors TK domains (see Figure 52–4). The amino-terminal portion of FGF23 binds the FGFR, while the carboxy-terminal segment binds to klotho. This ternary complex (FGFR, klotho, and FGF23) then dimerizes, thereby enabling intracellular TK autophosphorylation of FGFR. Intracellular signaling is achieved primarily through ERKs and PLC.

Regulation of Secretion. Fibroblast growth factor 23 is released from bone into the circulation in response to elevations of serum phosphate, PTH, and 1,25 vitamin D. The sensing mechanism responding to these changes and its control over FGF23 secretion is unknown. Iron deficiency also influences FGF23 levels. FGF23 is normally released by both as iFGF23 and inactive cFGF23 fragments. In healthy individuals, low serum iron is associated with elevated cFGF23 levels, but not with iFGF23. The ratio of iFGF23:cFGF23 varies appreciably in disease states and in inherited disorders of FGF23 metabolism (Wolf and White, 2014).

Physiological Functions and Mechanism of Action

Effects on Kidney. Fibroblast growth factor 23 increases phosphate excretion and lowers vitamin D formation. The phosphaturic action of FGF23 is mediated by FGFR1c and to a lesser degree by FGFR4 (Gattineni et al., 2014). FGF23 and PTH reduce phosphate by mechanisms that involve endocytosis and downregulation of NPT2A and NPT2C, the principal Na-phosphate cotransporters that mediate phosphate retrieval from the tubular fluid. *Notably, although FGF23 and PTH exert parallel inhibitory actions on phosphate excretion, they have opposite effects on vitamin D metabolism. PTH stimulates the formation of the active form*

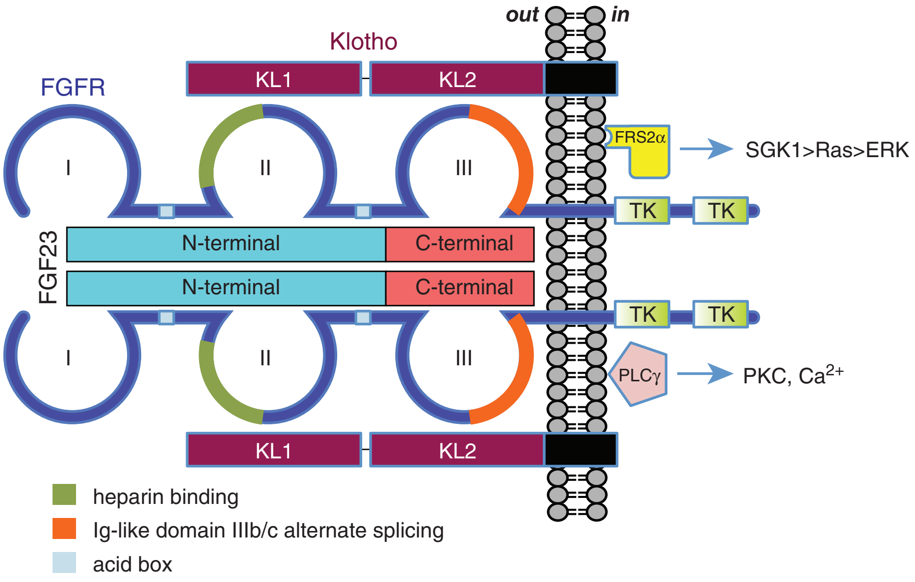


Figure 52–4 *FGF23-FGFR-Klotho complex.* FGF23 binding to the FGFR + klotho complex promotes dimerization of the ternary complex. FGFRs possess three Ig-like domains (I, II, III). Alternative splicing in domain III gives rise to FGFR1/2/3 isoforms. FGFR1c is believed to be the FGF23 receptor. FGF23 requires α-klotho to bind and activate FGFR1. FGF23 consists of a larger N-terminal region and shorter C-terminal segment. Intracellular proteolysis and inactivation of FGF23 occur at a site separating the two domains. Klotho is a transmembrane protein with two large extracellular KL1 and KL2 domains that can be cleaved by a disintegrin and metalloproteinase 10 and 17 (ADAM10/17). Full-length klotho and the shed extracellular domain of KL have distinct functions. Notably, full-length klotho is a coreceptor for FGFR1. Intracellular TK domains on FGFRs mediate downstream signaling, primarily through parallel pathways involving FRS2α, SGK1, ERK1/2 for FGF23 (Andrukhova et al., 2012), and PLCγ (which activates signaling by Ca^{2+}-activating PKC) (Ornitz and Itoh, 2015).

of vitamin D, whereas FGF23 reduces vitamin D levels by augmenting its metabolism to inactive forms.

Exogenous FGF23 administration reduces serum P_i and calcitriol synthesis. Although no clinical agents based on FGF23 have yet been developed, bioactive fragments or FGF23 inhibitors might become useful in counterbalancing the hyperphosphatemic actions of vitamin D therapy. The novel recombinant human IgG monoclonal antibody KRN23, which binds FGF23 and inhibits its activity, can increase P_i reabsorption and serum concentrations of P_i and calcitriol in patients with XLH (Carpenter et al., 2014; Imel et al., 2015).

Soluble klotho, a circulating cleavage product of membrane-associated klotho, when administered to mice, increased serum levels of FGF23 and reduced bone mineral content with a concomitant increase in fracture incidence (Smith et al., 2012). These findings suggest that a scavenging antibody directed to the soluble klotho fragment might provide an additional means to reduce FGF23 levels in patients with secondary hyperparathyroidism or chronic kidney disease-mineral bone disease (CKD-MBD).

Vitamin D

HISTORY

Prior to the discovery of vitamin D, a high percentage of urban children living in temperate zones developed rickets. Some researchers believed that the disease was due to lack of fresh air and sunshine; others claimed a dietary factor was responsible. Mellanby and Huldschinsky showed both notions to be correct; addition of cod liver oil to the diet or exposure to sunlight prevented or cured the disease. In 1924, it was found that ultraviolet irradiation of animal rations was as efficacious at curing rickets as was irradiation of the animal itself. These observations led to the elucidation of the structures of chole- and ergo-calciferol and eventually to the discovery that these compounds require further processing in the body to become active. The discovery of metabolic activation is attributable primarily to studies conducted in the laboratories of DeLuca and Kodicek (DeLuca, 1988).

Chemistry and Occurrence

Vitamin D is a hormone rather than a vitamin, and it plays an active role in Ca^{2+} homeostasis. The biological actions of vitamin D are mediated by the vitamin D receptor (VDR) a nuclear receptor. Vitamin D is the name applied to two related fat-soluble substances, vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). Vitamin D_3 has a potency about 10-fold greater than that of vitamin D_2. This difference is likely attributable to the longer $t_{1/2}$ of vitamin D_3 and lower affinity of vitamin D_2 metabolites for the vitamin D–binding protein (Jones et al., 2014), dispelling the long-held notion that there is no practical difference between vitamin D_2 and vitamin D_3. The total concentration of serum 25-hydroxyvitamin D (D_2 + D_3) is now accepted as the clinical parameter for assessing vitamin D status and functional adequacy of vitamin D treatments.

The primary provitamin found in animal tissues is *7-dehydrocholesterol*, which is synthesized in the skin. Exposure of the skin to sunlight converts 7-dehydrocholesterol to *cholecalciferol* (vitamin D_3). *Ergosterol*, present only in plants and fungi, is the provitamin for *ergocalciferol* (vitamin D_2). Vitamin D_2 is the active constituent of a number of commercial vitamin preparations and is in irradiated bread and irradiated milk.

Human Requirements and Units

Although sunlight provides adequate vitamin D supplies in the equatorial belt, in temperate climates, insufficient cutaneous solar radiation, especially in winter, may necessitate dietary vitamin D supplementation (Faurschou et al., 2012). Serum levels of vitamin D vary widely, likely reflecting genetic background, diet, latitude, time spent out of doors, body size, developmental stage, and state of health, as well as plasma levels of *vitamin D–binding protein*, a specific α globulin. The actions of vitamin D may differ with the expression of components of the synthetic and action pathways of vitamin D. Other factors contributing to the rise of vitamin D deficiency may include diminished consumption of vitamin D–fortified foods owing to concerns about fat intake; reduced intake of dairy products; an increased prevalence and duration of exclusive breastfeeding (human milk is a poor source of vitamin D); and increased use of sunscreens and decreased exposure to sunlight to reduce the risk of skin cancer and prevent premature aging from exposure to ultraviolet radiation. The recommended amount of sunscreen and SPF advised by the World Health Organization may abolish endogenous vitamin D production (Faurschou et al., 2012). The U.S. Institute of Medicine suggests achieving a serum level for 25-OH vitamin D of 50 nmol/L (20 ng/mL). The most recent recommended daily intakes of vitamin D and calcium are shown in Table 52–1.

Metabolic Activation

Vitamin D requires modification to become biologically active. The primary active metabolite, 1α,25-dihydroxy vitamin D (*calcitriol*), is the product of successive hydroxylations (Figure 52–5).

25-Hydroxylation of Vitamin D. The initial hydroxylation occurs in the *liver* to generate 25-OH-cholecalciferol (25-OHD_3, or *calcifediol*) and 25-OHD_2 (ergocalciferol), respectively. 25-OHD_3 is the major circulating form of vitamin D_3; it has a biological $t_{1/2}$ of 19 days, and normal steady-state concentrations are 15 to 50 ng/mL, whereas 25-OHD_2 has a $t_{1/2}$ of 13 days.

1α-Hydroxylation of 25-OHD. After production in the liver, 25-OHD enters the circulation and is carried by vitamin D–binding globulin. Final activation occurs primarily in the *kidney*, where the enzyme 25-hydroxyvitamin D-1α-hydroxylase (*CYP27B1*) in the proximal tubules converts 25-OHD_3 to *calcitriol*. This process is highly regulated (see Figures 52–3 and 52–5). Dietary deficiency of vitamin D, calcium, or phosphate stimulates 1α-hydroxylation of 25-OHD_3, increasing the formation of biologically active $1,25(OH)_2D_3$. In contrast, when Ca^{2+} concentrations are elevated, 25-OHD_3 is inactivated by 24-hydroxylation. Similar reactions occur with 25-OHD_2 (ergocalciferol). Calcitriol controls 1α-hydroxylase activity by a negative-feedback mechanism that involves a direct action on the kidney, as well as inhibition of PTH secretion. The plasma $t_{1/2}$ of calcitriol is estimated at 3 to 5 days in humans.

24-Hydroxylation of Calcitriol. The 25(OH) vitamin D–24-hydroxylase enzyme *CYP24A1* catalyzes several steps of $1,25(OH)_2D_3$ degradation. *CYP24A1* is upregulated by FGF23 and calcitriol and downregulated by PTH.

Physiological Functions and Mechanism of Action

Calcitriol augments absorption and retention of Ca^{2+} and phosphate and thereby helps to maintain normal concentrations of Ca^{2+} and phosphate in plasma. Calcitriol facilitates absorption of Ca^{2+} and phosphate in the small intestine, interacts with PTH to enhance their mobilization from bone, and decreases their renal excretion. The actions of calcitriol are mediated by the nuclear receptor VDR, a member of the steroid and thyroid hormone nuclear receptor superfamily. Calcitriol binds to cytosolic VDRs within target cells; the VDR-hormone complex translocates to the nucleus and interacts with DNA to modify gene transcription. Calcitriol also exerts rapid, nongenomic effects. These actions also involve the VDR but at an alternative site where calcitriol binds in a planar configuration.

Calcium is absorbed predominantly from the duodenum. In the absence of calcitriol, GI calcium absorption is inefficient and involves passive diffusion via a paracellular pathway. Ca^{2+} absorption is potently augmented by calcitriol. It is likely that calcitriol enhances all three steps involved in intestinal Ca^{2+} absorption (Kellett, 2011):

- Entry across mucosal membranes mediated by TRPV6 and $Ca_V1.3$ Ca^{2+} channels
- Diffusion through the enterocytes
- Active extrusion across serosal plasma membranes

Calcitriol upregulates the synthesis of FGF23, calbindin-D_{9K}, calbindin-D_{28K}, and the serosal plasma membrane Ca^{2+}-ATPase. Calbindin-D_{9K}

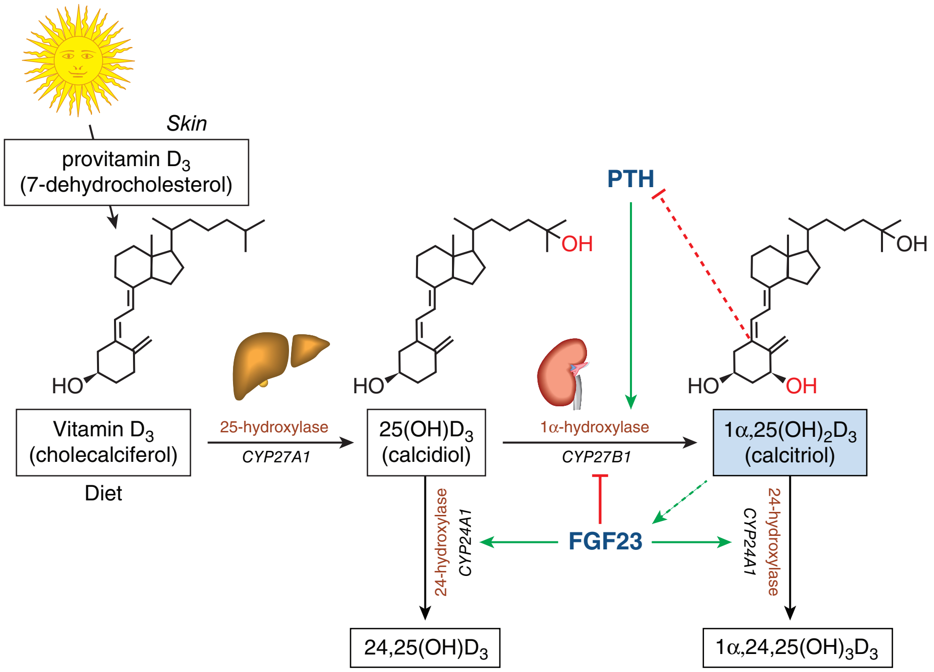


Figure 52–5 *Vitamin D metabolism.* Vitamin D (cholecalciferol) is formed in the skin by solar ultraviolet irradiation of 7-dehydrocholesterol or provided in the diet or by supplements. Sequential hydroxylation at position 25 (red) in the liver to 25(OH)D_3 (calcidiol) and at position 1 (red) in the kidneys produces biologically active 1α,25$(OH)_2D_3$ (calcitriol). Metabolism of calcidiol and calcitriol by 24-hydroxylase reduces serum levels of 1α,25$(OH)_2D_3$. PTH promotes the formation of 1α,25$(OH)_2D_3$, while FGF23 reduces 1α,25$(OH)_2D_3$ levels by stimulating 24-hydroxylation by *CYP24A1* and inhibiting 1α-hydroxylase (*CYP27B1*). Elevated calcitriol levels decrease PTH synthesis by parathyroid glands and stimulate FGF23 release from osteocytes.

HISTORY

Copp observed in 1962 that perfusion of canine parathyroid and thyroid glands with hypercalcemic blood caused transient hypocalcemia that occurred significantly earlier than that caused by total parathyroidectomy. He concluded that the parathyroid glands secreted a calcium-lowering hormone (calcitonin) in response to hypercalcemia and in this way normalized plasma Ca^{2+} concentrations. The physiological relevance of calcitonin has been challenged vigorously: Calcitonin normally circulates at remarkably low levels; surgical removal of the thyroids has no appreciable effect on calcium metabolism; and conditions associated with profound elevations of serum calcitonin concentration are not accompanied by hypocalcemia (Hirsch and Baruch, 2003). The primary interest in calcitonin arises from its pharmacological use in treating Paget disease and hypercalcemia and in its diagnostic use as a tumor marker for medullary carcinoma of the thyroid.

enhances the extrusion of Ca^{2+} by the Ca^{2+}-ATPase; the precise function of calbindin-D_{28K} is unsettled.

The primary role of calcitriol is to stimulate intestinal absorption of Ca^{2+}, which in turn indirectly promotes bone mineralization. Hence, PTH and calcitriol act independently to enhance bone resorption. Osteoblasts, the cells responsible for bone formation, express VDR, and calcitriol induces production of several osteoblast proteins, including osteocalcin, a vitamin K–dependent protein that contains γ-carboxyglutamic acid residues, and IL-1, a lymphokine that promotes bone resorption. Thus, the current view is that calcitriol is a bone-mobilizing hormone but not a bone-forming hormone. In a healthy scenario, osteoblast and osteoclast activities are coupled. Osteoporosis is a disease in which that coupling is disturbed; osteoblast responsiveness to calcitriol is profoundly impaired, osteoclast activity predominates, and bone resorption exceeds formation.

Other Effects of Calcitriol. Effects of calcitriol extend well beyond calcium homeostasis. Receptors for calcitriol are distributed widely throughout the body. Calcitriol affects maturation and differentiation of mononuclear cells and influences cytokine production and immune function. Calcitriol inhibits epidermal proliferation, promotes epidermal differentiation, and is used as a treatment of plaque psoriasis (see Chapter 75).

Calcitonin

Calcitonin is a hypocalcemic hormone whose actions generally oppose those of PTH. The thyroid parafollicular C cells produce and secrete calcitonin. Calcitonin is the most potent peptide inhibitor of osteoclast-mediated bone resorption and helps to protect the skeleton during periods of "calcium stress," such as growth, pregnancy, and lactation. Calcitonin acts through the CTR, a GPCR that links to G_s and G_q.

Regulation of Secretion

Calcitonin is a single-chain peptide of 32 amino acids with a disulfide bridge linking cys1 and cys7. Serum [Ca^{2+}] concentrations regulate the biosynthesis and secretion of calcitonin. Calcitonin secretion increases when serum Ca^{2+} is high and decreases when plasma Ca^{2+} is low. Thus, PTH secretion decreases and calcitonin release increases as serum calcium concentrations rise (Figure 52–6). The circulating concentrations of calcitonin are low, normally less than 15 and 10 pg/mL for males and females, respectively. The circulating $t_{1/2}$ of calcitonin is about 10 min. Abnormally elevated levels of calcitonin are characteristic of thyroid C cell hyperplasia and MTC. The calcitonin gene is localized on human chromosome 11p and contains six exons; differential splicing of the exons leads to tissue-specific production of calcitonin, katacalcin, and CGRP.

Bone Physiology

The skeleton is the primary structural support for the body and provides a protected environment for hematopoiesis. It contains both a large, mineralized matrix and a highly active cellular compartment.

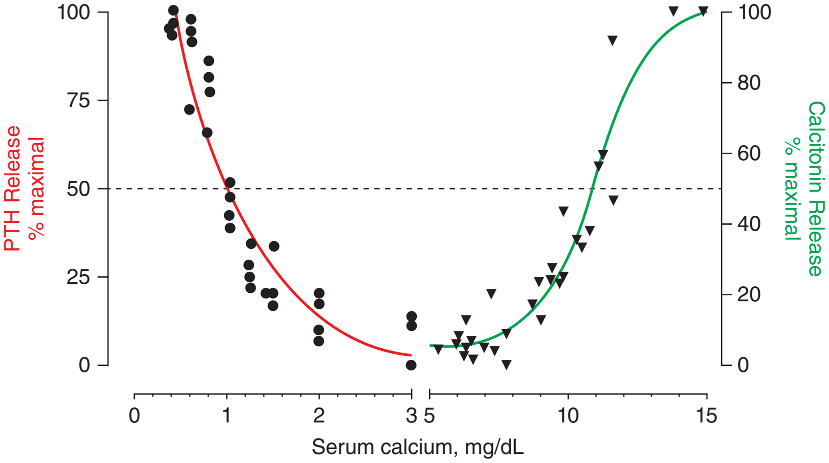


Figure 52–6 *Inverse relations between PTH and calcitonin release.* As serum calcium falls below its set point of about 1.2 mM, PTH secretion increases as a means to defend calcium homeostasis. Conversely, as calcium levels rise, PTH secretion is inhibited, while release of calcitonin increases. (Replotted from Imanishi et al., 2002; Torres et al., 1991.)

Bone Mass

Bone mineral density and fracture risk in later years reflect the maximal bone mineral content at skeletal maturity (peak bone mass) and the subsequent rate of bone loss. Major increases in bone mass, accounting for about 60% of final adult levels, occur during adolescence, mainly during years of highest growth velocity. Inheritance accounts for much of the variance in bone acquisition; other factors include circulating estrogen and androgens, physical activity, and dietary calcium. Bone mass peaks during the third decade, remains stable until age 50, and then declines progressively. In women, loss of estrogen at menopause accelerates the rate of bone loss. *Primary regulators of adult bone mass include physical activity, reproductive endocrine status, and calcium intake. Optimal maintenance of BMD requires sufficiency in all three areas, and deficiency of one is not compensated by excessive attention to another.*

Bone Remodeling

Once new bone is laid down, it is subject to a continuous process of breakdown and renewal called *remodeling*, by which bone mass is adjusted throughout adult life. Remodeling is carried out by myriad independent "bone-remodeling units" throughout the skeleton. In response to physical or biochemical signals, recruitment of marrow precursor cells to the bone surface results in their fusion into the characteristic multinucleated *osteoclasts* that resorb, or excavate, a cavity into the bone. Osteoclast production is regulated by osteoblast-derived cytokines (e.g., IL-1 and IL-6). One important mechanism is RANK and its natural ligand, RANKL (previously called *osteoclast differentiation factor*). On binding to RANK, RANKL induces osteoclast formation (Figure 52–7). RANKL initiates the activation of mature osteoclasts, as well as the differentiation of osteoclast precursors. Osteoblasts produce osteoprotegerin (OPG) which acts as a decoy ligand that inhibits osteoclast production by competing effectively with RANKL for binding to RANK. Under conditions favoring increased bone resorption, such as estrogen deprivation, OPG is suppressed, RANKL binds to RANK, and osteoclast production increases. When estrogen sufficiency is reestablished, OPG increases and competes effectively with RANKL for binding to RANK.

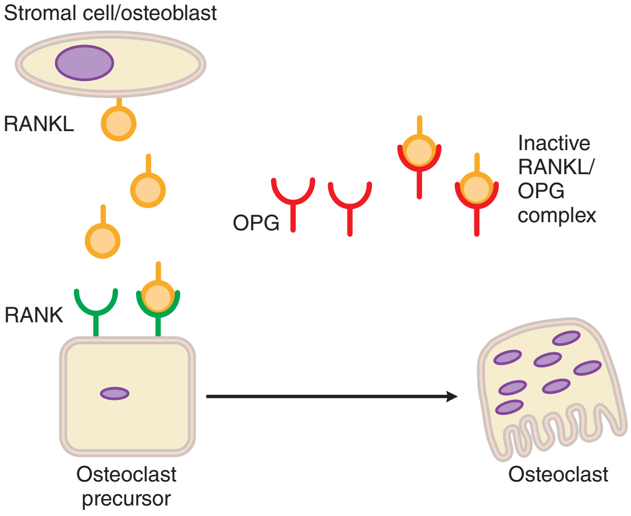


Figure 52–7 *Osteoclast formation.* Receptor for activating RANKL, acting on RANK, promotes osteoclast formation and subsequent resorption of bone matrix. OPG, a decoy receptor, binds to RANKL, reducing its interaction with RANK and thereby inhibiting osteoclast differentiation.

The resorption phase is followed by invasion of preosteoblasts into the base of the resorption cavity. These cells become osteoblasts and elaborate new bone matrix constituents that help form *osteoid*. Once the newly formed osteoid reaches a thickness of about 20 μM, mineralization begins. A complete remodeling cycle normally requires about 6 months. Small bone deficits persist on completion of each cycle, reflecting inefficient remodeling dynamics. Consequently, lifelong accumulation of remodeling deficits underlies the well-documented phenomenon of age-related bone loss, a process that begins shortly after growth stops. *Alterations in remodeling activity represent the final pathway through which diverse stimuli, such as dietary sufficiency, exercise, hormones, and drugs, affect bone balance.*

Disorders of Mineral Homeostasis and Bone

Abnormal Calcium Metabolism

Hypercalcemia

In an outpatient setting, the most common cause of hypercalcemia is primary hyperparathyroidism, which results from hypersecretion of PTH by one or more parathyroid glands. Symptoms and signs of primary hyperparathyroidism include fatigue, exhaustion, weakness, polydipsia, polyuria, joint pain, bone pain, constipation, depression, anorexia, nausea, heartburn, nephrolithiasis, and hematuria. This condition frequently is accompanied by significant hypophosphatemia owing to the effects of PTH in diminishing renal tubular phosphate reabsorption.

Hypercalcemia in hospitalized patients is caused most often by a systemic malignancy, either with or without bony metastasis. PTHrP is a primitive, highly conserved protein that may be abnormally expressed in malignant tissue. PTHrP interacts with the PTHR in target tissues, thereby causing the hypercalcemia and hypophosphatemia seen in humoral hypercalcemia of malignancy (Grill et al., 1998). In some patients with lymphomas, hypercalcemia results from overproduction of 1,25-dihydroxyvitamin D by the tumor cells owing to stimulation of 25(OH) vitamin D–1α-hydroxylase.

Vitamin D excess may cause hypercalcemia if sufficient 25-OHD is present to stimulate intestinal Ca^{2+} hyperabsorption, leading to hypercalcemia and suppressing PTH and 1,25-dihydroxyvitamin D levels. Measurement of 25-OHD is diagnostic. Occasionally, patients with *hyperthyroidism* show mild hypercalcemia, presumably owing to increased bone turnover. *Immobilization* may lead to hypercalcemia in growing children and young adults but rarely causes hypercalcemia in older individuals unless bone turnover is already increased, as in Paget disease or hyperthyroidism. Hypercalcemia sometimes is noted in adrenocortical deficiency, as in Addison disease, or following removal of a hyperfunctional adrenocortical tumor. Hypercalcemia occurs following renal transplantation owing to persistent hyperfunctioning parathyroid tissue that resulted from the previous renal failure. Serum assays for PTH, PTHrP, and 25-OH- and 1,25-$(OH)_2D$ permit accurate diagnosis in the great majority of cases.

Hypocalcemia

Combined deprivation of Ca^{2+} and vitamin D, as observed with malabsorption states, readily promotes hypocalcemia. When caused by malabsorption, hypocalcemia is accompanied by low concentrations of phosphate, total plasma proteins, and magnesium. Mild hypocalcemia (i.e., serum Ca^{2+} in the range of 8–8.5 mg/dL [2–2.1 mM]) is usually asymptomatic. Patients exhibit greater symptoms if the hypocalcemia develops acutely.

Symptoms of hypocalcemia include tetany and related phenomena, such as paresthesias, increased neuromuscular excitability, laryngospasm, muscle cramps, and tonic-clonic convulsions. In chronic *hypoparathyroidism*, ectodermal changes (e.g., consisting of loss of hair, grooved and brittle fingernails, defects of dental enamel, and cataracts) occur. Psychiatric symptoms such as emotional lability, anxiety, depression, and delusions often are present. Hypoparathyroidism is most often a consequence of thyroid or neck surgery but also may be due to genetic or autoimmune disorders. *Pseudohypoparathyroidism* is a family of various hypocalcemic and hyperphosphatemic disorders. Pseudohypoparathyroidism results from resistance to PTH; this resistance is due to mutations in $G_s\alpha$ (*GNAS1*), which normally mediates hormone-induced adenylyl cyclase activation (Bastepe, 2008). Multiple hormonal abnormalities have been associated with the *GNAS1* mutation, but none is as severe as the deficient response to PTH.

Disturbed Phosphate Metabolism

Dietary inadequacy rarely causes phosphate depletion. Sustained use of antacids, however, can severely limit phosphate absorption and result in clinical phosphate depletion, manifest as malaise, muscle weakness, and osteomalacia (see Chapter 53). *Osteomalacia* is characterized by undermineralized bone matrix and may occur when sustained phosphate depletion is caused by inhibiting its absorption in the GI tract (as with aluminum-containing antacids) or by excess renal excretion owing to PTH action. *Hyperphosphatemia* occurs commonly in CKD. The increased phosphate level reduces the serum Ca^{2+} concentration, which in turn activates the parathyroid gland CaSR, stimulates PTH secretion, and exacerbates the hyperphosphatemia. The CaSR agonists *cinacalcet* and *etelcalcetide* suppress PTH secretion and are approved as treatment of secondary hyperparathyroidism in adult patients with CKD receiving chronic dialysis therapy. Another CaSR agonist, *evocalcet*, is under investigation for treatment of secondary hyperparathyroidism in adult dialysis patients, and early clinical evidence suggests similar PTH lowering to *cinacalcet* (Fukagawa et al., 2018). In addition, *cinacalcet* is approved for treatment of hypercalcemia secondary to parathyroid cancer and for primary hyperparathyroidism in patients unable to be managed surgically. Potential new therapies targeting NPT2A with the small-molecule inhibitor PF-06869206 have been reported (Clerin et al., 2020).

The newly approved recombinant human monoclonal antibody to FGF23, *burosumab*, is extremely effective in correcting the hypophosphatemia associated with XLH, the most common form of hereditary rickets and osteomalacia. Monthly administration provides prolonged restoration of serum phosphate by reducing urinary phosphate excretion without adversely altering serum PTH or calcium levels. *Burosumab* is approved for treatment of XLH in children (Carpenter et al., 2018) and adults (Insogna et al., 2018) and for tumor-induced osteomalacia (Imanishi et al., 2021).

Disorders of Vitamin D

Hypervitaminosis D

The acute or long-term administration of excessive amounts of vitamin D or enhanced responsiveness to normal amounts of the vitamin leads to derangements in calcium metabolism. In adults, hypervitaminosis D results from overtreatment of hypoparathyroidism and from faddist use of excessive doses. The amount of vitamin D necessary to cause hypervitaminosis varies widely. As a rough approximation, continued daily ingestion of 50,000 units or more may result in poisoning. The initial signs and symptoms of vitamin D toxicity are those associated with hypercalcemia.

Vitamin D Deficiency

Vitamin D deficiency results in inadequate absorption of Ca^{2+} and phosphate. The consequent decrease of plasma Ca^{2+} concentration stimulates PTH secretion, which acts to restore plasma Ca^{2+} at the expense of bone. FGF23 increases as well. Plasma concentrations of phosphate remain subnormal because of the phosphaturic effect of increased circulating PTH and FGF23. In children, the result is failure to mineralize newly formed bone and cartilage matrix, causing the defect in growth known as *rickets*. In adults, vitamin D deficiency results in osteomalacia, a disease characterized by generalized accumulation of undermineralized bone matrix. Muscle weakness, particularly of large proximal muscles, is typical and may reflect both hypophosphatemia and inadequate vitamin D action on muscle. Gross deformity of bone occurs only in advanced stages of the disease. Circulating 25-OHD concentrations less than 8 ng/mL are highly predictive of osteomalacia.

Metabolic Rickets and Osteomalacia

The disorders of metabolic rickets and osteomalacia are characterized by abnormalities in calcitriol synthesis or response. Variants include the following:

- *Hypophosphatemic vitamin D–resistant rickets*: Usually, this is an X-linked disorder (XLH) of calcium and phosphate metabolism. Patients experience clinical improvement when treated with large doses of vitamin D, usually in combination with inorganic phosphate.
- *Vitamin D–dependent rickets*, also called *VDDR-1* or *PDDR*: This is an autosomal recessive disease caused by an inborn error of vitamin D metabolism involving defective conversion of 25-OHD to calcitriol due to mutations in CYP1α (1α-hydroxylase).
- *HVDDR*, also called *vitamin D–dependent rickets type II*: This is an autosomal recessive disorder that is characterized by hypocalcemia, osteomalacia, rickets, and total alopecia. Multiple heterogeneous mutations of the VDR cause this variant.
- *CKD-MBD (renal rickets)*: Refers to the disordered bone morphology that attends CKD. The variant is characterized by abnormalities of bone turnover, mineralization, volume, linear growth, or strength, as well as underlying defects in mineral ion, PTH, or vitamin D metabolism.

Osteoporosis

Osteoporosis is a condition of low bone mass and microarchitectural disruption that results in fractures with minimal trauma. Many women (30%–50%) and men (15%–30%) suffer a fracture related to osteoporosis. Characteristic sites of fracture include vertebral bodies, the distal radius, and the proximal femur, but osteoporotic individuals have generalized skeletal fragility, and fractures at sites such as ribs and long bones also are common. Fracture risk increases exponentially with age, and spine and hip fractures are associated with reduced survival.

Osteoporosis can be categorized as *primary* or *secondary*. *Primary osteoporosis* represents two different conditions: *type I osteoporosis*, characterized by loss of trabecular bone owing to estrogen lack at menopause, and *type II osteoporosis*, characterized by loss of cortical and trabecular bone in men and women due to long-term remodeling inefficiency, dietary inadequacy, and activation of the parathyroid axis with age. *Secondary osteoporosis* is due to systemic illness or chronic use of medications such as glucocorticoids or *phenytoin*. The most successful approaches to secondary osteoporosis are prompt resolution of the underlying cause and drug discontinuation. Whether primary or secondary, osteoporosis is associated with characteristic disordered bone remodeling, so the same therapies can be used in both conditions.

Paget Disease

Single or multiple sites of disordered bone remodeling characterize Paget disease. It affects up to 2% to 3% of the population more than 60 years of age. The primary pathological abnormality is increased bone resorption followed by exuberant bone formation. However, the newly formed bone is disorganized and of poor quality, resulting in characteristic bowing, stress fractures, and arthritis of joints adjoining the involved bone. The altered bone structure can produce secondary problems, such as deafness, spinal cord compression, high-output cardiac failure, and pain. Malignant degeneration to osteogenic sarcoma is a rare but lethal complication of Paget disease.

Chronic Kidney Disease–Mineral Bone Disease

Bone disease is a frequent consequence of CKD and dialysis treatment. Pathologically, lesions are typical of hyperparathyroidism (osteitis fibrosa), vitamin D deficiency (osteomalacia), or a mixture of both. The underlying pathophysiology reflects increased serum phosphate and decreased calcium, leading to the loss of bone.

Pharmacological Treatment of Disorders of Mineral Ion Homeostasis and Bone Metabolism

Hypercalcemia

Hypercalcemia can be life threatening. Such patients frequently are severely dehydrated because hypercalcemia compromises renal concentrating mechanisms. Thus, fluid resuscitation with large volumes of isotonic saline must be early and aggressive (6–8 L/day). Agents that augment Ca^{2+} excretion, such as loop diuretics (see Chapter 25), may help to counteract the effect of plasma volume expansion by saline but are contraindicated until volume is repleted.

Corticosteroids administered at high doses (e.g., 40–80 mg/day of *prednisone*) may be useful when hypercalcemia results from sarcoidosis, lymphoma, or hypervitaminosis D (see Chapter 50). The response to steroid therapy is slow; from 1 to 2 weeks may be required before plasma Ca^{2+} concentration falls. *Calcitonin* may be useful in managing hypercalcemia. Reduction in Ca^{2+} can be rapid, although “escape” from the hormone commonly occurs within several days. The recommended starting dose is 4 units/kg of body weight administered subcutaneously every 12 h; if there is no response within 1 to 2 days, the dose may be increased to a maximum of 8 units/kg every 12 h. If the response after 2 more days still is unsatisfactory, the dose may be increased to a maximum of 8 units/kg every 6 h. *Calcitonin* can lower serum calcium by 1 to 2 mg/dL.

Intravenous *bisphosphonates* (*pamidronate, zoledronate*) have proven very effective in the management of hypercalcemia (see further material for discussion of bisphosphonates). These agents potently inhibit osteoclastic bone resorption. *Pamidronate* is given as an intravenous infusion of 60 to 90 mg over 4 to 24 h. With *pamidronate*, resolution of hypercalcemia occurs over several days, and the effect usually persists for several weeks. *Zoledronate* has largely superseded *pamidronate* because of its more rapid normalization of serum Ca^{2+} and longer duration of action.

Plicamycin (*mithramycin*; discontinued in the U.S.) is a cytotoxic antibiotic that also decreases plasma Ca^{2+} concentrations by inhibiting bone resorption. Reduction in plasma Ca^{2+} concentrations occurs within 24 to 48 h when a relatively low dose of this agent is given (15–25 μg/kg of body weight) to minimize the high systemic toxicity of the drug; indeed, its toxicity generally precludes its use.

Once the hypercalcemic crisis has resolved, or in patients with milder calcium elevations, long-term therapy is initiated. Parathyroidectomy remains the only definitive treatment of primary hyperparathyroidism. As described further in this chapter, a calcium mimetic that stimulates the CaSR is an effective therapeutic option for hyperparathyroidism. Therapy of hypercalcemia of malignancy ideally is directed at the underlying cancer. When this is not possible, parenteral bisphosphonates often will maintain Ca^{2+} levels within an acceptable range.

Hypocalcemia and Other Therapeutic Uses of Calcium

Calcium is used in the treatment of calcium deficiency states and as a dietary supplement. Hypoparathyroidism is treated primarily with vitamin D and various calcium salts. *Calcium chloride* ($CaCl_2 \cdot 2H_2O$) contains 27% Ca^{2+}; it is valuable in the treatment of hypocalcemic tetany and laryngospasm. The salt is given intravenously and *must never be injected* into tissues. Injections of calcium chloride are accompanied by peripheral vasodilation and a cutaneous burning sensation. The usual intravenous preparation is a 10% solution (equivalent to 1.36 mEq Ca^{2+}/mL). The rate of injection should be slow (not more than 1 mL/min) to prevent cardiac arrhythmias from a high concentration of Ca^{2+}. The injection may induce a moderate fall in blood pressure owing to vasodilation.

Calcium gluceptate injection (a 22% solution; 18 mg or 0.9 mEq of Ca^{2+}/mL; not available in the U.S.) is administered intravenously at a dose of 5 to 20 mL for the treatment of severe hypocalcemic tetany. *Calcium gluconate* injection (a 10% solution; 9.3 mg of Ca^{2+}/mL) given intravenously is the treatment of choice for severe hypocalcemic tetany. Patients with moderate-to-severe hypocalcemia are typically treated by intravenous infusion of calcium gluconate at a dose of 10 to 15 mg of Ca^{2+}/kg of body weight over 4 to 6 h. Because the usual 10-mL vial of a 10% solution contains only 93 mg Ca^{2+}, many vials are needed. Treatment with intravenous Ca^{2+}, administered as calcium gluconate (10–30 mL of a 10% solution), also may be lifesaving in patients with extreme hyperkalemia (serum K^+ >7 mEq/L).

Additional FDA-approved uses of intravenous Ca^{2+} include treatment of black widow spider envenomation and management of magnesium toxicity. The intramuscular route should not be employed because abscess formation at the injection site may result.

For control of milder hypocalcemic symptoms, oral medication suffices, frequently in combination with vitamin D or one of its active metabolites. Calcium carbonate is relatively inexpensive and well tolerated, so it is prescribed most frequently. *Calcium carbonate* and *calcium acetate* are used to restrict phosphate absorption in patients with CKD and oxalate absorption in patients with inflammatory bowel disease. Recombinant full-length rhPTH(1–84) and a modified long-acting form of human PTH (LA-PTH) are novel therapies for managing chronic hypoparathyroidism that improve quality of life (Cusano et al., 2015).

Vitamin D

The physiology and corresponding mechanism of action of vitamin D were described previously in this chapter.

ADME

Vitamin D is absorbed from the small intestine. Bile is essential for adequate absorption of vitamin D and is also the primary route of vitamin D excretion. Patients who have intestinal bypass surgery or inflammation of the small intestine may fail to absorb vitamin D sufficiently to maintain normal levels; hepatic or biliary dysfunction also may seriously impair vitamin D absorption. Absorbed vitamin D circulates in the blood in association with vitamin D–binding protein. The vitamin disappears from plasma with a $t_{1/2}$ of 20 to 30 h but is stored in fat depots for prolonged periods.

Therapeutic Uses for Vitamin D

The major therapeutic uses of vitamin D are:

- Prophylaxis and cure of nutritional rickets
- Treatment of metabolic rickets and osteomalacia, particularly in the setting of CKD
- Treatment of hypoparathyroidism
- Prevention and treatment of osteoporosis
- Dietary supplementation

Nutritional Rickets. Nutritional rickets results from inadequate exposure to sunlight or deficiency of dietary vitamin D. The incidence of this condition in the U.S. is now increasing. Infants and children receiving adequate amounts of vitamin D–fortified food do not require additional vitamin D; however, breastfed infants or those fed unfortified formula should receive 400 units of vitamin D daily as a supplement (see Table 52–1) (Wagner and Greer, 2008), usually administered with vitamin A, for which purpose a number of balanced vitamin A and D preparations are available. *Because the fetus acquires more than 85% of its calcium stores during the third trimester, premature infants are especially susceptible to rickets and may require supplemental vitamin D*. Treatment of fully developed rickets requires a larger dose of vitamin D than that used prophylactically. One thousand units daily will normalize plasma Ca^{2+} and phosphate concentrations in about 10 days, with radiographic evidence of healing in about 3 weeks. However, a larger dose of 3000 to 4000 units daily often is prescribed for more rapid healing, particularly when severe thoracic rickets compromises respiration.

Treatment of Osteomalacia and CKD-MBD. Osteomalacia, distinguished by undermineralization of bone matrix, occurs commonly during sustained phosphate depletion. Patients with CKD are at risk for developing osteomalacia but also may develop a complex bone disease called CKD-MBD, formerly known as *renal osteodystrophy*. In this setting, bone metabolism is stimulated by an increase in PTH and by a delay in bone mineralization that is due to decreased renal synthesis of calcitriol. In CKD-MBD, low BMD may be accompanied by high-turnover bone lesions, typically seen in patients with uncontrolled hyperparathyroidism or by low bone remodeling activity seen in patients with adynamic bone disease.

The therapeutic approach to the patient with CKD-MBD depends on its skeletal manifestation. In high-turnover (hyperparathyroid) or mixed high-turnover disease with deficient mineralization, dietary phosphate restriction, generally in combination with a phosphate binder, is recommended. Administration of calcium-containing phosphate binders along with calcitriol may contribute to oversuppression of PTH secretion and likewise result in adynamic bone disease. The increased calcium burden associated with calcium-based phosphate binders likely contributes to the increased incidence of vascular calcification in patients with CKD.

Non–calcium-containing phosphate binders are highly effective alternatives to traditional calcium-based agents. *Sevelamer hydrochloride* is a nonabsorbable polymer that acts as a nonselective anion exchanger. The drug is modestly water soluble, and only trace amounts are absorbed from the GI tract. *Sevelamer* not only effectively lowers serum phosphate concentration in hemodialysis patients but also binds bile acids and, to a lesser extent, low-density lipoprotein cholesterol and fat-soluble vitamins. Side effects of *sevelamer hydrochloride* include vomiting, nausea, diarrhea, dyspepsia, and metabolic acidosis.

Sevelamer carbonate is equivalent to *sevelamer hydrochloride* in terms of safety and tolerability, with a lower likelihood of inducing metabolic acidosis. *Lanthanum carbonate* is a poorly permeable trivalent cation that is highly effective in treating the hyperphosphatemia associated with CKD-MBD but is associated with GI side effects.

Two iron-based phosphate binders also are commercially available. *Sucroferric oxyhydroxide* is a polynuclear iron(III)–oxyhydroxide compound that binds phosphate by ligand exchange. The drug exhibits similar phosphate control efficacy as *sevelamer* with a lower daily pill burden. *Ferric citrate* also exhibits comparable phosphate control efficacy to *sevelamer* and to *calcium acetate*. In addition, *ferric citrate* delivers a significant amount of iron, resulting in increased erythropoietic parameters and the possibility of iron overload with chronic dosing. Diarrhea is a common side effect of both iron-based phosphate binders (Shah et al., 2015).

Niacin and *nicotinic acid* lower serum phosphate and have been proposed as alternatives to the use of *sevelamer*. Extended-release *niacin* does not improve cardiovascular outcomes and is associated with greater all-cause mortality. Although *nicotinic acid* reduces hyperphosphatemia, *sevelamer* exhibits greater efficacy in controlling hyperphosphatemia as well as the Ca·P product (Ahmadi et al., 2012; Kalil et al., 2015).

Hypoparathyroidism. Vitamin D and its analogues are the mainstay of the therapy of hypoparathyroidism. Dihydrotachysterol (DHT), a reduced form of vitamin D_2, has a faster onset, shorter duration of action, and greater effect on bone mobilization than does vitamin D and traditionally has been a preferred agent; however, it is no longer available in the U.S. Although most hypoparathyroid patients respond to any form of vitamin D, *calcitriol* may be preferred for temporary treatment of hypocalcemia while awaiting effects of a slower-acting form of vitamin D.

Prevention and Treatment of Osteoporosis. This is described separately further in the chapter.

Dietary Supplementation. See Table 52–1.

Adverse Effects of Vitamin D Therapy

The primary toxicity associated with *calcitriol* reflects its potent effect to increase intestinal absorption of Ca^{2+} and phosphate, along with the potential to mobilize osseous Ca^{2+} and phosphate. Hypercalcemia, with or without hyperphosphatemia, commonly complicates *calcitriol* therapy and may limit its use at doses that effectively suppress PTH secretion. Noncalcemic vitamin D analogues provide alternative interventions, although they do not obviate the need to monitor serum Ca^{2+} and phosphorus concentrations. Hypervitaminosis D is treated by immediate withdrawal of the vitamin, a low-calcium diet, administration of glucocorticoids, and vigorous fluid support; forced saline diuresis with loop diuretics is also useful. With this regimen, the plasma Ca^{2+} concentration falls to normal, and Ca^{2+} in soft tissue tends to be mobilized. Conspicuous improvement in kidney function occurs unless kidney damage has been severe.

Available Vitamin D Analogues

Cholecalciferol (vitamin D_3) and *calcitriol* (1,25-dihydroxycholecalciferol) are available for oral administration or injection. Several derivatives of vitamin D are also used therapeutically.

Doxercalciferol (1α-hydroxyvitamin D_2), a prodrug that first must be activated by hepatic 25-hydroxylation, is approved for use in treating secondary hyperparathyroidism. *DHT* is a reduced form of vitamin D_2. In the liver, DHT is converted to its active form, 25-OH dihydrotachysterol. DHT is effective in mobilizing bone mineral at high doses; it therefore can be used to maintain plasma Ca^{2+} in hypoparathyroidism. DHT is well absorbed from the GI tract and maximally increases serum Ca^{2+} concentration after 2 weeks of daily administration. The hypercalcemic effects typically persist for 2 weeks but can last twice that long. DHT (not marketed in the U.S.) is available for oral administration in doses ranging from 0.2 to 1 mg/day (average 0.6 mg/day).

Ergocalciferol (calciferol) is vitamin D_2. It is available for oral administration. *Ergocalciferol* is indicated for the prevention of vitamin D deficiency and the treatment of familial hypophosphatemia, hypoparathyroidism, and vitamin D–resistant rickets type II, typically in doses of 50,000 to 200,000 units/day in conjunction with calcium supplements. *1α-Hydroxycholecalciferol* (1-OHD_3, alfacalcidol) is a synthetic vitamin D_3 derivative that is already hydroxylated in the 1α position and is rapidly hydroxylated by 25-hydroxylase to form 1,25-$(OH)_2D_3$. It is equivalent to *calcitriol* in assays for stimulation of intestinal absorption of Ca^{2+} and bone mineralization; it does not require renal activation. It is available in the U.S. for experimental purposes.

Analogues of Calcitriol. Several vitamin D analogues suppress PTH secretion by the parathyroid glands but have less or negligible hypercalcemic activity. They therefore offer a safer and more effective means of controlling secondary hyperparathyroidism.

Calcipotriene (Calcipotriol). *Calcipotriol* is a synthetic derivative of *calcitriol* with a modified side chain. *Calcipotriol* is less than 1% as active as *calcitriol* in regulating Ca^{2+} metabolism. *Calcipotriol* has been studied extensively as a treatment of psoriasis and is available for topical use (see Chapter 75).

Paricalcitol. *Paricalcitol* (1,25-dihydroxy-19-norvitamin D_2) is a synthetic *calcitriol* derivative that lacks the exocyclic C19 and has a vitamin D_2 rather than vitamin D_3 side chain. It reduces serum PTH levels without producing hypercalcemia or altering serum phosphorus (Mazzaferro et al., 2014). *Paricalcitol* administered orally or intravenously is FDA-approved for treating secondary hyperparathyroidism in patients with CKD.

Maxacalcitol. Known variously as 1,25-dihydroxy-22-oxavitamin D_3, OCT, and 22-oxacalcitriol, *maxacalcitol* differs from *calcitriol* only in the substitution of C-22 with an O atom. *Oxacalcitriol* has a low affinity for vitamin D–binding protein; thus, more of the drug circulates in the free (unbound) form and is metabolized more rapidly than *calcitriol*, with a consequent shorter $t_{1/2}$. *Oxacalcitriol* is a potent suppressor of PTH gene expression and shows very limited activity on intestine and bone. It is a useful compound in patients with overproduction of PTH in CKD. *Oxacalcitriol* is not available in the U.S.

Calcitonin

Mechanism of Action

The CTR, a GPCR that couples to multiple G proteins, mediates *calcitonin*'s actions. The hypocalcemic and hypophosphatemic effects of *calcitonin* are caused predominantly by direct inhibition of osteoclastic bone resorption (Henriksen et al., 2010). The *calcitonin* peptide family also includes CGRP, the closely related peptide *adrenomedullin, intermedin*, and *amylin*. CGRP and adrenomedullin are potent endogenous vasodilators.

Diagnostic Use

Calcitonin is a sensitive and specific marker for the presence of MTC, a neuroendocrine malignancy originating in thyroid parafollicular C cells.

Therapeutic Use

Calcitonin lowers plasma Ca^{2+} and phosphate concentrations in patients with hypercalcemia. *Calcitonin* is administered through injection or nasal spray. Although *calcitonin* is effective for up to 6 h in the initial treatment of hypercalcemia, patients become refractory after a few days. This is likely due to receptor downregulation (Henriksen et al., 2010). Use of *calcitonin* does not substitute for aggressive fluid resuscitation, and the bisphosphonates are the preferred agents. *Calcitonin* is effective in disorders of increased skeletal remodeling, such as Paget disease, and in some patients with osteoporosis. For Paget disease, *calcitonin* generally is administered by subcutaneous injection because intranasal delivery is relatively ineffective owing to limited bioavailability. After initial therapy at 100 units/day, the dose typically is reduced to 50 units three times a week. Side effects of *calcitonin* include nausea, hand swelling, urticaria, and, rarely, intestinal cramping. Hypersensitivity reactions, including anaphylaxis, have also been reported.

Bisphosphonates

Chemistry

Bisphosphonates are analogues of pyrophosphate that contain two phosphonate groups attached to a geminal (central) carbon that replaces the oxygen in pyrophosphate (Figure 52–8). These agents form a three-dimensional structure capable of chelating divalent cations such as Ca^{2+} and have a strong affinity for bone, targeting especially bone surfaces undergoing remodeling. *First-generation bisphosphonates* (*medronate*, *clodronate*, and *etidronate*) contain minimally modified side chains or possess a chlorophenol group (*tiludronate*) and are the least-potent agents. *Second-generation aminobisphosphonates* (e.g., *alendronate* and *pamidronate*) contain a nitrogen group in the side chain and are 10 to 100 times more potent than first-generation compounds. *Third-generation bisphosphonates* (e.g., *risedronate* and *zoledronate*) contain a nitrogen atom within a heterocyclic ring and are up to 10,000 times more potent than first-generation agents (Ebetino et al., 2011).

Mechanism of Action

Bisphosphonates directly inhibit bone resorption. They concentrate at sites of active remodeling, remain in the matrix until the bone is remodeled, and then are released in the acid environment of the resorption lacunae and induce apoptosis in osteoclasts. Although bisphosphonates prevent hydroxyapatite dissolution, their antiresorptive action is due to direct inhibitory effects on osteoclasts rather than strictly physiochemical effects (Cremers and Papapoulos, 2011). The antiresorptive activity apparently involves two primary mechanisms: osteoclast apoptosis and inhibition of components of the cholesterol biosynthetic pathway.

ADME

All oral bisphosphonates are poorly absorbed from the intestine. They have remarkably limited bioavailability (<1% [*alendronate*, *risedronate*] to 6% [*etidronate*, *tiludronate*]), which is further reduced by food and medications containing divalent cations such as calcium supplements, antacids, and iron. Hence, these drugs should be administered with a full glass of water following an overnight fast and at least 30 min before breakfast. Bisphosphonates distribute extensively into bone, undergo negligible hepatic clearance, and are excreted unchanged by the kidneys. Renal excretion of bisphosphonates declines proportionally with kidney function, and they are not recommended for patients with a creatinine clearance of less than 30 mL/min (Cremers and Papapoulos, 2011; Ott, 2015).

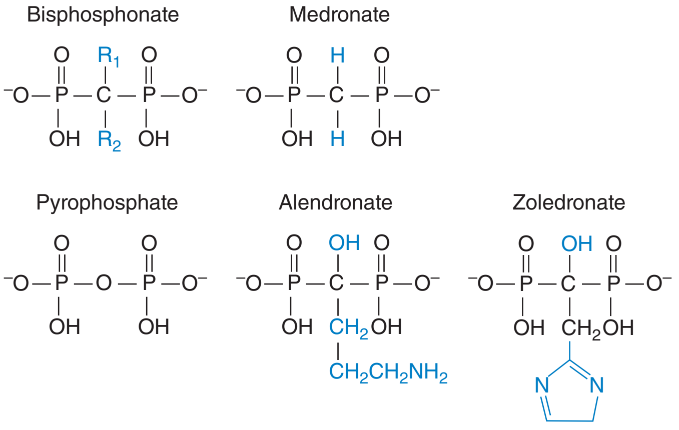


Figure 52–8 *Pyrophosphate and bisphosphonates.* The substituents (R_1 and R_2) on the central carbon of the bisphosphonate parent structure are shown in blue. Examples of a first-generation bisphosphonate (*medronate*), a second-generation aminobisphosphonate (*alendronate*), and a third-generation bisphosphonate (*zoledronate*) are shown.

Therapeutic Uses

Bisphosphonates are used extensively in conditions characterized by osteoclast-mediated bone resorption, including Paget disease, tumor-associated osteolysis, and hypercalcemia. In particular, much interest is focused on the role of bisphosphonates in the treatment of osteoporosis, including postmenopausal osteoporosis and steroid-induced osteoporosis. Bisphosphonate treatment is associated with increased BMD and protection against fracture. Bisphosphonates may also have direct antitumor action by inhibiting oncogene activation through their antiangiogenic effects. Randomized clinical trials of bisphosphonates in patients with breast cancer suggested that these agents delay or prevent development of metastases as a component of endocrine adjuvant therapy (Early Breast Cancer Trialists' Collaborative, 2015). Oral bisphosphonates have not been used widely in children or adolescents because of uncertainty of long-term effects of bisphosphonates on the growing skeleton.

Adverse Effects

Oral bisphosphonates can cause heartburn, esophageal irritation, or esophagitis. Other GI side effects include abdominal pain and diarrhea. Symptoms often abate when patients take the medication after an overnight fast, with tap or filtered water (not mineral water), and remain upright. Patients with active upper GI disease should not be given oral bisphosphonates. Initial parenteral infusion of *pamidronate* may cause skin flushing, flu-like symptoms, muscle and joint aches and pains, nausea and vomiting, abdominal discomfort, and diarrhea (or constipation) but mainly when given in higher concentrations or at faster rates than those recommended. These symptoms are short lived and generally do not recur with subsequent administration.

Zoledronate can cause severe hypocalcemia and has been associated with nephrotoxicity, deterioration of kidney function, and potential kidney disease. Infusion of *zoledronate*, 4 mg, should be performed over at least 15 min; patients should have standard laboratory and clinical parameters of kidney function assessed prior to treatment and periodically thereafter to monitor for deterioration in kidney function. Bisphosphonate use also is associated with osteonecrosis of the jaw (a rare event, with an incidence of ~2 in 100,000 patient-years in which the precise causal role of bisphosphonates has not been elucidated) as well as stress fractures in the lateral cortex of the femoral shaft (most commonly associated with *alendronate* and rarely with *zoledronate*) (Reid, 2015).

Available Bisphosphonates

Etidronate sodium is used for treatment of Paget disease and may be used parenterally to treat hypercalcemia (although largely supplanted for this use by *amidronate* and *zoledronate*). *Pamidronate* (available in the U.S. only for parenteral administration) is approved for management of hypercalcemia associated with malignancy and Paget disease and for prevention of bone loss in breast cancer and multiple myeloma; it also is effective in other skeletal disorders. For treatment of hypercalcemia, *pamidronate* may be given as an intravenous infusion of 60 to 90 mg over 2 to 24 h.

Several newer bisphosphonates have been approved for treatment of Paget disease. These include *tiludronate*, *alendronate*, and *risedronate*. *Tiludronate* standard dosing is 400 mg/day orally for 3 months. *Tiludronate* in recommended doses does not interfere with bone mineralization, unlike *etidronate*. *Zoledronate* is approved for treating Paget disease; administered as a single 5-mg infusion, *zoledronate* decreases bone turnover markers for 6 months with no loss of therapeutic effect. *Zoledronate* is widely used for prevention of osteoporosis in patients with prostate and breast cancer receiving hormonal therapy. It reduces both vertebral and nonvertebral fractures. A 4-mg formulation is available for intravenous treatment of hypercalcemia of malignancy, multiple myeloma, or bone metastasis resulting from solid tumors. The potent bisphosphonate *ibandronate* is approved for the prevention and treatment of postmenopausal osteoporosis. The recommended oral dose is 2.5 mg daily or 150 mg once monthly.

For patients in whom oral bisphosphonates cause severe esophageal distress, *intravenous zoledronate* and *ibandronate* offer skeletal protection without causing adverse GI effects. For treatment of osteoporosis, *ibandronate* (3 mg) is given intravenously every 3 months. *Zoledronate* is the first bisphosphonate to be approved for once-yearly intravenous treatment of osteoporosis (5 mg annually).

Parathyroid Hormone

Continuous administration of PTH or high-circulating PTH levels achieved in primary hyperparathyroidism causes bone demineralization and osteopenia. However, *intermittent* PTH administration promotes bone growth. Although hypoparathyroidism was the last classic endocrine-deficiency disease to have the missing hormone as an available treatment option, PTH analogues are now available to these patients.

Chemistry

As described previously, PTH is a single-polypeptide chain of 84 amino acids with a molecular mass of about 9500 Da. The classic biological activity of PTH is associated with the N-terminal portion of the peptide; residues 1 to 27 are required for optimal binding to the PTHR and hormone activity. Methionine residues at positions 8 and 18 may be oxidized, reducing PTH biological activity (Ursem et al., 2020). Such oxidized PTH is not detected by routine clinical analysis and may contribute to discrepancies between apparent circulating PTH levels and its action (Hocher et al., 2012; Zeng et al., 2020). Nonetheless, its contribution to skeletal turnover in chronic kidney disease is negligible (Ursem et al., 2021). Currently available PTH analogues include *teriparatide*, a synthetic human 34–amino acid amino-terminal PTH fragment [hPTH(1–34)], and a full-length replica of endogenous PTH, recombinant human PTH consisting of 84 amino acids [rhPTH(1–84)] (Kim and Keating, 2015). The PTHrP(1–34) analogue *abaloparatide* is FDA-approved for treating postmenopausal osteoporosis in women at high risk for fractures (Sleeman and Clements, 2019). Comprehensive skeletal evaluation of BMD in postmenopausal osteoporosis is required prior to initiating treatment. Consensus opinion recommends bisphosphonates in women without contraindications as the primary intervention for reducing hip, nonvertebral, and vertebral fractures (Black and Rosen, 2016). *Teriparatide* (see below) reduces the risk of nonvertebral and vertebral fractures. Preparations of human recombinant PTH (rhPTH) are not currently FDA- or EMA-approved.

Mechanism of Action

The physiological functions and mechanism of action of PTH were described previously in the chapter.

ADME

These agents are peptides and are administered by subcutaneous injection (see Drugs Available). Pharmacokinetics and systemic actions of *teriparatide* on mineral metabolism are the same as for PTH. Serum PTH concentrations peak at 30 min after the injection and are undetectable within 3 h, whereas the serum Ca^{2+} concentration peaks at 4 to 6 h after administration. *Teriparatide* bioavailability averages 95%. The drug's volume of distribution is approximately 0.1 L/kg. The elimination of *teriparatide* proceeds by nonspecific enzymatic mechanisms in the liver, followed by renal excretion. *Teriparatide* systemic clearance averages 62 L/h in women and 94 L/h in men. The elimination $t_{1/2}$ of serum *teriparatide* is about 1 h when administered subcutaneously versus 5 min when administered intravenously.

Abaloparatide is supplied in an injector pen with 30 daily doses. The peptide is rapidly absorbed (bioavailability = 36%), achieving peak concentrations approximately 30 min following subcutaneous injection. Elimination $t_{1/2}$ is approximately 1.7 h; clearance is, presumably, by proteolytic hydrolysis, with renal elimination of peptide fragments.

Therapeutic Uses

Teriparatide [hPTH(1–34)] and *abaloparatide* [PTHrP(1–34)] are the only anabolic agents currently available that increase new bone formation. They are approved for use in treating severe osteoporosis in patients at a high risk for fracture. In postmenopausal women with osteoporosis, *teriparatide* increases BMD and reduces the risk of vertebral and nonvertebral fractures. Candidates for treatment with *teriparatide* and *abaloparatide* include women who have a history of osteoporotic fracture, who have multiple risk factors for fracture, or who failed or are intolerant of previous osteoporosis therapy. Men with primary or hypogonadal osteoporosis are also candidates for treatment with these agents.

Adverse Effects

Adverse effects include hypercalcemia (the incidence with *abaloparatide* is lower than observed with *teriparatide*; Miller et al., 2016), exacerbation of nephrolithiasis, and elevation of serum uric acid levels. Development of osteosarcoma has been a serious concern in patients treated with *teriparatide*; however, postmarketing surveillance suggests that there is no causal association between *teriparatide* use and osteosarcoma (Andrews et al., 2012). Nevertheless, *teriparatide* and *abaloparatide* carry black-box warnings, and use should be limited to no more than 2 years and avoided in patients who are at increased baseline risk for osteosarcoma (including those with Paget disease of bone, unexplained elevations of alkaline phosphatase, open epiphyses, or prior radiation therapy involving the skeleton). Orthostatic hypotension may occur shortly after injection of *abaloparatide*.

Drugs Available

Teriparatide is administered by once-daily subcutaneous injection of 20 μg into the thigh or abdomen. *Abaloparatide* is administered at a starting dose of 80 μg by subcutaneous injection into the periumbilical region of the abdomen. The site of administration should be rotated, but the time at which the injection is made should be the same each day. Subcutaneous administration of *abaloparatide* reduced the risk of new vertebral and nonvertebral fractures over a period of 18 months (Miller et al., 2016). Shorter studies demonstrated increased lumbar spine and hip density that were greater than those achieved with *teriparatide* (Leder et al., 2015).

Long-acting formulations of PTH (LA-PTH) currently are in development (Maeda et al., 2013). Such formulations may offer an advantage over *teriparatide*, which is limited by its short (4- to 6-h) duration of effect on serum Ca^{2+} concentrations. Another agent that consists of the N-terminal biologically active region of PTH linked to a collagen-binding domain exhibited sustained increases in BMD by more than 10% for 1 year in rodents after single-dose administration (Ponnapakkam et al., 2012).

A novel class of drugs that inhibits the CaSR (*calcilytics*) stimulates the secretion of PTH and decrease renal excretion of Ca^{2+}. The calcilytic *ronacaleret*, investigated for potential treatment of postmenopausal osteoporosis, was less effective than *teriparatide*, and its development was subsequently halted (Fitzpatrick et al., 2012). The role of calcilytics in the treatment of diseases involving hypocalcemia or hypercalciuria continues to be explored (Nemeth and Shoback, 2013).

Calcium-Sensing Receptor Mimetics

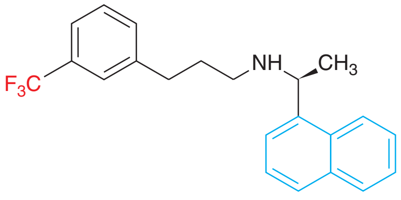
Calcimimetics are drugs that mimic the stimulatory effect of Ca^{2+} on the CaSR to inhibit PTH secretion by the parathyroid glands. *Cinacalcet* and *etelcalcetide*, the only approved drugs in the class currently, offer a pharmacotherapeutic alternative to surgery for the treatment of PTH hypersecretion diseases.

Chemistry

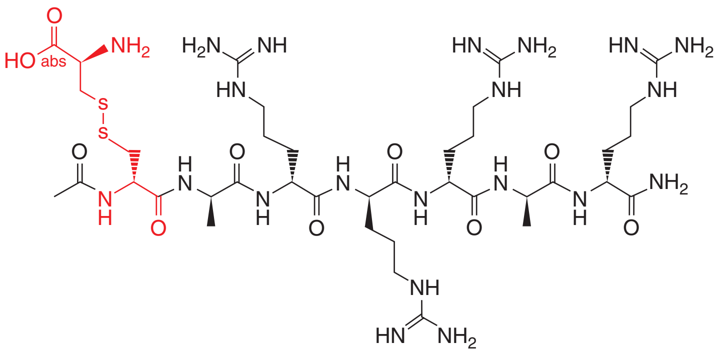
Cinacalcet is available as a hydrochloride and is formulated with one chiral center having an R-absolute configuration; the *R*-enantiomer is the more potent enantiomer and is primarily responsible for *cinacalcet*'s pharmacodynamic activity. *Etelcalcetide*, an octapeptide of D-amino acids, is available as a hydrochloride salt and is comparatively larger than *cinacalcet* (Figure 52–9).

Mechanism of Action

By enhancing the sensitivity of the CaSR to extracellular Ca^{2+}, calcimimetics lower the concentration of Ca^{2+} at which PTH secretion is suppressed. Inorganic di- and trivalent cations, along with polycations such as aminoglycosides (e.g., *streptomycin*, *gentamicin*, and *neomycin*) and polybasic amino acids (e.g., *polylysine*) are full agonists and are referred to as *type I calcimimetics*. They are able to activate the CaSR directly with no other cofactors. On the other hand, *cinacalcet* and *etelcalcetide* are positive allosteric CaSR modulators that require the presence of Ca^{2+} or other full agonists to enhance the sensitivity of activation without altering the maximal response and are designated *type II calcimimetics* (Cianferotti et al., 2015; Filopanti et al., 2013).



Cinacalcet



Etelcalcetide

Figure 52–9 *Structures of cinacalcet and etelcalcetide.* *Cinacalcet* is depicted as the free base. The compound is a phenyl-propylamine derivative with a 3-trifluoromethyl group (red) and naphthalene moiety (blue). *Etelcalcetide* is a short eight-residue peptide formed from D-amino acids. The N-terminal D-Cys is linked to an L-Cys by a disulfide (S-S) bond and is capped with an acetyl group. Formation of a covalent disulfide bond between the D-cysteine in *etelcalcetide* and Cys^{482} of the CaSR allosterically activates the CaSR (Nemeth et al., 2018).

ADME

Cinacalcet exhibits first-order absorption, with peak serum concentrations achieved 2 to 6 h after oral administration. Maximal effects on serum PTH occur 2 to 4 h after administration. It has an extraordinarily large volume of distribution of 1000 L and is metabolized by multiple hepatic CYPs, including CYPs 3A4, 2D6, and 1A2. Metabolites are eliminated by biliary (15%) and renal excretion (85%). *Cinacalcet* has an elimination $t_{1/2}$ of 30 to 40 h.

Etelcalcetide exhibits linear pharmacokinetics after intravenous administration. Plasma concentrations reach steady state approximately 8 weeks after thrice-weekly dosing; its elimination $t_{1/2}$ is 3 to 5 days in hemodialysis patients (Wu et al., 2018). *Etelcalcetide* is neither a substrate nor an inhibitor of CYPs or drug transport proteins. The drug is cleared by renal excretion in patients with normal kidney function; hemodialysis is the predominant elimination pathway in patients receiving hemodialysis (Wu et al., 2018).

Therapeutic Uses

Cinacalcet and *etelcalcetide* are approved for the treatment of secondary hyperparathyroidism in adults with CKD on dialysis. In addition, *cinacalcet* is approved for hypercalcemia in adults with parathyroid carcinoma and for hypercalcemia in adult patients with primary hyperparathyroidism who are not candidates for surgical parathyroidectomy. Treatment with *cinacalcet* or *etelcalcetide* lowers serum PTH (Block et al., 2017a, 2017b; Martin et al., 2014; Nemeth and Shoback, 2013). In patients with secondary hyperparathyroidism on dialysis, treatment with *cinacalcet* significantly decreases bone turnover and improves bone histology (Behets et al., 2015). Moreover, by lowering serum FGF23 concentrations, *cinacalcet* treatment decreases the rate of cardiovascular death and major cardiovascular events (Moe et al., 2015).

Adverse Effects

The principal adverse event common to *cinacalcet* and *etelcalcetide* is hypocalcemia. Thus, the drugs should not be used if the initial serum [Ca^{2+}] is less than 8.4 mg/dL; serum Ca^{2+} and phosphorus concentrations should be measured within 1 week, and PTH should be measured within 4 weeks after initiating therapy and after changing dosage. Seizure threshold is lowered by significant reductions in serum Ca^{2+}, so patients with a history of seizure disorders should be monitored especially closely. Finally, adynamic bone disease may develop if the PTH level is less than 100 pg/mL, and the drugs should be discontinued or the dose decreased if the PTH level falls below 150 pg/mL. *Etelcalcetide* also may increase risk for upper gastrointestinal bleeding. Overall, however, the safety profile of *etelcalcetide* appears similar to *cinacalcet* (Block et al., 2019).

Drug Interactions

Drug interactions can be anticipated with drugs that interfere with Ca^{2+} homeostasis or that hinder *cinacalcet* absorption. Potentially interfering drugs include vitamin D analogues, phosphate binders, bisphosphonates, *calcitonin*, glucocorticoids, *gallium*, and *cisplatin*. Caution is recommended when *cinacalcet* is coadministered with strong inhibitors of CYP3A4 (e.g., *ketoconazole*, *erythromycin*, or *itraconazole*). Because *cinacalcet* is a strong inhibitor of CYP2D6, dose adjustment may be required for concomitant medications that are CYP2D6 substrates (e.g., many β adrenergic receptor blockers, *flecainide*, *vinblastine*, and most tricyclic antidepressants). Conversely, *etelcalcetide* is neither a substrate nor an inhibitor of CYPs or drug transport proteins and has no known risks for drug-drug interactions (Wu et al., 2018).

Drugs Available

Cinacalcet is available in 30-, 60-, and 90-mg tablets. The recommended starting dose for treatment of secondary hyperparathyroidism in patients with CKD on dialysis is 30 mg once daily, with a maximum of 180 mg/day. For treatment of parathyroid carcinoma, a starting dose of 30 mg twice daily is recommended, with a maximum of 90 mg four times daily. The starting dose is titrated upward every 2 to 4 weeks to maintain the PTH level between 150 and 300 pg/mL (secondary hyperparathyroidism) or to normalize serum calcium (parathyroid carcinoma).

Etelcalcetide is available only as an injectable formulation and in single-dose 2.5-, 5-, and 10-mg vials. The recommended starting dose for treatment of secondary hyperparathyroidism in patients with CKD on dialysis is 5 mg administered by intravenous bolus injection three times per week at the end of the dialysis treatment. The dose may be titrated upward in 2.5- or 5-mg increments, based on PTH and corrected serum calcium response, no more frequently than every 4 weeks up to a maximum recommended dosage of 15 mg three times per week.

The investigational CaSR agonist *evocalcet* exhibits similar PTH lowering and a better gastrointestinal side effect profile compared to *cinacalcet* in adult dialysis patients treated for secondary hyperparathyroidism (Fukagawa et al., 2018). The drug is currently not available for use in the U.S.

Fluoride

Fluoride is discussed because of its effects on dentition and bone and its toxic properties.

Mechanism of Action

Sodium fluoride enhances osteoblast activity and increases bone volume. These effects may be bimodal, with low doses stimulating and higher doses suppressing osteoblasts. However, the apparent effects of *fluoride* in osteoporosis are slight compared with those achieved with PTH or others. *Fluoride* can inhibit several enzyme systems and diminish tissue respiration and anaerobic glycolysis.

ADME

Fluoride is obtained from the ingestion of plants and water, with absorption taking place largely in the intestine. A second route of absorption is through the lungs, and inhalation of *fluoride* present in dusts and gases constitutes the major route of industrial exposure. *Fluoride* is distributed widely in organs and tissues but is concentrated in bone and teeth, and the skeletal burden is related to intake and age. Bone deposition reflects skeletal turnover; growing bone shows greater deposition than mature bone. The kidneys are the major sites of *fluoride* excretion. Small amounts of *fluoride* also appear in sweat, milk, and intestinal secretions.

Therapeutic Use

Because it is concentrated in the bone, the radionuclide ^{18}F has been used in skeletal imaging. *Sodium fluoride* is a mainstay of therapy for the prevention of dental carries.

Fluoride and Dental Caries. Supplementation of water *fluoride* content to 1.0 ppm is a safe and practical intervention that substantially reduces the incidence of caries in permanent teeth. There are partial benefits for children who begin drinking fluoridated water at any age; however, optimal benefits are obtained at ages before permanent teeth erupt. Topical application of *fluoride* solutions by dental personnel appears to be effective on newly erupted teeth and can reduce the incidence of caries by 30% to 40%. Dietary *fluoride* supplements should be considered for children less than 12 years of age whose drinking water contains less than 0.7 ppm *fluoride*. Adequate incorporation of *fluoride* into teeth hardens the outer layers of enamel and increases resistance to demineralization. The *fluoride* salts usually employed in dentifrices are *sodium fluoride* and *stannous fluoride*. *Sodium fluoride* also is available in a variety of preparations for oral and topical use.

Regulation of the *fluoride* concentration of community water supplies periodically encounters vocal opposition, including allegations of putative adverse health consequences of fluoridated water. Careful examination of these issues indicates that cancer and all-cause mortalities do not differ significantly between communities with fluoridated and nonfluoridated water.

Acute Poisoning

Acute *fluoride* poisoning usually results from accidental ingestion of *fluoride*-containing insecticides or rodenticides. Initial symptoms (salivation, nausea, abdominal pain, vomiting, and diarrhea) are secondary to the local action of *fluoride* on the intestinal mucosa. Systemic symptoms are varied and severe: increased irritability of the CNS consistent with the Ca^{2+}-binding effect of *fluoride* and the resulting hypocalcemia; hypotension, presumably owing to central vasomotor depression as well as direct cardiotoxicity; and stimulation and then depression of respiration. Death can result from respiratory paralysis or cardiac failure. The lethal dose of *sodium fluoride* for humans is about 5 g, although there is considerable variation. Treatment includes the intravenous administration of glucose in saline and gastric lavage with limewater (0.15% calcium hydroxide solution) or other Ca^{2+} salts to precipitate the *fluoride*. *Calcium gluconate* is given intravenously for tetany; urine volume is kept high with vigorous fluid resuscitation.

Chronic Poisoning

In humans, the major manifestations of chronic ingestion of excessive *fluoride* are osteosclerosis and mottled enamel. Osteosclerosis is characterized by increased bone density secondary both to elevated osteoblastic activity and to the replacement of hydroxyapatite by the denser fluoroapatite. The degree of skeletal involvement varies from changes that are barely detectable radiologically to marked cortical thickening of long bones, numerous exostoses scattered throughout the skeleton, and calcification of ligaments, tendons, and muscle attachments. In its severest form, it is a disabling and crippling disease.

Mottled enamel, or dental fluorosis, was first described more than 60 years ago. In very mild mottling, small, opaque, paper-white areas are scattered irregularly over the tooth surface. In severe cases, discrete or confluent, deep brown– to black-stained pits give the tooth a corroded appearance. Mottled enamel results from a partial failure of the enamel-forming ameloblasts to elaborate and lay down enamel. Mottling is one of the first visible signs of excess *fluoride* intake during childhood. Continuous use of water containing about 1 ppm of *fluoride* may result in very mild mottling in 10% of children; at 4 to 6 ppm, the incidence approaches 100%, with a marked increase in severity. Severe dental fluorosis formerly occurred in regions where local water supplies had a very high *fluoride* content (e.g., Pompeii, Italy, and Pike's Peak, CO). Current regulations in the U.S. require lowering the *fluoride* content of the water supply or providing an alternative source of acceptable drinking water for affected communities. Sustained consumption of water with a *fluoride* content of 4 mg/L (4 ppm) is associated with deficits in cortical bone mass and increased rates of bone loss over time.

Integrated Approach to Prevention and Treatment of Osteoporosis

Osteoporosis is a major and growing public health problem in developed nations. Approximately 50% of women and 25% of men more than 50 years of age will experience an osteoporosis-related fracture. Important reductions in fracture risk can be achieved with attention to health (muscle-strengthening exercise; avoiding smoking and excessive alcohol use) and nutrition (i.e., increased dietary calcium or calcium or vitamin D supplements). Pharmacological agents used to manage osteoporosis act by decreasing the rate of bone resorption and thereby slowing the rate of bone loss (antiresorptive therapy) or by promoting bone formation (anabolic therapy). Because bone remodeling is a coupled process, antiresorptive drugs ultimately decrease the rate of bone formation and therefore do not promote substantial gains in BMD. Increases in BMD during the first years of antiresorptive therapy represent a constriction of the remodeling space to a steady-state level, after which BMD reaches a new plateau (Figure 52–10).

Pharmacological treatment of osteoporosis is aimed at restoring bone strength and preventing fractures. Antiresorptive drugs (such as the bisphosphonates, estrogen, the SERM *raloxifene*, and, to some extent, *calcitonin*) inhibit osteoclast-mediated bone loss, thereby reducing bone turnover. Although the administration of estrogen to women at menopause is a powerful intervention to preserve bone and protect against fracture, the detrimental effects of HRT have mandated a major reexamination of treatment options (see further and Chapter 48). In addition to antiresorptive agents, the FDA has approved the hPTH(1–34) fragment (*teriparatide*) and the hPTHrP(1–34) fragment (*abaloparatide*) for use in treating postmenopausal women with osteoporosis. *Teriparatide* is also approved for use in increasing bone mass in men with primary or hypogonadal osteoporosis.

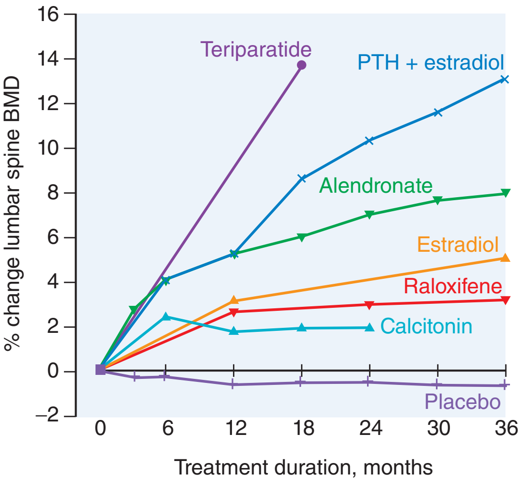


Figure 52–10 *Relative efficacy of different therapeutic interventions on BMD of the lumbar spine.* *Teriparatide* (40 µg) (Neer et al., 2001), PTH (25 µg) + *estradiol*, *alendronate* (10 mg), *estradiol* (0.625 mg/d), *raloxifene* (120 mg), and *calcitonin* (200 IU). Typical results with placebo treatment underscore the inexorable bone loss without intervention. Some of the indicated treatment interventions involved combination therapy, and absolute comparisons should not be made.

Antiresorptive Agents

Bisphosphonates

Bisphosphonates are the most frequently used drugs for the prevention and treatment of osteoporosis. Second- and third-generation oral bisphosphonates, *alendronate* and *risedronate*, have sufficient potency to suppress bone resorption at doses that do not inhibit mineralization. *Alendronate*, *risedronate*, and *ibandronate* are used for prevention and treatment of osteoporosis and for the treatment of glucocorticoid-associated osteoporosis.

Monoclonal Antibodies

Denosumab. RANK ligand (RANKL) binds to its cognate receptor RANK on the surface of precursor and mature osteoclasts and stimulates these cells to mature and resorb bone. OPG, which competes with RANK for binding to RANKL, is the physiological inhibitor of RANKL. *Denosumab* is a human monoclonal antibody that binds with high affinity to RANKL, mimicking the effect of OPG and thereby reducing the binding of RANKL to RANK. *Denosumab* blocks osteoclast formation and activation. It increases BMD and decreases bone turnover markers when given subcutaneously, 60 mg once every 6 months. Osteonecrosis of the jaw and fractures of the femoral shaft have been reported with *denosumab* use. It is contraindicated in the setting of preexisting hypocalcemia.

Burosumab. The recombinant human monoclonal anti-FGF23 antibody *burosumab* is effective in correcting the hypophosphatemia associated with XLH and osteomalacia. Monthly administration provides prolonged restoration of serum phosphate by reducing urinary phosphate excretion without adversely altering serum PTH or calcium levels. *Burosumab* is approved for treatment of XLH in children (Carpenter et al., 2018) and adults (Insogna et al., 2018) and for tumor-induced osteomalacia (Imanishi et al., 2021). It significantly improves biochemical markers of bone formation and resorption, as well as histomorphometric measures of osteomalacia in adults with XLH when administered at 1 mg/kg every 4 weeks (Insogna et al., 2018, 2019). Nephrocalcinosis has been reported during *burosumab* treatment. The drug is contraindicated with oral phosphate and active vitamin D analogues, when serum phosphorus is within or above the normal limits, and in patients with severe kidney impairment or end-stage kidney disease.

Romosozumab. *Romosozumab* is a monoclonal antibody that binds sclerostin and relatively rapidly increases bone formation (McClung, 2018). In a clinical trial, initiation of *denosumab* maintained or increased bone mineral density following cessation of *romosozumab* (Cosman et al., 2016).

Selective Estradiol Receptor Modulators

Considerable work has been undertaken to develop estrogenic compounds with tissue-selective activities. *Raloxifene* acts as an estrogen agonist on bone and liver, is inactive on the uterus, and acts as an antiestrogen on the breast (see Chapter 48). In postmenopausal women, *raloxifene* stabilizes and modestly increases BMD and reduces the risk of vertebral compression fracture (Komm and Mirkin, 2014). *Raloxifene* is approved for both the prevention and the treatment of osteoporosis. Adverse effects of *raloxifene* include worsening of vasomotor symptoms. The drug is also associated with an increased risk of deep vein thrombosis and pulmonary embolism, so it is contraindicated in adults with a history of venous thromboembolism.

Estrogen

Postmenopausal status or estrogen deficiency at any age significantly increases a patient's risk for osteoporosis and fractures. Likewise, overwhelming evidence supports the positive impact of estrogen replacement on the conservation of bone and protection against osteoporotic fracture after menopause (see Chapter 48). However, the Women's Health Initiative studies indicated that HRT significantly increases risks of heart disease and breast cancer; consequently, HRT is now reserved only for the short-term relief of vasomotor symptoms associated with menopause.

Calcium

The rationale for using supplemental calcium to protect bone varies with time of life. For preteens and adolescents, adequate substrate calcium is required for bone accretion. Higher Ca^{2+} intake during the third decade of life is positively related to the final phase of bone acquisition. There is controversy about the role of calcium during the early years after menopause, when the primary basis for bone loss is estrogen withdrawal. In elderly subjects, supplemental calcium suppresses bone turnover and improves BMD. Patients may increase calcium by dietary means and may choose from many palatable, low-cost calcium preparations. The most frequently prescribed is carbonate, which should be taken with meals to facilitate dissolution and absorption. Traditional dosing of calcium is about 1000 mg/day, nearly the amount present in a quart of milk. Adults more than 50 years of age need 1200 mg of calcium daily. More may be necessary to overcome endogenous intestinal calcium losses, but daily intakes of 2000 mg or more frequently are reported to be constipating.

Vitamin D and Its Analogues

Modest supplementation with vitamin D (400–800 IU/day) may improve intestinal Ca^{2+} absorption, suppress bone remodeling, and improve BMD in individuals with marginal or deficient vitamin D status. Supplemental vitamin D in combination with calcium reduces fracture incidence.

The use of *calcitriol* to treat osteoporosis is distinct from ensuring vitamin D nutritional adequacy. Here, the rationale is to suppress parathyroid function directly and reduce bone turnover. Higher doses of *calcitriol* appear to be more likely to improve BMD, but at the risk of developing hypercalciuria and hypercalcemia; therefore, close scrutiny of patients and dose are required. Restriction of dietary calcium may reduce toxicity during *calcitriol* therapy.

Calcitonin

Calcitonin inhibits osteoclastic bone resorption and modestly increases bone mass in patients with osteoporosis, most prominently in patients with high intrinsic rates of bone turnover. *Calcitonin* nasal spray (200 units/day) reduces the incidence of vertebral compression fractures by about 40% in osteoporotic women (Chesnut et al., 2000).

Thiazide Diuretics

Although not strictly antiresorptive, thiazides reduce urinary Ca^{2+} excretion and constrain bone loss in patients with hypercalciuria. *Hydrochlorothiazide*, 25 mg once or twice daily, may reduce urinary Ca^{2+} excretion substantially. Effective doses of thiazides for reducing urinary Ca^{2+} excretion generally are lower than those necessary for blood pressure control (see Chapters 29 and 32).

Anabolic Agents

Teriparatide, Abaloparatide, rhPTH

Teriparatide and *abaloparatide* increase new bone formation. *Teriparatide* and *abaloparatide* are approved by the FDA for treating osteoporosis for up to 2 years in both men and postmenopausal women at high risk for fractures. *Teriparatide* increases predominantly trabecular bone at the lumbar spine and femoral neck; it has less-significant effects at cortical sites. *Teriparatide* is approved at the 20-μg dose, administered once daily by subcutaneous injection in the thigh or abdominal wall. The most common adverse effects of *teriparatide* include injection-site pain, nausea, headaches, leg cramps, and dizziness. *Abaloparatide* is administered at a starting dose of 80 μg by subcutaneous injection into the periumbilical region of the abdomen. Subcutaneous administration of *abaloparatide* reduced the risk of new vertebral and nonvertebral fractures over 18 months. Differences in PTHR binding between *teriparatide* and *abaloparatide* permit the use of the higher dose of *abaloparatide* and may explain its greater bone formation with lower stimulation of bone resorption (Miller et al., 2016).

Preparations of rhPTH are not currently approved for use in the U.S. or E.U.

Combination Therapies

Osteoporosis

Because *teriparatide* stimulates bone formation, whereas bisphosphonates reduce bone resorption, it was predicted that therapy combining the two would enhance the effect on BMD more than treatment with either one alone. However, addition of *alendronate* to PTH treatment provided no additional benefit for BMD and reduced the anabolic effect of PTH in both women and men. Sequential treatment with PTH(1–84) followed by *alendronate* increases vertebral BMD to a greater degree than *alendronate* or estrogen alone.

Paget Disease

Although most patients with Paget disease require no treatment, factors such as severe pain, neural compression, progressive deformity, hypercalcemia, high-output congestive heart failure, and repeated fracture risk are considered indications for treatment. Bisphosphonates and *calcitonin* decrease the elevated biochemical markers of bone turnover, such as plasma alkaline phosphatase activity and urinary excretion of hydroxyproline. An initial course of bisphosphonate typically is given once daily or once weekly for 6 months. With treatment, most patients experience a decrease in bone pain over several weeks. Such treatment may induce long-lasting remission. If symptoms recur, additional courses of therapy can be effective.

Optimal therapy for Paget disease varies among patients. Bisphosphonates are the standard therapy. Intravenous *pamidronate* induces long-term remission following a single infusion. *Zoledronate* exhibits greater response rates and a longer median duration of complete response. Compared with *calcitonin*, bisphosphonates have the advantage of oral administration, lower cost, lack of antigenicity, and generally fewer side effects.

Drug Facts for Your Personal Formulary: *Agents Affecting Mineral Ion Homeostasis and Bone Turnover*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Vitamin D Analogues		
Ergocalciferol	• Vitamin D deficiency • Nutritional rickets • Vitamin D–resistant rickets • Familial hypophosphatemia • Hypoparathyroidism • Osteomalacia/osteoporosis	• Vitamin D_2 • May cause hypercalcemia
Cholecalciferol		• Vitamin D_3 • May cause hypercalcemia
Doxercalciferol	• Secondary hyperparathyroidism in patients with CKD	• 1-Hydroxylated ergocalciferol (1-OH-D_2) • "Activated" in the liver by 25-hydroxylation • May cause hypercalcemia, hypercalciuria, or hyperphosphatemia
Alfacalcidol	• Secondary hyperparathyroidism in patients with CKD	• 1-Hydroxylated cholecalciferol (1-OH-D_3) • "Activated" in the liver by 25-hydroxylation • May cause hypercalcemia, hypercalciuria, or hyperphosphatemia
Dihydrotachysterol	• Familial hypophosphatemia • Hypoparathyroidism • Osteoporosis • Secondary hyperparathyroidism in patients with CKD	• Reduced form of ergocalciferol • "Activated" in the liver by 25-hydroxylation • May cause hypercalcemia, hypercalciuria, or hyperphosphatemia • Not available in the U.S.
Calcifediol	• Hypocalcemia • Secondary hyperparathyroidism in patients with CKD	• 25-Hydroxylated form of cholecalciferol • "Activated" in the kidney by 1-hydroxylation • Not available in the U.S.
Calcitriol	• Hypocalcemia • Secondary hyperparathyroidism in patients with CKD • Hypoparathyroidism	• 1,25-Dihydroxylated form of cholecalciferol • Activated form of vitamin D • May cause hypercalcemia, hypercalciuria, or hyperphosphatemia
Paricalcitol	• Secondary hyperparathyroidism in patients with CKD	• 1,25-Dihydroxy-19-norvitamin D_2 • Minimal effects on serum calcium and phosphorus
Maxacalcitol	• Secondary hyperparathyroidism in patients with CKD	• 1,25-Dihydroxy-22-oxavitamin D_3 • Shorter $t_{1/2}$ than calcitriol • Potent suppressor of PTH gene expression • Not marketed in the U.S.
Calcipotriol	• Psoriasis	• Negligible effects on serum calcium • For topical application only
Phosphate-Binding Agents • Taken with meals to reduce the amount of dietary phosphate absorbed		
Calcium carbonate	• Treatment and prevention of CKD-MBD	• Inexpensive, well tolerated, commonly used • 40% elemental calcium
Calcium acetate	• Treatment and prevention of CKD-MBD	• Well tolerated, commonly used • 25% elemental calcium
Sevelamer hydrochloride	• Treatment and prevention of CKD-MBD	• Nonabsorbable polymer that acts as a nonselective anion exchanger • Risk of metabolic acidosis
Sevelamer carbonate	• Treatment and prevention of CKD-MBD	• Same polymeric structure as sevelamer hydrochloride, with chloride replaced by carbonate • Decreased risk of metabolic acidosis
Lanthanum carbonate	• Treatment and prevention of CKD-MBD	• Risk of gastrointestinal obstruction and ileus • Contraindicated in bowel obstruction
Sucroferric oxyhydroxide (oral formulation)	• Treatment and prevention of CKD-MBD	• Polynuclear iron(III)–oxyhydroxide compound that binds phosphate by ligand exchange • Negligible absorption of iron • Injectable formulation is used for iron replacement therapy
Ferric citrate	• Treatment and prevention of CKD-MBD	• Iron absorption may lead to increased systemic iron parameters and toxicity

Drug Facts for Your Personal Formulary: *Agents Affecting Mineral Ion Homeostasis and Bone Turnover (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Bisphosphonates • Inhibit osteoclast-mediated bone resorption		
Etidronate	• Paget disease • Heterotopic ossification • Hypercalcemia	• Esophagitis, esophageal ulcers or erosions reported with oral administration • Contraindicated in those with abnormalities that delay esophageal emptying • Risk of nephrotoxicity • Osteonecrosis of the jaw reported
Clodronate	• Paget disease • Treatment and prevention of osteoporosis • Hypercalcemia of malignancy • Prevention of bone loss in breast cancer and multiple myeloma	• Risk of nephrotoxicity • Osteonecrosis of the jaw reported • Not available in the U.S.
Tiludronate	• Paget disease	• Esophagitis, esophageal ulcers or erosions reported with oral administration • Caution in creatinine clearance <35 mL/min • Osteonecrosis of the jaw reported
Pamidronate	• Paget disease • Hypercalcemia of malignancy • Prevention of bone loss in breast cancer and multiple myeloma	• 10–100 times more potent than etidronate • Risk of nephrotoxicity • Osteonecrosis of the jaw reported • Fractures of the femoral shaft reported • Available in the U.S. only for parenteral administration
Alendronate	• Paget disease • Treatment and prevention of osteoporosis	• 10–100 times more potent than etidronate • Esophagitis, esophageal ulcers or erosions reported with oral administration • Contraindicated in those with abnormalities that delay esophageal emptying • Osteonecrosis of jaw, fractures of femoral shaft reported
Ibandronate	• Treatment and prevention of osteoporosis	• Esophagitis, esophageal ulcers or erosions reported with oral administration • Contraindicated in those with abnormalities that delay esophageal emptying • Risk of nephrotoxicity • Osteonecrosis of jaw, fractures of the femoral shaft, anaphylaxis reported
Risedronate	• Paget disease • Treatment and prevention of osteoporosis	• Third-generation agent • 10,000 times more potent than etidronate • Esophagitis, esophageal ulcers or erosions reported with oral administration • Contraindicated in those with abnormalities that delay esophageal emptying • Osteonecrosis of jaw, fractures of the femoral shaft reported • Many dosing regimens (daily to 2 months)
Zoledronate	• Paget disease • Treatment and prevention of osteoporosis • Hypercalcemia of malignancy • Adjunctive treatment of bone metastases from solid tumors and osteolytic lesions of multiple myeloma	• Third-generation agent • 10,000 times more potent than etidronate • Contraindicated in hypocalcemia and creatinine clearance <35 mL/min • May cause severe hypocalcemia • Risk of nephrotoxicity • Osteonecrosis of jaw, fractures of femoral shaft, anaphylaxis reported • Annual dosing for postmenopausal use
Parathyroid Hormone Analogues		
Teriparatide *[hPTH(1–34)]* Abaloparatide *[hPTHrP(1–34)]*	• Treatment of osteoporosis	• Anabolic agents • Increase new bone formation • Use should be limited to ≤2 years • Should not be used in patients who are at increased baseline risk for osteosarcoma
rhPTH	• Adjunctive treatment of hypocalcemia in patients with hypoparathyroidism	Not currently approved for use in U.S. or E.U.
Long-acting PTH	• Treatment of hypoparathyroidism	• Increases serum calcium concentrations in rodents for almost 24 h • Investigational use only

Drug Facts for Your Personal Formulary: *Agents Affecting Mineral Ion Homeostasis and Bone Turnover (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Calcium-Sensing Receptor Mimetics		
Cinacalcet	• Secondary hyperparathyroidism in adults with CKD on dialysis • Hypercalcemia in adults with parathyroid carcinoma • Hypercalcemia in adults with primary hyperparathyroidism who are not candidates for surgical parathyroidectomy	• May cause severe hypocalcemia • Concomitant use of strong inhibitors of CYP3A4 should be avoided • Dose adjustment may be required for concomitant medications that are CYP2D6 substrates
Etelcalcetide	• Secondary hyperparathyroidism in adults with CKD on dialysis	• May cause severe hypocalcemia • May worsen heart failure • Upper GI bleeding reported
Evocalcet	• Secondary hyperparathyroidism in adults with CKD on dialysis	• May cause severe hypocalcemia • Not available in the U.S.
Miscellaneous Agents		
Calcitonin	• Paget disease • Hypercalcemia • Postmenopausal osteoporosis	• Direct inhibitor of osteoclastic bone resorption • Anaphylaxis/hypersensitivity reported
Denosumab	• Treatment and prevention of osteoporosis • Treatment to increase bone mass in adults at high risk for fracture receiving cancer therapy	• Human monoclonal antibody that binds with high affinity to RANKL • Contraindicated in setting of preexisting hypocalcemia • Osteonecrosis of the jaw reported • Fractures of the femoral shaft reported
Raloxifene	• Treatment and prevention of osteoporosis	• Selective estrogen receptor modulator • Contraindicated in adults with history of venous thromboembolism; increased risk of deep vein thrombosis and pulmonary embolism
Hydrochlorothiazide	• Osteoporosis • Hypercalciuria	• Reduce urinary calcium excretion • Constrain bone loss in patients with hypercalciuria
Burosumab	• Treatment of X-linked hypophosphatemia • Treatment of FGF23-related hypophosphatemia in tumor-induced osteomalacia	• Human monoclonal antibody that binds to and inhibits FGF23 activity • Contraindicated with oral phosphate and active vitamin D analogues • Contraindicated when serum phosphorus is within or above the normal limits • Contraindicated in patients with severe kidney impairment or end-stage kidney disease • Hyperphosphatemia and nephrocalcinosis reported
Fluoride		
Sodium fluoride	• Prophylaxis of dental caries	• Childhood consumption of fluoridated drinking water reduces incidence of caries in permanent teeth • Topical application can reduce the incidence of caries by 30%–40%

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Section VI

Gastrointestinal Pharmacology

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Chapter 53

Pharmacotherapy for Gastric Acidity, Peptic Ulcers, and Gastroesophageal Reflux Disease

Keith A. Sharkey and Wallace K. MacNaughton

PHYSIOLOGY OF GASTRIC SECRETION
- Parietal Cell H^+,K^+-ATPase
- Gastric Defenses Against Acid

PROTON PUMP INHIBITORS
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H_2 RECEPTOR ANTAGONISTS
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POTASSIUM-COMPETITIVE ACID BLOCKERS
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AGENTS THAT ENHANCE MUCOSAL DEFENSE
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THERAPEUTIC STRATEGIES FOR SPECIFIC ACID-PEPTIC DISORDERS
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- Peptic Ulcer Disease
- Treatment of *Helicobacter pylori* Infection
- NSAID-Related Ulcers
- Stress-Related Ulcers
- Zollinger-Ellison Syndrome
- Functional Dyspepsia
- Functional Esophageal Disorders

The stomach has a number of critical functions in the processes of digestion: storage, digestion, and defense. The volume of the stomach is quite small at rest, but the gastric musculature can undergo receptive relaxation to accommodate a meal volume of 1 to 2 L. Food is broken down in the presence of acid by the grinding actions of the thick muscular coats of the stomach, and the contents then pass in a regulated manner into the duodenum. Gastric acid not only serves to facilitate digestion, but it also provides an effective antimicrobial milieu that facilitates defense against pathogens.

Gastric acid and pepsin in the stomach normally do not produce damage or symptoms of acid-peptic diseases because of intrinsic defense mechanisms. The stomach is protected by a number of factors, collectively referred to as “mucosal defense,” many of which are stimulated by the local generation of prostaglandins (PGs) and nitric oxide (NO). If these defenses are disrupted, a gastric or duodenal ulcer may form. The treatment and prevention of acid-related disorders are accomplished by decreasing gastric acidity and enhancing mucosal defense. The appreciation that an infectious agent, *Helicobacter pylori*, plays a key role in the pathogenesis of acid-peptic diseases revolutionized approaches to prevention and therapy of these common disorders.

Barriers to the reflux of gastric contents into the esophagus comprise the primary esophageal defense. If these protective barriers fail and reflux occurs, dyspepsia or erosive esophagitis may result. Therapies are directed at decreasing gastric acidity, enhancing the tone of the lower esophageal sphincter, and stimulating esophageal motility (see Chapter 54).

Physiology of Gastric Secretion

Gastric acid secretion is a complex and continuous process: Neuronal (acetylcholine [ACh], gastrin-releasing peptide [GRP]); paracrine (histamine); and endocrine (gastrin) factors regulate the secretion of H^+ by parietal cells (acid-secreting cells) (Figure 53–1). Their specific receptors (M_3, BB_2, H_2, and CCK_2, respectively) are on the basolateral membrane of parietal cells in the body and fundus of the stomach. Some of these receptors are also present on ECL cells, where they regulate the release of histamine. The H_2 receptor is a G protein-coupled receptor (GPCR) that activates the G_s–adenylyl cyclase–cyclic AMP–PKA pathway (see Chapters 3 and 43). ACh and gastrin signal through GPCRs that couple to the G_q-PLC-IP_3-Ca^{2+} pathway in parietal cells; GRP uses the same signaling pathway to activate gastrin secretion from G cells. In parietal cells, the cyclic AMP and the Ca^{2+}-dependent pathways activate H^+,K^+-ATPase (the proton pump), which exchanges H^+ and K^+ across the parietal cell membrane. This pump generates the largest ion gradient known in vertebrates, with an intracellular pH of about 7.3 and an intracanalicular pH of about 0.8.

The important structures for CNS stimulation of gastric acid secretion are the dorsal motor nucleus of the vagus, the hypothalamus, and the nucleus of the solitary tract. Efferent fibers originating in the dorsal motor nuclei descend to the stomach via the vagus nerve and synapse with ganglion cells of the enteric nervous system (ENS). ACh release from postganglionic vagal fibers directly stimulates gastric acid secretion through muscarinic M_3 receptors on the basolateral membrane of parietal cells. The CNS predominantly modulates the activity of the ENS via ACh, stimulating gastric acid secretion in response to the sight, smell, taste, or anticipation of food (the “cephalic” phase of acid secretion). ACh also indirectly affects parietal cells by increasing the release of histamine from the ECL cells in the fundus of the stomach and of gastrin from G cells in the gastric antrum (Engevik et al., 2020).

The ECL cells, the source of gastric histamine, are usually near parietal cells. Histamine acts as a paracrine mediator, diffusing from its site of release to nearby parietal cells, where it activates H_2 receptors to stimulate gastric acid secretion.

Gastrin, produced by antral G cells, is the most potent inducer of acid secretion. Multiple pathways stimulate gastrin release, including CNS

Abbreviations

ACh: acetylcholine
cAMP: cyclic adenosine monophosphate (cyclic AMP)
CCK: cholecystokinin
CNS: central nervous system
CYP: cytochrome P450
DU: duodenal ulcer
ECL: enterochromaffin-like cell
ENS: enteric nervous system
GERD: gastroesophageal reflux disease
GI: gastrointestinal
GPCR: G protein-coupled receptor
GRP: gastrin-releasing peptide
GU: gastric ulcer
HIST: histamine
IP_3: inositol 1,4,5-trisphosphate
NO: nitric oxide
NSAID: nonsteroidal anti-inflammatory drug
OTC: over the counter
PG: prostaglandin
PK: protein kinase
PLC: phospholipase C
PPI: proton pump inhibitor
SARS-CoV-2 (COVID-19): severe acute respiratory syndrome coronavirus 2 (coronavirus disease 2019)
SST: somatostatin

activation, local distention, and chemical components of the gastric contents. In addition to releasing ACh, some vagal fibers to the stomach also release GRP (a 27–amino acid peptide); GRP activates the BB_2 bombesin receptor on G cells, activating the G_q-PLC-IP_3-Ca^{2+} pathway and causing secretion of gastrin. Gastrin stimulates acid secretion indirectly by inducing the release of histamine by ECL cells; a direct effect on parietal cells also plays a lesser role.

Somatostatin, produced by antral D cells, inhibits gastric acid secretion. Acidification of the gastric luminal pH to less than 3 stimulates somatostatin release, which in turn suppresses gastrin release in a negative-feedback loop. Somatostatin-producing cells are decreased in patients with *H. pylori* infection, and the consequent reduction of somatostatin's inhibitory effect may contribute to excess gastrin production.

Parietal Cell H^+,K^+-ATPase

H^+,K^+-ATPase is the enzyme responsible for secreting protons into the lumen of the gastric gland (Engevik et al., 2020). It is a heterodimeric protein composed of two subunits that are the products of two genes. The *ATP4A* gene encodes the α subunit that contains the catalytic sites of the enzyme and forms the membrane pore, and the *ATP4B* gene encodes the β subunit of the H^+,K^+-ATPase, which contains an N-terminal cytoplasmic domain, a transmembrane domain, and a highly glycosylated extracellular domain. Hydronium ions bind to three active sites present in the α subunit, and secretion involves a conformational change that allows the movement of protons. This movement is balanced by the transport of K^+. The stoichiometry of transport is pH dependent, varying between two H^+ and two K^+ per molecule of ATP to one of each under more acidic conditions. Inhibiting the H^+,K^+-ATPase (or proton pump) is the mainstay of modern pharmacotherapy for acid-related disorders.

Gastric Defenses Against Acid

The extremely high concentration of H^+ in the gastric lumen requires robust defense mechanisms to protect the esophagus, stomach, and proximal small intestine (Wallace, 2008). The primary esophageal defense is the gastroesophageal junction—the lower esophageal sphincter in association with the diaphragm and angle of His—which prevents reflux of acidic gastric contents into the esophagus. The stomach protects itself from acid damage by a number of mechanisms that require adequate mucosal blood flow. One key defense is the secretion of a mucous layer that helps to protect gastric epithelial cells by trapping secreted bicarbonate at the cell surface. Gastric mucus is soluble when secreted but quickly forms an insoluble gel that coats the mucosal surface of the stomach, slows ion diffusion, and prevents mucosal damage by macromolecules such as pepsin. Mucus production is stimulated by PGs E_2 and I_2, which also directly inhibit gastric acid secretion by parietal cells. Thus, drugs that inhibit PG formation (e.g., NSAIDs, ethanol) decrease mucus secretion and predispose to the development of acid-peptic disease. The proximal part of the duodenum is protected from gastric acid through the production of bicarbonate, primarily from mucosal Brunner's glands.

Figure 53–1 outlines the rationale and pharmacological basis for the therapy of acid-peptic disease. The PPIs are used most commonly, followed by the histamine H_2 receptor antagonists.

Proton Pump Inhibitors

The most potent suppressors of gastric acid secretion are inhibitors of the gastric H^+,K^+-ATPase or proton pump (Figure 53–2). These drugs diminish the daily production of acid (basal and stimulated) by 80% to 95% (Shin and Sachs, 2008).

Mechanism of Action and Pharmacology

Six PPIs are available for clinical use: *omeprazole* and its S-isomer, *esomeprazole*, *lansoprazole* and its R-enantiomer, *dexlansoprazole*, *rabeprazole*, and *pantoprazole*. All PPIs have equivalent efficacy at comparable doses.

Proton pump inhibitors are prodrugs that require activation in an acid environment. After absorption into the systemic circulation, the prodrug diffuses into the parietal cells of the stomach and accumulates in the acidic secretory canaliculi. Here, it is activated by proton-catalyzed formation of a tetracyclic sulfenamide (see Figure 53–2), trapping the drug so that it cannot diffuse back across the canalicular membrane. The activated form then binds covalently with sulfhydryl groups of cysteines in the H^+,K^+-ATPase, irreversibly inactivating the pump molecule. Acid secretion resumes only after new pump molecules are synthesized and inserted into the luminal membrane, providing a prolonged (up to 24- to 48-h) suppression of acid secretion, despite the much shorter plasma $t_{1/2}$ of about 0.5 to 3 h of the parent compounds. Because they block the final step in acid production, the PPIs effectively suppress stimulated acid production, regardless of the physiological stimulus, as well as basal acid production.

The amount of H^+,K^+-ATPase increases after fasting; therefore, PPIs should be given before the first meal of the day. In most individuals, once-daily dosing is sufficient to achieve an effective level of acid inhibition, and a second dose, which is occasionally necessary, can be administered before an evening meal. Rebound acid hypersecretion occurs following prolonged treatment with PPIs, and clinical studies suggest that rebound after ceasing treatment can provoke symptoms such as dyspepsia.

To prevent degradation of PPIs by acid in the gastric lumen and improve oral bioavailability, oral dosage forms are supplied in different formulations:

- Enteric-coated pellets within gelatin capsules (*rabeprazole*)
- Delayed-release tablets (*lansoprazole*, *pantoprazole, rabeprazole*)
- Delayed-release capsules (*dexlansoprazole*, *esomeprazole*, *omeprazole, lansoprazole*)
- Delayed-release oral suspension (*esomeprazole, omeprazole, pantoprazole*)

The delayed-release and enteric-coated preparations dissolve only at alkaline pH, which improves the oral bioavailability of these acid-labile drugs. Patients for whom the oral route of administration is not available can be treated parenterally with *esomeprazole sodium, omeprazole sodium*, or *pantoprazole*.

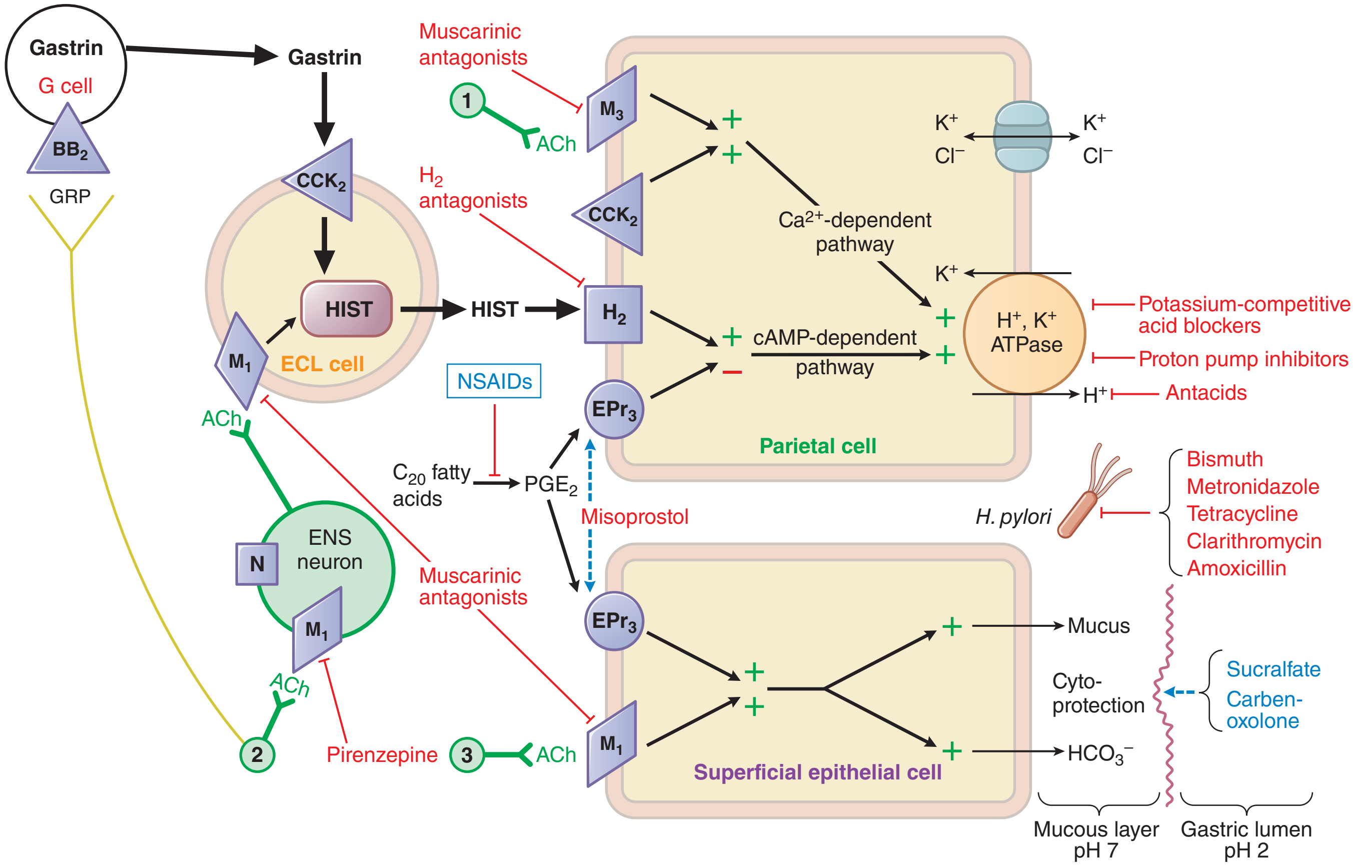

Gastrin
G cell
BB$_2$
GRP
Gastrin
CCK$_2$
HIST
M$_1$
ECL cell
ACh
HIST
Muscarinic antagonists
①
ACh
M$_3$
H$_2$ antagonists
CCK$_2$
H$_2$
NSAIDs
EPr$_3$
C$_{20}$ fatty acids
PGE$_2$
Misoprostol
N
ENS neuron
M$_1$
Muscarinic antagonists
ACh
②
Pirenzepine
③
ACh
EPr$_3$
M$_1$
+
+
Ca^{2+}-dependent pathway
K$^+$
Cl$^-$
K$^+$
Cl$^-$
K$^+$
H$^+$, K$^+$ ATPase
+
cAMP-dependent pathway
+
−
+
H$^+$
Parietal cell
Potassium-competitive acid blockers
Proton pump inhibitors
Antacids
H. pylori
Bismuth
Metronidazole
Tetracycline
Clarithromycin
Amoxicillin
+
+
+
+
Mucus
Cyto-protection
HCO$_3^-$
Sucralfate
Carben-oxolone
Superficial epithelial cell
Mucous layer pH 7
Gastric lumen pH 2

Figure 53–1 *Pharmacologist's view of gastric secretion and its regulation: the basis for therapy of acid-peptic disorders.* Shown are the interactions among neural input and a variety of enteroendocrine cells: an ECL cell that secretes histamine, a ganglion cell of the ENS, a G cell that secretes gastrin, a parietal cell that secretes acid, and a superficial epithelial cell that secretes mucus and bicarbonate. Physiological pathways, shown in solid black, may be stimulatory (+) or inhibitory (–). **1** and **3** indicate possible inputs from postganglionic cholinergic fibers; **2** shows neural input from the vagus nerve. Physiological agonists and their respective membrane receptors include ACh and its muscarinic (M) and nicotinic (N) receptors; GRP and its receptor, the BB$_2$ bombesin receptor; gastrin and its receptor, the CCK$_2$; HIST and the H$_2$ receptor; and PGE$_2$ and the EP$_3$ receptor. A red line with a T bar indicates sites of pharmacological antagonism. A light blue dashed arrow indicates a drug action that mimics or enhances a physiological pathway. Shown in red are drugs used to treat acid-peptic disorders. NSAIDs can induce ulcers via inhibition of cyclooxygenase. Not shown is a physiological pathway that reduces acid secretion: a D cell that secretes SST, which inhibits G-cell release of gastrin.

ADME

Because an acidic pH in the parietal cell acid canaliculi is required for drug activation and food stimulates acid production, these drugs ideally should be given about 30 min before meals. Concurrent administration of food may reduce somewhat the rate of absorption of PPIs, but this effect is not thought to be clinically significant. Once in the small bowel, PPIs are rapidly absorbed, highly protein bound, and extensively metabolized by hepatic CYPs, particularly CYP2C19 and CYP3A4. Asians and Oceanians are more likely than Caucasians or Africans to have the CYP2C19 genotype that correlates with reduced metabolism of PPIs (25%–30% Asians, ~60% Oceanians vs. ~15% Caucasians or Africans), which may contribute to heightened efficacy or toxicity in this ethnic group (Lima et al., 2021). For chronic use of first-generation PPIs (*omeprazole, lansoprazole,* and *pantoprazole*) after efficacy has been achieved, reduction in the dose is recommended for those individuals with a CYP2C19 genotype that predicts reduced function (Lima et al., 2021).

Because not all pumps and all parietal cells are active simultaneously, maximal suppression of acid secretion requires several doses of the PPIs. For example, it may take 2 to 5 days of therapy with once-daily dosing to achieve the about 70% inhibition of proton pumps that is seen at steady state. More frequent initial dosing (e.g., twice daily) will reduce the time to achieve full inhibition but has not been shown to improve patient outcome. The resulting proton pump inhibition is irreversible; thus, acid secretion is suppressed for 24 to 48 h, or more, until new proton pumps are synthesized and incorporated into the luminal membrane of parietal cells. Chronic renal failure does not lead to drug accumulation with once-a-day dosing of the PPIs. Hepatic disease substantially reduces the clearance of *esomeprazole* and *lansoprazole*. Thus, in patients with severe hepatic disease, dose reduction is recommended for *esomeprazole* and *lansoprazole*.

Therapeutic Uses and Adverse Effects

Prescription PPIs are primarily used to promote healing of gastric and duodenal ulcers and to treat gastroesophageal reflux disease (GERD), including erosive esophagitis, which is either complicated or unresponsive to treatment with H$_2$ receptor antagonists. They are also used in conjunction with antibiotics for the eradication of *H. pylori*. PPIs also are the mainstay in the treatment of pathological hypersecretory conditions, including the Zollinger-Ellison syndrome. *Lansoprazole, pantoprazole*, and *esomeprazole* are approved for treatment and prevention of recurrence of NSAID-associated gastric ulcers in patients who continue NSAID use. It is not clear if PPIs affect the susceptibility to NSAID-induced damage and bleeding in the small and large intestine. All PPIs are approved for reducing the risk of duodenal ulcer recurrence associated with *H. pylori* infections. Over-the-counter *omeprazole, esomeprazole,* and *lansoprazole* are approved for the self-treatment of acid reflux. Therapeutic applications of the PPIs are discussed further in the section Therapeutic Strategies for Specific Acid-Peptic Disorders.

The PPIs generally cause remarkably few adverse effects and have a strong safety record (Malfertheiner et al., 2017a; Reimer, 2013). The most common side effects are headache, nausea, abdominal pain, constipation, flatulence, and diarrhea. Subacute myopathy, arthralgias, interstitial

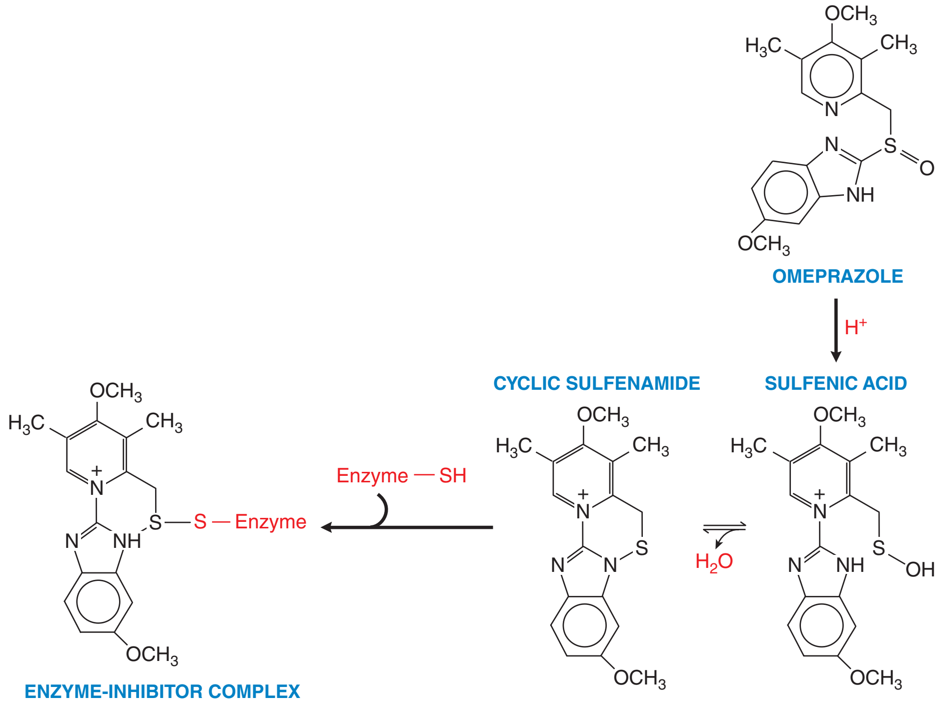


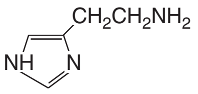
Figure 53–2 *Activation of a PPI from its prodrug form.* *Omeprazole* is converted to a sulfenamide in the acidic secretory canaliculi of the parietal cell. The sulfenamide interacts covalently with sulfhydryl groups in the proton pump, thereby irreversibly inhibiting its activity. *Lansoprazole, rabeprazole*, and *pantoprazole* undergo analogous conversions.

nephritis, pharyngitis, and skin rashes also have been reported. PPIs are metabolized by hepatic CYPs and therefore may interfere with the elimination of other drugs cleared by this route. PPIs have been observed to interact with *warfarin* (*esomeprazole, lansoprazole, omeprazole*, and *rabeprazole*); *diazepam* (*esomeprazole* and *omeprazole*); *atazanavir* or *nelfinavir* (*esomeprazole, dexlansoprazole, lansoprazole, omeprazole, pantoprazole*, and *rabeprazole*); and *cyclosporine* (*omeprazole* and *rabeprazole*). Among the PPIs, only *omeprazole* inhibits CYP2C19 (thereby decreasing the clearance of *disulfiram*, *phenytoin*, and other drugs) and induces the expression of CYP1A2 (thereby increasing the clearance of *imipramine*, several antipsychotic drugs, *tacrolimus*, and *theophylline*). There is some evidence that PPIs can inhibit conversion of *clopidogrel* (at the level of CYP2C19) to the active anticoagulating form, but this is controversial (Huang et al., 2012). *Pantoprazole* is less likely to result in this interaction; concurrent use of *clopidogrel* and PPIs (mainly *pantoprazole*) significantly reduces GI bleeding without increasing adverse cardiac events (see Chapter 36). Another drug interaction is between *methotrexate* and PPI therapy because PPIs can competitively inhibit *methotrexate* elimination and thereby increase *methotrexate* levels.

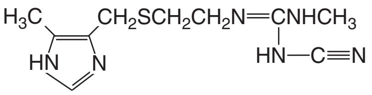
Chronic treatment with PPIs decreases the absorption of vitamin B_{12} (cobalamin), but the clinical relevance of this effect is not completely clear. Determination of serum vitamin B_{12} levels might be considered in long-term PPI users, especially those on high-dose PPIs and if they also have dietary restrictions that may limit vitamin B_{12} intake (see Chapter 45 for details of the importance of vitamin B_{12} to human health). Loss of gastric acidity also may affect the bioavailability of such drugs as *ketoconazole*, ampicillin esters, and iron salts. There is an association between PPI use and hypomagnesemia, with some guidelines recommending monitoring of magnesium levels in patients receiving long-term PPI therapy, particularly those also using diuretics or those with malabsorption disorders (Malfertheiner et al., 2017a; Nehra et al., 2018). Chronic use of PPIs has been reported to be associated with an increased risk of bone fracture and with increased susceptibility to certain infections (e.g., hospital-acquired pneumonia, community-acquired *Clostridium difficile*, spontaneous bacterial peritonitis in patients with ascites). Hypergastrinemia is more frequent and more severe with PPIs than with H_2 receptor antagonists and associated with this is ECL hyperplasia, fundic gland polyposis, and atrophic gastritis. This hypergastrinemia may predispose to rebound hypersecretion of gastric acid on discontinuation of therapy and may promote the growth of GI tumors, although the risk appears very low (Malfertheiner et al., 2017a; Nehra et al., 2018). There have been associations made between long-term PPI use and increased risk of small intestinal bacterial overgrowth, chronic kidney disease, and dementia. These studies are not yet supported by well-controlled prospective trials, and the evidence for these significant adverse effects remains limited (Freedberg et al., 2017; Malfertheiner et al., 2017a; Nehra et al., 2018). Recently, a dose relationship between the use of PPIs and more severe infection and secondary infections in patients with the SARS-CoV-2 (COVID-19) virus has been described (Almario et al., 2021; Pranata et al., 2021). This finding highlights the importance of gastric acid for gastrointestinal defense and that PPI use should be limited to the lowest effective doses and only when indicated clinically.

H_2 Receptor Antagonists

The arrival of selective histamine H_2 receptor antagonists was a landmark in the treatment of acid-peptic disease. Before the availability of the H_2 receptor antagonists, the standard of care was simply acid neutralization in the stomach lumen, generally with inadequate results. The long history of safety and efficacy with the H_2 receptor antagonists led to their availability without a prescription. Increasingly, however, PPIs are replacing the H_2 receptor antagonists in clinical practice.



HISTAMINE



CIMETIDINE

TABLE 53–1 ■ INTRAVENOUS DOSES OF H_2 RECEPTOR ANTAGONISTS

	CIMETIDINE	RANITIDINE	FAMOTIDINE
Intermittent bolus	300 mg every 6–8 h	50 mg every 6–8 h	20 mg every 12 h
Continuous infusion	37.5–100 mg/h	6.25–12.5 mg/h	1.7 mg/h

Mechanism of Action and Pharmacology

The H_2 receptor antagonists inhibit acid production by reversibly competing with histamine for binding to H_2 receptors on the basolateral membrane of parietal cells (Black, 1993). Four different H_2 receptor antagonists, which differ mainly in their pharmacokinetics and propensity to cause drug interactions, were available in the U.S. until recently: *cimetidine*, *ranitidine*, *famotidine*, and *nizatidine*. However, due to contamination issues, some preparations of *ranitidine* and *nizatidine* have been withdrawn from use. These drugs are less potent than PPIs but still suppress 24-h gastric acid secretion by about 70%. Suppression of basal and nocturnal acid secretion is about 70%; because suppression of nocturnal acid secretion is important in the healing of duodenal ulcers, evening dosing of an H_2 receptor antagonist is adequate therapy in most cases. There is little evidence for the use of H_2 receptor antagonists for the treatment of bleeding ulcers, and they are no longer recommended for this purpose. All four H_2 receptor antagonists are available as prescription and over-the-counter formulations for oral administration. Intravenous and intramuscular preparations of *cimetidine*, *ranitidine*, and *famotidine* also are available for use in critically ill patients (Table 53–1).

ADME

The H_2 receptor antagonists are rapidly absorbed after oral administration, with peak serum concentrations within 1 to 3 h. Absorption may be enhanced by food or decreased by antacids, but these effects probably are unimportant clinically. Therapeutic levels are achieved rapidly after intravenous dosing and are maintained for 4 to 5 h (*cimetidine*), 6 to 8 h (*ranitidine*), or 10 to 12 h (*famotidine*). The $t_{1/2}$ values of these agents after oral administration in adults range from 1 to 3 h; cimetidine clearance is faster in children, reducing its $t_{1/2}$ by about 30%. Only a small fraction of these drugs is protein bound. The kidneys excrete these drugs and their metabolites by filtration and renal tubular secretion, and it is important to reduce drug doses in patients with decreased creatinine clearance. Neither hemodialysis nor peritoneal dialysis clears significant amounts of these drugs. Hepatic metabolism accounts for a small fraction of clearance (from <10% to ~35%), but liver disease *per se* is generally not an indication for dose adjustment.

Therapeutic Uses and Adverse Effects

The major therapeutic indications for H_2 receptor antagonists are to promote healing of gastric and duodenal ulcers, to treat uncomplicated GERD, and to prevent the occurrence of stress ulcers. For more information about the therapeutic applications of H_2 receptor antagonists, see Therapeutic Strategies for Specific Acid-Peptic Disorders.

The H_2 receptor antagonists generally are well tolerated, with a low incidence of adverse effects. Side effects are minor and include diarrhea, headache, drowsiness, fatigue, muscular pain, and constipation. Less-common side effects include those affecting the CNS (confusion, delirium, hallucinations, slurred speech, and headaches), which occur primarily with intravenous administration of the drugs or in elderly subjects. Several reports have associated H_2 receptor antagonists with various blood disorders, including thrombocytopenia and vitamin B_{12} deficiency (Feldman and Burton, 1990; Lam et al., 2013). Gynecomastia and impotence have been reported to occur with cimetidine in a dose- and time-dependent fashion and resolve when the drug is discontinued (Jensen et al., 1983). H_2 receptor antagonists cross the placenta and are excreted in breast milk. Although no major teratogenic risk has been associated with these agents, caution is warranted when they are used in pregnancy.

All agents that inhibit gastric acid secretion may alter the rate of absorption and subsequent bioavailability of the H_2 receptor antagonists (see Antacids section). Drug interactions with H_2 receptor antagonists occur mainly with *cimetidine*, and its use has decreased markedly. *Cimetidine* inhibits CYPs (e.g., CYP1A2, CYP2C9, and CYP2D6) and thereby can increase the levels of a variety of drugs that are substrates for these enzymes. *Ranitidine* also interacts with hepatic CYPs, but with an affinity of only 10% of that of *cimetidine*. *Famotidine* and *nizatidine* are even safer in this regard. Slight increases in blood alcohol concentration may result from concomitant use of H_2 receptor antagonists and alcohol.

Tolerance and Rebound With Acid-Suppressing Medications

Tolerance to the acid-suppressing effects of H_2 receptor antagonists may develop within 3 days of starting treatment and may be resistant to increased doses of the medications (Sandevik et al., 1997). Diminished sensitivity to these drugs may result from the effect of the secondary hypergastrinemia to stimulate histamine release from ECL cells.

Potassium-Competitive Acid Blockers

While PPIs were a significant advancement in the treatment of acid peptic diseases and GERD, there are many gastrointestinal conditions, such as nonerosive reflex disease and erosive esophagitis, where there remains significant unmet clinical need for more effective therapies. Potassium-competitive acid blockers have been developed over the last 30 years and have been found to rapidly suppress acid secretion. Currently, this drug class is available only in Asia. Clinical trials of one potassium-competitive acid blocker, *vonoprazan*, are currently being conducted in the U.S. and Europe.

Mechanism of Action and Pharmacology

There are currently three potassium-competitive acid blockers available for clinical use, the pyrimidine derivative *revaprazan*, the benzimidazole derivative *tegoprazan*, and the pyrrole derivative *vonoprazan* (Figure 53–3).

The potassium-competitive acid blockers are weak bases that bind competitively and reversibly to the potassium binding site of the H^+/K^+-ATPase following protonation (Engevik et al., 2020). The large size of these molecules prevents the access of K^+ cations to their binding site, thus blocking activation of the H^+/K^+-ATPase. They accumulate to a much higher concentration than PPIs in the canaliculi of the parietal cell. After oral administration, this class of drugs achieves a high plasma concentration that results in early onset of action. They are also able to bind to both the active and inactive forms of the H^+/K^+-ATPase, resulting in a faster and longer duration of acid suppression than is observed for PPIs. The potential advantage of the K^+-competitive agents is their immediate full effect from the first dose, their long half-life, and their long duration of effect, resulting in greater nocturnal acid suppression (Abdel-Aziz et al., 2020; Shibli et al., 2020). Of the three K^+-competitive acid blockers, *vonoprazan* has been studied to the greatest extent.

ADME

The K^+-competitive acid blockers are rapidly absorbed in the fed or fasted condition, reaching maximum serum concentrations in 0.5 to 2 h. These drugs dissociate only slowly from their target, which increases their duration of action and results in a maximal effect in 1 day (compared to 3–5 days for PPIs). Since K^+-competitive acid blockers do not require acid-catalyzed activation, they have similar efficacy of acid suppression irrespective of meals. The elimination of these agents is independent of CYP2C19 activity, making them less susceptible than PPIs to interindividual variation. *Vonoprazan* is mainly metabolized in the liver via

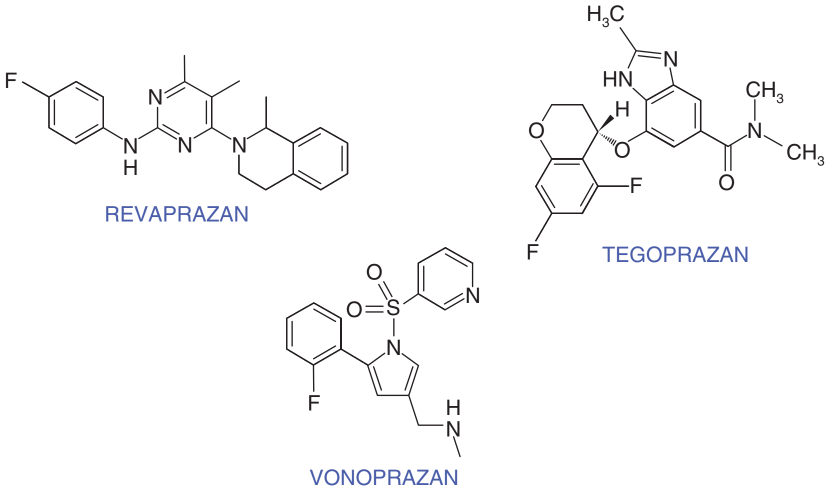


Figure 53–3 *The structures of potassium-competitive acid blockers.* *Revaprazan* is a pyrimidine derivative, *tegoprazan* is a benzimidazole derivative, and *vonoprazan* is a pyrrole derivative.

CYP3A4/5. The elimination $t_{1/2}$ values of these agents after oral administration in adults range from 2 to 9 h.

Therapeutic Uses and Adverse Effects

Revaprazan is approved in India and South Korea for the treatment of gastric ulcer, gastritis, and duodenal ulcer (200 mg daily); *tegoprazan* is approved in South Korea for erosive esophagitis and nonerosive reflux disease (50 mg daily). *Vonoprazan* fumarate is approved in Japan for the treatment of gastroduodenal ulcers, healing and prevention of erosive esophagitis, gastric protection in patients taking *aspirin* or NSAIDs, and eradication of *H. pylori* infection (10–20 mg daily).

Potassium-competitive acid blockers appear to be generally safe and well tolerated. In clinical trials of *vonoprazan*, the most frequent adverse effects were diarrhea, nasopharyngitis, dyspepsia, headache, and abdominal pain.

Agents That Enhance Mucosal Defense

Misoprostol

Misoprostol (15-deoxy-16-hydroxy-16-methyl-PGE$_1$) is a synthetic analogue of PGE$_1$ that is FDA-approved to prevent NSAID-induced mucosal injury.

Mechanism of Action and Pharmacology

Prostaglandin E$_2$ and prostacyclin (PGI$_2$) are the major PGs synthesized by the gastric mucosa. Contrary to their cyclic AMP–elevating effects on many cells via EPr$_2$ and EPr$_4$ receptors, these prostanoids bind to the EPr$_3$ receptor on parietal cells and stimulate the G$_i$ pathway, thereby decreasing intracellular cyclic AMP and gastric acid secretion. PGE$_2$ also can prevent gastric injury by cytoprotective effects that include stimulation of mucin and bicarbonate secretion and increased mucosal blood flow. Acid suppression appears to be the most important effect clinically (Wolfe and Sachs, 2000).

Because NSAIDs diminish PG formation by inhibiting cyclooxygenase, synthetic PG analogues offer a logical approach to counteract NSAID-induced damage.

ADME

Misoprostol is rapidly absorbed after oral administration and is rapidly and extensively deesterified to form misoprostol acid, the principal and active metabolite of the drug. A single dose inhibits acid production within 30 min; the therapeutic effect peaks at 60 to 90 min and lasts for up to 3 h. Food and antacids decrease the rate of *misoprostol* absorption. The free acid is excreted mainly in the urine, with an elimination $t_{1/2}$ of 20 to 40 min.

Therapeutic Uses and Adverse Effects

Misoprostol is rarely used because of its side effects (Rostom et al., 2009); see Chapters 41 and 48 concerning non-GI effects of *misoprostol*. The degree of inhibition of gastric acid secretion by *misoprostol* is directly related to dose; oral doses of 100 to 200 μg significantly inhibit basal acid secretion (up to 85%–95% inhibition) or food-stimulated acid secretion (up to 75%–85% inhibition). The usual recommended dose for ulcer prophylaxis is 200 μg four times a day.

Diarrhea, with or without abdominal pain and cramps, occurs in up to 30% of patients who take *misoprostol*. Apparently dose related, it typically begins within the first 2 weeks after therapy is initiated and often resolves spontaneously within a week; more severe cases may necessitate drug discontinuation. *Misoprostol* can cause clinical exacerbations of inflammatory bowel disease (see Chapter 55). *Misoprostol* is contraindicated for reducing the risk of NSAID-induced ulcers in women of childbearing potential unless the patient is at high risk of complications from gastric ulcers associated with use of the NSAID. It is also completely contraindicated during pregnancy because it can increase uterine contractility and abort a pregnancy (see Chapter 48).

Sucralfate

Mechanism of Action and Pharmacology

In the presence of acid-induced damage, pepsin-mediated hydrolysis of mucosal proteins contributes to mucosal erosion and ulcerations. This process can be inhibited by sulfated polysaccharides. *Sucralfate* consists of the octasulfate of sucrose to which Al(OH)$_3$ has been added. In an acid environment (pH <4), *sucralfate* undergoes extensive cross-linking to produce a viscous, sticky polymer that adheres to epithelial cells and ulcer craters for up to 6 h after a single dose. In addition to inhibiting hydrolysis of mucosal proteins by pepsin, *sucralfate* may have other cytoprotective effects, including stimulation of local production of PGs and epidermal growth factor (Szabo, 2014). *Sucralfate* also binds bile salts; thus, some clinicians use *sucralfate* to treat individuals with the syndromes of biliary esophagitis or gastritis (the existence of which is controversial).

Therapeutic Uses and Adverse Effects

Sucralfate is no longer used to treat peptic acid disease because PPIs are more effective. However, it is used for the initial treatment of GERD in pregnancy because it is poorly absorbed and for the treatment of mucositis secondary to cancer therapy (Lalla et al., 2014). Because increased gastric pH may be a factor in the development of nosocomial pneumonia in critically ill patients, *sucralfate* may offer an advantage over PPIs and H$_2$ receptor antagonists for the prophylaxis of stress ulcers. *Sucralfate* is also used in conditions associated with mucosal inflammation/ulceration that may not respond to acid suppression, including oral mucositis (radiation and aphthous ulcers) and bile reflux gastropathy. Administered by rectal enema, *sucralfate* also has been used for radiation proctitis and solitary rectal ulcers. Because it is activated by acid, *sucralfate* should be taken on an empty stomach 1 h before meals. Use of antacids within 30 min of a dose of *sucralfate* should be avoided. The dose of *sucralfate* is 1 g three times daily for the treatment of GERD in pregnancy and 1 g four to six times daily for prophylaxis of stress ulcers for a maximum of 14 days. For the prevention of radiation-induced enteropathy in patients receiving radiation therapy to the pelvis, *sucralfate* is given as an oral dose of 500 mg twice daily. For the treatment of chronic radiation-induced proctitis, it can be given as an enema.

The most common side effect of *sucralfate* is constipation (~2%). *Sucralfate* should be avoided in patients with renal failure who are at risk for aluminum overload (Marks, 1991). Likewise, aluminum-containing antacids should not be combined with *sucralfate* in these patients. *Sucralfate* forms a viscous layer in the stomach that may inhibit absorption of other drugs, including *phenytoin, digoxin, cimetidine, ketoconazole*, and fluoroquinolone antibiotics. *Sucralfate* therefore should be taken at least 2 h after the administration of other drugs. The "sticky" nature of the viscous gel produced by *sucralfate* in the stomach also may be responsible for the development of bezoars in some patients.

Antacids

Mechanism of Action and Pharmacology

There are far more effective and persistent agents than antacids, but their price, accessibility, and rapid action make them popular with consumers as OTC medications, and they can be used for the acute treatment of acid reflux ("heartburn") and esophagitis (see discussion that follows). Many factors, including palatability, determine the effectiveness and choice of antacid. Although sodium bicarbonate effectively neutralizes acid, it is very water soluble and rapidly absorbed from the stomach, and the alkali and sodium loads may pose a risk for patients with cardiac or renal failure. $CaCO_3$ rapidly and effectively neutralizes gastric H^+, but the release of CO_2 from bicarbonate- and carbonate-containing antacids can cause belching, nausea, abdominal distention, and flatulence. Calcium also may induce rebound acid secretion, necessitating more frequent administration. Combinations of Mg^{2+} (rapidly reacting) and Al^{3+} (slowly reacting) hydroxides provide a relatively balanced and sustained neutralizing capacity and are preferred by most experts. Magaldrate, a hydroxymagnesium aluminate complex, is converted rapidly in gastric acid to $Mg(OH)_2$ and $Al(OH)_3$, which are absorbed poorly and thus provide a sustained antacid effect. Although fixed combinations of Mg^{2+} and Al^{3+} theoretically counteract the adverse effects of each other on the bowel (Al^{3+} can relax gastric smooth muscle, producing delayed gastric emptying and constipation; Mg^{2+} exerts the opposite effects), such balance is not always achieved in practice. *Simethicone,* a surfactant that may decrease foaming and hence esophageal reflux, is included in many antacid preparations. However, other fixed combinations, particularly those with *aspirin*, that are marketed for "acid indigestion" are potentially unsafe in patients predisposed to gastroduodenal ulcers and should not be used.

Therapeutic Uses and Adverse Effects

Antacids are used for the relief of mild GERD symptoms that occur infrequently in adults, older children, and adolescents. They are given orally 1 and 3 h after meals and at bedtime. In general, antacids should be administered in suspension form because this formulation probably has greater neutralizing capacity than powder or tablet dosage forms. Antacids are cleared from the empty stomach in about 30 min. However, the presence of food is sufficient to elevate gastric pH to approximately 5 for about 1 h and to prolong the neutralizing effects of antacids for about 2 to 3 h.

Antacids vary in the extent to which they are absorbed and hence in their systemic effects. In general, most antacids can elevate urinary pH by about 1 pH unit. Antacids that contain Al^{3+}, Ca^{2+}, or Mg^{2+} are absorbed less completely than are those that contain $NaHCO_3$. With renal insufficiency, absorbed Al^{3+} can contribute to osteoporosis, encephalopathy, and proximal myopathy. About 15% of orally administered Ca^{2+} is absorbed, causing transient hypercalcemia. The hypercalcemia from as little as 3 to 4 g of $CaCO_3$ per day can be problematic in patients with uremia. In the past, when large doses of $NaHCO_3$ and $CaCO_3$ were administered commonly with milk or cream for the management of peptic ulcer, the *milk-alkali syndrome* (alkalosis, hypercalcemia, and renal insufficiency) occurred frequently. Today, this syndrome is rare and generally results from the chronic ingestion of large quantities of Ca^{2+} (five to forty 500-mg tablets per day of calcium carbonate) taken with milk.

By altering gastric and urinary pH, antacids may affect a number of drugs (e.g., thyroid hormones, *allopurinol*, and imidazole antifungals) by altering rates of dissolution and absorption, bioavailability, and renal elimination. Al^{3+} and Mg^{2+} antacids also are notable for their propensity to chelate other drugs present in the GI tract and thereby decrease their absorption. Most interactions can be avoided by taking antacids 2 h before or after ingestion of other drugs.

Other Acid Suppressants and Cytoprotectants

The M_1 muscarinic receptor antagonists *pirenzepine* and *telenzepine* (see Chapter 11) can reduce basal acid production by 40% to 50%. The ACh receptor on the parietal cell itself is of the M_3 subtype, and these drugs are believed to suppress neural stimulation of acid production via actions on M_1 receptors of intramural ganglia (see Figure 53–1). Because of their relatively poor efficacy, significant and undesirable anticholinergic side effects, and risk of blood disorders (*pirenzepine*), they are rarely used today.

Rebamipide is used for ulcer therapy in India, parts of Asia, and Russia. It exerts cytoprotective effects by increasing PG generation in gastric mucosa and by scavenging reactive oxygen species. *Ecabet*, which appears to increase the formation of PGE_2 and PGI_2, also is used for ulcer therapy, mostly in Japan. *Carbenoxolone*, a derivative of glycyrrhizic acid found in licorice root, has been used with modest success for ulcer therapy in Europe. Unfortunately, *carbenoxolone* inhibits the type I isozyme of 11β-hydroxysteroid dehydrogenase, which protects the mineralocorticoid receptor from activation by cortisol in the distal nephron; it therefore causes hypokalemia and hypertension due to excessive mineralocorticoid receptor activation (see Chapter 50). Bismuth compounds (see Chapter 54) are frequently prescribed in combination with antibiotics to eradicate *H. pylori* and prevent ulcer recurrence. Bismuth compounds bind to the base of the ulcer, promote mucin and bicarbonate production, and have significant antibacterial effects. In the colon, bismuth salts combine with hydrogen sulfide to form bismuth sulfide, which turns the stools black.

Therapeutic Strategies for Specific Acid-Peptic Disorders

Gastroesophageal Reflux Disease

Although most cases of acid reflux or gastroesophageal regurgitation follow a relatively benign course, these symptoms, often referred to as nonerosive reflux disease, can still be troubling (Boeckxstaens et al., 2014). More severe GERD is erosive esophagitis, characterized by endoscopically visible mucosal damage. This can lead to stricture formation and Barrett's metaplasia (replacement of squamous by intestinal columnar epithelium), which is associated with a small but significant risk of adenocarcinoma. The goals of GERD therapy are complete resolution of symptoms and healing of esophagitis (Altan et al., 2012). PPIs clearly are more effective than H_2 receptor antagonists in achieving these goals (Figure 53–4).

In general, the optimal dose for each patient is determined based on symptom control. Strictures associated with GERD also respond better to PPIs than to H_2 receptor antagonists. One of the complications of GERD, Barrett's esophagus, appears to be more refractory to therapy because neither acid suppression nor antireflux surgery has been shown convincingly to produce regression of metaplasia.

Regimens for the treatment of GERD with PPIs and histamine H_2 receptor antagonists are listed in Table 53–2. Although some patients with mild GERD symptoms may be managed by nocturnal doses of H_2 receptor antagonists, twice-daily dosing usually is required. Antacids are insufficient and are recommended only for the patient with mild,

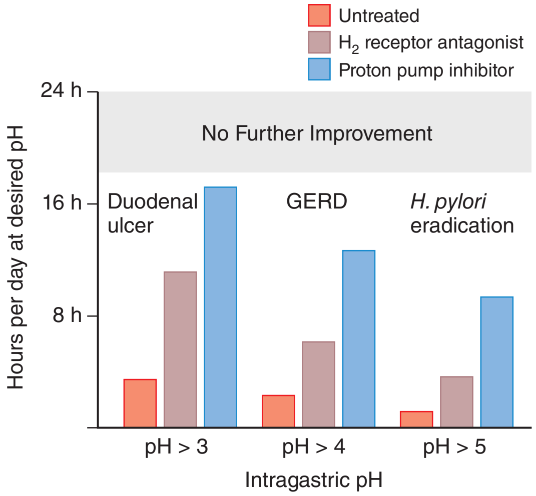


Figure 53–4 *Comparative success of therapy with PPIs and H_2 antagonists.* Data show the effects of a PPI (given once daily) and an H_2 receptor antagonist (given twice daily) in elevating gastric pH to the target ranges (i.e., pH 3 for duodenal ulcer, pH 4 for GERD, and pH 5 for antibiotic eradication of *H. pylori*).

infrequent episodes of acute acid reflux. In general, prokinetic agents (see Chapter 54) are not particularly useful for the treatment of refractory GERD, either alone or in combination with acid-suppressant medications. There is reasonable evidence that PPIs and, to a lesser extent, H_2 receptor antagonists are safe and effective for the treatment of GERD in children (Tighe et al., 2014).

Severe Symptoms and Nocturnal Acid Breakthrough

In patients with severe symptoms or extraintestinal manifestations of GERD, twice-daily dosing with a PPI may be needed. However, it is difficult, if not impossible, to render patients achlorhydric, and two-thirds or more of subjects will continue to make acid, particularly at night. This phenomenon, called *nocturnal acid breakthrough*, has been invoked as a cause of refractory symptoms in some patients with GERD. However, decreases in gastric pH at night while on therapy generally are not associated with acid reflux into the esophagus, and the rationale for suppressing nocturnal acid secretion remains to be established. Patients with continuing symptoms on twice-daily PPIs are often treated by adding an H_2 receptor antagonist at night. Although this can further suppress acid production, the effect is short lived, probably due to the development of tolerance (Fackler et al., 2002).

Therapy for Extraintestinal Manifestations of GERD

Acid reflux has been implicated in a variety of atypical symptoms, including noncardiac chest pain, asthma, laryngitis, chronic cough, and other

TABLE 53–2 ■ ANTISECRETORY DRUG REGIMENS FOR TREATMENT OF GERD

DRUG	ADULT DOSAGE	PEDIATRIC DOSAGE
H_2 receptor antagonists[a]		
Cimetidine	400 mg 4 times daily or 800 mg twice daily for 12 weeks	20–40 mg/kg/day divided every 6 h for 8–12 weeks
Famotidine	10–20 mg twice daily for up to 12 weeks	0.5 mg/kg/day at bedtime or divided every 12 h (infants <3 months)[b]
Nizatidine	150 mg twice daily	<12 years: 5–10 mg/kg/day[c] divided every 12 h >12 years: 150 mg twice daily
Ranitidine	150 mg twice daily	5–10 mg/kg/day divided, every 8–12 h
Proton pump inhibitors		
Esomeprazole magnesium Esomeprazole sodium Esomeprazole strontium	20–40 mg daily for 4–8 weeks 20–40 mg daily (IV)[e] 24.65 or 49.3 mg daily for 4–8 weeks	1–11 years: 10 mg/day, >12 years, 20 mg/day up to 8 weeks IV[d,e]: 0.5 mg/kg daily (infants >1 month); children: 10 mg daily (<55 kg); 20 mg daily (>55 kg)
Dexlansoprazole	30 mg daily for 4 weeks (nonerosive GERD); erosive GERD: 60 mg daily up to 6 months, then 30 mg daily up to 6 months (maintenance therapy)	60 mg/day for 8 weeks then 30 mg/g for 6 months (erosive esophagitis) 30 mg/day for 4 weeks (GERD)
Lansoprazole	15 mg (nonerosive GERD) or 30 mg (erosive GERD) daily up to 8 weeks	15–30 mg daily[d] for up to 12 weeks
Omeprazole	20 mg daily	5–20 mg daily[d]
Pantoprazole	40 mg daily (erosive GERD)	20–40 mg daily[d] for up to 8 weeks
Rabeprazole	20 mg daily (erosive GERD)	Children 1–11 years old: 5–10 mg daily up to 12 weeks >12 years: 20 mg daily up to 8 weeks
Potassium competitive acid blockers		
Revaprazan	200 mg daily	
Tegoprazan	50 mg daily	
Vonoprazan	10–20 mg daily	

[a] Not for erosive disease.

[b] For children and adolescents, individualize treatment duration and dose based on clinical response or pH determination (gastric or esophageal) and endoscopy. For infants, employ conservative measures (e.g., thickened feedings) and limit therapy to 8 weeks.

[c] Indicates off-label use.

[d] Varies by weight.

[e] Used when oral PPI cannot be given; short-term use only.

ear, nose, and throat conditions. PPIs (at higher doses) have been used with some success in certain patients with these disorders.

GERD and Pregnancy

Acid reflux is estimated to occur in 30% to 50% of pregnancies, with an incidence approaching 80% in some populations (Richter, 2003). In the vast majority of cases, GERD ends soon after delivery and thus does not represent an exacerbation of a preexisting condition. Because of its high prevalence and the fact that it can contribute to the nausea of pregnancy, treatment often is required. Treatment choice in this setting is complicated by the paucity of safety data about use during pregnancy for the most commonly used drugs. The FDA has ceased to use a lettered risk classification system (A–D and X, progressing from no risk to high risk/do not use in pregnancy), preferring a more flexible and descriptive system customized for each drug. In the old system, most drugs used to treat GERD were considered safe for conservative use during pregnancy (old category B), except for *omeprazole* (old category C), which was to be used only when the benefits outweighed the risks. In the new system, physicians, pharmacists, and patients should consult the package insert for the most recent information on use in pregnancy. For cases of GERD during pregnancy and breastfeeding, literature reviews (Ali and Egan, 2007; Thélin and Richter, 2020) suggest a conservative progression of treatments, starting with antacids, alginates, or *sucralfate*, agents that are considered the first-line drugs in this setting. If symptoms persist, H_2 receptor antagonists can be used, with *cimetidine* having the most established track record in this setting, and with *nizatidine* to be avoided due to adverse data from animal studies. PPIs are reserved for women with intractable symptoms or complicated reflux disease; considering available data, *lansoprazole* and *pantoprazole* seem to be the safest choices.

Pediatric GERD

Reflux disease in infants and children is increasing at an alarming rate (Vandenplas, 2014). Children over 10 years can be diagnosed and treated similarly to adults, but infants and very young children require careful diagnosis to rule out cow's milk allergy or eosinophilic esophagitis. Many nonpharmacologic approaches can be used to alleviate some of the very troubling symptoms of this condition, which may not be due to acid reflux. If acid reduction is indicated, PPIs are more effective than H_2 receptor antagonists; however, the therapeutic efficacy of PPIs in newborns and infants is low, and there is an increased risk of adverse effects, including respiratory tract infections and gastroenteritis, which should be carefully considered. It is likely PPIs are overused in the treatment of pediatric GERD.

Peptic Ulcer Disease

Peptic ulcer disease is best viewed as an imbalance between mucosal defense factors (bicarbonate, mucin, PGs, NO, and other peptides and growth factors) and injurious factors (acid and pepsin) (Hunt et al., 2015; Wallace, 2008). On average, patients with *duodenal ulcers* produce more acid than do control subjects, particularly at night (basal secretion). Although patients with *gastric ulcers* have normal or even diminished acid production, ulcers rarely, if ever, occur in the complete absence of acid. Presumably, weakened mucosal defense and reduced bicarbonate production contribute to the injury from the relatively lower levels of acid in these patients. *H. pylori* and exogenous agents such as NSAIDs interact in complex ways to cause an ulcer. Up to 60% of peptic ulcers are associated with *H. pylori* infection of the stomach. This infection may lead to impaired production of somatostatin by D cells and, in time, cause decreased inhibition of gastrin production, resulting in increased acid production and reduced duodenal bicarbonate production. Table 53–3 summarizes current recommendations for drug therapy of gastroduodenal ulcers.

The PPIs relieve symptoms of duodenal ulcers and promote healing more rapidly than do H_2 receptor antagonists, although both classes of drugs are effective in this setting (see Figure 53–4). A peptic ulcer represents a chronic disease, and recurrence within 1 year is expected in most patients who do not receive prophylactic acid suppression. With the appreciation that *H. pylori* plays a major etiopathogenic role in most peptic ulcers, prevention of relapse is focused on eliminating this organism from the stomach. Intravenous *esomeprazole* (80 mg IV over 30 min, followed by 8 mg/h continuous infusion for a total of 72 h, then 40 mg orally or another single daily dose oral PPI, for an appropriate duration; off-label use) and *pantoprazole* (off-label use) are the preferred therapy in patients with acute bleeding ulcers (Laine and Jensen, 2012; Wong and Sung, 2013). The theoretical benefit of maximal acid suppression in this setting is to accelerate healing of the underlying ulcer. In addition, a higher gastric pH enhances clot formation and retards clot dissolution.

The NSAIDs also are frequently associated with peptic ulcers and bleeding. The effects of these drugs are mediated systemically; in the stomach, NSAIDs suppress mucosal PG synthesis (particularly PGE_2 and PGI_2) and thereby reduce mucus production and cytoprotection (see Figure 53–1). Thus, *minimizing NSAID use is an important adjunct to gastroduodenal ulcer therapy.*

TABLE 53–3 ■ REGIMENS FOR TREATING GASTRODUODENAL ULCERS IN ADULTS[a]

DRUG	ACTIVE ULCER	MAINTENANCE THERAPY
Proton pump inhibitors[b]		
Esomeprazole magnesium	NSAID risk reduction: 20 or 40 mg daily for up to 6 months	
Esomeprazole strontium	NSAID risk reduction: 24.65 or 49.3 mg daily for up to 6 months	
Lansoprazole	15 mg (DU) daily for 4 weeks 15 mg (NSAID risk reduction) daily for up to 12 weeks 30 mg (GU including NSAID associated) daily for up to 8 weeks	15 mg daily 30 mg daily[c]
Omeprazole	20 mg (DU and GU) daily for 4–8 weeks	20 mg daily[c]
Pantoprazole	20 mg (NSAID risk reduction) daily[c] 20 mg (GU) daily[c]	20 mg daily[c]
Rabeprazole	20 mg (DU for up to 4 weeks; GU[c]) daily	
Prostaglandin analogue		
Misoprostol	200 μg four times daily (NSAID-associated ulcer prevention)[d]	

[a] There is little evidence for the use of H_2 receptor antagonists for the treatment of bleeding ulcers.
[b] Deslansoprazole is not labeled for the treatment of active ulcers.
[c] Off-label use.
[d] Only misoprostol 800 μg/day has been directly shown to reduce the risk of ulcer complications such as perforation, hemorrhage, or obstruction. (Rostom A, Moayyedi P, Hunt R. Canadian Association of Gastroenterology Consensus Group. Canadian consensus guidelines on long-term nonsteroidal anti-inflammatory drug therapy and the need for gastroprotection: benefits versus risks. *Aliment Pharmacol Ther* **2009**, 29:481–496.)

Treatment of *Helicobacter pylori* Infection

Helicobacter pylori, a gram-negative rod, has been associated with gastritis and the subsequent development of gastric and duodenal ulcers, gastric adenocarcinoma, and gastric B-cell lymphoma (Suerbaum and Michetti, 2002). Because of the critical role of *H. pylori* in the pathogenesis of peptic ulcers, eradicating this infection is standard care in patients with gastric or duodenal ulcers (Malfertheiner et al., 2013). Provided that patients are not taking NSAIDs, this strategy almost completely eliminates the risk of ulcer recurrence. Eradication of *H. pylori* also is indicated in the treatment of mucosa-associated lymphoid tissue lymphomas of the stomach, which can regress significantly after such treatment. *H. pylori* eradication is also indicated for treatment of chronic atrophic gastritis and presence of intestinal metaplasia/dysplasia (with positive *H. pylori* biopsies).

Five important considerations influence the selection of an eradication regimen (Table 53–4) (Chey et al., 2017; Malfertheiner et al., 2017b):

- Single-antibiotic regimens are ineffective in eradicating *H. pylori* infection and lead to microbial resistance. Combination therapy with two or three antibiotics (plus acid-suppressive therapy) is associated with the highest rate of *H. pylori* eradication.
- A PPI significantly enhances the effectiveness of *H. pylori* antibiotic regimens containing *amoxicillin* and *clarithromycin* (see Figure 53–4).
- A regimen of 10 to 14 days of treatment appears to be better than shorter treatment regimens.
- Poor patient compliance is linked to the medication-related side effects experienced by as many as half of patients taking triple-agent regimens and to the inconvenience of three- or four-drug regimens administered several times per day. Packaging that combines the daily doses into one convenient unit is available and may improve patient compliance.
- The emergence of resistance to *clarithromycin* and *metronidazole* increasingly is recognized as an important factor in the failure to eradicate *H. pylori*. In the presence of *in vitro* evidence of resistance to *metronidazole*, *amoxicillin* should be used instead. In areas with a high frequency of resistance to *clarithromycin* and *metronidazole*, a 14-day quadruple-drug regimen (three antibiotics combined with a PPI) generally is effective therapy.

TABLE 53–4 ■ THERAPY OF *HELICOBACTER PYLORI* INFECTION

Triple therapy × 10–14 days: PPI + clarithromycin 500 mg + amoxicillin 1 g twice a day (metronidazole 500 mg twice a day can be substituted for amoxicillin)
Quadruple therapy × 10–14 days: PPI + metronidazole 250 mg + bismuth subsalicylate 300 mg + tetracycline 500 mg four times daily
Or
Sequential therapy: PPI + amoxicillin 1 g twice a day for 5 days followed by PPI + clarithromycin 500 mg and tinidazole/metronidazole 500 mg twice a day for 5 days
Or
PPI + amoxicillin 1 g twice a day + levofloxacin 250 or 500 mg twice a day for 10 days
PPI *daily dosages*:
Omeprazole: 20 mg twice a day (triple therapy); 40 mg daily (dual therapy)
Lansoprazole: 30 mg twice a day (triple therapy); 30 mg three times daily for 14 days (dual therapy with amoxicillin)
Rabeprazole: 20 mg twice a day for 7 days
Pantoprazole: 40 mg twice a day[a]
Esomeprazole magnesium: 40 mg daily (triple therapy)
Esomeprazole strontium: 49.3 mg daily (triple therapy)

[a] Off-label use.
Data from Chey et al., 2017.

NSAID-Related Ulcers

Chronic NSAID users have a 2% to 4% risk of developing a symptomatic ulcer, GI bleeding, or perforation. Ideally, NSAIDs should be discontinued in patients with an ulcer if possible. Healing of ulcers despite continued NSAID use is possible with the use of acid-suppressant agents, usually at higher doses and for a considerably longer duration than standard regimens (e.g., ≥8 weeks). PPIs are superior to H_2 receptor antagonists and misoprostol in promoting the healing of active ulcers and in preventing recurrence of gastric and duodenal ulcers in the setting of continued NSAID administration (Rostom et al., 2009). The FDA has approved fixed-dose combinations of NSAIDs with a PPI or H_2 antagonist; these combinations are intended to lower the risk of ulcers in patients who regularly use NSAIDs for arthritic pain.

Stress-Related Ulcers

Stress ulcers are ulcers of the stomach or duodenum that occur in the context of a profound illness or trauma requiring intensive care (Bardou et al., 2015). The etiology of stress-related ulcers differs somewhat from that of other peptic ulcers, involving acid and mucosal ischemia. Because of limitations on the oral administration of drugs in many patients with stress-related ulcers, intravenous H_2 receptor antagonists have been used extensively to reduce the incidence of GI hemorrhage due to stress ulcers. Now that intravenous preparations of PPIs are available, they are appropriate to consider. However, there is some concern over the risk of pneumonia secondary to gastric colonization by bacteria in an alkaline milieu. In this setting, *sucralfate* appears to provide reasonable prophylaxis against bleeding without increasing the risk of aspiration pneumonia.

Zollinger-Ellison Syndrome

Patients with Zollinger-Ellison syndrome develop pancreatic or duodenal gastrinomas that stimulate the secretion of very large amounts of acid, sometimes in the setting of multiple endocrine neoplasia, type I (Krampitz and Norton, 2013). This can lead to severe gastroduodenal ulceration and other consequences of uncontrolled hyperchlorhydria. PPIs are the drugs of choice, usually given at about twice the routine dosage for peptic ulcers (*omeprazole* 60 mg daily, *esomeprazole* 80 mg daily, *lansoprazole* 60 mg daily, *rabeprazole* 60 mg daily, or *pantoprazole* 120 mg daily); some patients need two to three times these doses to control acid secretion. However, once control of acid secretion has been achieved, dose reduction is usually possible. PPIs are well tolerated and safe even at very high doses. If PPIs are unable to control gastric acid secretion, the long-acting somatostatin analogue *octreotide* (off-label indication) can be given to inhibit secretion of gastrin. This is not a first-line agent due to unpredictable response rates and the side effects of the treatment.

Functional Dyspepsia

The term *functional dyspepsia* refers to ulcer-like symptoms in patients who lack overt gastroduodenal ulceration (Masuy et al., 2019). Functional dyspepsia can be subdivided into postprandial distress syndrome and epigastric pain syndrome, based on the presence of symptoms related to meals. It is defined as the presence of one or more of the following: postprandial fullness, early satiation, epigastric pain or burning, and no evidence of structural disease. It may be associated with gastritis (with or without *H. pylori*) or with NSAID use, but the pathogenesis of this syndrome remains controversial.

The PPIs appear to be moderately effective in the treatment of patients with epigastric pain syndrome and are a first-line therapy (Masuy et al., 2019). In general, twice-daily PPIs are no better than once-daily PPIs. The dosing is as for GERD (Table 53–2). H_2 receptor antagonists are only marginally effective for the treatment of functional dyspepsia. Because central mechanisms may contribute to functional dyspepsia either through visceral hypersensitivity or other mechanisms, tricyclic antidepressants such as *amitriptyline* or *desipramine* (10–25 mg at night) (see Chapter 18) can be considered in patients with functional dyspepsia whose symptoms persist despite PPI therapy for 8 weeks. Prokinetic agents such as *metoclopramide* (see Chapter 54) are a first-line therapy for the treatment of postprandial distress syndrome. The novel gastroprokinetic agent *acotiamide* has been applied to postprandial distress syndrome with symptom improvements noted in most trials (Masuy et al., 2019), and the $5HT_{1A}$ serotonin receptor agonist *buspirone* that relaxes the gastric fundus (see Chapter 15) improved gastric accommodation and GI symptoms in patients with functional dyspepsia. Antacids are not generally helpful for the treatment of functional dyspepsia.

Functional Esophageal Disorders

Functional esophageal disorders are disorders that cause esophageal symptoms and that are diagnosed based on negative results on standard esophageal tests, thereby excluding structural disorders, motility disorders like achalasia, and GERD (Amarasinghe and Sifrim, 2014). There are four of these common disorders: (1) functional heartburn, (2) functional chest pain, (3) functional dysphagia, and (4) globus. PPI therapy (off-label use) as outlined previously is routinely used for the initial treatment of functional heartburn, functional chest pain, and globus. As in functional dyspepsia, central mechanisms contribute to these disorders and similar approaches follow for the treatment of functional heartburn and functional chest pain if PPI therapy is ineffective, including the use of tricyclic antidepressants or selective serotonin reuptake inhibitors. For the treatment of globus, *gabapentin* or *pregabalin* is used.

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Drug Facts for Your Personal Formulary: *Antisecretory Agents and Gastroprotectives*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Proton Pump Inhibitors		
Dexlansoprazole	• Gastroesophageal reflux disease • Erosive esophagitis	• Generally well tolerated • Possible interaction with clopidogrel (controversial) • Increased incidence of osteoporosis-related fractures of hip, wrist, or spine • Diarrhea • Interstitial nephritis • May cause cyanocobalamin (vitamin B_{12}) deficiency with daily long-term use (>3 years)
Esomeprazole Lansoprazole Omeprazole Pantoprazole	• Gastric ulcers • Duodenal ulcers • Erosive esophagitis • Gastroesophageal reflux disease • *Helicobacter pylori* eradication • Zollinger-Ellison syndrome	• OTC forms for acid reflux • Generally well tolerated • Possible interaction with clopidogrel (controversial) • Increased incidence of osteoporosis-associated fractures of hip, wrist, or spine • Diarrhea • Interstitial nephritis • May cause cyanocobalamin (vitamin B_{12}) deficiency with daily long-term use (>3 years) • Interactions with diagnostic investigations for neuroendocrine tumors
Rabeprazole	• Gastroesophageal reflux disease • *Helicobacter pylori* eradication • Zollinger-Ellison syndrome	• Generally well tolerated • Possible interaction with clopidogrel (controversial) • Increased incidence of osteoporosis-associated bone fractures of hip, wrist, or spine • Diarrhea • Interstitial nephritis
H_2 Receptor Antagonists		
Cimetidine Famotidine Nizatidine Ranitidine	• Gastric ulcer (to promote healing) • Duodenal ulcer (to promote healing) • Gastroesophageal reflux disease	• No longer recommend for treating active ulcers • Generally well tolerated • Beware of drug interactions with cimetidine
Potassium-Competitive Acid Blockers		
Revaprazan Tegoprazan Vonoprazan	• Gastric ulcer • Duodenal ulcer • Gastroesophageal reflux disease • *Helicobacter pylori* eradication (vonoprazan)	• Generally well tolerated • Only available in Asia
Mucosal Defensive Agents		
Misoprostol	• Ulcer prophylaxis	• Rarely used because of side effects (especially ↑ uterine contractility) • Not be used in women of childbearing potential or in pregnancy • Diarrhea; exacerbation of inflammatory bowel disease • Marketed in combination with diclofenac
Sucralfate	• Initial treatment of GERD in pregnancy	• Generally well tolerated • Constipation
Antacids	• Acid reflux • Esophagitis	• OTC; generally well tolerated; produce rapid but temporary effects • Na^+ and Al^{+3} loads: potential problems in cardiovascular and renal disease

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Chapter 54

Gastrointestinal Motility and Water Flux, Emesis, and Biliary and Pancreatic Disease

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- Small Intestinal Bacterial Overgrowth

Gastrointestinal Motility

The gastrointestinal (GI) tract is in a continuous contractile, absorptive, and secretory state. The control of this state is complex, with contributions by the muscle and epithelium, the enteric nervous system (ENS), the autonomic nervous system (ANS), microbial mediators, innate and adaptive immune cells and their mediators, and local enteroendocrine and circulating hormones. Of these, the master regulator of physiological gut function is the ENS (Figure 54–1) (Fung and Vanden Berghe, 2020; Furness, 2012; Sharkey et al., 2018; Spencer and Hu, 2020).

The ENS is an extensive collection of nerves and glial cells that constitutes the third division of the ANS. It is the only part of the ANS that is truly capable of autonomous function if separated from the CNS. The ENS lies within the wall of the GI tract and is organized into two connected networks of neurons, nerve fibers, and glial cells: the *myenteric (Auerbach) plexus*, found between the circular and longitudinal muscle layers, and the *submucosal (Meissner) plexus*, located in the submucosa (Furness, 2012; Sharkey, 2015). The former is largely responsible for motor control, whereas the latter regulates secretion, ion and fluid transport, and blood flow. There is extensive two-way neuronal communication between the two plexuses to regulate these processes.

To prevent the unwanted translocation of toxins, antigens, commensal bacteria, and other potentially pathogenic components of the luminal contents, an elaborate "intestinal barrier" has developed. This consists of three components:

1. A physical barrier comprising the epithelial cells and the junctional proteins that maintain the integrity of this monolayer
2. An immune barrier, which comprises both innate and adaptive immune cells and specialized epithelial cells (microfold cells) that overlie Peyer's patches
3. A secretory barrier, which includes the secretion of antimicrobial peptides, secretory IgA, mucus, and water, driven by luminally directed ion transport

The secretory and immune components of the intestinal barrier are regulated by ENS and ANS neural mechanisms that integrate the control of these components of barrier function with digestive processes in the gut (Martin et al., 2018; Odenwald and Turner, 2017).

Generation and Regulation of GI Motor and Secretory Activity

The ENS is responsible for the largely autonomous nature of most GI motor and secretory activity. This activity is organized into relatively distinct "programs" that respond to input from the local environment of the gut, as well as the ANS-CNS. Each program consists of a series of complex, but coordinated, patterns of secretion and movement that show regional and temporal variation (Deloose et al., 2012). The fasting program of motor activity in the gut is called the MMC (migrating myoelectric complex when referring to electrical activity and migrating motor complex when referring to the accompanying contractions) and consists of a series of four phasic activities: I, quiescence; II, increasing frequencies of action potentials and smooth muscle contractions; III, peak contractile activity; and IV, declining activity toward a renewal of phase I. Phase II of the MMC is associated with the release of the peptide hormone motilin. Motilin agonists stimulate motility in the proximal gut. The most characteristic, phase III, consists of clusters of rhythmic contractions that occupy short segments of the intestine for a period of 6 to 10 min before proceeding caudally (toward the anus). One MMC cycle (i.e., all four phases) takes about 80 to 110 min. The MMC occurs in the fasting state, helping to sweep debris caudad in the gut and limiting the overgrowth of commensal luminal bacteria. The MMC is interrupted by

Abbreviations

ACh: acetylcholine
ANS: autonomic nervous system
CCK: cholecystokinin
CFTR: cystic fibrosis transmembrane conductance regulator
CTZ: chemoreceptor trigger zone
CYP: cytochrome P450
DOR: delta opioid receptor
ECG: electrocardiogram
ENaC: epithelial sodium channel
ENS: enteric nervous system
FDA: U.S. Food and Drug Administration
GC: guanyl cyclase
GERD: gastroesophageal reflux disease
GI: gastrointestinal
GLP: glucagon-like peptide
GPCR: G protein-coupled receptor
HIV: human immunodeficiency virus
5HT: serotonin, 5-hydroxytryptamine
IBS: irritable bowel syndrome
KOR: kappa opioid receptor
MOR: mu opioid receptor
NEP: neutral endopeptidase
NHE: Na$^+$-H$^+$ exchanger
NK: neurokinin
NO: nitric oxide
NTS: nucleus of the solitary tract
OTC: over-the-counter
PEG: polyethylene glycol
Pgp: P-glycoprotein (MDR1, ABCB1)
PK: protein kinase (e.g., PKA, PKC)
PONV: postoperative nausea and vomiting
QT: ECG interval (duration of ventricular depolarization and repolarization)
SLC: solute carrier transporter
SSRI: selective serotonin reuptake inhibitor
SST: somatostatin
TMEM: transmembrane protein
USP: U.S. Pharmacopeia
VIP: vasoactive intestinal peptide

the fed program in intermittently feeding animals such as humans. The fed program consists of high-frequency (12–15/min) contractions that either are propagated for short segments (propulsive) or are irregular and not propagated (mixing).

Peristalsis is a series of reflex responses to a bolus in the lumen of a given segment of the intestine; the ascending excitatory reflex results in contraction of the circular muscle on the oral side of the bolus, whereas the descending inhibitory reflex results in relaxation on the anal side. The net pressure gradient moves the bolus caudad. Motor neurons receive input from ascending and descending interneurons (which constitute the relay and programming systems), which are of two broad types, excitatory and inhibitory. The primary neurotransmitter of the excitatory motor neurons is acetylcholine (ACh). The principal neurotransmitter of the inhibitory motor neurons is nitric oxide (NO), although important contributors may include ATP, vasoactive intestinal peptide (VIP), and pituitary adenylyl cyclase–activating peptide (PACAP). Enterochromaffin cells, the major population of enteroendocrine cells, scattered throughout the epithelium of the intestine, release serotonin (5HT) to initiate many gut reflexes by acting locally on enteric neurons (Mawe and Hoffman, 2013). Excessive release of 5HT in the gut wall (e.g., by chemotherapeutic agents) leads to vomiting by actions of 5HT on vagal nerve endings in the proximal small intestine. Compounds targeting the 5HT system are important modulators of motility, secretion, and emesis.

Other cell types are also important in the regulation of GI motility, including interstitial cells of Cajal and various other enteroendocrine cell populations. Interstitial cells of Cajal, which are distributed in networks within the gut wall, are responsible for setting the electrical rhythm and the pace of contractions in various regions of the gut (Huizinga and Chen, 2014). These cells also modulate excitatory and inhibitory neuronal communication to the smooth muscle. Enteroendocrine cell populations release locally acting hormones, such as ghrelin, cholecystokinin (CCK), motilin, and glucagon-like peptide-1 (GLP-1), all of which influence GI motility, before (e.g., ghrelin) or after meals (e.g., CCK and GLP-1) (Psichas et al., 2015).

Excitation-Contraction Coupling in GI Smooth Muscle

Control of tension in GI smooth muscle is dependent on the intracellular Ca^{2+} concentration (Sanders et al., 2012). There are basically two types of excitation-contraction coupling in these cells. *Ionotropic receptors* can mediate changes in membrane potential, which in turn activate voltage-gated Ca^{2+} channels to trigger an influx of Ca^{2+} (electromechanical coupling); *metabotropic receptors* activate various signal transduction pathways to release Ca^{2+} from intracellular stores (pharmacomechanical coupling). Inhibitory receptors act via PKA and PKG and lead to hyperpolarization, decreased cytosolic [Ca^{2+}], and reduced interaction of actin and myosin. As an example, NO may induce relaxation via activation of the guanylyl cyclase–cyclic GMP pathway and cause the opening of several types of K$^+$ channels.

Functional and Motility Disorders of the Bowel

Gastrointestinal motility disorders are a heterogeneous group of conditions (Black et al., 2020; Faure et al., 2012). Common motility disorders include achalasia of the esophagus (impaired relaxation of the lower esophageal sphincter associated with defective esophageal peristalsis that results in dysphagia and regurgitation), gastroparesis (delayed gastric emptying), GERD (gastroesophageal reflux disease, chronic reflux of gastric contents into the esophagus due to an increased frequency of transient lower esophageal sphincter relaxations, ineffective esophageal peristalsis, or gastric dysmotility), intestinal pseudoobstruction (myopathic and neuropathic forms of intestinal dysmotility), Hirschsprung disease, anorectal dysfunction, and others. These disorders can be congenital, idiopathic, or secondary to systemic diseases (e.g., diabetes mellitus or scleroderma). Motility disorders also traditionally include functional GI conditions, such as irritable bowel syndrome (IBS), functional dyspepsia, and noncardiac chest pain (Black et al., 2020). These disorders of brain-gut interaction are characterized by the presence of visceral hypersensitivity from the gut associated with GI motor abnormalities and other symptoms. For most of these disorders, treatment remains empirical and symptom based, reflecting limited understanding of the pathophysiology involved in most cases.

Prokinetic Agents and Other Stimulants of GI Motility

Prokinetic agents are medications that enhance coordinated GI motility and transit of material in the GI tract (Acosta and Camilleri, 2015; Bharucha and Lacy, 2020; Gudsoorkar and Quigley, 2020; Pittayanon et al., 2019; Tack and Camilleri, 2018). These agents appear to enhance the release of excitatory neurotransmitter at the nerve-muscle junction without interfering with the normal physiological pattern and rhythm of motility, or they mimic the effects of motilin. By contrast, activation of muscarinic receptors with the older cholinomimetic agents (see Chapter 11) or secondary to acetylcholinesterase inhibitors (see Chapter 12) enhances contractions in a relatively uncoordinated fashion that produces little or no net propulsive motor activity.

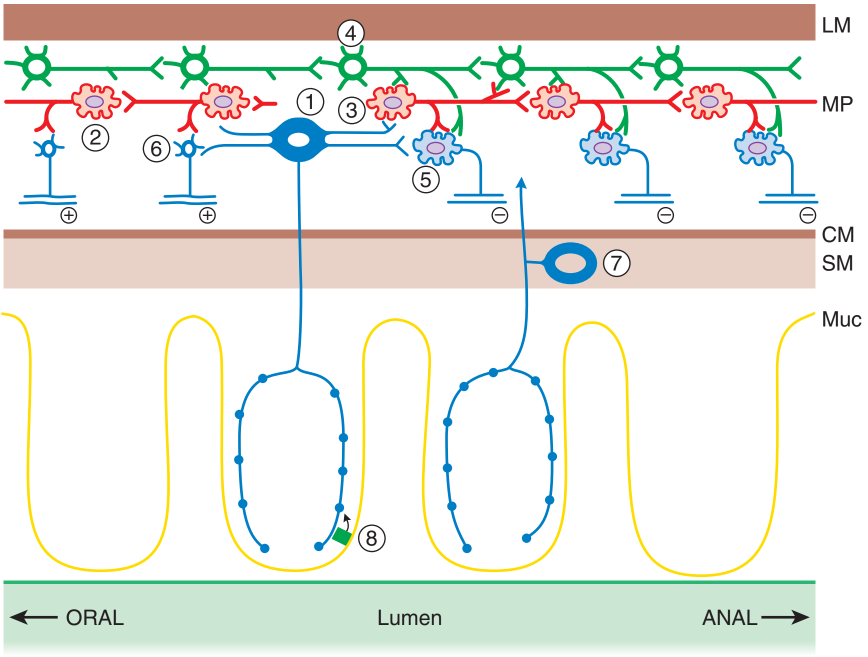
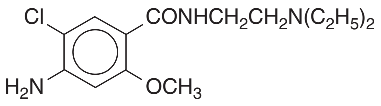


Figure 54–1 *The neuronal network that initiates and generates peristalsis* Mucosal stimulation leads to the release of serotonin by enterochromaffin cells (8), which excites the intrinsic primary afferent neurons (1), which then communicate with ascending (2) and descending (3) interneurons in the local reflex pathways. The reflex results in contraction at the oral end via the excitatory motor neuron (6) and aboral relaxation via the inhibitory motor neuron (5). The migratory myoelectric complex (see text) is shown here as being conducted by a different chain of interneurons (4). Another intrinsic primary afferent neuron with its cell body in the submucosa also is shown (7). CM, circular muscle; LM, longitudinal muscle; MP, myenteric plexus; Muc, mucosa; SM, submucosa.

Dopamine Receptor Antagonists

Dopamine is present in significant amounts in the GI tract and has several inhibitory effects on motility, including reduction of lower esophageal sphincter and intragastric pressures. These effects, which result from suppression of ACh release from myenteric motor neurons, are mediated by D_2 dopaminergic receptors. Dopamine receptor antagonists are effective as prokinetic agents; they have the additional advantage of relieving nausea and vomiting by antagonism of dopamine receptors in the chemoreceptor trigger zone (CTZ) of the brainstem. Examples are *metoclopramide* and *domperidone* (Acosta and Camilleri, 2015; Reddymasu et al., 2007).

Metoclopramide



METOCLOPRAMIDE

Mechanism of Action and Pharmacology. *Metoclopramide* and other substituted benzamides are derivatives of *para*-aminobenzoic acid and are structurally related to *procainamide*. The mechanisms of action of *metoclopramide* are complex and involve $5HT_4$ receptor agonism, vagal and central $5HT_3$ antagonism, and possible sensitization of muscarinic receptors on smooth muscle, in addition to dopamine receptor antagonism. Administration of *metoclopramide* results in coordinated contractions that enhance transit. The effects are confined largely to the upper digestive tract, where it increases lower esophageal sphincter tone and stimulates antral and small intestinal contractions. *Metoclopramide* has no clinically significant effects on large-bowel motility (Acosta and Camilleri, 2015).

ADME. *Metoclopramide* is absorbed rapidly after oral ingestion, undergoes sulfation and glucuronide conjugation by the liver, and is excreted principally in the urine, with a $t_{1/2}$ of 4 to 6 h. Peak concentrations occur within 1 h after a single oral dose; the duration of action is 1 to 2 h.

Therapeutic Uses and Adverse Effects. *Metoclopramide* is a first-line therapy in patients with gastroparesis, who may experience improvements of gastric emptying. Because of adverse effects related to drug exposure, the recommended duration of use is less than 12 weeks. *Metoclopramide* injection is used as an adjunctive measure in medical or diagnostic procedures such as upper endoscopy or contrast radiography of the GI tract (single IV dose of 10 mg). Its greatest utility lies in its ability to ameliorate the nausea and vomiting that often accompany GI dysmotility syndromes, such as gastroparesis. *Metoclopramide* is available in oral dosage forms (tablets and solution) and as a parenteral preparation for intravenous, subcutaneous, or intramuscular administration. An intranasal formulation has also recently been developed and approved for diabetic gastroparesis (one spray is equivalent to a 10-mg oral dose). The initial regimen is 5 to 10 mg orally, 15 to 30 min before each meal and at bedtime, titrating up to the lowest effective dose. A dose of up to 40 mg/day given in divided doses can generally be tolerated. The onset of action is within 30 to 60 min. In patients with severe nausea, an initial dose of 10 mg can be given intramuscularly (onset of action 10–15 min), subcutaneously, or intravenously (onset of action 1–3 min). *Metoclopramide* is used as an adjunctive agent for the prevention of *cisplatin*-induced delayed emesis and for emesis failing first-line treatments. For prevention of chemotherapy-induced emesis, *metoclopramide* can be given as an infusion of 1 to 2 mg/kg administered over at least 15 min, beginning 30 min before the chemotherapy is begun and repeated as needed every 2 h for two doses, then every 3 h for three doses. *Metoclopramide* is not recommended for the treatment of GERD or in children because of significant safety concerns (see discussion that follows) and limited efficacy.

The major side effects of *metoclopramide* include extrapyramidal effects. Dystonias, usually occurring acutely after intravenous administration, and parkinsonian-like symptoms that may occur several weeks after initiation of therapy generally respond to treatment with anticholinergic or antihistaminic drugs and reverse on discontinuation of *metoclopramide*. Tardive dyskinesia also can occur with chronic treatment and may be irreversible. Extrapyramidal effects appear to occur more commonly in children and young adults and at higher doses. *Metoclopramide* also can cause anxiety, depression, and prolonged QT intervals. It may also cause galactorrhea by blocking the inhibitory effect of dopamine on prolactin release (seen infrequently in clinical practice). Methemoglobinemia has been reported occasionally in premature and full-term neonates receiving *metoclopramide*.

Domperidone

Mechanism of Action and Pharmacology. In contrast to *metoclopramide*, *domperidone* predominantly antagonizes the D_2 receptor without major involvement of other receptors, but otherwise, its mechanism of action is similar (Reddymasu et al., 2007).

ADME. *Domperidone* is rapidly absorbed, yielding peak concentrations in 30 min. The drug undergoes metabolism via hepatic CYP3A4, N-dealkylation, and hydroxylation; it has a $t_{1/2}$ of 7 h. The metabolites are excreted in the feces (~two-thirds) and urine (~one-third).

Therapeutic Uses and Adverse Effects. *Domperidone* is available for use in the U.S. only through an expanded access to investigational drugs with the U.S. Food and Drug Administration (FDA), but it is readily available in many other countries. For the treatment of gastroparesis, *domperidone* has modest prokinetic activity in doses of 10 mg three times a day, which can be increased to 20 mg three times a day if needed. Although it does not readily cross the blood-brain barrier to cause extrapyramidal side effects, *domperidone* exerts effects in the parts of the CNS that lack this barrier, such as those regulating emesis, temperature, and prolactin release. *Domperidone* does not appear to have any significant effects on lower GI motility. Like *metoclopramide*, it has limited efficacy in children. There is an increased risk of serious ventricular arrhythmias, including sudden cardiac death, in association with *domperidone* use, especially in older persons (>60 years) and at doses above 30 mg/day. Like *metoclopramide*, it can also elevate prolactin levels, presenting as galactorrhea, gynecomastia, amenorrhea, or impotence.

Serotonin Receptor Agonists

Serotonin (5HT) plays an important role in the normal motor and secretory function of the gut (see Chapter 13) (Gershon and Tack, 2007; Mawe and Hoffman, 2013). Indeed, more than 90% of the total 5HT in the body exists in the GI tract. Enterochromaffin cells produce most of this 5HT and rapidly release it in response to chemical and mechanical stimulation (e.g., food boluses; chemotherapeutic agents such as *cisplatin*; certain microbial toxins; adrenergic, cholinergic, and purinergic receptor agonists). 5HT triggers the peristaltic reflex (see Figure 54–1) by stimulating intrinsic sensory neurons in the myenteric plexus (via 5HT$_{1p}$ and 5HT$_4$ receptors), as well as extrinsic vagal and spinal sensory neurons (via 5HT$_3$ receptors). In addition, stimulation of submucosal intrinsic afferent neurons activates secretomotor reflexes, resulting in epithelial secretion.

The 5HT receptors occur on other neurons in the ENS, where they can be either stimulatory (5HT$_3$ and 5HT$_4$) or inhibitory (5HT$_{1A}$). In addition, serotonin stimulates the release of other neurotransmitters. Thus, 5HT$_1$ stimulation of the gastric fundus results in release of NO and reduction in smooth muscle tone. 5HT$_4$ stimulation of excitatory motor neurons enhances ACh release at the neuromuscular junction, and both 5HT$_3$ and 5HT$_4$ receptors facilitate interneuronal signaling. Developmentally, 5HT acts as a neurotrophic factor for enteric neurons via the 5HT$_{2B}$ and 5HT$_4$ receptors. Reuptake of serotonin by enteric neurons and epithelium is mediated by the same transporter (SERT) as 5HT reuptake by serotonergic neurons in the CNS. This reuptake also is blocked by SSRIs (see Figures 15–4 and 18–1), which explains the common side effect of diarrhea that accompanies the use of these agents (Gershon, 2013).

Because of the importance of 5HT on gut motor function, it became a major target for drug development. However, the availability of serotonergic prokinetic drugs has been restricted in recent years because of serious adverse cardiac events (Tack et al., 2012).

Cisapride

Mechanism of Action and Pharmacology. *Cisapride* is a 5HT$_4$ agonist that stimulates adenylyl cyclase activity in neurons. It also has weak 5HT$_3$ antagonistic properties and may directly stimulate smooth muscle. *Cisapride* was a commonly used prokinetic agent; however, it is no longer generally available and is rarely used because of its potential to induce serious and occasionally fatal cardiac arrhythmias, including ventricular tachycardia, ventricular fibrillation, and torsades de pointes. These arrhythmias result from a prolonged QT interval through an interaction with pore-forming subunits of the human ether-a-go-go related gene, the *HERG* K^+ channel (see Chapter 34).

ADME. *Cisapride* is metabolized by hepatic CYP3A4. It has an onset of action of 30 to 60 min and a $t_{1/2}$ of 6 to 12 h.

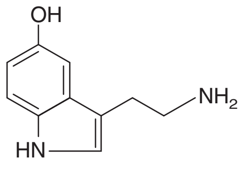
Therapeutic Uses and Adverse Effects. *Cisapride* is available only through an investigational, limited-access program for patients with GERD, gastroparesis, intestinal pseudoobstruction, refractory severe chronic constipation, and neonatal enteral feeding intolerance who have failed all standard therapeutic modalities and who have undergone a thorough diagnostic evaluation, including an electrocardiogram (ECG). It has modest prokinetic activity in doses of 10 to 20 mg four times a day, given 30 min before meals. *Cisapride* is contraindicated in patients with a history of prolonged QT interval, renal failure, ventricular arrhythmias, ischemic heart disease, congestive heart failure, respiratory failure, uncorrected electrolyte abnormalities, or concomitant medications known to prolong the QT interval. Other side effects of *cisapride* include abdominal discomfort and diarrhea.

Prucalopride

Mechanism of Action and Pharmacology. *Prucalopride* is a specific 5HT$_4$ receptor agonist (Figure 54–2) that facilitates cholinergic neurotransmission. It acts throughout the length of the intestine, increasing oral-cecal transit and colonic transit without affecting gastric emptying in healthy volunteers.

ADME. *Prucalopride* has a time to peak action of 2 to 3 h and a $t_{1/2}$ of 24 h. It is primarily excreted in the urine as the unchanged drug.

Therapeutic Uses and Adverse Effects. Given once daily in doses of 1 to 4 mg orally, the drug improves bowel habits, significantly increases the number of spontaneous, complete bowel movements, reduces the severity of symptoms, and improves quality of life in patients with severe chronic constipation. *Prucalopride* is approved for use in adults with chronic constipation in whom laxatives fail to provide adequate relief. Cardiovascular risks do not seem to be elevated, but patients should be monitored



5HT

LIGAND	SPECIFICITY
Alosetron	5HT$_3$ antagonist
Metoclopramide	5HT$_4$ agonist; 5HT$_3$ antagonist; D_2 antagonist
Prucalopride	5HT$_4$ agonist
Tegaserod	5HT$_4$ partial agonist

Figure 54–2 *Serotonergic agents modulating GI motility.*

(Diederen et al., 2015). The drug carries a warning for suicidal ideation, although no causal association has been established based on observations in clinical trials. Patients should be closely monitored and counseled to be aware of changes in mood or behavior. Headache, abdominal pain, nausea, and diarrhea are the most common adverse effects reported in clinical trials. *Prucalopride* is contraindicated in patients with intestinal perforation or obstruction, obstructive ileus, and inflammatory conditions of the GI tract such as Crohn's disease, ulcerative colitis, and toxic megacolon/megarectum.

Tegaserod

Mechanism of Action and Pharmacology. *Tegaserod* is an aminoguanidine indole that is structurally related to serotonin and is a partial $5HT_4$ agonist with negligible affinity for other receptor subtypes (see Figure 54–2). *Tegaserod* has multiple effects on the GI tract: It stimulates motility and accelerates transit in the esophagus, stomach, small bowel, and ascending colon; it also stimulates Cl^- secretion. The clinical efficacy of *tegaserod* has been proven only in female patients with constipation-predominant IBS. *Tegaserod* is indicated in women under the age of 65 who fail first-line therapies. *Tegaserod* is also an option for female patients with IBS with mixed bowel habits who fail other therapies.

ADME. *Tegaserod* has a time to peak action of about 1 h and a $t_{1/2}$ of 5 to 8 h. The drug is metabolized via hydrolysis and direct glucuronidation. It is primarily excreted in the feces as the unchanged drug, with the remainder excreted in the urine as metabolites.

Therapeutic Uses and Adverse Effects. Given in doses of 6 mg orally, twice a day and 30 min before meals, the drug improves bowel habits. It should be discontinued if adequate symptom control is not obtained in 4 to 6 weeks. *Tegaserod* carries a number of warnings and precautions. It is contraindicated in patients with a history of myocardial infarction, stroke, transient ischemic attacks or angina, ischemic colitis, severe renal impairment, moderate or severe hepatic impairment, bowel obstruction, or gallbladder disease. Like *prucalopride*, *tegaserod* carries a warning for suicidal ideation, based on observations in clinical trials. Patients should be closely monitored and counseled to be aware of changes in mood or behavior. Diarrhea is one of the most common adverse effects of *tegaserod*. Volume depletion, hypotension, and syncope associated with diarrhea have been reported. Other adverse effects reported in clinical trials include headache, abdominal pain, nausea, flatulence, dyspepsia, and dizziness.

Motilin and Macrolide Antibiotics

Mechanism of Action and Pharmacology

Motilin, a 22–amino acid peptide hormone secreted by enteroendocrine M cells and by some enterochromaffin cells of the upper small bowel, is a potent contractile agent of the upper GI tract. Motilin levels fluctuate in association with the MMC and appear to be responsible for the amplification, if not the actual induction, of phase III activity. Motilin receptors are G protein-coupled receptors (GPCRs) found on smooth muscle cells and enteric neurons.

The effects of motilin can be mimicked by *erythromycin*, a property shared to varying extents by other macrolide antibiotics (e.g., *azithromycin*, *clarithromycin*, etc.; see Chapter 60). In addition to its motilin-like effects, which are most pronounced at higher doses (250–500 mg), *erythromycin* at lower doses (e.g., 40–80 mg) also may act by other poorly defined mechanisms that may involve cholinergic facilitation. *Erythromycin* has multiple effects on upper GI motility, increasing lower esophageal pressure and stimulating gastric and small-bowel contractility. By contrast, it has little or no effect on colonic motility. At doses higher than 3 mg/kg, it can produce a spastic type of contraction in the small bowel, resulting in cramps, impairment of transit, and vomiting.

ADME

Erythromycin is metabolized by demethylation in the liver by CYP3A4. The time to peak action is about 0.5 to 2.5 h (ethylsuccinate formulation), and it has a $t_{1/2}$ of 2 h. It is primarily excreted in the feces.

Therapeutic Uses and Adverse Effects

Erythromycin is used as a prokinetic agent in patients with diabetic gastroparesis, where it can improve gastric emptying in the short term. *Erythromycin*-stimulated gastric contractions can be intense and result in "dumping" of relatively undigested food into the small bowel. This potential disadvantage can be exploited clinically to clear the stomach of undigestible residue such as bezoars or blood after a GI bleed prior to endoscopy. Rapid development of tolerance (~28 days) to the prokinetic effect of *erythromycin*, possibly by downregulation of the motilin receptor, and the agent's antibiotic effects (undesirable in this context) limit the use of *erythromycin* as a prokinetic agent. A standard dose of *erythromycin* for gastric stimulation is 1.5 to 3 mg/kg intravenous infusion every 6 h in a hospital setting or 125 mg orally every 12 h (Acosta and Camilleri, 2015). For small-bowel stimulation, a smaller dose (e.g., 3 mg/kg IV every 8 h) may be more useful; higher doses may retard the motility. Tachyphylaxis to *erythromycin* and potential side effects limit its use in the management of gastroparesis. Concerns about GI toxicity, ototoxicity, pseudomembranous colitis, and the induction of resistant strains of bacteria, QT prolongation, and sudden death, particularly when used in patients taking medications that inhibit CYP3A4, limit the use of *erythromycin* to acute situations or in circumstances where patients are resistant to other medications.

Other macrolides (e.g., *azithromycin* and *clarithromycin*) accelerate gastric emptying, but there are no clinical trials compared to other medications or placebo indicating any benefit. Moreover, their additional cost, potential for risk, and antibiotic resistance preclude consideration for their use in motility disorders.

Miscellaneous Agents for Stimulating Motility

The hormone CCK is released from the intestine in response to meals and delays gastric emptying, causes contraction of the gallbladder, stimulates pancreatic enzyme secretion, increases intestinal motility, and promotes satiety. The C-terminal octapeptide of CCK, *sincalide*, is useful for stimulating the gallbladder or pancreas and for accelerating barium transit through the small bowel for diagnostic testing of these organs. It is given by intravenous injection or infusion and has an onset of action of 5 to 15 min.

Currently, there are a number of agents under evaluation that stimulate motility whose mechanisms of action are based on well-established neurohumoral mechanisms (Tack and Camilleri, 2018). These include novel motilin receptor agonists and $5HT_4$ agonists (*velusetrag* and *naronapride*).

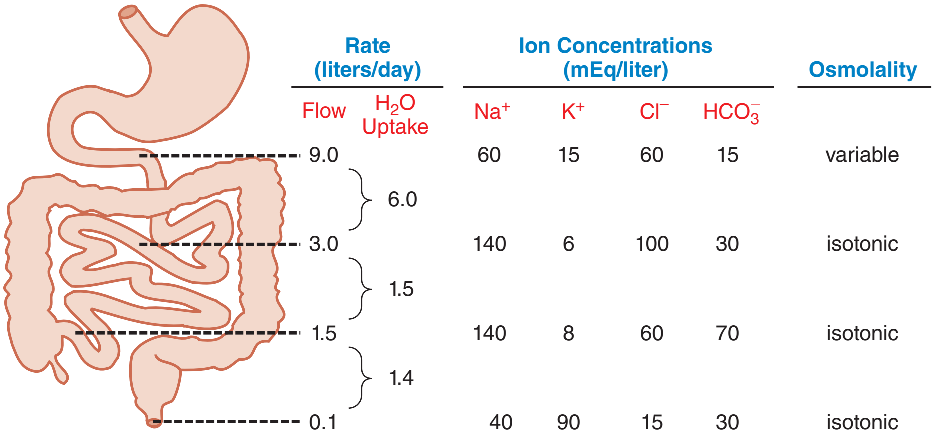
Agents That Suppress Motility

Smooth muscle relaxants such as organic nitrates, type 5 phosphodiesterase inhibitors, and Ca^{2+} channel antagonists produce temporary, if partial, relief of symptoms in motility disorders such as jackhammer esophagus, in which the lower esophageal sphincter fails to relax, resulting in severe difficulty in swallowing (Pandolfino and Gawron, 2015). Preparations of botulinum toxin (*onabotulinumtoxinA*), injected directly into the lower esophageal sphincter via an endoscope, in doses of 100 units given in four equal portions, inhibit ACh release from nerve endings and can produce partial paralysis of the sphincter muscle, with significant improvements in symptoms and esophageal clearance (Zhao and Pasricha, 2003). Other GI conditions in which botulinum toxin A has been used include gastroparesis, sphincter of Oddi dysfunction, and anal fissures, although currently there are no strong trial data to support its efficacy in these conditions.

Laxatives, Cathartics, and Therapy for Constipation

Overview of GI Water and Electrolyte Flux

Water normally accounts for 70% to 85% of total stool weight. Net stool fluid content reflects a balance between luminal input (ingestion of fluids and luminally directed secretion of water and electrolytes) and output



Rate (liters/day)		Ion Concentrations (mEq/liter)				Osmolality
Flow	H_2O Uptake	Na^+	K^+	Cl^-	HCO_3^-	
9.0		60	15	60	15	variable
	6.0					
3.0		140	6	100	30	isotonic
	1.5					
1.5		140	8	60	70	isotonic
	1.4					
0.1		40	90	15	30	isotonic

Figure 54–3 *Typical volume and composition of fluid that traverses the small and large intestines daily.* Of the 9 L of fluid typically presented to the small intestine each day, 2 L are from the diet and 7 L are from secretions (salivary, gastric, pancreatic, and biliary). The absorptive capacity of the colon is 4 to 5 L/day.

(absorption) along the length of the GI tract. The daily challenge for the gut is to extract water, minerals, and nutrients from the luminal contents, leaving behind sufficient fluid for proper expulsion of waste via the process of defecation.

Normally, about 8 to 9 L of fluid enter the small intestine daily from exogenous and endogenous sources (Figure 54–3). Net absorption of the water occurs in the small intestine in response to osmotic gradients that result from the uptake and secretion of ions and the absorption of nutrients (mainly sugars and amino acids), with only about 1 to 1.5 L crossing the ileocecal valve. The colon then extracts most of the remaining fluid, leaving about 100 mL of fecal water daily. Under normal circumstances, these quantities are within the range of the total absorptive capacity of the small bowel (~16 L) and colon (4–5 L). Neurohumoral mechanisms, pathogens, and drugs can alter secretion and absorption of fluid by the intestinal epithelium (Figure 54–4). Altered motility also contributes to this process. With decreased motility and excess fluid removal, feces can become inspissated and impacted, leading to constipation. When the capacity of the colon to absorb fluid is exceeded, diarrhea occurs.

Constipation: General Principles of Pathophysiology and Treatment

Patients use the term *constipation* not only for decreased frequency but also for difficulty in initiation, passage of firm or small-volume feces, or a feeling of incomplete evacuation.

Constipation has many reversible or secondary causes, including lack of dietary fiber, side effects of drugs, hormonal disturbances, neurogenic disorders, and systemic illnesses. In most cases of chronic constipation, no specific cause is found. Up to 60% of patients presenting with constipation have normal colonic transit. These patients either have IBS or define constipation in terms other than stool frequency. In the rest, attempts usually are made to categorize the underlying pathophysiology either as a disorder of delayed colonic transit because of an underlying defect in colonic motility or, less commonly, as an isolated disorder of defecation or evacuation (outlet disorder) due to dysfunction of the neuromuscular apparatus of the rectoanal region.

Colonic motility is responsible for mixing luminal contents to promote absorption of water and moving them from proximal to distal segments by means of propulsive contractions (Dinning et al., 2009). Mixing in the colon is accomplished in a way similar to that in the small bowel: by short- or long-duration, stationary (nonpropulsive) contractions. In any given patient, the predominant factor often is not obvious. Consequently, the pharmacological approach to constipation remains empirical and is usually based on physiologic principles.

Treatment of constipation includes both nonpharmacologic and pharmacologic approaches. Most guidelines recommend a diet rich in fiber (20–35 g daily), adequate fluid intake, and appropriate bowel habits and training as primary measures for subjects affected by constipation. Dietary and lifestyle factors, such as low intake of dietary fibers and physical inactivity, can predispose individuals to develop constipation (Bharucha and Lacy, 2020; Camilleri et al., 2017). Many drug classes cause constipation (Bharucha and Lacy, 2020). In order to correct this, stopping or reducing the doses of medications that cause constipation may be an option. Current evidence supports the use of osmotic or stimulant laxatives as first treatment strategies in patients with functional and chronic constipation (Bharucha and Lacy, 2020). Stimulant laxatives are frequently recommended in patients who do not respond to osmotic laxatives (Camilleri et al., 2017). When stimulant laxatives are used, they should be administered at the lowest effective dosage and for the shortest period in order to achieve regular bowel evacuations and to avoid abuse. Treatment cycles may have to be repeated because of the chronic nature of the condition. In addition to perpetuating dependence on drugs, the laxative habit may give rise to chronic diarrhea leading to excessive loss of water and electrolytes and abdominal pain. Secondary aldosteronism may occur if volume depletion is prominent. Steatorrhea, protein-losing enteropathy with hypoalbuminemia, and osteomalacia due to excessive loss of calcium in the stool have been reported.

Laxatives frequently are employed before surgical, radiological, and endoscopic procedures where an empty colon is desirable. The terms *laxatives*, *cathartics*, *purgatives*, *aperients*, and *evacuants* often are used interchangeably. There is a distinction, however, between *laxation* (the evacuation of formed fecal material from the rectum) and *catharsis* (the evacuation of unformed, usually watery, fecal material from the entire colon). Most of the commonly used agents promote laxation, but some are cathartics that act as laxatives at low doses.

Laxatives relieve constipation and promote evacuation of the bowel via:

- Enhancing retention of intraluminal fluid by hydrophilic or osmotic mechanisms
- Decreasing net absorption of fluid by effects on small- and large-bowel fluid and electrolyte transport
- Altering motility by inhibiting segmenting (nonpropulsive) contractions or stimulating propulsive contractions

Laxatives can be classified based on their actions (Table 54–1) or by the pattern of effects produced by the usual clinical dosage (Table 54–2), with some overlap between classifications.

A variety of laxatives, both osmotic agents and stimulants, increase the activity of NO synthase and the biosynthesis of platelet-activating factor (PAF; see Chapter 41) in the gut. PAF is a phospholipid proinflammatory mediator that stimulates colonic secretion and GI motility (Izzo et al., 1998). NO also may stimulate intestinal secretion and inhibit segmenting contractions in the colon, thereby promoting laxation. Agents that reduce

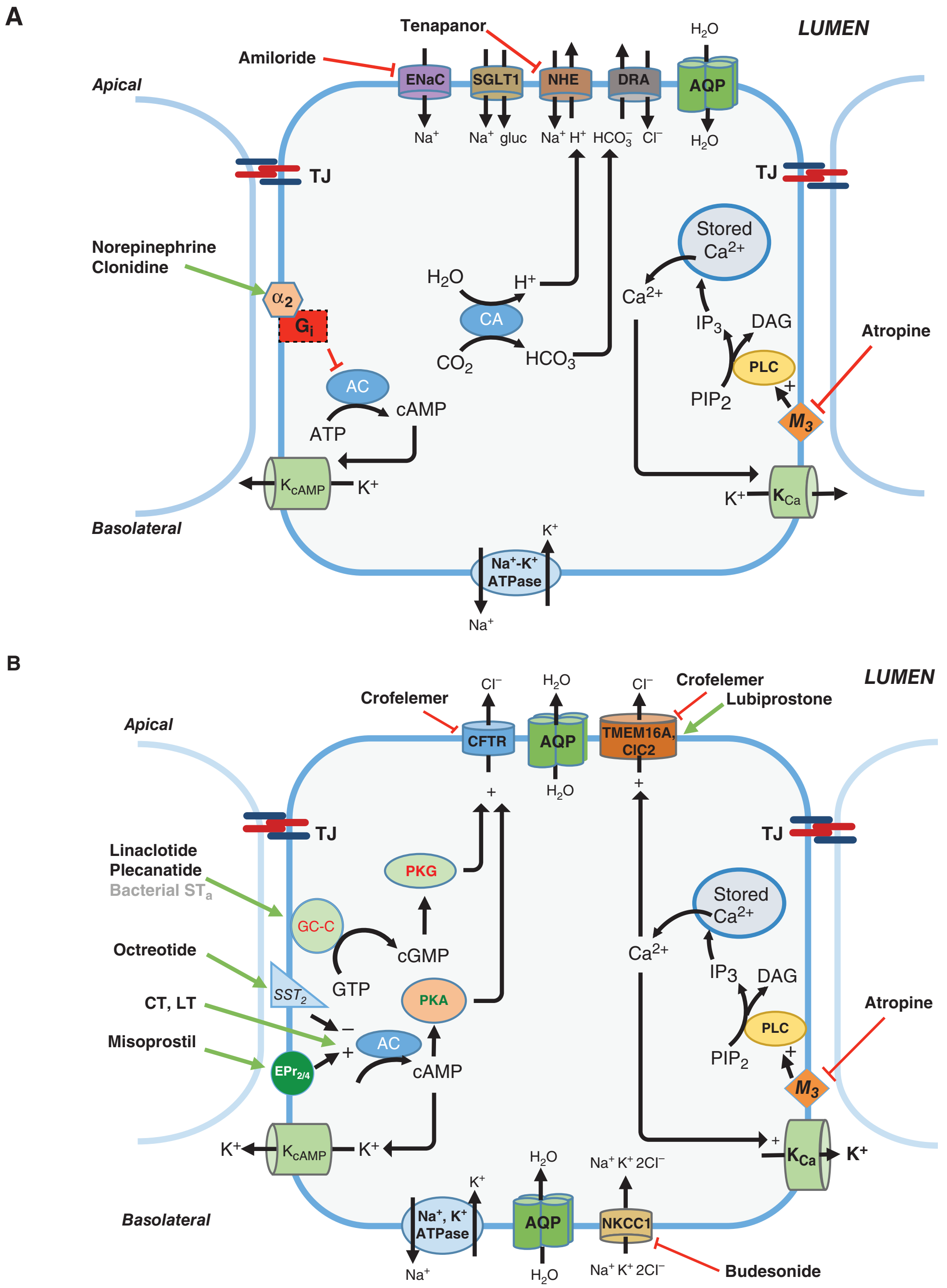

A
LUMEN
Apical
Tenapanor
Amiloride
ENaC
SGLT1
NHE
DRA
AQP
H_2O
Na^+
Na^+ gluc
Na^+ H^+ HCO_3^- Cl^-
H_2O
TJ
TJ
Norepinephrine
Clonidine
α_2
G_i
AC
ATP
cAMP
K_{cAMP}
K^+
H_2O
H^+
CA
CO_2
HCO_3
Stored Ca^{2+}
Ca^{2+}
IP_3
DAG
PLC
PIP_2
+
M_3
Atropine
K^+
K_{Ca}
Basolateral
K^+
Na^+-K^+ ATPase
Na^+
B
LUMEN
Apical
Crofelemer
Cl^-
H_2O
Cl^-
Crofelemer
Lubiprostone
CFTR
AQP
TMEM16A, ClC2
H_2O
+
+
TJ
TJ
Linaclotide
Plecanatide
Bacterial ST_a
PKG
GC-C
cGMP
GTP
Octreotide
SST_2
CT, LT
PKA
–
+
Misoprostil
$EPr_{2/4}$
AC
cAMP
Stored Ca^{2+}
Ca^{2+}
IP_3
DAG
PLC
PIP_2
+
M_3
Atropine
K^+
K_{cAMP}
K^+
+
K_{Ca}
K^+
Basolateral
K^+
Na^+, K^+ ATPase
Na^+
H_2O
AQP
H_2O
Na^+ K^+ $2Cl^-$
NKCC1
Na^+ K^+ $2Cl^-$
Budesonide

Figure 54–4 *Mechanism of action of drugs that alter intestinal epithelial absorption and secretion.* **A.** *Agents affecting intestinal epithelial absorption*. Absorption is driven by Na^+,K^+-ATPase in absorptive enterocytes, which creates the Na^+ gradient that facilitates Na^+ absorption through the epithelial sodium channel (ENaC) or through coupled transporters such as the Na^+-glucose cotransporter SGLT1 (SLC5A1) and members of the NHE family. ENaC is blocked by *amiloride* and similar compounds. Both NHE and the bicarbonate-Cl^- exchanger (SLC26A3, known as DRA, ***d**own**r**egulated in **a**denoma*), depend on the action of carbonic anhydrase (CA), which generates H^+ and HCO_3^- from water and CO_2 in the cytosol. *Tenapanor* inhibits NHE3, the most abundant isoform of NHE expressed in the intestine, reducing sodium absorption, which leads to fluid secretion into the lumen. Water enters the cell through apical aquaporin (AQP) water channels. As in secretion, regulation of K^+ channels by cyclic AMP and Ca^{2+} is essential. Thus, drugs that act on α_2 adrenergic receptors (e.g., *clonidine*) will reduce adenylyl cyclase activity and lower enterocyte cyclic AMP levels, thereby reducing absorption. **B.** *Agents affecting intestinal epithelial secretion*. Secretion is driven in secretory enterocytes by the Na^+ gradient established by the Na^+,K^+-ATPase. This Na^+ gradient drives the symporter NKCC1 (SLC12A2), which allows for the accumulation of Cl^- in the cell. Regulation of chloride channels in the apical (luminal) membrane drives Cl^- secretion. Vectorial movement of chloride drives the secretion of water through the paracellular route and through AQPs. Chloride secretion is rapidly regulated through phosphorylation of CFTR by the cyclic nucleotide–dependent protein kinases, PKA and PKG. Thus, drugs that stimulate adenylyl cyclase (i.e., *misoprostol*, acting through prostanoid EP_2 or EP_4 receptors) or guanylate cyclase C (GC-C: *linaclotide, plecanatide*) will stimulate Cl^- and water secretion. Several bacterial toxins cause water efflux and diarrhea by these mechanisms: cholera toxin (CT) and heat-labile *Escherichia coli* toxin (LT) stimulate cyclic AMP synthesis in the enterocyte by ADP-ribosylating $G\alpha_s$, blocking its GTPase activity and leading to constitutive activation of adenylyl cyclase; the heat-stable enterotoxins (e.g., ST_a) stimulate GC-C, the membrane-bound isoform of guanylyl cyclase. Drugs that inhibit adenylyl cyclase (e.g., *octreotide*, acting at SST_2 receptors) inhibit secretion. Calcium-dependent chloride channels (e.g., the Ca^{2+}-activated Cl^- channel TMEM16A (Lam et al., 2021); the ClC_2 chloride channel [Koster et al., 2020]) are regulated by increases in cytosolic Ca^{2+}, such as that induced by activation of muscarinic M_3 receptors that atropine blocks. Increases in cytosolic cyclic AMP and Ca^{2+} also regulate cyclic AMP–dependent and Ca^{2+}-dependent K^+ channels; this regulation is essential in maintaining the Na^+ gradient necessary to facilitate secretion. Apical chloride channels (CFTR, TMEM16A) can also be inhibited or activated by drugs such as *crofelemer* (inhibitor) or *lubiprostone* (activator). Drugs such as *budesonide* inhibit NKCC1 function and thereby reduce secretion.

TABLE 54–1 ■ CLASSIFICATION OF LAXATIVES

1. **Luminally active agents**
   - Hydrophilic colloids; bulk-forming agents (bran, psyllium, etc.)
   - Osmotic agents (nonabsorbable inorganic salts or sugars)
   - Stool-wetting agents (surfactants) and emollients (docusate, mineral oil)
2. **Nonspecific stimulants or irritants (with effects on fluid secretion and motility)**
   - Diphenylmethanes (bisacodyl and sodium picosulfate)
   - Anthraquinones (senna and cascara)
   - Castor oil
3. **Prokinetic agents (acting primarily on motility)**
   - $5HT_4$ receptor agonists
   - Dopamine receptor antagonists
   - Motilides (erythromycin)
4. **Prosecretory agents**
   - ClC-2 chloride channel activator
   - Guanyl cyclase C activators
   - Sodium-hydrogen exchange 3 inhibitor

the expression of NO synthase or its activity can prevent the laxative effects of castor oil, cascara, magnesium sulfate, and bisacodyl (but not senna).

Dietary Fiber and Supplements

Bulk, softness, and hydration of feces depend on the fiber content of the diet. Fiber is the part of food that resists enzymatic digestion and reaches the colon largely unchanged. Colonic bacteria ferment fiber to varying degrees, depending on its chemical nature and water solubility. Fermentation of fiber has two important effects:

1. Production of short-chain fatty acids that are trophic for colonic epithelium
2. Increase in bacterial mass

Although fermentation of fiber generally decreases stool water, short-chain fatty acids may have a prokinetic effect, and increased bacterial mass may contribute to increased stool volume. However, fiber that is not fermented can attract water and increase stool bulk. The net effect on bowel movement therefore varies with different compositions of dietary fiber (Table 54–3). In general, insoluble, poorly fermentable fibers, such as lignin, are most effective in increasing stool bulk and transit.

TABLE 54–2 ■ CLASSIFICATION AND COMPARISON OF REPRESENTATIVE LAXATIVES

LAXATIVE EFFECT AND LATENCY IN USUAL CLINICAL DOSAGE		
SOFTENING OF FECES, 1–3 DAYS	**SOFT OR SEMIFLUID STOOL, 6–8 h**	**WATERY EVACUATION, 1–3 h**
Bulk-forming laxatives	***Stimulant laxatives***	**Osmotic laxatives**[a]
Bran	Diphenylmethane derivatives	Magnesium sulfate
Psyllium preparations	Bisacodyl	Milk of magnesia
Methylcellulose		Magnesium citrate
Calcium polycarbophil		
Surfactant/osmotic laxatives	***Anthraquinone derivatives***	***Castor oil***
Docusates	Senna	
Poloxamers	Cascara sagrada	
Lactulose		

[a] Employed in high dosage for rapid cathartic effect and in lower dosage for laxative effect.

TABLE 54–3 ■ PROPERTIES OF DIFFERENT DIETARY FIBERS

TYPE OF FIBER	WATER SOLUBILITY	% FERMENTED
Nonpolysaccharides		
Lignin	Poor	0
Cellulose	Poor	15
Noncellulose polysaccharides		
Hemicellulose	Good	56–87
Mucilages and gums	Good	85–95
Pectins	Good	90–95

In general, insoluble, poorly fermentable fibers, such as lignin, are most effective in increasing stool bulk and transit.

Bran, the residue left when flour is made from cereal grains, contains more than 40% dietary fiber. Wheat bran, with its high lignin content, is most effective at increasing stool weight (a dose of 1–3 g up to three times a day). Fruits and vegetables contain more *pectins* and *hemicelluloses*, which are more readily fermentable and produce less effect on stool transit. *Psyllium husk*, derived from the seed of the plantago herb (*Plantago ovata*; known as ispaghula or isabgol in many parts of the world), is a component of many commercial products for constipation. Psyllium husk contains a hydrophilic mucilloid that undergoes significant fermentation in the colon, leading to an increase in colonic bacterial mass; the usual dose is 2.5 to 4 g (1–3 teaspoons full in 250 mL of fruit juice), titrated upward until the desired goal is reached. A variety of semisynthetic celluloses—such as methylcellulose (~2 g three times a day) and the hydrophilic resin calcium polycarbophil (1–2 g/day), a polymer of acrylic acid resin—also are available. These poorly fermentable compounds absorb water and increase fecal bulk. Malt soup extract, an extract of malt from barley grains that contains small amounts of polymeric carbohydrates, proteins, electrolytes, and vitamins, is another orally administered bulk-forming agent. The onset of action of these bulk-forming laxatives is generally between 12 and 72 h. Bloating is the most common side effect of soluble fiber products (perhaps due to colonic fermentation) but usually decreases with time (Lacy et al., 2014).

Osmotically Active Agents

Polyethylene Glycol–Electrolyte Solutions. Long-chain PEGs (MW ~3350 Da; also known as macrogol) are poorly absorbed and retained in the lumen because of their high osmotic nature (Paré and Fedorak, 2014). Orally administered high-molecular-weight macrogols undergo no or minimal absorption. When used in high volume, aqueous solutions of PEGs with electrolytes produce an effective catharsis and have replaced oral sodium phosphates as the most widely used preparations for colonic cleansing prior to radiological, surgical, and endoscopic procedures.

Usually, 240 mL of this solution is taken every 10 min until 4 L is consumed or the rectal effluent is clear. To avoid net transfer of ions across the intestinal wall, these preparations contain an isotonic mixture of sodium sulfate, sodium bicarbonate, sodium chloride, and potassium chloride. The osmotic activity of the polyethylene glycol (PEG) molecules retains the added water, and the electrolyte concentration ensures little or no net ionic shifts. A powder form of PEG 3350 is now available as an over-the-counter (OTC) product for the treatment of occasional constipation and for the treatment of more chronic constipation; the PEG preparation is suitable because it has such a benign side effect profile. The usual dose is 8.5 to 34 g of powder per day in 8 oz of water, with an expected onset of action of 1 to 4 days. These laxatives may cause nausea, cramping, and bloating. In elderly patients or those with preexisting electrolyte abnormalities, it is important to monitor K^+ concentrations since PEG preparations can cause electrolyte imbalances.

Saline Laxatives. Laxatives containing magnesium cations or phosphate anions commonly are called *saline laxatives*: magnesium sulfate, magnesium hydroxide, magnesium citrate, and sodium phosphate. Their cathartic action is believed to result from osmotic water retention, which then stimulates peristalsis. Other mechanisms may contribute, including the production of inflammatory mediators.

Magnesium-containing laxatives may stimulate the release of CCK, which leads to intraluminal fluid and electrolyte accumulation and to increased intestinal motility. For every additional milliequivalent of Mg^{2+} in the intestinal lumen, fecal weight increases by about 7 g. The usual dose of Mg^{2+} salts contains 40 to 120 mEq of Mg^{2+} and produces 300 to 600 mL of stool within 0.5 to 6 h. The most common side effect is diarrhea, which is dose related. In patients with impaired renal function, hypermagnesemia may lead to renal failure.

Phosphate salts are better absorbed than Mg^{2+}-based agents and therefore need to be given in larger doses to induce catharsis. However, because of the risks of acute phosphate nephropathy, oral phosphates are not recommended for the treatment of constipation and should be completely avoided in patients at risk (the elderly, patients with known bowel pathology or renal dysfunction, and patients on angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and NSAIDs).

The Mg^{2+}-containing preparations must be used with caution or avoided in patients with renal insufficiency, cardiac disease, or preexisting electrolyte abnormalities and in patients on diuretic therapy.

Nondigestible Sugars and Alcohols

Lactulose is a synthetic disaccharide of galactose and fructose that resists intestinal disaccharidase activity. This and other nonabsorbable sugars such as *sorbitol* and *mannitol* are hydrolyzed in the colon to short-chain fatty acids, which stimulate colonic propulsive motility by osmotically drawing water into the lumen. Sorbitol and lactulose are equally efficacious in the treatment of constipation caused by opioids and *vincristine*, of constipation in the elderly, and of idiopathic chronic constipation. They are available as 70% solutions, which are given in doses of 15 to 30 mL at night, with increases as needed up to 60 mL/day in divided doses. Effects may not be seen for 24 to 48 h after dosing begins. Abdominal discomfort or distention and flatulence are relatively common but usually subside with continued administration.

Lactulose also is used to treat hepatic encephalopathy. Patients with severe liver disease have an impaired capacity to detoxify ammonia coming from the colon, where it is produced by bacterial metabolism of fecal urea. The drop in luminal pH that accompanies hydrolysis to short-chain fatty acids in the colon results in “trapping” of the ammonia by its conversion to the polar ammonium ion. Combined with the increases in colonic transit, this therapy significantly lowers circulating ammonia levels. The therapeutic goal in this condition is to give enough lactulose (usually 20–30 g three to four times per day) to produce two to three soft stools a day with a pH of 5 to 5.5.

Stool-Wetting Agents and Emollients

Docusate. Docusate salts are anionic surfactants that lower the surface tension of the stool to allow mixing of aqueous and fatty substances, softening the stool and permitting easier defecation. These agents also stimulate intestinal fluid and electrolyte secretion (possibly by increasing mucosal cyclic AMP) and alter intestinal mucosal permeability. *Docusate sodium* (dioctyl sodium sulfosuccinate, 100 mg twice per day) and *docusate calcium* (dioctyl calcium sulfosuccinate, 240 mg/day) are well tolerated but have marginal efficacy in most cases of chronic constipation.

Mineral Oil. Mineral oil is a mixture of aliphatic hydrocarbons obtained from petrolatum. The oil is indigestible and absorbed only to a limited extent. When mineral oil is taken orally for 2 to 3 days, it penetrates and softens the stool and may interfere with resorption of water. The side effects of mineral oil preclude its regular use and include interference with absorption of fat-soluble substances (e.g., vitamins), elicitation of foreign-body reactions in the intestinal mucosa and other tissues, and leakage of oil past the anal sphincter. Rare complications such as lipid pneumonitis due to aspiration also can occur. Thus, “heavy” mineral oil should not be taken at bedtime, and “light” (topical) mineral oil should never be administered orally.

Stimulant (Irritant) Laxatives

Stimulant laxatives have direct effects on enterocytes, enteric neurons, and GI smooth muscle and probably induce limited low-grade inflammation in the small and large bowel to promote accumulation of water and electrolytes and stimulate intestinal motility (Lacy et al., 2014; Paré and Fedorak, 2014). This group includes *diphenylmethane derivatives*, *anthraquinones*, and *ricinoleic acid*.

Diphenylmethane Derivatives. ***Bisacodyl.*** *Bisacodyl* is marketed as enteric-coated and regular tablets and as a suppository for rectal administration. The usual oral daily dose of *bisacodyl* is 10 mg (to a maximum of 30 mg) for adults and 5 to 10 mg for children ages 6 to 12 years old. The drug requires hydrolysis by endogenous esterases in the bowel for activation, so the laxative effects after an oral dose usually are produced in 6 to 10 h. Suppositories (10 mg) work within 15 to 60 min. Due to the possibility of developing an atonic nonfunctioning colon, *bisacodyl* should not be used for more than 10 consecutive days. *Bisacodyl* is mainly excreted in the stool. Only 5% is absorbed and excreted in the urine as a glucuronide. Overdosage can lead to catharsis and fluid and electrolyte deficits. The diphenylmethanes can damage the mucosa and initiate an inflammatory response in the small bowel and colon; they can also cause colonic ischemia.

Sodium Picosulfate. *Sodium picosulfate* is a diphenylmethane derivative that is hydrolyzed by colonic bacteria to its active form and acts locally only in the colon. It is used as a preparation agent prior to endoscopic procedures. The most commonly reported adverse reactions are abdominal pain, bloating, and diarrhea. *Sodium picosulfate* should not be taken on a continuous daily basis as prolonged use may lead to fluid and electrolyte imbalances such as hypokalemia and to enteropathy. *Sodium picosulfate* is contraindicated in patients with reduced glomerular filtration rate, bowel perforation, and toxic megacolon. Caution should be exercised in patients with cardiac arrhythmias and those with renal impairment. *Phenolphthalein*, once among the most popular components of laxatives, has been withdrawn from the market in the U.S. because of potential carcinogenicity. *Oxyphenisatin* was withdrawn due to hepatotoxicity.

Anthraquinone Laxatives. These derivatives of plants such as aloe, cascara, and senna share a tricyclic anthracene nucleus modified with hydroxyl, methyl, or carboxyl groups to form monoanthrones, such as rhein and frangula. For medicinal use, monoanthrones (oral mucosal irritants) are converted to more innocuous dimeric (dianthrones) or glycoside forms. This process is reversed by bacterial action in the colon to generate the active forms.

Senna. Senna is obtained from the dried leaflets on pods of *Cassia acutifolia* or *Cassia angustifolia* and contains the rhein dianthrone glycosides sennoside A and B. From 15 to 30 mg are given as a single dose or a divided dose twice daily, resulting in an onset of action within 6 to 12 h. Chronic use of senna may lead to melanosis coli, and adverse effects include tolerance, nausea and vomiting, and abdominal cramping.

Cascara sagrada. Cascara sagrada is obtained from the bark of the buckthorn tree and contains the glycosides barbaloin and chrysaloin. The synthetic monoanthrone *danthron* was withdrawn from the U.S. market because of concerns over possible carcinogenicity. The FDA has categorized aloe and cascara sagrada products sold for laxation as not generally recognized as safe and effective for OTC use because of a lack of scientific information about potential carcinogenicity. This judgment is medically prudent but may provoke wistfulness among Joyceans, who recall that cascara sagrada, the *sacred bark*, worked well for Leopold Bloom, in Dublin, on the morning of June 16, 1904:

> Midway, his last resistance yielding, he allowed his bowels to ease themselves quietly as he read, reading still patiently that slight constipation of yesterday quite gone. Hope it’s not too big to bring on piles again. No, just right. So. Ah! Costive. One tabloid of cascara sagrada. Life might be so. (Joyce, 1922)

Castor Oil. A bane of childhood since the time of the ancient Egyptians, castor oil is derived from the bean of the castor plant, *Ricinus communis*. The castor bean is the source of an extremely toxic protein, ricin, as well as the oil (chiefly of the triglyceride of ricinoleic acid). The triglyceride is hydrolyzed in the small bowel by the action of lipases into glycerol and the active agent, *ricinoleic acid*, which acts primarily in the small intestine to stimulate secretion of fluid and electrolytes and speed intestinal transit. When taken on an empty stomach, as little as 4 mL of castor oil may produce a laxative effect within 1 to 3 h; however, the usual dose for a cathartic effect is 15 to 60 mL for adults. Because of its unpleasant taste and its potential toxic effects on intestinal epithelium and enteric neurons, castor oil is not recommended now.

Enemas and Suppositories. Enemas are employed either by themselves or as adjuncts to bowel preparation regimens to empty the distal colon or rectum of retained solid material. Bowel distention by any means will produce an evacuation reflex in most people, and almost any form of enema, including normal saline solution, can achieve this. Specialized enemas contain additional substances that are either osmotically active or irritants; however, their safety and efficacy have not been studied. Repeated enemas with hypotonic solutions can cause hyponatremia while repeated enemas with sodium phosphate–containing solution can cause hypocalcemia.

Glycerin. Glycerin is absorbed when given orally but acts as a hygroscopic agent and lubricant when given rectally. The resultant water retention stimulates peristalsis and usually produces a bowel movement in less than an hour. Glycerin is for rectal use only and is given in a single daily dose as a 2- or 3-g rectal suppository or as 5 to 15 mL of an 80% solution in enema form. Rectal glycerin may cause local discomfort, burning, or hyperemia and (minimal) bleeding. *CEO-TWO* suppositories contain sodium bicarbonate and potassium bitartrate and make use of rectal distension to initiate laxation. When administered rectally, the suppository produces CO_2, which initiates a bowel movement in 5 to 30 min.

Prokinetic and Secretory Agents for Constipation

The term *prokinetic* is reserved for agents that enhance GI transit via interaction with specific receptors involved in the regulation of motility (Acosta and Camilleri, 2015; Gudsoorkar and Quigley, 2020; Tack and Camilleri, 2018).

The potent $5HT_4$ receptor agonist *prucalopride* (1–4 mg/day) may be useful for the treatment of chronic constipation. *Misoprostol*, a synthetic prostaglandin analogue, is primarily used for protection against gastric ulcers resulting from the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and for the medical termination of pregnancy (see Chapters 41, 48, and 53). Prostaglandins can stimulate colonic contractions, particularly in the descending colon, an effect that may explain the diarrhea that limits the usefulness of *misoprostol* as a gastroprotectant and *misoprostol*'s utility in patients with intractable constipation. Doses of 200 μg daily or every other day can be effective when used with PEG. *Misoprostol* should not be used in women who could become pregnant because it induces labor. It can also increase menstrual bleeding. *Colchicine*, a microtubule formation inhibitor used for gout (see Chapter 42), has also been shown to be effective in constipation (1 mg/day), but its toxicity limits widespread use.

Four recently introduced secretory agents, *lubiprostone*, *linaclotide*, *plecanatide*, and *tenapanor*, with novel mechanisms of action restricted to the gut lumen have demonstrated effectiveness in the treatment of chronic constipation in adults.

Lubiprostone. ***Mechanism of Action and Pharmacology.*** *Lubiprostone* is a prostanoid activator of ClC-2 Cl^- channels. The drug appears to bind to the EP_4 prostaglandin receptor for PGE_2, a GPCR that couples to G_s, activating adenylyl cyclase and leading to enhanced apical Cl^- conductance. The drug promotes the secretion of a chloride-rich fluid, thereby improving stool consistency and promoting increased frequency by reflexly activating motility (Wilson and Schey, 2015).

Therapeutic Uses and Adverse Effects. A dose of 8 μg twice daily was found to be effective in constipation-predominant IBS, although higher doses (24 μg twice daily) are given for chronic constipation and opioid-induced constipation (see discussion that follows). The drug is poorly bioavailable, acting only in the lumen of the bowel. Side effects of *lubiprostone* include nausea (in up to 30% of patients), headache, diarrhea, allergic reactions, and dyspnea.

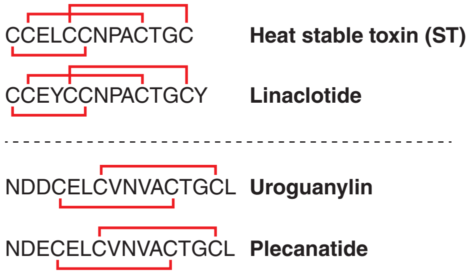


Figure 54–5 *Endogenous, bacterial, and pharmacological activators of intestinal guanylyl cyclase.* Amino acids are represented by the single letter amino acid code; the red connection lines represent intrachain disulfide bonds joining pairs of cysteines. In the intestinal tract, the naturally occurring ligands uroguanylin and bacterial heat stable toxin (ST) activate a membrane-spanning isoform of guanylyl cyclase, GC-C; these two peptides form the basis for two pharmacological activators of GC-C, *linaclotide* and *plecanatide*. *Plecanatide* is a synthetic analogue of uroguanylin; each has an aspartate in the N-terminal region that facilitates interaction with GC-C at acidic pH. *Linaclotide* is a synthetic analog of ST; both have rigid structure conferred by three pairs of disulfide bonds. The rigidity of ST and *linaclotide* renders their actions relatively pH independent (Data from Waldman and Camilleri, 2018).

Linaclotide. ***Mechanism of Action and Pharmacology.*** Another class of secretory agent is *linaclotide*, a 14–amino acid peptide agonist of the C isoform of membrane-spanning guanyl cyclase (GC-C). In the intestinal epithelium, GC-C is activated physiologically by guanylin and uroguanylin, pathologically by heat-stable bacterial toxins that cause diarrhea, and pharmacologically by *linaclotide*, which is a synthetic analog of a heat stable bacterial toxin (Figures 54-4 and 54–5). Activation of GC-C results in increased synthesis of cyclic GMP, resulting in enhanced chloride and bicarbonate secretion into the intestinal lumen, leading in turn to water secretion and enhanced motility. Some cellular cyclic GMP may be exported and reduce visceral pain by an action on primary afferent nerves innervating the GI tract (Yu and Rao, 2014).

Therapeutic Uses and Adverse Effects. This compound is approved in the treatment of constipation-predominant IBS and chronic constipation in adults at doses of 290 and 145 μg daily, respectively. Common side effects include diarrhea (which can be severe), gas, abdominal pain, and headaches. *Linaclotide* is contraindicated in children under 6 years old and is not recommended for older children.

Plecanatide. ***Mechanism of Action and Pharmacology.*** *Plecanatide* is a 16–amino acid synthetic analogue of uroguanylin and acts via the same basic mechanism, activation of GC-C, as does *linaclotide* (see Figure 54–5).

Therapeutic Uses and Adverse Effects. This agent is approved for the treatment of chronic idiopathic constipation in adults at a dose of 3 mg daily, with or without food. The most common adverse reaction is diarrhea (5%; severe in 0.6%). *Plecanatide* is contraindicated in children less than 6 years old and not advised in older children up to 18 years of age.

Tenapanor. ***Mechanism of Action and Pharmacology.*** *Tenapanor* is an inhibitor of isoform 3 of the Na^+-H^+ exchanger (NHE), the most abundantly expressed isoform of the NHE in the GI tract. Treatment with *tenapanor* reduces the absorption of Na^+ and enhances intestinal fluid volume and transit.

Therapeutic Uses and Adverse Effects. This agent is approved for the treatment of constipation-predominant IBS in adults at a dose of 50 mg orally twice daily. Diarrhea, abdominal distension, flatulence, and dizziness were the most frequent side effects. Severe diarrhea was reported in 2.5% of patients treated with *tenapanor*. It is contraindicated in children less than 6 years old and not advised in older children up to 18 years of age.

Opioid-Induced Constipation

Opioid analgesics can cause severe constipation. Laxatives are the first-line strategy for opioid-induced constipation, but they are frequently ineffective. *Lubiprostone*, described above, is one recent alternative. Another promising alternative strategy is the prevention of opioid-induced constipation with peripherally acting MOR (mu opioid receptor) antagonists that specifically target the underlying reason for this condition, without limiting centrally produced analgesia and limiting the symptoms of opioid withdrawal (Crocket et al., 2019; Farmer et al., 2019; see also Chapter 23).

Methylnaltrexone

Mechanism of Action and Pharmacology. The peripherally restricted MOR antagonist *methylnaltrexone* is approved for the treatment of opioid-induced constipation. The efficacy of this compound has been shown in randomized placebo-controlled trials (Crocket et al., 2019; Farmer et al., 2019).

ADME. In patients who respond to the drug, its onset of action is 30 to 60 min. It is excreted largely unchanged in the urine and feces but does undergo some hepatic metabolism including sulfation. The time to peak plasma concentration is 30 min, and the $t_{1/2}$ is about 8 h.

Therapeutic Uses and Adverse Effects. *Methylnaltrexone* is given as a subcutaneous injection (12 mg/day) in adults with chronic noncancer pain after discontinuing other laxatives. In advanced illness (palliative care), dosing varies according to body weight (0.15 mg/kg), with dosing every other day to a maximum of daily injection if required. When administered repeatedly every other day for 2 weeks, bowel movements occurred in about 50% of patients, compared with 8% to 15% of patients receiving placebo. Abdominal pain, flatulence, and nausea frequently accompany this treatment. Serious diarrhea sometimes occurs that requires discontinuing therapy. Patients with known or suspected GI obstruction are at increased risk of perforation. Opioid withdrawal may be precipitated in patients with a compromised blood-brain barrier.

Naldemedine

Mechanism of Action and Pharmacology. *Naldemedine* is a peripherally restricted opioid antagonist. It is a derivative of *naltrexone* made more polar and larger in mass with the addition of a side chain. It is also a substrate for the ATP-dependent efflux transporter P-glycoprotein (Pgp, MDR1, ABCB1; see Tables 4–1 and 4–4). These two properties limit its access to the CNS.

ADME. Following oral administration in the fasted state, *naldemedine* is rapidly absorbed, reaching peak concentrations in approximately 45 min. Food prolongs the time to peak (to ~2.5 h) but does not reduce the overall extent of absorption. *Naldemedine* is metabolized by hepatic CYP3A and is excreted in both the urine (57%) and feces (35%); its $t_{1/2}$ is 11 h.

Therapeutic Uses and Adverse Effects. *Naldemedine* is approved for the treatment of opioid-induced constipation in adult patients with chronic noncancer pain; dose is 0.2 mg/day orally. Common adverse reactions are abdominal pain, diarrhea, nausea, vomiting, and gastroenteritis. *Naldemedine* is contraindicated in patients with known or suspected obstruction of the GI tract. Opioid withdrawal may be precipitated with the use of this compound.

Naloxegol

Mechanism of Action and Pharmacology. *Naloxegol* comprises the MOR antagonist *naloxone* conjugated to a PEG polymer. This limits blood-brain barrier permeability because it is a substrate for the Pgp efflux transporter, so it behaves as a peripherally restricted MOR antagonist. It is approved for the treatment of opioid-induced constipation. Randomized placebo-controlled trials have demonstrated the efficacy of this compound (Crocket et al., 2019; Farmer et al., 2019).

ADME. The drug is given orally on an empty stomach and is rapidly absorbed. The time to peak plasma concentration is about 2 h with a secondary peak occurring 0.4 to 3 h after the first. *Naloxegol* is metabolized primarily by hepatic CYP3A, and metabolites are excreted in the feces (68%) and urine (16%). The plasma $t_{1/2}$ is variable (6–11 h).

Therapeutic Uses and Adverse Effects. *Naloxegol*, approved for opioid-induced constipation in adults, is given orally 12.5 or 25 mg once per day after discontinuing other laxatives. Diarrhea, abdominal pain, flatulence, nausea, and vomiting are the major adverse reactions. Precautions are the same as for *methylnaltrexone*: Patients with known or suspected GI obstruction are at increased risk of perforation, and opioid withdrawal may be precipitated in patients with a compromised blood-brain barrier.

Naloxone and Oxycodone

Mechanism of Action and Pharmacology. This fixed-ratio (2:1 of *oxycodone:naloxone*) combination drug is given orally to relieve opioid-induced constipation when opioid pain relief is still required. The *naloxone* displaces *oxycodone* from the MOR in the GI tract without limiting the degree of central analgesia (Farmer et al., 2019). This combination drug carries with it the risks inherent with all opioids, including addiction and respiratory depression. Full details of this agent are given in Chapter 23.

Therapeutic Uses and Adverse Effects. *Naloxone-oxycodone* is approved in Canada and other countries for opioid-induced constipation in adults but currently not for this purpose in the U.S. This oral medication dosing is for pain control and is individualized. A single dose of 40 mg *oxycodone*/20 mg *naloxone* every 12 h should not be exceeded. Adverse GI reactions include nausea and vomiting, constipation, and diarrhea.

Other Agents for Opioid-Induced Constipation

In clinical trials, the MOR antagonist *alvimopan* (see separate discussion below) increased spontaneous bowel movements and improved other symptoms of opioid-induced constipation without compromising analgesia. *Alvimopan* is approved for use in the U.S. for postoperative bowel recovery. However, due to significant cardiovascular adverse events, this drug is not FDA-approved for opioid-induced constipation, and further phase III trials are under way. In addition, the GC-C agonist *linaclotide* (see previous discussion) is in clinical trials for this condition.

Postoperative Ileus

Postoperative ileus refers to the intolerance to oral intake and nonmechanical obstruction of the bowel that occurs 3 to 5 days after abdominal and nonabdominal surgery. The pathogenesis is complex and is a combination of activation of neural inhibitory reflexes involving sympathetic nerves, enteric MOR, and the activation of local inflammatory mechanisms that reduce smooth muscle contractility (Bragg et al., 2015). The condition is exacerbated by opioids, which are the mainstay of postoperative analgesia. Prokinetic agents typically do not have much effect in this condition, but a new therapeutic agent, *alvimopan*, has been introduced to reduce GI recovery time after surgery.

Alvimopan

Mechanism of Action and Pharmacology. *Alvimopan* is an orally active, peripherally restricted MOR antagonist that is approved to accelerate the time to upper and lower GI recovery following partial large- or small-bowel resection surgery with primary anastomosis (Curran et al., 2008).

ADME. The drug is hydrolyzed by the gut flora to the active amide. The active metabolite is further metabolized by hepatic glucuronidases. The peak plasma concentration of the active metabolite occurs in about 36 h. The drug's $t_{1/2}$ is 10 to 18 h; the drug is excreted in the urine and feces.

Therapeutic Uses and Adverse Effects. The drug is given 30 min to 5 h prior to surgery (12 mg) and then twice daily for up to a maximum of 7 days or until discharge, not to exceed 15 doses. Adverse effects include hypokalemia, dyspepsia, anemia, back pain, and urinary retention. Because of the risk of myocardial infarctions and neoplasia, this drug is available only through a restricted-access program in the U.S.

Antidiarrheal Agents

Diarrhea: General Principles and Approach to Treatment

Diarrhea (Greek and Latin: *dia*, “through,” and *rheein*, “to flow or run”) does not require any definition to people who suffer from “the too rapid evacuation of too fluid stools.” Diarrhea is usually defined as excessive fluid weight, with 200 g/day representing the upper limit of normal stool water weight for healthy adults in the Western world. Because stool weight is largely determined by stool water, most cases of diarrhea result from disorders of intestinal water and electrolyte transport.

An appreciation and knowledge of the underlying causative processes of diarrhea facilitates effective treatment (Thiagarajah et al., 2015). From a mechanistic perspective, diarrhea can be caused by an increased osmotic load within the intestine (resulting in retention of water within the lumen), excessive secretion of electrolytes and water into the intestinal lumen, exudation of protein and fluid from the mucosa, and altered intestinal motility resulting in rapid transit (and decreased fluid absorption). In most instances, multiple processes are affected simultaneously, leading to a net increase in stool volume and weight accompanied by increases in fractional water content.

Many patients with sudden onset of diarrhea have a *benign*, self-limited illness requiring no treatment or evaluation. Acute diarrhea is frequently due to infection with bacteria, viruses, or protozoa. In more severe cases of diarrhea and in infants and small children, dehydration and electrolyte imbalances are the principal risk. *Oral rehydration therapy* therefore is a cornerstone for patients with acute illnesses resulting in significant diarrhea. This therapy exploits the fact that nutrient-linked cotransport of water and electrolytes remains intact in the small bowel in most cases of acute diarrhea. Na^+ absorption links to glucose uptake by the enterocyte, which is followed by movement of water in the same direction. A balanced mixture of glucose and electrolytes in volumes matched to losses therefore can prevent dehydration. This can be provided by many commercial premixed formulas using glucose-electrolyte or rice-based physiological solutions.

Pharmacotherapy of diarrhea in adults should be reserved for patients with significant or persistent symptoms (Menees et al., 2012). Nonspecific antidiarrheal agents typically do not address the underlying pathophysiology responsible for the diarrhea. Many of these agents act by decreasing intestinal motility and should be avoided in acute diarrheal illnesses caused by invasive organisms. In such cases, these agents may mask the clinical picture, delay clearance of organisms, and increase the risk of systemic invasion by the infectious organisms.

Empiric Antibiotic Therapy

The use of empiric antibiotic therapy for acute diarrhea (therapy given in the absence of diagnostic evaluation) must be carefully balanced with the risks. In patients with suspected or proven enterohemorrhagic *Escherichia coli*, antibiotics should be avoided because of the risk of hemolytic uremic syndrome. Similarly, in patients with suspected *Clostridium difficile*, other antibiotics should be discontinued if possible. Treatment of traveler's diarrhea, bacterial diarrhea, and those with more severe conditions is appropriate under some conditions, based on the severity of diarrhea and the duration of the symptoms (Steffen et al., 2015). The first-line therapy for acute (most commonly, traveler's) diarrhea in adults is oral *fluoroquinolone* antibiotics (see Chapter 57 for specific drug details): *ciprofloxacin* (500 mg twice daily for up to 3 days), *norfloxacin* (400 mg twice daily for up to 3 days), *ofloxacin* (200 mg twice daily for up to 3 days), or *levofloxacin* (500 mg daily for up to 3 days). *Azithromycin* (500 mg/day for 1–3 days or a maximum of 1000-mg single dose), *rifaximin* (200 mg three times per day for up to 3 days), and *rifamycin* (388 mg twice daily for 3 days) are alternative therapeutic agents. *Trimethoprim/sulfamethoxazole* is no longer recommended for prevention or treatment of traveler's diarrhea because of increasing resistance worldwide among likely pathogens. In children, the treatment of traveler's diarrhea remains controversial. *Azithromycin* (10 mg/kg to a maximum of 500-mg single dose) is the preferred treatment of children with traveler's diarrhea.

Bismuth Subsalicylate

Mechanism of Action and Pharmacology. Bismuth compounds are used to treat a variety of GI disorders, although their mechanism of action remains poorly understood (Menees et al., 2012). *Bismuth subsalicylate* is a popular OTC preparation that consists of trivalent bismuth and salicylate suspended in a mixture of magnesium aluminum silicate clay. In the low pH of the stomach, the *bismuth subsalicylate* reacts with hydrochloric acid to form bismuth oxychloride and salicylic acid.

Bismuth is thought to have antisecretory, anti-inflammatory, and antimicrobial effects. Bismuth also relieves nausea and abdominal cramps. The clay in *bismuth subsalicylate* and generic formulations may have some additional benefits in diarrhea, but this is not clear. *Bismuth subsalicylate* is used for the prevention and treatment of traveler's diarrhea, but it also is effective in other forms of episodic diarrhea and in acute gastroenteritis.

Therapeutic Uses and Adverse Effects. A recommended dose of the *bismuth subsalicylate* (30 mL of regular-strength liquid or two tablets) contains approximately equal amounts of bismuth and salicylate (262 mg each). For control of indigestion, nausea, or diarrhea, the dose is repeated every 30 to 60 min, as needed, up to eight times a day. Dark stools (sometimes mistaken for melena) and black staining of the tongue in association with bismuth compounds are caused by bismuth sulfide formed in a reaction between the drug and bacterial sulfides in the GI tract. Although 99% of the bismuth passes unaltered and unabsorbed into the feces, the salicylate is absorbed in the stomach and small intestine. Thus, the product carries the same warning regarding Reye's syndrome as other salicylates and may also cause CNS side effects, hearing loss, and tinnitus.

Probiotics

The GI tract contains a vast and complex commensal microflora necessary for health, presented at length in Chapter 6. Alterations in the balance or composition of the microflora are responsible for antibiotic-associated diarrhea and possibly other disease conditions (see Chapter 55). Probiotic preparations containing a variety of bacterial strains have shown some degree of benefit in acute diarrheal conditions, antibiotic-associated diarrhea, and infectious diarrhea (Menees et al., 2012). In clinical trials, preparations containing *Lactobacillus* GG and *Saccharomyces boulardii* have been found to be effective for these conditions.

Antimotility and Antisecretory Agents

Opioids. Opioids continue to be widely used in the treatment of diarrhea. They act by several different mechanisms, mediated principally through either MORs or DORs on enteric nerves, epithelial cells, and muscle (see Chapter 23). These mechanisms include effects on intestinal motility (via MOR), intestinal secretion (via DOR, the delta opioid receptor), and absorption (via MOR and DOR). Commonly used antidiarrheals such as *diphenoxylate*, *difenoxin*, and *loperamide* act principally via peripheral MOR and are preferred over opioids that penetrate the CNS.

Loperamide. ***Mechanism of Action and Pharmacology.*** *Loperamide*, a compound with MOR activity, is an orally active antidiarrheal agent (Hanauer, 2008; Menees et al., 2012). The drug is 40 to 50 times more potent than *morphine* as an antidiarrheal agent and penetrates the CNS poorly. It increases small intestinal and mouth-to-cecum transit times. *Loperamide* also increases anal sphincter tone. In addition, *loperamide* has antisecretory activity against cholera toxin and some forms of *E. coli* toxin, presumably by acting on G_i-linked receptors to counter the stimulation of adenylyl cyclase activity by the toxins.

ADME. *Loperamide* is available OTC in capsule, solution, and chewable tablet forms. It acts quickly after an oral dose, with peak plasma levels achieved within 3 to 5 h. It has a $t_{1/2}$ of about 11 h and undergoes extensive hepatic metabolism.

Therapeutic Uses and Adverse Effects. The usual adult dose is 4 mg initially followed by 2 mg after each subsequent loose stool, up to 16 mg/day. If there is clinical improvement and acute diarrhea does not occur within 48 h, *loperamide* should be discontinued. Recommended maximum daily doses for children are 3 mg for ages 2 to 5 years, 4 mg for ages 6 to 8 years, and 6 mg for ages 8 to 12 years. *Loperamide* is not recommended for use in children younger than 2 years. *Loperamide* is effective against traveler's diarrhea, used alone or in combination with antibiotics. It is used as adjunct treatment in many forms of chronic diarrheal disease (initially as for acute diarrhea, but with typical divided daily doses of 4–8 mg/day), with few adverse effects. *Loperamide* is more effective in treating diarrhea than *diphenoxylate*. Overdosage, however, can result in constipation, CNS depression (especially in children), and paralytic ileus. In addition, the FDA has placed a black box warning on the drug, noting that exceeding the recommended dosage can result in cardiac events including torsades de pointes, cardiac arrest, and death. In patients with active inflammatory bowel disease involving the colon (see Chapter 55), *loperamide* should be used with great caution, if at all, to avoid development of toxic megacolon.

Diphenoxylate and Difenoxin. ***Mechanism of Action and Pharmacology.*** *Diphenoxylate* and its active metabolite *difenoxin* (diphenoxylic acid) are related structurally to *meperidine*. As antidiarrheal agents, *diphenoxylate* and *difenoxin* are somewhat more potent than *morphine* (Menees et al., 2012). Both drugs are listed as schedule V controlled substances by the Drug Enforcement Agency, and both are coformulated with *atropine* to discourage habituation.

ADME. Both compounds are extensively absorbed after oral administration, with peak levels achieved within 1 to 2 h. *Diphenoxylate* is rapidly deesterified to *difenoxin*, which is eliminated with a $t_{1/2}$ of about 12 h.

Therapeutic Uses and Adverse Effects. Both drugs are indicated for the treatment of diarrhea. The usual dosage for adults is two tablets initially (*diphenoxylate* or *difenoxin*), then one tablet every 3 to 4 h, not to exceed 20 mg/day (*diphenoxylate*) or 8 mg/day (*difenoxin*). Acute diarrhea usually improves in 48 h if the medication is effective. If chronic diarrhea does not improve within 10 days at the maximum daily dose, then these agents are not likely to be effective. *Diphenoxylate* is also sold as an oral solution (2.5 mg per 5 mL), which is recommended if used cautiously in children. For children, the initial dose is 0.3 to 0.4 mg/kg per day in four divided doses to a maximum of 10 mg/day. Once symptoms are controlled, dosing should be reduced; if no effect is seen in 48 h, the drug is unlikely to be effective. Both drugs can produce CNS effects when used in higher doses (40–60 mg/day) and thus have a potential for abuse or addiction. They are available in preparations containing small doses of *atropine* (considered subtherapeutic) to discourage abuse and deliberate overdosage: 25 μg of *atropine* sulfate per tablet with either 2.5 mg *diphenoxylate* hydrochloride or 1 mg of *difenoxin* hydrochloride. With excessive use or overdose, constipation and (in inflammatory conditions of the colon) toxic megacolon may develop. In high doses, these drugs cause CNS effects as well as anticholinergic effects from the *atropine* (nausea, dry mouth, blurred vision, etc.) (see Chapter 11).

Other Opioids. Opioids used for diarrhea include *codeine* (in doses of 30 mg given three or four times daily) and opium-containing compounds. *Paregoric* (camphorated opium tincture) contains the equivalent of 2 mg of *morphine* per 5 mL (0.4 mg/mL); *deodorized tincture of opium*, which is 25 times stronger, contains the equivalent of 50 mg of *morphine* per 5 mL (10 mg/mL). The two tinctures sometimes are confused in prescribing and dispensing, resulting in dangerous overdoses. The antidiarrheal dose of opium tincture for adults is 0.6 mL (equivalent to 6 mg *morphine*) four times daily; the adult dose of *paregoric* is 5 to 10 mL (equivalent to 2–4 mg *morphine*) one to four times daily. *Paregoric* is used in children at a dose of 0.25 to 0.5 mL/kg (equivalent to 0.1–0.2 mg *morphine*/kg) one to four times daily.

Enkephalins. Enkephalins are endogenous opioids that are important enteric neurotransmitters; they can inhibit intestinal secretion without affecting motility. *Racecadotril* is an example.

Racecadotril. ***Mechanism of Action and Pharmacology.*** *Racecadotril* (acetorphan), a prodrug, is rapidly converted in the body to thiorphan, a dipeptide inhibitor of enkephalinase (a neutral endopeptidase [NEP]; EC 3.4.24.11) that does not penetrate the CNS. By inhibiting peripheral enkephalin degradation, thiorphan potentiates the effects of endogenous enkephalins on the MOR in the GI tract to produce an antidiarrheal effect, acting predominantly as an antisecretory agent (Thiagarajah et al., 2015). In addition to enkephalins, substrates of NEP include neuropeptide Y, atrial and brain natriuretic peptides, substance P, and neurotensin, among others (Erdös and Skidgel, 1989). Thus, inhibition of enkephalinase activity could elevate the levels of these messengers as well, complicating interpretation of *racecadotril*'s effects on physiological systems.

Therapeutic Uses and Adverse Effects. *Racecadotril* is indicated for acute diarrhea. It is given orally as a 100-mg initial dose, which is repeated every 8 h as needed until diarrhea stops, for up to 7 days maximum. In children, it is given with oral rehydration solution according to body weight (1.5 mg/kg every 8 h), until symptoms improve or for a maximum of 7 days. This drug is available in many countries, but not the U.S., and is efficacious and safe in children with acute diarrhea. It produces less constipation than *loperamide* and has minimal other adverse effects (headache, itching).

α_2 Adrenergic Receptor Agonists

Mechanism of Action and Pharmacology. The α_2 adrenergic receptor agonists such as *clonidine* can interact with specific receptors on enteric neurons and enterocytes, thereby stimulating absorption and inhibiting secretion of fluid and electrolytes and increasing intestinal transit time. These agents may have a role for use by diabetics with chronic diarrhea.

Therapeutic Uses and Adverse Effects. Oral *clonidine* (beginning at 0.6 mg three times daily) has been used in diabetic patients with chronic diarrhea; the use of a topical preparation may result in plasma levels of the drug that are steadier. *Clonidine* also may be useful in patients with diarrhea caused by opiate withdrawal. Side effects such as hypotension, depression, and perceived fatigue may be dose limiting in susceptible patients (see Chapter 14 for details of the pharmacology of *clonidine*).

Octreotide and Somatostatin

Mechanism of Action and Pharmacology. *Octreotide* (see Chapter 46) is an octapeptide analogue of somatostatin (SST) that is effective in inhibiting the severe secretory diarrhea brought about by hormone-secreting tumors of the pancreas and the GI tract. *Octreotide* inhibits secretion of 5HT and various GI peptides. Its greatest utility may be in the "dumping syndrome" seen in some patients after gastric surgery and pyloroplasty, in whom *octreotide* inhibits the release of hormones (triggered by rapid passage of food into the small intestine) that are responsible for distressing local and systemic effects. *Octreotide* is widely available. SST is available in some countries, but not the U.S.

ADME. *Octreotide* has a $t_{1/2}$ of 1 to 2 h and is administered either subcutaneously or intravenously as a bolus dose. The time to peak is 0.4 h after subcutaneous injection and 1 h after intramuscular injection. It is metabolized in the liver and excreted in the urine. SST has a plasma $t_{1/2}$ of 1 to 2 min.

Therapeutic Uses and Adverse Effects. Standard initial therapy with *octreotide* is 50 to 100 μg, given subcutaneously two or three times a day, with titration to a maximum dose of 500 μg three times daily, based on clinical and biochemical responses. A long-acting preparation of *octreotide* acetate enclosed in biodegradable microspheres is available for use in the treatment of diarrhea associated with carcinoid tumors and VIP-secreting tumors, as well as in the treatment of acromegaly (see Chapter 46). This preparation is injected intramuscularly once per month in a dose of 20 mg. Side effects of *octreotide* depend on the duration of therapy: Transient nausea, bloating, or pain at sites of injection may occur in the short term, with the potential for gallstone formation and hypo- or hyperglycemia occurring in the long term. However, there are also numerous other side effects, including cardiovascular, endocrine, and CNS.

Variceal Bleeding. SST and *octreotide* are effective in reducing hepatic blood flow, hepatic venous wedge pressure, and azygos blood flow. These agents constrict the splanchnic arterioles by a direct action on vascular smooth muscle and by inhibiting the release of peptides contributing to the hyperdynamic circulatory syndrome of portal hypertension. *Octreotide* also may act through the ANS. For patients with variceal bleeding, therapy with *octreotide* usually is initiated while the patient is waiting for endoscopy (a 50-μg bolus dose followed by 50 μg hourly for 2–5 days) (Bhutta and Garcia-Tsao, 2015). Because of its short $t_{1/2}$ (1–2 min), SST can be given only by intravenous infusion (a 250-μg bolus dose followed by 250 μg hourly for 2–5 days). Higher doses (up to 500 μg/h) are more efficacious and can be used for patients who continue to bleed on the lower dose.

Intestinal Dysmotility. *Octreotide* has complex and apparently conflicting effects on GI motility, including inhibition of antral motor activity and colonic tone. However, *octreotide* also can rapidly induce phase III activity of the migrating motor complex in the small bowel to produce longer and faster contractions than those occurring spontaneously. Its use has been shown to result in improvement in selected patients with scleroderma and small-bowel dysfunction.

Pancreatitis. Both SST and *octreotide* inhibit pancreatic secretion and have been used for the prophylaxis and treatment of acute pancreatitis (Li et al., 2011). The rationale for their use is to rest the pancreas so inflammation by the continuing production of proteolytic enzymes is not aggravated, to reduce intraductal pressures, and to ameliorate pain. However, clinical trials have demonstrated that neither agent is effective in the treatment of acute pancreatitis, although *octreotide* confers some benefit when given prophylactically to prevent postendoscopic retrograde cholangiopancreatography pancreatitis.

Telotristat Ethyl

Mechanism of Action and Pharmacology. This drug reduces diarrhea associated with carcinoid tumors by inhibiting tryptophan hydroxylase, the rate-limiting enzyme of 5HT biosynthesis. 5HT secretion stimulates fluid secretion and motility in the GI tract.

ADME. *Telotristat ethyl* is absorbed after oral administration and converted to the active agent *telotristat* by the action of carboxylesterases. Peak plasma levels of *telotristat* occur 1 to 3 h after ingestion. Clearance occurs with a $t_{1/2}$ of 5 h; elimination is via the feces.

Therapeutic Uses and Adverse Effects. *Telotristat* is given in combination with somatostatin analogue therapy for the treatment of diarrhea in carcinoid syndrome. A dose of 250 mg three times per day may be given to adult patients who are not adequately controlled by somatostatin analogue therapy alone. The main adverse effects are constipation, nausea, headache, increased gamma glutamyl transferase levels, depression, peripheral edema, flatulence, reduced appetite, and pyrexia.

Berberine

Berberine is a plant alkaloid that has complex pharmacological actions that include antimicrobial effects, stimulation of bile flow, inhibition of ventricular tachyarrhythmias, and possible antineoplastic activity. It is used most commonly to treat bacterial diarrhea and cholera but is also apparently effective against intestinal parasites (Menees et al., 2012). The antidiarrheal effects in part may be related to its antimicrobial activity, as well as its ability to inhibit smooth muscle contraction and delay intestinal transit by antagonizing the effects of ACh (by competitive and noncompetitive mechanisms) and blocking the entry of Ca^{2+} into cells. In addition, it inhibits intestinal secretion. *Berberine* is not FDA-approved for use in the U.S.

Bulk-Forming and Hydroscopic Agents

Hydrophilic and poorly fermentable colloids or polymers such as *carboxymethylcellulose* and calcium polycarbophil absorb water and increase stool bulk (calcium polycarbophil absorbs 60 times its weight in water). They usually are used for constipation but are sometimes useful in acute episodic diarrhea and in mild chronic diarrhea in patients with IBS. Some of these agents also may bind bacterial toxins and bile salts.

Another bulk-forming agent is *dextranomer and hyaluronic acid*. *Dextranomer* microspheres are a network of dextran-sucrose beads with exposed hydroxy groups. When this complex is applied to an exudative wound surface, the exudate is drawn by capillary forces generated by the swelling of the beads. The sodium hyaluronate provides viscosity and facilitates injection of the *dextranomer*. This agent is licensed (as a device) for the treatment of fecal incontinence in adults. It is given as four 1-mL submucosal injections in the anal canal, which can be repeated after at least 4 weeks if the first treatment is inadequate. The major adverse effects include injection area pain and bleeding.

Bile Acid Sequestrants

Cholestyramine, *colestipol*, and *colesevelam* effectively bind bile acids and some bacterial toxins (Menees et al., 2012). *Cholestyramine* is useful in the treatment of bile salt–induced diarrhea, as in patients with resection of the distal ileum or after cholecystectomy. In these patients, excessive concentrations of bile salts reach the colon and stimulate water and electrolyte secretion. Patients with extensive ileal resection (usually >100 cm) eventually develop net bile salt depletion, which can produce steatorrhea because of inadequate micellar formation required for fat absorption. In such patients, the use of *cholestyramine* aggravates the diarrhea. In patients having persistent diarrhea despite treatment, bile-acid malabsorption may be a contributing factor giving rise to bile salt–induced diarrhea. *Cholestyramine* and *colesevelam* can be given as an off-label use at a dose of 4 to 12 g of the dried resin per day. If successful, the dose may be titrated down to achieve the desired stool frequency. The use of these agents is limited by GI side effects, including bloating, flatulence, abdominal discomfort, and constipation, as well as malabsorption of fat-soluble vitamins and drug-drug interactions.

Crofelemer

Mechanism of Action and Pharmacology. *Crofelemer* is a purified oligomeric proanthocyanidin from "dragon's blood," the reddish latex-like sap of a South American euphorbia. This botanic extract is used for the treatment of diarrhea associated with antiretroviral therapy for HIV/AIDS (Crutchley et al., 2010). It is not approved for infectious or other diarrheas. This drug has minimal systemic absorption and works by inhibiting the cystic fibrosis transmembrane conductance regulator (CFTR, a cyclic AMP–stimulated Cl^- channel) and Ca^{2+}-activated chloride ion channels on the luminal aspect of the enterocyte, thereby reducing the water loss associated with chloride secretion into the lumen.

Therapeutic Uses and Adverse Effects. This drug is given orally (125 mg twice daily) to adults. Infectious diarrhea must be ruled out before treatment. The main adverse effects include upper respiratory tract infections, cough, flatulence, nausea, joint and back pain, and some other GI effects.

Irritable Bowel Syndrome

Irritable bowel syndrome affects up to 10% to 15% of the population in the U.S. and most other Western countries. Patients may complain of a variety of symptoms, the most characteristic of which is recurrent abdominal pain associated with altered bowel movements. IBS appears to result from a varying combination of disturbances in visceral motor and sensory function, often associated with significant affective disorders (Khan and Chang, 2010; Mayer et al., 2014). The disturbances in bowel function can be either constipation or diarrhea or both at different times. Considerable evidence suggests a specific enhancement of visceral (as opposed to somatic) sensitivity to noxious, as well as physiological, stimuli in this syndrome (Dekel et al., 2013; Mayer et al., 2014).

Many patients can be managed with fiber supplementation and dietary restrictions, notably by avoiding fermentable oligo-di-monosaccharides and polyols (FODMAPs), lactose, or gluten; however, many cannot. Treatment of bowel symptoms (either diarrhea or constipation) is predominantly symptomatic and nonspecific, using the agents discussed previously. An important role for serotonin in IBS has been suggested based on its involvement in sensitization of nociceptor neurons in inflammatory conditions and its role in the control of motility and secretion (Dekel et al., 2013). This has led to the development of specific receptor modulators for the treatment of IBS, such as the $5HT_3$ antagonist *alosetron* and the $5HT_4$ agonist *prucalopride* (see Figure 54–2).

An effective class of agents for IBS has been the tricyclic antidepressants (see Chapter 18), which can have neuromodulatory and analgesic properties independent of their antidepressant effect (Dekel et al., 2013). Tricyclic antidepressants have a proven track record in the management of chronic "functional" visceral pain in adults (off-label use). *Amitriptyline, nortriptyline, imipramine*, or *desipramine* can be used at lower doses than those used to treat depression. Starting doses of 10 to 25 mg *amitriptyline*, *nortriptyline,* or *imipramine* or 12.5 to 25 mg *desipramine* at bedtime should be given for 3 to 4 weeks because of their delayed onset of action; doses can be increased if tolerated and the patient is responsive to treatment. Although changes in mood usually do not occur at these doses, there may be some diminution of anxiety and restoration of sleep patterns. SSRIs (see Chapters 15 and 18) have fewer side effects and have been advocated particularly for patients with functional constipation because SSRIs can increase bowel movements and even cause diarrhea. However, they probably are not as effective as tricyclic antidepressants in the management of visceral pain. Antidepressant use in children is not strongly supported by clinical trials. α_2 Adrenergic agonists, such as *clonidine* (see Chapter 14), also can increase compliance and reduce distention-induced pain.

Alosetron

Mechanism of Action and Pharmacology

The $5HT_3$ receptor participates in sensitization of spinal sensory neurons, vagal signaling of nausea, and peristaltic reflexes. The clinical effect of $5HT_3$ antagonism is a general reduction in GI contractility with decreased colonic transit, along with an increase in fluid absorption. *Alosetron*, a potent antagonist of the $5HT_3$ receptor, was initially withdrawn from the U.S. market because of an unusually high incidence of ischemic colitis (up to 3 per 1000 patients), leading to surgery and even death in a small number of cases. Nevertheless, the FDA has reapproved this drug under a limited distribution system for women with severe diarrhea-predominant IBS (Camilleri, 2013). The manufacturer requires a prescription program that includes physician certification and an elaborate patient education and consent protocol before dispensing.

ADME

Alosetron is rapidly absorbed from the GI tract; its duration of action (~10 h) is longer than expected from its $t_{1/2}$ of 1.5 h. It is metabolized by hepatic CYPs and is excreted in the urine and feces.

Therapeutic Uses and Adverse Effects

The drug should be started at 1 mg/day divided into two doses for the first 4 weeks and, if tolerated, advanced to a maximum of 1 mg twice daily if necessary. If the response is inadequate after 4 weeks of 1 mg twice-daily dosing, treatment should be discontinued. The most serious adverse reactions are constipation and ischemic colitis, and therapy must be discontinued immediately in patients who develop those symptoms. Other adverse reactions include nausea and vomiting, GI discomfort and pain, diarrhea, flatulence, hemorrhoids, and others.

Additional $5HT_3$ antagonists currently available in the U.S. are approved for nausea and vomiting (see later in this chapter and Chapter 15).

Eluxadoline

Mechanism of Action and Pharmacology

Eluxadoline is a mixed MOR agonist, DOR antagonist, and KOR (kappa opioid receptor) agonist. It acts locally to reduce abdominal pain and diarrhea without producing constipation in patients with IBS. This opioid drug is FDA-approved for the treatment of diarrhea-predominant IBS in adults (Hornby, 2015).

ADME

Eluxadoline's time to peak C_p is 1.5 to 2 h; its $t_{1/2}$ is 3.7 to 6 h. The route of *eluxadoline*'s metabolism is not well established. The drug and its metabolic products are excreted in the feces.

Therapeutic Uses and Adverse Effects

In patients with diarrhea-predominant IBS with a gallbladder, the therapeutic dose is 100 mg twice daily with food; the dose may be decreased to 75 mg twice daily in patients unable to tolerate the 100-mg dose. In patients without a gallbladder, *eluxadoline* is dosed at 75 mg twice daily to reduce the risk of sphincter of Oddi spasm and the potential complication of pancreatitis. Patients with known or suspected biliary duct obstruction, sphincter of Oddi disease or dysfunction, a history of pancreatitis, or structural diseases of the pancreas should not be given *eluxadoline*. There are also risks from constipation, and the drug should be discontinued if severe constipation occurs. There is some potential for addiction. The major adverse reactions to the drug are constipation, nausea, and abdominal pain.

Rifaximin

Mechanism of Action and Pharmacology

Antibiotics should not be used routinely in patients with IBS, but the FDA has approved the bacterial RNA synthesis inhibitor *rifaximin*, a poorly absorbed derivative of *rifamycin*, for diarrhea-predominant IBS (Saadi and McCallum, 2013).

ADME

Rifaximin is not suitable for treating systemic bacterial infections because of limited systemic exposure after oral administration. Most of an oral dose of *rifaximin* is recovered as unchanged drug in the feces. The half-life and AUC of the portion of the administered dose that enters the systemic circulation are increased in IBS patients ($t_{1/2}$ is 6 h), and the drug is cleared by the action of hepatic CYP3A; metabolites are excreted in the feces.

Therapeutic Uses and Adverse Effects

In patients with diarrhea-predominant IBS, the therapeutic dose is 550 mg three times daily for 2 weeks. Patients may be re-treated with this regimen twice if symptoms recur. Adverse reactions include nausea, peripheral edema, dizziness, fatigue, the development of ascites, and elevation in serum alanine aminotransferase. If diarrhea worsens after treatment with *rifaximin*, then an evaluation for development of a severe infectious diarrhea or *C. difficile* enterocolitis should be performed.

Antispasmodics

Anticholinergic agents ("spasmolytics" or "antispasmodics") are used in patients with IBS but should not be used over the long term. The most common agents of this class available in the U.S. are nonspecific antagonists of the muscarinic receptor (see Chapter 11) and include the tertiary amines *dicyclomine* and *hyoscyamine* and the quaternary ammonium compounds *glycopyrrolate* and *methscopolamine* (off-label use). The advantage of the last two compounds is that they have a limited propensity to cross the blood-brain barrier and hence have a lower risk for neurological side effects such as light-headedness, drowsiness, or nervousness. These agents typically are given on either an as-needed basis or before meals to prevent the pain and fecal urgency that occur in some patients with IBS.

Dicyclomine is given in 20-mg doses orally every 6 h, increasing to 40 mg every 6 h unless limited by side effects. *Hyoscyamine* is available as sublingual tablets, orally disintegrating tablets, immediate-release oral capsules, tablets, elixir, and drops (all administered as 0.125–0.25 mg every 4 h as needed), as extended-release forms for oral use (0.25–0.375 mg every 12 h, or 0.375 mg every 8 h, as needed), and as an injection for intramuscular, intravenous, or subcutaneous use (0.25–0.5 mg every 4 h as needed). *Glycopyrrolate* is rarely used but is available as immediate-release tablets, as an oral solution, and as an injectable; the oral dose is 1 to 2 mg two or three times daily, not to exceed 6 mg/day. *Methscopolamine* is provided as 2.5- and 5-mg tablets; the dose is 2.5 mg a half hour before meals and 2.5 to 5 mg at bedtime.

Other Drugs

Cimetropium and *acotiamide* are muscarinic antagonists that are effective in patients with IBS but are not available in the U.S. *Acotiamide* appears to be a promising agent for the treatment of postprandial distress syndrome, one of two major forms of functional dyspepsia (Zala et al., 2015). *Otilonium bromide* is a quaternary ammonium salt with antimuscarinic effects that also appears to block Ca^{2+} channels and neurokinin NK_2 receptors; it is not available in the U.S. *Mebeverine hydrochloride*, a derivative of hydroxybenzamide, appears to have a direct effect on the smooth muscle cell, blocking K^+, Na^+, and Ca^{2+} channels. *Mebeverine* is used outside the U.S. as an antispasmodic agent. It is given orally, 100 to 135 mg three times daily or 200 mg twice daily, before meals.

Antinauseants and Antiemetics

Nausea and Vomiting

Emesis and the sensation of nausea that frequently accompanies it are generally viewed as components of a protective reflex that serve to rid the stomach and intestine of toxic substances (emesis) and prevent their further ingestion (nausea serves as an unconditioned aversive stimulus for learning and memory) (Horn, 2008; Hornby, 2001). Vomiting is a complex process that appears to be coordinated by a central emesis center in the lateral reticular formation of the brainstem adjacent to both the CTZ in the area postrema on the floor of the fourth ventricle and the nucleus of the solitary tract (NTS). The lack of a blood-brain barrier at the CTZ permits monitoring of blood and cerebrospinal fluid constantly for toxic substances and relaying information to the emesis center to trigger nausea and vomiting. The emesis center also receives information from the gut, principally by the vagus nerve (via the NTS) and by splanchnic afferents via the spinal cord. Two other important inputs to the emesis center come from the cerebral cortex (particularly in anticipatory nausea or vomiting) and the vestibular apparatus (in motion sickness). The CTZ has high concentrations of receptors for serotonin ($5HT_3$), dopamine (D_2), ACh (muscarinic M_1), neurokinin (NK_1), cannabinoid (CB_1), and opioids. The NTS is rich in receptors for enkephalin, histamine, and ACh and expresses $5HT_3$ receptors. Myriad neurotransmitter agonists for these receptors are involved in nausea and vomiting (Figure 54–6). Antiemetics generally are classified according to the predominant receptor on which they are proposed to act (Table 54–4). For treatment and prevention of the nausea and emesis associated with cancer chemotherapy, several antiemetic agents from different pharmacological classes may be used in combination (Table 54–5).

Nausea is distinct from emesis and is a frequent side effect of medications as well as a common feature of diseases that range from CNS disorders to GI disorders to infection. The brain centers involved in the sensation of nausea are located in higher brain regions than the emetic centers and include the insular, anterior cingulate, orbitofrontal, somatosensory, and prefrontal cortices. Most drugs used to treat emesis are relatively poor at preventing nausea (Andrews and Sanger, 2014).

$5HT_3$ Receptor Antagonists

Mechanism of Action and Pharmacology. The $5HT_3$ antagonists are the most effective drugs for the treatment of chemotherapy-induced and PONV (postoperative nausea and vomiting) in adults and children (Andrews and Sanger, 2014; Berger et al., 2017; Navari, 2013). However, they are less effective at suppressing acute nausea than they are at suppressing acute vomiting, and they are ineffective at reducing instances of delayed (24 h later) nausea and vomiting and anticipatory nausea and vomiting.

Ondansetron is the prototypical drug in this class. Other agents in this class include the first-generation antagonists *granisetron*, *dolasetron* (not available in the U.S. or Canada), and *tropisetron* (not available in the U.S.) and the second-generation antagonist *palonosetron*. *Palonosetron* has higher receptor affinity, a longer $t_{1/2}$, and demonstrated superiority over first-generation antagonists (Navari, 2014).

The $5HT_3$ receptors are present in several critical sites involved in emesis, including vagal afferents, the NTS (which receives signals from vagal afferents), and the area postrema itself (see Figure 54–6). Serotonin is released by the enterochromaffin cells of the small intestine in response to chemotherapeutic agents and stimulates vagal afferents (via $5HT_3$ receptors) to initiate the vomiting reflex. The highest concentrations of $5HT_3$ receptors in the CNS are found in the NTS and CTZ, and antagonists of $5HT_3$ receptors also may suppress nausea and vomiting by acting at these sites.

ADME. These agents are absorbed well from the GI tract and have a rapid onset of action. *Ondansetron* is extensively metabolized in the liver by CYP1A2, CYP2D6, and CYP3A4, followed by glucuronide or sulfate conjugation. The $t_{1/2}$ is 3 to 6 h. Patients with hepatic dysfunction have reduced plasma clearance, and some adjustment in the dosage is advisable. *Granisetron* also is metabolized predominantly by the liver by the CYP3A family and has a $t_{1/2}$ of 6 to 9 h, depending on the route of administration. *Dolasetron* is converted rapidly by plasma carbonyl reductase to its active metabolite, hydrodolasetron. A portion of this compound then undergoes subsequent biotransformation by CYP2D6 and CYP3A4 in the liver, while about one-third of it is excreted unchanged in the urine. The $t_{1/2}$ of the active metabolite hydrodolasetron is 6 to 8 h. *Palonosetron* is metabolized principally by CYP2D6; the metabolized and the unchanged forms are excreted in the urine in roughly equal proportions. The $t_{1/2}$ after intravenous injection is about 40 h in adults. The antiemetic effects of these drugs persist long after they disappear from the circulation, suggesting their continuing interaction at the receptor level; these drugs require only once a day administration to be effective.

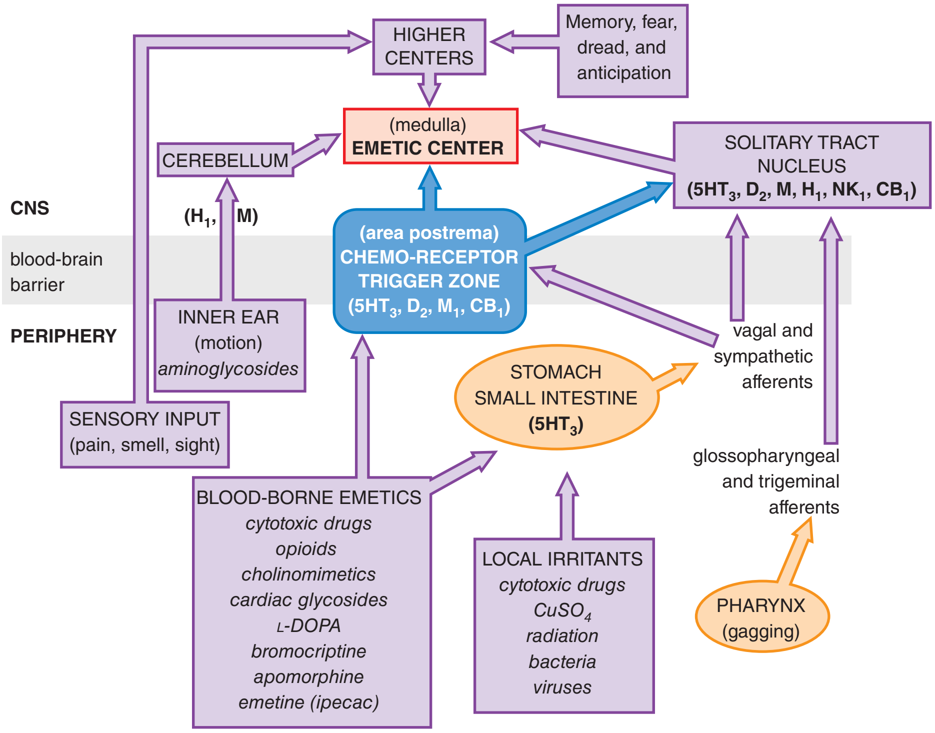

HIGHER CENTERS
Memory, fear, dread, and anticipation
(medulla) EMETIC CENTER
CEREBELLUM
SOLITARY TRACT NUCLEUS (5HT$_3$, D$_2$, M, H$_1$, NK$_1$, CB$_1$)
CNS
(H$_1$, M)
(area postrema) CHEMO-RECEPTOR TRIGGER ZONE (5HT$_3$, D$_2$, M$_1$, CB$_1$)
blood-brain barrier
PERIPHERY
INNER EAR (motion) aminoglycosides
vagal and sympathetic afferents
STOMACH SMALL INTESTINE (5HT$_3$)
SENSORY INPUT (pain, smell, sight)
glossopharyngeal and trigeminal afferents
BLOOD-BORNE EMETICS cytotoxic drugs opioids cholinomimetics cardiac glycosides L-DOPA bromocriptine apomorphine emetine (ipecac)
LOCAL IRRITANTS cytotoxic drugs CuSO$_4$ radiation bacteria viruses
PHARYNX (gagging)

Figure 54–6 *Pharmacologist's view of emetic stimuli.* Many signaling pathways lead from the periphery to the emetic center. Stimulants of these pathways are noted in *italics*. These pathways involve specific neurotransmitters and their receptors (**bold** type). Receptors are shown for dopamine (D_2), ACh (muscarinic, M), histamine (H_1), cannabinoids (CB_1), substance P (NK_1), and $5HT_3$. Some of these receptors also may mediate signaling in the emetic center.

TABLE 54–4 ■ GENERAL CLASSIFICATION OF ANTIEMETIC AGENTS

ANTIEMETIC CLASS	EXAMPLES	MOST EFFECTIVE AGAINST
5HT$_3$ receptor antagonists[a]	Ondansetron	Cytotoxic drug-induced emesis
Centrally acting dopamine receptor antagonists	Metoclopramide[b] Promethazine[c]	
Cannabinoid receptor agonists	Dronabinol, nabilone	
Neurokinin receptor antagonists	Aprepitant	Cytotoxic drug-induced emesis (delayed vomiting)
Histamine H$_1$ antagonists	Cyclizine	Vestibular emesis (motion sickness)
Muscarinic receptor antagonists	Hyoscine (scopolamine)	

[a] The most effective agents for chemotherapy-induced nausea and vomiting are the 5HT$_3$ antagonists and metoclopramide. In addition to their use as single agents, they are often combined with other drugs to improve efficacy and reduce incidence of side effects. See Table 54–5.
[b] Also has some peripheral activity at 5HT$_3$ receptors.
[c] Also has some antihistaminic and anticholinergic activity.

TABLE 54–5 ■ ANTIEMETIC AGENTS IN CANCER CHEMOTHERAPY[a]

Low risk of emesis: *Prechemotherapy* • Dexamethasone • Metoclopramide ± diphenhydramine • Prochlorperazine ± lorazepam	*Postchemotherapy (delayed emesis)* • None
Moderate risk of emesis: *Prechemotherapy* • 5HT$_3$ antagonist + dexamethasone • Aprepitant + 5HT$_3$ antagonist + dexamethasone • Fosaprepitant + 5HT$_3$ antagonist + dexamethasone • Rolapitant + 5HT$_3$ antagonist + dexamethasone • Netupitant/palonosetron + dexamethasone • Olanzapine + palonosetron + dexamethasone	*Postchemotherapy (delayed emesis)* • Dexamethasone *or* 5HT$_3$ antagonist monotherapy (days 2 and 3) • Aprepitant ± dexamethasone (days 2 and 3) • ± Dexamethasone (days 2 and 3) • ± Dexamethasone (days 2 and 3) • ± Dexamethasone (days 2 and 3) • Olanzapine (days 2 and 3)
High risk of emesis: *Prechemotherapy* • Aprepitant + 5HT$_3$ antagonist + dexamethasone • Fosaprepitant + 5HT$_3$ antagonist + dexamethasone • Rolapitant + 5HT$_3$ antagonist + dexamethasone • Netupitant/palonosetron + dexamethasone • Olanzapine + palonosetron + dexamethasone • Aprepitant *or* fosaprepitant + • 5HT$_3$ antagonist + dexamethasone + olanzapine	*Postchemotherapy (delayed emesis)* • Aprepitant (days 2 and 3) ± dexamethasone (days 2–4) • Dexamethasone (day 2), dexamethasone twice daily (days 3 and 4) • Dexamethasone (days 2–4) • Dexamethasone (days 2–4) • Olanzapine (days 2–4) • Aprepitant (days 2 and 3) if given on day 1 • + dexamethasone (days 2–4) + olanzapine (days 2–4)

[a] *Specific recommendations and doses are tailored to the patient and the chemotherapeutic regimen. For updated information, see the National Cancer Institute website (see Cancer Topics: Nausea and Vomiting).* Some patients profit from cannabinoids (dronabinol, nabilone) with or without a phenothiazine or dexamethasone.
NCCN Guidelines Insights: Antiemesis, Version 2. 2017 (Berger et al., 2017).

Therapeutic Uses and Adverse Effects. These agents are most effective in treating chemotherapy-induced nausea and in treating nausea secondary to upper abdominal irradiation. They also are effective against hyperemesis of pregnancy and PONV, but not against motion sickness. Unlike other agents in this class, *palonosetron* may be helpful in delayed emesis, perhaps reflecting its long $t_{1/2}$. These agents are available as tablets, oral solution, and intravenous preparations for injection. *Palonosetron*, in combination with the NK$_1$ receptor antagonist *netupitant* (see further discussion), is recently FDA approved for the treatment of acute and delayed nausea and vomiting. This combination is highly effective when combined with the corticosteroid *dexamethasone* (see further discussion). For patients on cancer chemotherapy, these drugs can be given in a single intravenous dose (Table 54–6) infused over 15 min, beginning 30 min before chemotherapy, or in two or three divided doses, with the first usually given 30 min before and subsequent doses at various intervals after chemotherapy. The drugs also can be used intramuscularly (*ondansetron* only) or orally. *Granisetron* is available as a transdermal formulation that is applied 24 to 48 h before chemotherapy and worn for up to 7 days (see Table 54–6). For the treatment of PONV, *ondansetron* is considered a gold standard (Gan et al., 2020). It is given as a 4-mg dose intravenously or 8-mg tablet orally. Other 5HT$_3$ antagonists are also used for the treatment of PONV: *Dolasetron* is given as a 12.5-mg dose intravenously, *granisetron* 0.35 to 3 mg intravenously, and *palonosetron* 0.075 mg intravenously (Gan et al., 2020).

In general, these drugs are very well tolerated, with the most common adverse effects being constipation or diarrhea, headache, and light-headedness. ECG interval changes (QT prolongation) are a feature of the first-generation antagonists. The injectable form of *dolasetron* is contraindicated for prophylactic therapy for chemotherapy-induced nausea and vomiting; the oral form is associated with a lower risk of QT prolongation, but the risk is still present. *Palonosetron* does not appear to increase QT intervals (Gonullu et al., 2012). These drugs have also been associated with serotonin syndrome and should be used cautiously if patients are taking other medications, such as SSRIs, that could increase 5HT levels.

Dopamine Receptor Antagonists

Mechanism of Action and Pharmacology. The principal mechanism of action of dopamine receptor antagonists is D$_2$ receptor antagonism at the CTZ, reducing excitatory neurotransmitter release (Andrews and Sanger, 2014; Navari, 2013).

Phenothiazines. *Prochlorperazine* and, to a lesser extent, *chlorpromazine* (see Chapter 19) are among the most commonly used "general-purpose" antinauseants and antiemetics in adults and children. These drugs are not uniformly effective in cancer chemotherapy-induced emesis, but they possess antihistaminic and anticholinergic activities that are of value in other forms of nausea and vomiting, such as motion sickness and that of GI origin. These drugs are available as tablets, injectables, or suppositories. Typical dosing of *prochlorperazine* is 5 to 10 mg orally every 6 to 8 h, 5 to 10 mg intramuscularly, 2.5 to 10 mg intravenously every 3 to 4 h (maximum 40 mg/day), or 25 mg rectally every 12 h. The main adverse effects are extrapyramidal reactions, including dystonia, cardiac effects, and hypotension. These drugs are contraindicated due to increased mortality in elderly patients with dementia-related psychosis.

Benzamides. The prokinetic benzamide agents (see previous discussion) are moderately useful antiemetics but are no longer the drugs of

TABLE 54–6 ■ $5HT_3$ ANTAGONISTS IN CHEMOTHERAPY-INDUCED NAUSEA/EMESIS

DRUG	CHEMICAL NATURE	RECEPTOR INTERACTIONS	$t_{1/2}$	ADULT DOSE
Ondansetron	Carbazole derivative	$5HT_3$ antagonist, weak $5HT_4$ antagonist	3–4 h	16–24 mg (orally); 8–16 mg (IV)
Granisetron	Indazole	$5HT_3$ antagonist	5–9 h	2 mg (orally); 0.01 mg/kg (max 1 mg IV), 10 mg (subcutaneously); 3.1 mg/24 h transdermal patch
Dolasetron (not approved in the U.S.)	Indole moiety	$5HT_3$ antagonist	7–8 h	100 mg (orally)
Palonosetron	Isoquinoline	$5HT_3$ antagonist; highest affinity for $5HT_3$ receptor in class	37–48 h	0.25 mg (IV)

IV, intravenous.
NCCN Guidelines Insights: Antiemesis, Version 2.2017 (Berger et al., 2017).

choice for acute chemotherapy-induced nausea and vomiting due to their lack of efficacy and side effect profile. However, the antiemetic actions add to their value in the treatment of GI motor disturbances, and *metoclopramide* is a useful treatment of delayed emesis. *Amisulpride* is a benzamide derivative and an atypical antipsychotic (see Chapter 19). It was recently approved in the U.S. in adults for the prevention of PONV, either alone or in combination with an antiemetic of a different class, and to treat PONV in those who have received antiemetic prophylaxis with an agent of a different class or have not received prophylaxis. It is given as a single intravenous 5-mg dose to prevent PONV or as a single intravenous 10-mg dose to treat this condition. *Amisulpride* is contraindicated in patients with preexisting arrhythmias/cardiac conduction disorders, electrolyte abnormalities, congestive heart failure, or renal impairment and in patients taking other drugs (e.g., *ondansetron*) known to prolong the QT interval.

Butyrophenones. The butyrophenone *droperidol* is used for the treatment of PONV, and *haloperidol* is used as an adjunctive agent for nausea and vomiting in a palliative setting in cancer patients and for the acute treatment of cannabinoid hyperemesis syndrome (off-label). *Droperidol* is given as a single intravenous 0.625- to 1.25-mg dose at the end of surgery to prevent PONV. Its use has declined due to black box warnings (for much higher doses). However, the risks at the doses used to treat PONV appear low (Gan et al., 2020). *Haloperidol* is given as a single intravenous or intramuscular 5-mg dose for the treatment of cannabinoid hyperemesis syndrome and at 0.5 to 5 mg (by various routes of administration) for cancer patients. Potential adverse effects include QT prolongation, sedation, extrapyramidal symptoms, neuroleptic malignant syndrome, and hypotension. See Chapter 19 for further details of these drugs.

Olanzapine. *Olanzapine* is an atypical (second-generation) antipsychotic that is a dopamine ($D_{1–4}$) and $5HT_2$ receptor antagonist (see Chapters 15 and 19). It is an effective agent for the prevention of chemotherapy-associated delayed nausea or vomiting (off-label use; used in combination with a corticosteroid and $5HT_3$ antagonist) (Fonte et al., 2015). It is also gaining attention for the treatment of refractory non–chemotherapy-induced nausea and vomiting. It is given orally, 10 mg once daily for 3 to 5 days, beginning on day 1 of chemotherapy or 5 mg once daily for 2 days before chemotherapy, followed by 10 mg once daily (beginning on the day of chemotherapy) for 3 to 8 days. The adverse reactions are extensive and include many CNS, cardiovascular, and metabolic side effects that are described in Chapter 19.

Antihistamines

Histamine H_1 antagonists are primarily useful for motion sickness and PONV. They act on vestibular afferents and within the brainstem. *Cyclizine*, *meclizine, promethazine*, and *diphenhydramine* are examples of this class of agents. *Cyclizine* has additional anticholinergic effects that may be useful for patients with abdominal cancer. Sedation is always a common side effect of these drugs. For a detailed discussion of these drugs, see Chapter 43.

Anticholinergic Agents

The most commonly used muscarinic receptor antagonist for motion sickness is *scopolamine* (hyoscine), which can be injected as the hydrobromide, but usually is administered as the free base in the form of a transdermal patch (1.5 mg every 3 days). Its principal utility is in the prevention and treatment of motion sickness, with some activity in postoperative nausea and vomiting. In general, however, anticholinergic agents have no role in chemotherapy-induced nausea. The principal side effects are dry mouth, visual disturbances, and drowsiness.

Neurokinin Receptor Antagonists

Mechanism of Action and Pharmacology. The nausea and vomiting associated with emetogenic chemotherapy (see Chapters 69–73) has two components: an acute phase that universally is experienced (within 24 h after chemotherapy) and a delayed phase that affects only some patients (on days 2–5). $5HT_3$ receptor antagonists are not very effective against delayed emesis. However, antagonists of the NK_1 receptors, the receptors for the neuropeptide substance P, such as *aprepitant* (and its parenteral formulation *fosaprepitant*), have antiemetic effects in delayed nausea and improve the efficacy of standard antiemetic regimens in patients receiving multiple cycles of chemotherapy (Aapro et al., 2015). A new, highly selective NK_1 antagonist, *rolapitant*, with an exceptionally long plasma $t_{1/2}$ (180 h) is FDA-approved for the prevention of chemotherapy-induced delayed emesis.

Aprepitant. The NK_1 antagonist *aprepitant* is typically given with a $5HT_3$ antagonist and *dexamethasone*.

ADME. After absorption, *aprepitant* is bound extensively to plasma proteins (>95%); it is metabolized primarily by hepatic CYP3A4 and is excreted in the stools; its $t_{1/2}$ is 9 to 13 h. *Aprepitant* has the potential to interact with other substrates of CYP3A4, requiring adjustment of other drugs, including *dexamethasone*, *methylprednisolone* (the dose of which may need to be reduced by 50%), and *warfarin*.

Therapeutic Uses and Adverse Effects. *Aprepitant* is contraindicated in patients receiving *cisapride, terfenadine, astemizole*, or *pimozide*, in whom life-threatening QT prolongation has been reported. *Aprepitant* is supplied in 40-, 80-, and 125-mg capsules and is administered for 3 days for highly or moderately emetogenic chemotherapy, along with a $5HT_3$ antagonist and *dexamethasone*. The injectable form, *fosaprepitant*, in a dose of 150 mg, may be substituted for the first dose of *aprepitant* at the start of the 3-day regimen. The recommended adult dosage of *aprepitant* for moderately and highly emetogenic chemotherapy is 125 mg administered 1 h before chemotherapy on day 1, followed by 80 mg once daily in the morning on days 2 and 3 of the treatment regimen. *Aprepitant* and *fosaprepitant* are used for the treatment of PONV given orally (*aprepitant,* 40–125 mg) or intravenously (*fosaprepitant*, 150 mg) (Gan et al., 2020).

Rolapitant. *Rolapitant* is a potent NK_1 receptor antagonist that is administered with a $5HT_3$ antagonist and *dexamethasone* to help prevent delayed-phase chemotherapy-induced nausea and vomiting.

ADME. After a single oral dose of 180 mg, *rolapitant* is well absorbed with peak C_p at 4 h and $t_{1/2}$ at about 180 h. *Rolapitant* is metabolized primarily by CYP3A4 to form an active metabolite, M19 (C4-pyrrolidine-hydroxylated *rolapitant*). M19 has a $t_{1/2}$ of about 158 h. *Rolapitant* is eliminated mainly via the hepatic/biliary route.

Therapeutic Uses and Adverse Effects. A single 180-mg dose is administered orally 1 to 2 h prior to chemotherapy (together with $5HT_3$ antagonist and *dexamethasone*). The adverse effects include neutropenia, hiccups, decreased appetite, and dizziness. *Rolapitant* is a moderate inhibitor of CYP2D6 and of the Pgp and BCRP transporters. *Rolapitant* is contraindicated in patients receiving drugs that are CYP2D6 substrates, such as *thioridazine* or *pimozide*. A significant increase in plasma concentrations of *thioridazine* may result in QT prolongation and torsades de pointes.

Netupitant and Palonesetron Combination. A combination NK_1 receptor antagonist plus $5HT_3$ receptor antagonist (*netupitant* and *palonesetron*) has been approved (Abramovitz and Gaertner, 2016).

ADME. This combination is well absorbed; the drugs have a similar time to peak C_p (5 h) and very long half-lives (*netupitant*, ~80 h; *palonesetron*, ~48 h). They are excreted in the feces and urine. *Netupitant* is extensively metabolized by CYP3A4 (major) and CYP2C9 and CYP2D6 (minor) to active metabolites. *Palonesetron* is about 50% metabolized in the liver to inactive metabolites.

Therapeutic Uses and Adverse Effects. A single capsule is administered orally about 1 h prior to chemotherapy (together with *dexamethasone*, at doses varying according to the type of chemotherapy). The adverse effects are the same as for the $5HT_3$ antagonists (see previous discussion).

Cannabinoids

Cannabis has been widely used medicinally, including for the treatment of nausea and vomiting. It is an effective treatment, especially for nausea, though its use is limited because of its psychotropic side effects (Sharkey et al., 2014). See Chapter 26 for further details of the pharmacology of cannabis and cannabinoids. Two cannabinoids are used for the treatment of nausea and vomiting, *dronabinol* and *nabilone*.

Dronabinol. ***Mechanism of Action and Pharmacology.*** *Dronabinol* (Δ-9-tetrahydrocannabinol) is a naturally occurring cannabinoid that can be synthesized chemically or extracted from the marijuana plant, *Cannabis sativa*. The mechanism of the antiemetic action of *dronabinol* is related to stimulation of the CB_1 subtype of cannabinoid receptors on neurons in and around the CTZ and brainstem emetic centers (see Figure 54–6) (Sharkey et al., 2014).

ADME. *Dronabinol* is a highly lipid-soluble compound that is absorbed readily after oral administration; its onset of action occurs within an hour, and peak levels are achieved within 2 to 4 h. It undergoes extensive first-pass metabolism with limited systemic bioavailability after single doses (only 10%–20%). The principal active metabolite is 11-OH-delta-9-tetrahydrocannabinol. These metabolites are excreted primarily via the biliary-fecal route, with only 10% to 15% excreted in the urine. Both *dronabinol* and its metabolites are highly bound (>95%) to plasma proteins. Because of its large volume of distribution, a single dose of *dronabinol* can result in detectable levels of metabolites for several weeks.

Therapeutic Uses and Adverse Effects. *Dronabinol* is a useful prophylactic agent in patients receiving cancer chemotherapy when other antiemetic medications are not effective. It also can stimulate appetite and has been used in patients with AIDS and anorexia. As an antiemetic agent, it is administered at an initial dose of 5 mg/m^2 given 1 to 3 h before chemotherapy and then every 2 to 4 h afterward for a total of four to six doses. If this is inadequate, incremental increases can be made up to a maximum of 15 mg/m^2 per dose. For other indications, the usual starting dose is 2.5 mg twice a day; this can be titrated up to a maximum of 20 mg/day.

Dronabinol has complex effects on the CNS, including a prominent central sympathomimetic activity. This can lead to palpitations, tachycardia, vasodilation, hypotension, and conjunctival injection (bloodshot eyes). Patient supervision is necessary because marijuana-like "highs" (e.g., euphoria, somnolence, detachment, dizziness, anxiety, nervousness, panic) can occur, as can more disturbing effects such as paranoid reactions and abnormalities of thinking. After abrupt withdrawal of *dronabinol*, an abstinence syndrome (irritability, insomnia, and restlessness) can occur. Because of its high affinity for plasma proteins, *dronabinol* can displace other plasma protein-bound drugs, the doses of which may have to be adjusted as a consequence. *Dronabinol* should be prescribed with great caution to persons with a history of substance abuse (alcohol, drugs) because it also may be abused by these patients.

Nabilone. ***Mechanism of Action and Pharmacology.*** *Nabilone* is a synthetic cannabinoid with a mode of action similar to that of *dronabinol*.

ADME. *Nabilone* is a highly lipid-soluble compound that is rapidly absorbed after oral administration; its onset of action occurs within an hour, and peak levels are achieved within 2 h. The $t_{1/2}$ is about 2 h for the parent compound and 35 h for metabolites. The metabolites are excreted primarily via the biliary-fecal route (60%), with only about 25% excreted in the urine.

Therapeutic Uses and Adverse Effects. *Nabilone* is a useful prophylactic agent in patients receiving cancer chemotherapy when other antiemetic medications are not effective. A dose (1–2 mg) can be given the night before chemotherapy; usual dosing starts 1 to 3 h before treatment and then every 8 to 12 h during chemotherapy and for 2 days following its cessation. The adverse effects are largely the same as for *dronabinol*, with significant CNS actions in more than 10% of patients. Cardiovascular, GI, and other side effects are also common and, together with the CNS actions, limit the usefulness of this agent.

Glucocorticoids and Anti-inflammatory Agents

Glucocorticoids such as *dexamethasone* can be useful adjuncts (see Table 54–5) in the treatment of nausea in patients with widespread cancer, possibly by suppressing peritumoral inflammation and prostaglandin production. A similar mechanism has been invoked to explain beneficial effects of nonsteroidal anti-inflammatory drugs in the nausea and vomiting induced by systemic irradiation (Chu et al., 2014). *Dexamethasone* is given at doses between 4 and 10 mg for the treatment of PONV (Gan et al., 2020) and between 12 and 20 mg for the treatment of chemotherapy-induced nausea and vomiting (Berger et al., 2017). For a detailed discussion of these drugs, see Chapter 50.

Benzodiazepines

Benzodiazepines, such as *lorazepam* and *alprazolam*, by themselves are not very effective antiemetics, but their sedative, amnesic, and anxiolytic effects can be helpful in reducing the anticipatory component of nausea and vomiting in patients. For a detailed discussion of these drugs, see Chapter 22.

Phosphorated Carbohydrate Solutions

Aqueous OTC solutions of *glucose, fructose*, and *orthophosphoric* are available to relieve nausea. These solutions are given orally (15–30 mL, adults; 5–10 mL, children; repeated every 15 min until the symptoms alleviate; no more than five doses may be taken). Their mechanisms of action are unclear.

Doxylamine Succinate and Pyridoxine

Mechanism of Action and Pharmacology. Nausea commonly occurs in the early stages of pregnancy. This may or may not be accompanied by vomiting. The management of this condition depends on the severity of symptoms, which usually resolve by midpregnancy regardless of their severity. *Pyridoxine* (vitamin B_6) improves mild-to-moderate nausea, and its efficacy is improved when it is combined with the histamine H_1 antagonist *doxylamine* (Fantasia, 2014). Considering the caveats associated with the use of antinausea medications during early pregnancy, readers may wish to review the history of this drug combination; see the work of Slaughter et al. (2014).

ADME. *Doxylamine* is metabolized in the liver by N-dealkylation. It has a $t_{1/2}$ of 10 to 12 h and is excreted in the urine. *Pyridoxine* is well absorbed and has a $t_{1/2}$ of 2 to 3 weeks.

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Therapeutic Uses and Adverse Effects. This drug-vitamin combination is given for the treatment of nausea and vomiting of pregnancy. Initially, two delayed-release tablets (a total of *doxylamine* 20 mg and *pyridoxine* 20 mg) are taken at bedtime. The dose may be increased to four tablets per day as needed for more severe nausea (one tablet in the morning, one tablet in the afternoon, two tablets at bedtime). The major side effects of this drug include drowsiness, dry mouth, light-headedness, and constipation.

Miscellaneous GI Disorders

Cystic Fibrosis, Chronic Pancreatitis, and Steatorrhea

Pancreatic Enzymes

Chronic pancreatitis is a debilitating syndrome that results in symptoms from loss of glandular function (exocrine and endocrine) and inflammation (pain). The goals of pharmacological therapy are prevention of malabsorption and palliation of pain (Trang et al., 2014). *Cystic fibrosis* is a genetic disorder that affects exocrine secretion. Exocrine pancreatic insufficiency occurs in most patients with more severe forms of cystic fibrosis. Pharmacological therapy is used to treat these patients (Somaraju and Solis-Moya, 2014).

Enzyme Formulations. Pancreatic enzymes (lipase, amylase, and proteases) are secreted together; hence, lipase can be used to titrate the doses of pancreatic enzyme supplements, which are typically prescribed based on the lipase content. Only *pancrelipase* is licensed for sale in the U.S. *Pancrelipase* products, of which there are six on the market, differ in their content of lipase, protease, and amylase and thus may not be interchangeable.

Replacement Therapy for Malabsorption. Fat malabsorption (*steatorrhea*) and protein maldigestion occur when the pancreas loses more than 90% of its ability to produce digestive enzymes. This occurs in chronic pancreatitis, following pancreatectomy, or in cystic fibrosis. The resultant diarrhea and malabsorption can be managed well if 90,000 USP (U.S. Pharmacopeia) units of pancreatic lipase are delivered to the duodenum during a 4-h period with and after meals. Alternatively, one can titrate the dosage to the fat content of the diet, with about 8000 USP units of lipase activity required for each 17 g of dietary fat. Available preparations of pancreatic enzymes contain 3000 to 40,000 USP units of lipase, 10,000 to 136,000 USP units of protease, and 15,000 to 218,000 USP units of amylase. In adults and children over 4 years, the initial dose of lipase is 500 USP units/kg per meal, increasing up to 2500 USP units/kg per meal. Children younger than 4 years have increased needs for lipase, and initial doses are higher. There are also special dosing regimens for breastfeeding infants. In all cases, lipase dosing should not exceed maximum recommendations and generally should not exceed 2500 USP units/kg per meal or 10,000 USP unit/kg per day.

Enzymes for Pain. Pain is the other cardinal symptom of chronic pancreatitis. The rationale for its treatment with pancreatic enzymes is based on the principle of negative-feedback inhibition of the pancreas by the presence of duodenal proteases. The release of CCK, the principal secretagogue for pancreatic enzymes, is triggered by CCK-releasing monitor peptide in the duodenum, which normally is denatured by pancreatic trypsin. In chronic pancreatitis, trypsin insufficiency leads to persistent activation of this peptide and an increased release of CCK, which is thought to cause pancreatic pain because of continuous stimulation of pancreatic enzyme output and increased intraductal pressure. Delivery of active proteases to the duodenum (which can be done reliably only with uncoated preparations) therefore is important for the interruption of this loop. Although enzymatic therapy has become firmly entrenched for the treatment of painful pancreatitis, the evidence supporting this practice is equivocal at best.

Adverse Effects. Despite the fact that the enzymes are not absorbed and are excreted in feces, there are adverse effects, which include headache and abdominal pain; however, pancreatic enzyme preparations are tolerated extremely well by patients. Hyperuricosuria in patients with cystic fibrosis can occur, and malabsorption of folate and iron has been reported.

Gallstones and Primary Biliary Cholangitis

Bile Acids

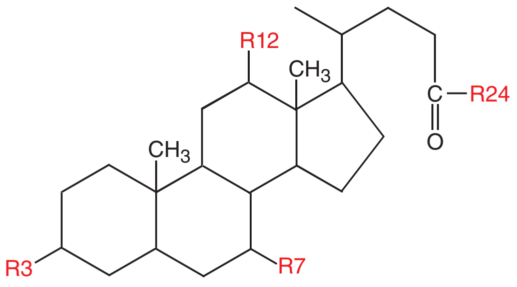
Bile acids and their conjugates are synthesized from cholesterol in the liver. Bile acids induce bile flow, feedback-inhibit cholesterol synthesis, promote intestinal excretion of cholesterol, and facilitate the emulsification and absorption of lipids and fat-soluble vitamins. After secretion into the biliary tract, bile acids are largely (95%) reabsorbed in the intestine, returned to the liver, and then again secreted in bile (enterohepatic circulation). Cholic acid, chenodeoxycholic acid, and deoxycholic acid constitute 95% of bile acids; lithocholic acid and ursodeoxycholic acid are minor constituents. The bile acids exist largely as glycine and taurine conjugates, the salts of which are called bile salts.

Traditional therapy for gallstones involves oral litholysis with *ursodeoxycholic acid* (*ursodiol*), but there is now evidence that inhibiting cholesterol synthesis (with statins) or intestinal cholesterol absorption (with *ezetimibe*) may have some beneficial effects to reduce gallstone formation (Portincasa et al., 2012). As for the treatment of gallstones, treatments for primary biliary cholangitis also involve the use of *ursodiol*. Recently, an alternative approach for the treatment for primary biliary cholangitis was developed based on agonism of the farnesoid X receptor by *obeticholic acid*. Activation of the farnesoid X receptor within hepatocytes results in potent suppression of bile acid synthesis (Gulamhusein and Hirschfield, 2020). New therapies are being developed for primary biliary cholangitis that include treatment with fibrates and peroxisome proliferator-activated receptor alpha (PPARα) agonists (Gulamhusein and Hirschfield, 2020).

Ursodeoxycholic Acid (Ursodiol). *Ursodeoxycholic acid* (*ursodiol*) (Figure 54–7) is a hydrophilic, dehydroxylated bile acid that is formed by epimerization of the bile acid chenodeoxycholic acid in the gut by intestinal bacteria.

Mechanism of Action and Pharmacology. *Ursodiol* is a naturally occurring hydrophilic bile acid. Litholytic bile acids such as *ursodiol*, when administered orally, alter relative concentrations of bile acids, decrease biliary lipid secretion, and reduce the cholesterol content of the bile so that it is less lithogenic. *Ursodiol* acts by replacing and/or displacing toxic concentrations of endogenous hydrophobic bile acids that tend to accumulate in cholestatic liver disease. *Ursodiol* may also have cytoprotective effects on hepatocytes and effects on the immune system that account for some of its beneficial effects in cholestatic liver diseases.

ADME. *Ursodiol* becomes a major biliary and plasma bile acid. Following oral administration, the majority of *ursodiol* is absorbed by



Bile Acid	R3	R7	R12	R24
Cholic acid	–OH	–OH	–OH	glycine (75%) taurine (24%) –OH (<1%)
Chenodeoxycholic acid	–OH	–OH	–H	
Deoxycholic acid	–OH	–H	–OH	
Lithocholic acid	$-SO_2^-$ / –OH	–H	–H	
Ursodeoxycholic acid	–OH	◄OH	–H	

Figure 54–7 *Major bile acids in adults.*

passive diffusion. In the liver, *ursodiol* is conjugated with glycine or taurine, then secreted into bile. *Ursodiol* is primarily excreted in the feces.

Therapeutic Uses and Adverse Effects. *Ursodiol* is given for the prevention and treatment of gallstones and for the treatment of primary biliary cholangitis (Portincasa et al., 2012; Tabibian and Lindor, 2015). For gallstone treatment, it is given orally 8 to 10 mg/kg per day in divided doses, for gallstone prevention 300 mg twice daily, and for primary biliary cholangitis 13 to 15 mg/kg per day in two to four divided doses with food. Adverse effects at these doses are generally uncommon but include headache, GI disturbances, and nausea. At higher than recommended doses, there may be serious adverse effects of *ursodiol*.

Obeticholic Acid. *Obeticholic acid* is a semisynthetic analogue of chenodeoxycholic acid.

Mechanism of Action and Pharmacology. *Obeticholic acid* is an agonist of the farnesoid X receptor, a nuclear receptor important for regulating bile acid and cholesterol metabolism. Farnesoid X receptors are expressed in the liver and intestine and are key regulators of bile acid synthesis, inflammation, and fibrosis. Activation of farnesoid X receptors regulates the *de novo* synthesis of bile acids from cholesterol, as well as increasing transport of bile acids out of the hepatocytes. Together these mechanisms reduce the size of the circulating bile acid pool while promoting secretion of bile, thereby reducing exposure to bile acids.

ADME. Patients are started at 5 mg daily for the first 3 months, which may be increased to 10 mg if tolerated, to a maximum dose of 10 mg daily. There is a dose adjustment for patients with advanced liver disease (5 mg weekly for the first 3 months, followed by 5 mg twice weekly to a maximum of 10 mg twice weekly). *Obeticholic acid* is conjugated with glycine or taurine in the liver and secreted into bile. The conjugates are absorbed in the small intestine, leading to enterohepatic recirculation. They can be deconjugated by enteric microbiota, leading to the conversion to *obeticholic acid* that can be reabsorbed or excreted in the feces.

Therapeutic Uses and Adverse Effects. *Obeticholic acid* is indicated for the treatment of primary biliary cholangitis in combination with *ursodiol* in adults with an inadequate response to *ursodiol* or as a monotherapy in adults unable to tolerate *ursodiol* (Gulamhusein and Hirschfield, 2020).

Obeticholic acid carries a black box warning: This drug can cause hepatic decompensation and liver failure in incorrectly dosed patients. Patients at an increased risk of hepatic decompensation must be monitored closely. The most common adverse reactions are pruritus, fatigue, abdominal pain and discomfort, rash, oropharyngeal pain, dizziness, constipation, arthralgia, thyroid function abnormality, and eczema.

Flatulence

"Gas" is a common but relatively vague GI complaint, used in reference not only to flatulence and eructation but also bloating or fullness. OTC and herbal preparations are popular. *Simethicone*, a mixture of siloxane polymers stabilized with silicon dioxide, is an inert, nontoxic surfactant. Because of its capacity to collapse bubbles by forming a thin layer on their surface, it is an effective antifoaming agent, but whether this accomplishes a therapeutic effect in the GI tract is not clear. *Simethicone* is available in chewable tablets, liquid-filled capsules, suspensions, and orally disintegrating strips, either by itself or in combination with other OTC medications, including antacids and other digestants. The usual dosage in adults is 40 to 125 mg four times daily after meals; the pediatric dose is 20 to 50 mg four times daily after meals and at bedtime, depending on the age of the child. Activated charcoal may be used alone or in combination with *simethicone* but has not been shown conclusively to have much benefit. An α-galactosidase OTC preparation is available to reduce gas from baked beans.

Short-Bowel Syndrome

Short-bowel syndrome is a malabsorption disorder caused by removal of the small intestine or rarely because of a congenital bowel abnormality. Short-bowel syndrome requires total parenteral nutrition, and treatments are aimed at reducing the need for this, including supplemented specialized diets and implementing a therapy based on physiologic principles of the actions of gut hormones.

Teduglutide

Mechanism of Action and Pharmacology. The gut hormone GLP-2 is secreted by L cells of the ileum and colon and is the only intestinotrophic

TABLE 54–7 ■ ORAL ANTIBIOTIC TREATMENTS FOR SMALL INTESTINAL BACTERIAL OVERGROWTH

ANTIBIOTIC	SITE OF ACTION	ADULT DOSE	PEDIATRIC DOSE	NOTES
Amoxicillin-clavulanate	Systemic	875 mg twice daily	25–30 mg/kg (amoxicillin)/dose 2–3 times daily	10-day course
Ciprofloxacin	Systemic	500 mg twice daily		Not routinely recommended for children 10-day course
Doxycycline	Systemic	100 mg 1–2 times daily	≥8 years and >45 kg: adult dosing	Not routinely recommended for young children 10-day course
Metronidazole	Systemic	250 mg 3 times daily	10 mg/kg per dose twice daily	10-day course
Norfloxacin	Systemic	400 mg twice daily		Not recommended for children 10-day course
Rifaximin	Nonabsorbable	550 mg 3 times daily	Children and adolescents ≥12 years: adult dose Children 3–11 years: 200 mg 3 times daily	14-day course
Tetracycline	Systemic	250 mg 4 times daily	≥8 years and >45 kg: adult dose	Not routinely recommended for young children 10-day course
Trimethoprim-sulfamethoxazole	Systemic	160/800 mg twice daily	4–5 mg/kg of trimethoprim component per dose twice daily	10-day course

gut peptide. Among other actions, it enhances growth of the intestinal mucosa through the release of mediators, including insulin-like growth factor 1. *Teduglutide* is a 33–amino acid GLP-2 analogue recently approved for the treatment of short-bowel syndrome (Jeppesen, 2015).

ADME. The drug has a $t_{1/2}$ of 1 to 2 h and is excreted in the urine. It is catabolized by dipeptidyl peptidase 4 but more slowly than the native peptide because of the substituted amino acid structure.

Therapeutic Uses and Adverse Effects. *Teduglutide* is administered subcutaneously once daily (0.05 mg/kg) to help improve intestinal absorption of nutrients and thereby reduce the need for parenteral support. Common side effects include abdominal pain, nausea, headache, and flu-like symptoms. There is also the potential for *teduglutide* to cause cancer of the bowel; therefore, it is not recommended for patients with active malignancies.

Small Intestinal Bacterial Overgrowth

Small intestinal bacterial overgrowth (SIBO) is caused by excessive colonization of the small bowel with aerobic and anaerobic bacteria that are normally present in the colon. A patient generally presents with bloating, flatulence, abdominal discomfort, or diarrhea. The diagnosis may be made using a breath test or small-bowel aspirate, but frequently antibiotic therapy is typically begun on an empiric basis if the patient has a known underlying condition (e.g., scleroderma) and presents with typical symptoms. *Rifaximin* is well tolerated and has been demonstrated to be effective in the treatment of SIBO (Pimentel et al., 2020). In adults, it is given at a dose of 550 mg three times daily for 14 days. However, due to its costs, alternative antibiotics are also used (Table 54–7) (Pimentel et al., 2020).

Drug Facts for Your Personal Formulary: *Antisecretory Agents and Gastroprotectives*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Prokinetic Agents (agents acting through specific receptors to regulate GI motility)		
MOR antagonist Alvimopan	• Postoperative ileus	• Myocardial infarction • Hypokalemia • Dyspepsia
$5HT_4$ receptor agonists Cisapride Prucalopride Tegaserod	• Gastroesophageal reflux disease • Gastroparesis • Intestinal pseudoobstruction • Severe constipation • Neonatal feeding intolerance	• Serious cardiac risks • Risk of suicidal ideation • Headache • Diarrhea
D_2 receptor antagonist Domperidone Metoclopramide (also $5HT_3$ receptor antagonist, $5HT_4$ receptor agonist)	• Gastroparesis • Prevention of nausea and vomiting	• Serious cardiac risks, especially in older persons • Limited pediatric use • Tardive dyskinesia • Limited pediatric use • Short-term use only
Motilin receptors Erythromycin (stimulates motilin receptors on GI smooth muscle cells)	• Gastroparesis	• Short-term use only • Ototoxicity • Pseudomembranous colitis • Cardiac risks
CCK peptide analogue Sincalide (C-terminal octapeptide of CCK)	• Intravenous injection • Gallbladder contraction • Pancreatic secretion • Intestinal motility • Accelerates barium transit through small bowel for diagnostic testing	• Nausea, vomiting, diarrhea • Sweating • light-headedness • Headache • May cause serious allergic reactions
Laxatives		
Dietary Fiber		
Psyllium Methylcellulose	• Increase fecal bulk	• Bloating
Stool-Softening Agents		
Docusate	• Constipation	• Marginal efficacy
Mineral oil	• Constipation	• Side effects preclude regular use • Interferes with absorption of fat-soluble vitamins • Oil leakage
Osmotically Active Agents		
Polyethylene glycol–electrolyte solutions	• Colonic cleansing prior to examination • Constipation (powder form)	• Nausea • Cramping and bloating
Saline laxatives–Mg^{2+}	• Constipation	• Diarrhea • Renal insufficiency may predispose to Mg-accumulation

Drug Facts for Your Personal Formulary: *Antisecretory Agents and Gastroprotectives (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Nondigestible sugars and alcohols Lactulose Sorbitol	• Constipation caused by opioids • Idiopathic chronic constipation • Lactulose also used to treat hepatic encephalopathy	• Abdominal discomfort • Flatulence
Stimulant Laxatives		
Diphenylmethane derivatives Bisacodyl Sodium picosulfate	• Constipation • Bowel cleansing prior to colonoscopy, x-ray examination or surgery	• Diarrhea • Abdominal pain • Possible electrolyte imbalances and hypokalemia with prolonged use • Local irritation caused by suppositories
Anthraquinone laxatives Senna	• Constipation	• Plant derivatives • Melanosis coli • Nausea and vomiting • Cramping
Ricinoleic acid Castor oil	• Act on small intestine • Stimulate secretion • Increase intestinal transit	• Potential toxic effect from ricin • Not clinically recommended
Enemas and suppositories Glycerin	• Bowel distension • Glycerin for rectal use	• Discomfort
Prosecretory Agents		
Guanylate cyclase-C agonist Linaclotide Plecanatide	• Opioid-induced constipation	• Contraindicated in children up to 6 years • Diarrhea
Cl^- channel activator Lubiprostone	• Chronic idiopathic constipation • Opioid-induced constipation • IBS with constipation	• Nausea • Diarrhea
NHE3 inhibitor Tenapanor	• IBS with constipation	• Diarrhea • Abdominal distension • Flatulence
Drugs for Opioid-Induced Constipation		
MOR antagonists Methylnaltrexone Naloxegol Naldemedine	• Opioid-induced constipation	• Peripheral MOR antagonist • Diarrhea • Abdominal pain • Nausea and vomiting • Flatulence
Opioid receptor agonist/antagonist Oxycodone:naloxone (2:1 ratio)	• Opioid-induced constipation	• Respiratory depression • Addiction • Nausea and vomiting • Constipation • Diarrhea
Antidiarrheal Agents		
$5HT_3$ receptor antagonist Alosetron	• Diarrhea-predominant IBS in women	• Ischemic colitis • Constipation
Antibiotics—empiric therapy Fluoroquinolone Ciprofloxacin Levofloxacin Norfloxacin Ofloxacin *Alternative antibiotics* Azithromycin Rifaximin	• Acute diarrhea • Traveler's diarrhea • Azithromycin: preferred treatment for children with traveler's diarrhea • Rifaximin: preferred for diarrhea-predominant IBS	• Avoid if *Escherichia coli* suspected • Avoid if *Clostridium difficile* suspected • Controversial in children (azithromycin preferred) • Nausea • Peripheral edema • Dizziness
Bile acid sequestrants Cholestyramine Colesevelam Colestipol	• Bile salt–induced diarrhea	• Bloating • Flatulence • Abdominal discomfort • Constipation

Drug Facts for Your Personal Formulary: *Antisecretory Agents and Gastroprotectives (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Bismuth subsalicylate	• Acute diarrhea • Nausea and abdominal cramping	• Dark stools; darkened tongue
α_2 adrenergic receptor agonist Clonidine	• Diabetic diarrhea	• Hypotension, depression • Drowsiness, fatigue
Crofelemer (plant derived)	• HIV/AIDS diarrhea	• Infectious diarrhea must not be suspected • Inhibits CFTR and reduces Cl^- secretion
MOR/KOR agonist DOR antagonist Eluxadoline	• Diarrhea-predominant IBS	• Pancreatitis • Sphincter of Oddi spasm • Constipation
SST receptor agonist Octreotide	• Severe secretory diarrhea due to GI tumors • Postgastrectomy dumping syndrome	• Sinus bradycardia, chest pain • Headache, abdominal pain • Nausea, diarrhea
Enkephalinase inhibitor Racecadotril	• Acute diarrhea	• Proven safety in children
Tryptophan hydroxylase inhibitor Telotristat ethyl	• Severe diarrhea due to carcinoid tumors	• Adverse effects: constipation, nausea, headache, depression
Antispasmodic Agents (Anticholinergics)		
Dicyclomine Glycopyrrolate Hyoscyamine Methscopolamine	• Abdominal and urgency in IBS	• Contraindicated in colitis, reflux esophagitis, and bowel obstruction • Dizziness, dry mouth • Nausea, blurred vision
Antiemetic Agents		
Antihistamines Cyclizine Diphenhydramine Meclizine Promethazine	• Motion sickness • Nausea and vomiting	• Sedation • Dry mouth • Promethazine is contraindicated in children <2 years old
Doxylamine succinate and pyridoxine (H_1 receptor antagonist and vitamin B_6)	• Nausea and vomiting of pregnancy	• Drowsiness, light-headedness • Dry mouth • Constipation
NK_1 antagonists Aprepitant Fosaprepitant Rolapitant	• Chemotherapy-induced nausea and vomiting • Postoperative nausea and vomiting	• Given with dexamethasone and a $5HT_3$ antagonist • Contraindicated in patients on cisapride, pimozide, or thioridazine • Fatigue, constipation, hiccups
$5HT_3$ antagonists Dolasetron Granisetron Ondansetron Palonesetron	• Chemotherapy-induced nausea and vomiting • Radiation-induced nausea and vomiting • Postoperative nausea and vomiting	• ECG effects • Serotonin syndrome • Headache • Constipation • Fatigue, malaise
$NK_1/5HT_3$ antagonists Netupitant Palonesetron	• Chemotherapy-induced nausea and vomiting	• Serotonin syndrome • Headache • Constipation, fatigue
Cannabinoid receptor agonists Dronabinol Nabilone	• Chemotherapy-induced nausea and vomiting	• Psychoactive • Many CNS side effects
Dopamine receptor antagonists Olanzapine ($5HT_{2A}$ D_{1-4}, H_1, α_1 adrenergic, and M receptor antagonists) Phenothiazines (D_2, H_1, $5HT_{2A}$, M, and α_1 receptor antagonists) Chlorpromazine Prochlorperazine Benzamide Amisulpride Butyrophenone (D_{2-5}, $5HT_{2A}$, $5HT_{2B}$, $5HT_7$, H_1, α_{1A-1C} adrenergic antagonists) Droperidol Haloperidol	• Chemotherapy-induced nausea and vomiting • Refractory nausea and vomiting • Postoperative nausea and vomiting • Cannabinoid hyperemesis syndrome (haloperidol)	• Prolonged QT interval and torsades de pointes • Somnolence • Hypotension • Increased mortality in elderly patients with dementia-related psychosis • Extrapyramidal reactions

Drug Facts for Your Personal Formulary: *Antisecretory Agents and Gastroprotectives (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Muscarinic receptor antagonist Scopolamine	• Motion sickness • Nausea and vomiting	• Cardiovascular actions • Constipation, drowsiness, dry mouth, blurred vision; many other side effects (see Chapter 11)
Miscellaneous Agents		
Pancreatic enzymes	• Malabsorption (postpancreatectomy; cystic fibrosis) • Pancreatitis pain	• Headache • Abdominal pain
Simethicone	• Flatulence, bloating	
Teduglutide (GLP-2 receptor analogue)	• Short-bowel syndrome	• Colonic polyps/malignancy • Pancreatitis, abdominal pain and distention • Nausea, headache
Obeticholic acid (farnesoid X receptor agonist)	• Primary biliary cholangitis	• Can cause hepatic decompensation and liver failure • Pruritis
Antibiotics for the Treatment of Small Intestinal Bacterial Overgrowth		
Amoxicillin-clavulanate Ciprofloxacin Doxycycline Metronidazole Norfloxacin Rifaximin Tetracycline Trimethoprim-sulfamethoxazole	• Treatment of small intestinal bacterial overgrowth	• Nausea, vomiting, headache • GI disturbances • Abdominal pain

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Chapter 55

Pharmacotherapy of Inflammatory Bowel Disease

Wallace K. MacNaughton and Keith A. Sharkey

INFLAMMATORY BOWEL DISEASE
- Pathogenesis of IBD

CLASSIFICATIONS OF DRUGS TO TREAT IBD
- Mesalamine-Based Therapy
- Glucocorticoids
- Immunomodulatory Agents
- Biological Therapies for IBD
- Other Small-Molecule Drugs to Treat IBD

MANIPULATING THE INTESTINAL MICROBIOME TO TREAT IBD
- Antibiotics and Probiotics
- Fecal Transplant as Therapy in IBD

SUPPORTIVE THERAPY IN IBD

PEDIATRIC IBD

THERAPY OF IBD DURING PREGNANCY

Inflammatory Bowel Disease

Inflammatory bowel disease is a spectrum of remitting and relapsing, chronic, inflammatory intestinal conditions. IBD causes significant GI symptoms that include diarrhea, abdominal pain, bleeding, anemia, and weight loss. IBD conventionally is divided into two major subtypes: ulcerative colitis *and* Crohn's disease.

Ulcerative colitis is characterized by confluent mucosal inflammation of the colon starting at the anal verge and extending proximally for a variable extent (e.g., proctitis, left-sided colitis, or pancolitis). *Crohn's disease*, by contrast, is characterized by transmural inflammation of any part of the GI tract but most commonly the area adjacent to the ileocecal valve. The inflammation in Crohn's disease is not necessarily confluent, frequently leaving "skip areas" of relatively normal mucosa. The transmural nature of the inflammation may lead to fibrosis and strictures or fistula formation. IBD is often associated with extraintestinal manifestations involving the joints, skin, or eyes (Ott and Scholmerich, 2013). IBD is also increasingly recognized to have comorbid psychological manifestations, notably anxiety and depression (Graff et al., 2009; Taft et al., 2017). Primary sclerosing cholangitis is a serious but infrequent extraintestinal manifestation of IBD, usually ulcerative colitis, in which inflammation and fibrostenosis occur in the intra- and extrahepatic biliary tree (Williamson and Chapman, 2014). Chronic, severe IBD is associated with an increased risk for the development of colorectal cancer (Beaugerie and Itzkowitz, 2015).

Pathogenesis of IBD

A summary of proposed pathogenic events and potential sites of therapeutic intervention is shown in Figure 55–1. Both diseases are associated with an aberrant immune response to the commensal microbiota of the gut in genetically susceptible individuals. Evidence of dysbiosis of the microbiome in IBD supports this theory (Lee and Chang, 2020). Nevertheless, Crohn's disease and ulcerative colitis result from distinct pathogenic mechanisms at the level of mucosal immune activation (Xavier and Podolsky, 2007). Histologically, the transmural lesions in *Crohn's disease* exhibit marked infiltration of lymphocytes and macrophages, granuloma formation, and submucosal fibrosis, whereas the superficial lesions in *ulcerative colitis* have lymphocytic and neutrophilic infiltrates.

Our understanding of the pathogenesis of both Crohn's disease and ulcerative colitis has increased dramatically in over the past two decades. The identification of disease susceptibility genes (McGovern et al., 2015) and the recognition of the role of the microbiome have led to an emerging area of therapeutic development that may yield new therapies (Cohen et al., 2019). However, current treatments focus on immune-based therapies because of the important role of cytokines in disease pathogenesis. Within the diseased bowel in *Crohn's disease*, the cytokine profile includes increased levels of IL-12, IL-23, IFN-γ, and TNF-α, findings characteristic of T_H1-mediated inflammatory processes. In contrast, the inflammatory response in *ulcerative colitis* resembles aspects of that mediated by the T_H2 pathway, including the involvement of IL-4 and IL-13. Understanding of the inflammatory processes has evolved with the description of regulatory T cells and proinflammatory T_H17 cells, a novel T-cell population that expresses IL-23 receptor as a surface marker and produces, among others, the proinflammatory cytokines IL-17, IL-21, IL-22, and IL-26. T_H17 cells seem to play a prominent role in intestinal inflammation, particularly in *Crohn's disease*.

Medical therapy for IBD is problematic. Because of the multifactorial nature of disease etiology, current therapy for IBD seeks to dampen the generalized inflammatory response. Regrettably, no agent can reliably accomplish this, and the response of an individual patient to a given drug may be limited and unpredictable. Recently, mucosal healing has become an important therapeutic aim, as opposed to simply the relief of symptoms. Specific goals of pharmacotherapy in IBD include controlling acute exacerbations of the disease, maintaining remission, and treating the disease based on the presence and nature of extraintestinal manifestations. The major therapeutic options are considered in the following material.

Classifications of Drugs to Treat IBD

Mesalamine-Based Therapy

First-line therapy for mild-to-moderate ulcerative colitis generally involves 5-ASA (Bressler et al., 2015). 5-ASA–based treatments have largely been abandoned for the maintenance of remission in Crohn's disease (Sandborn et al., 2007) due to the fact that their anti-inflammatory effects are targeted topically to the mucosa, with limited effects on deeper inflammation, which has implications for long-term outcomes. The archetype for this class of medications is *sulfasalazine*, which consists of *5-ASA* linked to *sulfapyridine* by an *azo* bond (Figure 55–2).

Mechanism of Action, Pharmacological Properties, and Therapeutic Uses

Sulfasalazine is an oral prodrug that effectively delivers 5-ASA to the distal GI tract (Figure 55–3). The *azo* linkage in *sulfasalazine* prevents

Abbreviations

APC: antigen-presenting cell
5-ASA: 5-aminosalicylic acid, mesalamine
CNS: central nervous system
COX: cyclooxygenase
FDA: Food and Drug Administration
GI: gastrointestinal
GPCR: G protein-coupled receptor
HGPRT: hypoxanthine-guanine phosphoribosyl transferase
HPA: hypothalamic-pituitary-adrenal (axis)
5HT: 5-hydroxytryptamine (serotonin)
IBD: inflammatory bowel disease
IFN: interferon
IL: interleukin
JAK: Janus kinase
MAO: monoamine oxidase
6-MMP: 6-methyl-mercaptopurine
NE: norepinephrine
NF-κB: nuclear factor-κB
NSAID: nonsteroidal anti-inflammatory drug
PPAR-γ: peroxisome proliferator-activated receptor gamma
S1P: sphingosine-1-phosphate
STAT: signal transducer and activator of transcription
TB: tuberculosis
TGF: transforming growth factor
T_H: T helper (lymphocyte)
TNF: tumor necrosis factor
TPMT: thiopurine methyltransferase
TYK2: tyrosine kinase 2
XO: xanthine oxidase

absorption in the stomach and small intestine, and the individual components are not liberated until colonic bacterial *azoreductases* cleave the bond for local effect (Peppercorn and Goldman, 1972). 5-ASA is the therapeutic moiety, with little, if any, contribution by *sulfapyridine,* a *sulfonamide antibiotic*. Although 5-ASA is a salicylate and can inhibit cyclooxygenase (COX), its mode of action does not appear to involve this activity; indeed, traditional NSAIDs may exacerbate IBD and are strongly contraindicated. Many potential sites of action (effects on immune function and inflammation) have been demonstrated *in vitro* for *sulfasalazine* and *mesalamine* (Perrotta et al., 2015), including activation of the production of IL-1 and TNF-α, inhibition of the lipoxygenase pathway, scavenging of free radicals and oxidants, activation of PPAR-γ, and inhibition of NF-κB, a transcription factor pivotal to production of inflammatory mediators. However, specific mechanisms of action underlying the efficacy of *sulfasalazine*/5-ASA in IBD have not been identified.

To preserve the therapeutic effect of 5-ASA without the adverse effects of *sulfapyridine*, several second-generation 5-ASA compounds have been developed. They are divided into two groups: *prodrugs* and *coated drugs*. Prodrugs contain the same azo bond as *sulfasalazine* but replace the linked *sulfapyridine* with either another 5-ASA (*olsalazine*) or an inert compound (*balsalazide*) (Jain et al., 2006). As is the case for *sulfasalazine*, colonic bacteria cleave *olsalazine* and *balsalazide* by azoreduction, producing two molecules of 5-ASA per molecule of *olsalazine* and equimolar quantities of 5-ASA (plus the carrier portion, 4-aminobenzoyl-b-alanine) from *balsalazide*. The alternative approaches employ *mesalamine* directly, using either a delayed-release formulation or a pH-sensitive coating. *Delayed-release mesalamine* releases drug throughout the GI tract, whereas *pH-sensitive mesalamine* is released in the small intestine and colon. These different distributions of drug delivery have potential therapeutic implications (Figure 55–4).

Oral *sulfasalazine* is effective in patients with mild or moderately active ulcerative colitis, with response rates of 60% to 80%. The usual initial dose is 500 to 1000 mg every 6 to 8 h and not more than a total of 4 g/day. The maintenance dose is 2000 mg/day. Doses as high as 6 g/day can be used but cause an increased incidence of side effects. For patients with severe colitis, *sulfasalazine* is of less-certain value, even though it is often added as an adjunct to systemic glucocorticoids. The drug plays a useful role in preventing relapses once remission has been achieved. Because they lack the dose-related side effects of *sulfapyridine*, the delayed and pH-dependent formulations can be used to deliver *mesalamine* with improved safety and tolerability. The doses of *mesalamine* used to treat active disease are 2.4 to 4.8 g/day for up to 8 weeks for induction, and current practice is to administer 5-ASA as a once-daily dose, which is as effective as a multiple daily dosing regimen (Feagan and Macdonald, 2012).

Topical preparations of *mesalamine* suspended in a wax matrix suppository or in a suspension enema are effective in active proctitis and distal ulcerative colitis, respectively. They appear to be superior to topical *hydrocortisone* in this setting, with response rates of 75% to 90%. *Mesalamine* enemas (4 g/60 mL) should be used at bedtime and retained for at least 8 h; the suppository (500 and 1000 mg) should be used two to three times a day with the objective of retaining it for at least 3 h. Response to local therapy with *mesalamine* may occur within 7 to 14 days; however, the usual course of therapy is from 8 to 16 weeks for induction of remission. Once remission has occurred, lower doses can be considered for maintenance, although increasingly, the dose used for induction is continued for maintenance.

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The pharmacokinetics of 5-ASA–based drugs are well described (Sandborn and Hanauer, 2003). About 20% to 30% of orally administered *sulfasalazine* (*mesalamine* prodrug) is absorbed in the small intestine. Much of this is taken up by the liver and excreted unmetabolized in the bile; the rest (~10%) is excreted unchanged in the urine. The remaining 70% reaches the colon, where, if cleaved completely by bacterial enzymes, it generates 400 mg *mesalamine* for every gram of the parent compound. Thereafter, the individual components of *sulfasalazine* follow different metabolic pathways. *Sulfapyridine* is absorbed rapidly from the colon and undergoes extensive hepatic metabolism, including acetylation and hydroxylation, and conjugation with glucuronic acid, prior to excretion in the urine. The acetylation phenotype of the patient determines plasma levels of *sulfapyridine* and the probability of side effects; rapid acetylators have lower systemic levels of the drug and fewer adverse effects. Only 25% of *mesalamine* is absorbed from the colon, and most of the drug is excreted in the stool. The small amount that is absorbed is acetylated in the intestinal mucosal wall and liver and then excreted in the urine. Intraluminal concentrations of *mesalamine* therefore are very high (~1500 μg/mL).

The pH-sensitive coatings limit gastric and small intestinal absorption of *mesalamine*. The pharmacokinetics of delayed-release formulations differ somewhat. The ethylcellulose-coated microgranules are released in the upper GI tract as discrete prolonged-release units of *mesalamine*. Acetylated *mesalamine* can be detected in the circulation within an hour after ingestion, indicating some rapid absorption, although some intact microgranules can later be detected in the colon. Because the delayed-release drug is released in the small bowel, a greater fraction of the 5-ASA in the delayed-release formulations is absorbed systemically compared with the other 5-ASA preparations.

Adverse Effects

Side effects of *sulfasalazine* occur in 10% to 45% of patients with ulcerative colitis and are related primarily to the sulfa moiety. Some are dose related, including headache, nausea, and fatigue; these can be minimized by giving the medication with meals or by decreasing the dose. Allergic reactions include rash, fever, Stevens-Johnson syndrome, hepatitis, pneumonitis, hemolytic anemia, and bone marrow suppression. *Sulfasalazine* reversibly decreases the number and motility of sperm but does not impair female fertility. *Sulfasalazine* inhibits intestinal folate absorption and therefore is usually administered with folate.

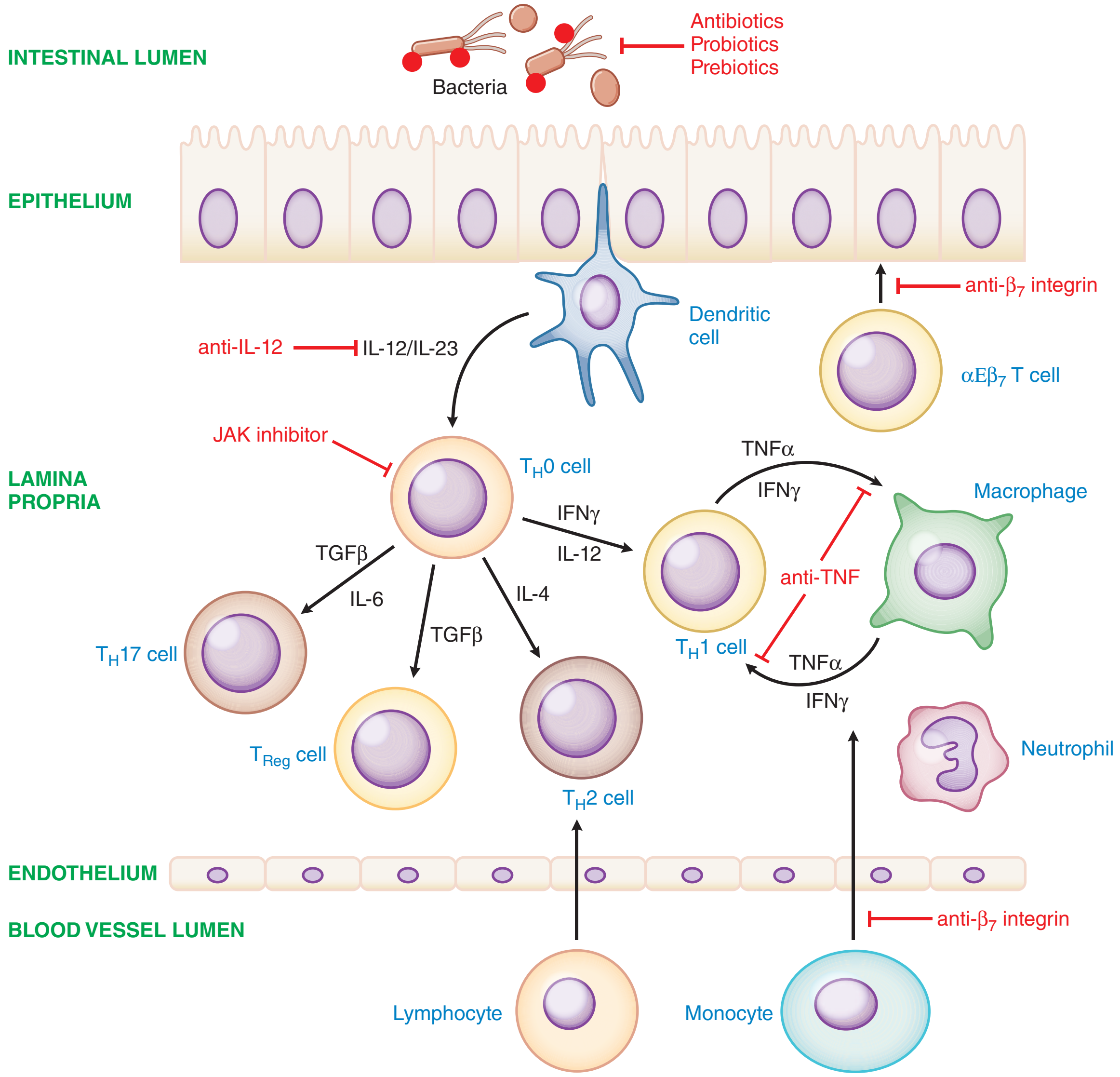
INTESTINAL LUMEN
Bacteria
Antibiotics
Probiotics
Prebiotics
EPITHELIUM
anti-β_7 integrin
Dendritic cell
anti-IL-12
IL-12/IL-23
$\alpha E \beta_7$ T cell
LAMINA PROPRIA
JAK inhibitor
T$_H$0 cell
TNFα
IFNγ
Macrophage
IFNγ
IL-12
TGFβ
IL-6
IL-4
TGFβ
anti-TNF
T$_H$17 cell
T$_H$1 cell
TNFα
IFNγ
T$_{Reg}$ cell
T$_H$2 cell
Neutrophil
ENDOTHELIUM
BLOOD VESSEL LUMEN
anti-β_7 integrin
Lymphocyte
Monocyte

Figure 55–1 *Proposed pathogenesis of IBD and target sites for pharmacological intervention.* Shown are the interactions among bacterial antigens in the intestinal lumen and immune cells in the intestinal wall. If the epithelial barrier is impaired, bacterial antigens can gain access to APCs such as dendritic cells in the lamina propria. These cells then present the antigen(s) to CD4$^+$ lymphocytes and also secrete cytokines such as IL-12 and IL-23, thereby inducing the differentiation of T$_H$1 cells in Crohn's disease (or, under the control of IL-4, T$_H$2 cells in ulcerative colitis). The balance of proinflammatory and anti-inflammatory events is also governed by regulatory T$_H$17 and T$_{Reg}$ cells, both of which serve to limit immune and inflammatory responses in the GI tract. T cells expressing αEβ7 integrin may interact with epithelial E-cadherin to alter epithelial barrier function. TGF-β and IL-6 are important cytokines that drive the expansion of the regulatory T-cell subsets. The T$_H$1 cells produce a characteristic array of cytokines, including IFN-γ and TNF-α, which in turn activate macrophages. Macrophages positively regulate T$_H$1 cells by secreting additional cytokines, including IFN-γ and TNF-α. Recruitment of a variety of leukocytes is mediated by activation of resident immune cells, including neutrophils. Cell adhesion molecules such as integrins are important in the infiltration of leukocytes, and novel biological therapeutic strategies aimed at blocking leukocyte recruitment are effective at reducing inflammation. General immunosuppressants (e.g., glucocorticoids, thioguanine derivatives, *methotrexate*, and *cyclosporine*) affect multiple sites of inflammation. More site-specific interventions involve intestinal bacteria (antibiotics, prebiotics, and probiotics) and therapy directed at TNF-α, IL-12/23, integrins, or members of the JAK signaling pathway.

Mesalamine formulations generally are well tolerated. Headache, dyspepsia, and skin rash are the most common side effects. Diarrhea appears to be particularly common with *olsalazine* (occurring in 10%–20% of patients). Nephrotoxicity, although rare, is a more serious concern. *Mesalamine* has been associated with interstitial nephritis; renal function should be monitored in all patients receiving these drugs. Pancreatitis, pericarditis, and pleuritis are less common adverse events that should be considered.

Both *sulfasalazine* and its metabolites cross the placenta but have not been shown to harm the fetus. The newer formulations also appear to be safe in pregnancy, but there have been some safety concerns about dibutyl phthalate, an inactive ingredient in the coating of some formulations, in the context of pregnancy.

Glucocorticoids

The glucocorticoids *prednisone, prednisolone, methylprednisolone, budesonide*, and *triamcinolone* are FDA-approved for the treatment of IBD. Numerous other corticosteroids are also used as short-term therapy in critical periods of ulcerative colitis and regional enteritis.

Mechanism of Action, Pharmacological Properties, and Therapeutic Uses

The effects of glucocorticoids on the inflammatory response are numerous, including downregulating expression of several cell adhesion molecules involved in inflammatory responses, reducing production of proinflammatory cytokines (e.g., TNF, IL-1, IL-8), and inhibiting transcription of genes for PLA2 and COX-2 (see Chapters 39, 50, 74, and 75).

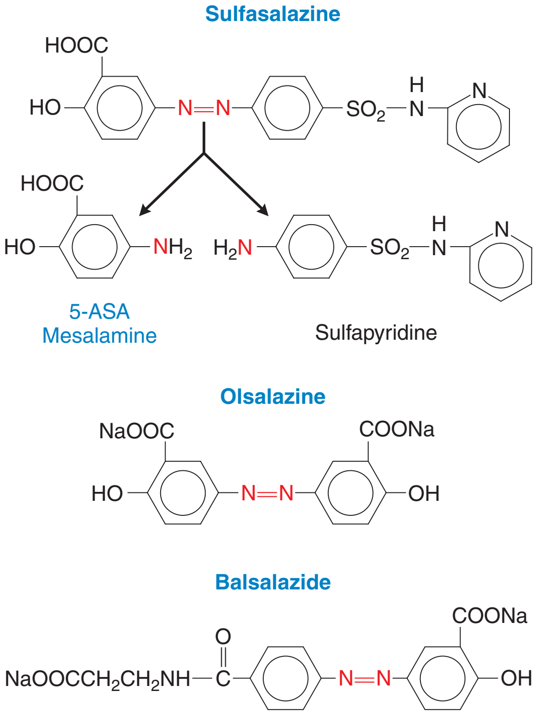

Figure 55–2 *Sulfasalazine and related agents.* The red N atoms indicate the diazo linkage that is cleaved to generate the active moiety, 5-ASA.

Glucocorticoids are indicated for moderate-to-severe IBD. Patients with IBD segregate into three general groups with respect to their response to glucocorticoids:

- *Glucocorticoid-responsive patients* improve clinically within 1 to 2 weeks and remain in remission as the steroids are tapered and then discontinued.
- *Glucocorticoid-dependent patients* respond to glucocorticoids but then experience a relapse of symptoms as the steroid dose is tapered or discontinued.
- *Glucocorticoid-unresponsive* or *"steroid-resistant" patients* do not improve, despite prolonged high-dose steroids.

Glucocorticoids induce a reduction in the inflammatory response and symptomatic remission in most patients with Crohn's disease, with improvement generally occurring within 5 days of initiating treatment; however, some patients require treatment for several weeks before remission occurs. Glucocorticoids are sometimes used for prolonged periods to control symptoms in corticosteroid-dependent patients, as these patients will often experience a recurrence of their disease when the glucocorticoid is withdrawn. A proportion of patients with IBD are steroid resistant, and the failure to respond to steroids with sustained remission (i.e., a disease relapse) should prompt consideration of adjunctive and alternative therapies, including immunosuppressive agents and biological therapies (Manz et al., 2012). Glucocorticoids are not a safe or practical means to maintain remission in either ulcerative colitis or Crohn's disease due to the high rate of adverse events associated with their prolonged use. The most commonly used glucocorticoid in Crohn's disease is *prednisone,* given orally. For more severe cases, glucocorticoids such as *methylprednisolone* or *hydrocortisone* are given intravenously. Rectal preparations can be used in treating IBD in the lower colon and rectum.

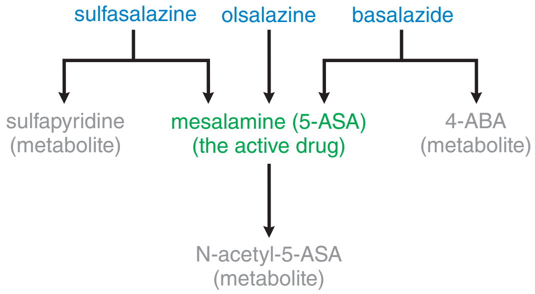

Figure 55–3 *Metabolic fates of the different oral formulations of 5-ASA.* Chemical structures are in Figure 55–2. 4-ABA, 4-aminobenzoyl-β-alanine.

Budesonide is a synthetic glucocorticoid. Compared to *prednisolone*, *budesonide* exhibits greater affinity (~15 times) for the glucocorticoid receptor. The agent is available in several delayed-release or enteric release formulations that are used for mild-to-moderate colonic or terminal ileal Crohn's disease or mild-to-moderate ulcerative colitis. *Budesonide*'s putative action is the delivery of therapeutic quantities of steroid to a specific portion of inflamed gut while minimizing systemic side effects owing to its local release and extensive first-pass hepatic metabolism to inactive derivatives such that systemic levels remain low. *Budesonide* is FDA approved for use in the short-term maintenance of remission (up to 3 months). Some older studies questioned the effectiveness of *budesonide* for this indication (Kuenzig et al., 2014). A multimatrix formulation of *budesonide* delays release until the local pH is greater than 7 (pH of distal ileum), thereby delivering the drug predominantly to the colon (Hoy, 2015; Maconi et al., 2020).

Glucocorticoid enemas are useful mainly in patients whose disease is limited to the rectum and left colon. *Betamethasone* and *budesonide* are available as retention enemas. Patients with distal disease usually respond within 3 to 7 days. Absorption, although less than with oral preparations, is still substantial (up to 50%–75%). *Hydrocortisone* also can be given once or twice daily as a 10% foam suspension that delivers 80 mg *hydrocortisone* per application; this formulation can be useful in patients with very short areas of distal proctitis and difficulty retaining enemas.

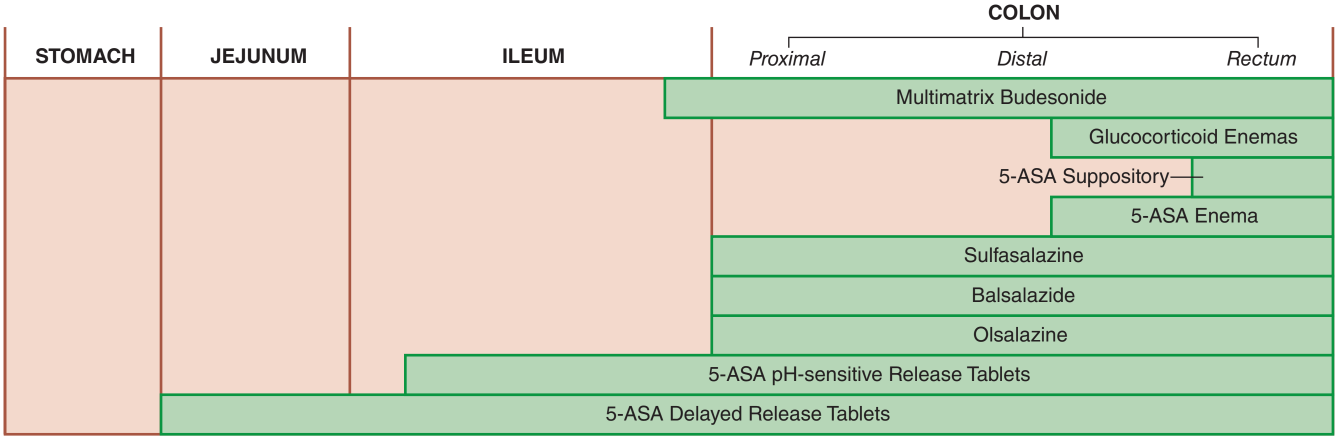

Figure 55–4 *Comparison of sites of release of multimatrix budesonide and various formulations of 5-ASA in the GI tract.*

ADME

Prednisone is the most commonly administered glucocorticoid and is used for the induction of remission of moderate-to-severe Crohn's disease (Benchimol et al., 2008). It is sometimes given as a first-line therapeutic to induce remission. *Prednisone* is most often administered orally but can be administered intravenously when patients present with severe, acute flares of disease. Initial doses in IBD are 40 to 60 mg of *prednisone* or equivalent per day; higher doses are generally no more effective. Most patients respond within 10 to 14 days, at which point the dose is reduced by 5 mg per week (tapered) over several weeks to months. *Prednisone* is absorbed at a rate of 50% to 90%; 65% to 90% of the absorbed drug is protein bound in the serum. It is metabolized to the active compound, *prednisolone*, in the liver. The $t_{1/2}$ for *prednisone* about 3.5 h, with metabolized drug excreted in the urine. Because of the complex nature of the mechanism of action of glucocorticoids, numerous drug interactions have been reported.

Budesonide for the treatment of IBD is administered orally at a dose of 9 mg/day for up to 8 weeks (Kane et al., 2002) followed by a 3-mg reduction every 4 to 6 weeks. There is usually no therapeutic benefit of continuing treatment beyond 3 months. The bioavailability of orally administered *budesonide* is limited (9%–21%) by its high first-pass metabolism by hepatic CYP3A4. The time to peak serum concentration is 7 to 19 h when given in capsule form. The $t_{1/2}$ is about 2 to 3.6 h but can be prolonged by agents that inhibit the activity of CYP3A4, such as the antifungal agent *ketoconazole* and the furanocoumarins in grapefruit juice. Excretion of metabolites is renal (60%) and fecal.

Triamcinolone, a widely used synthetic corticosteroid without significant mineralocorticoid activity, can provide temporary therapy for acute flare-ups of Crohn's disease and ulcerative colitis. *Triamcinolone* has high bioavailability (90%) when taken orally. The plasma $t_{1/2}$ is 3 to 5 h, but the receptor-bound drug within responsive cells prolongs the half-life of effectiveness to ~36 h. Chapters 74 and 75 cover the ophthalmic and dermatologic uses of *triamcinolone*.

Adverse Effects

The significant adverse events associated with conventional glucocorticoids such as *prednisone* limit their long-term use. These are numerous, but among the more common are skin and soft-tissue manifestations, including skin thinning and the development of Cushingoid features (weight redistribution and weight gain). Other side effects include cardiovascular events and psychiatric and cognitive effects. Conventional glucocorticoids can suppress the HPA axis, which can result in exogenous adrenal insufficiency when the drug is withdrawn abruptly. This effect necessitates the tapering of dose rather than quick withdrawal of the drug. The mechanisms underlying these and other adverse effects of conventional glucocorticoids are detailed in Chapter 46. *Budesonide* has a similar profile of adverse events, but with lower incidence due to its extensive first-pass hepatic metabolism.

Immunomodulatory Agents

Several drugs developed for cancer chemotherapy or as immunosuppressive agents in organ transplants are also used for treatment of IBD. Clinical experience has defined specific roles for each of these agents as mainstays in the pharmacotherapy of IBD. However, their potential for serious adverse effects mandates a careful assessment of risks and benefits in each patient.

Thiopurine Derivatives

The cytotoxic thiopurine derivatives *mercaptopurine* and *azathioprine* (see Chapters 39 and 70) are used off label to treat patients with severe IBD or those who are steroid resistant or steroid dependent (Coskun et al., 2016). Thiopurines are not used to treat mild-to-moderate disease (Solitano et al., 2020). These thiopurines impair purine biosynthesis and inhibit cell proliferation. Both are prodrugs: *Azathioprine* is converted to 6-mercaptopurine, which is subsequently metabolized to 6-thioguanine nucleotides, the putative active moieties (Figure 55–5).

Therapeutic Uses. These drugs are generally used interchangeably with appropriate dose adjustments, typically *azathioprine* (1.5–2.5 mg/kg per day) or *mercaptopurine* (1.5–2.0 mg/kg per day) in both Crohn's disease and ulcerative colitis as an adjunct to glucocorticoids and biologics (Nielsen et al., 2001). They help maintain remission in both diseases; they also may prevent or delay recurrence of Crohn's disease after surgical resection. Finally, they are used to treat fistulas in Crohn's disease. The clinical response to *azathioprine* or *mercaptopurine* may take weeks to months, such that other drugs with a more rapid onset of action (e.g., *mesalamine*, glucocorticoids, or biologics) are preferred in the acute setting.

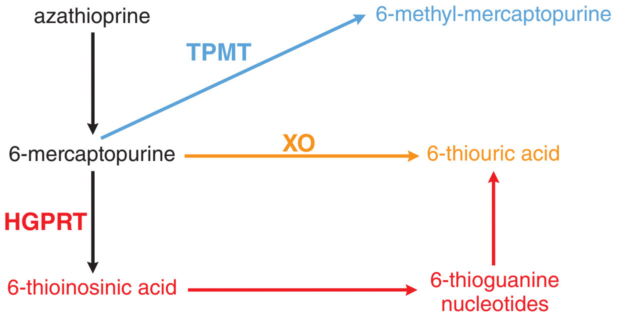

Figure 55–5 *Metabolism of azathioprine and 6-mercaptopurine.* The activities of these enzymes vary among humans because of genetic polymorphisms, explaining responses and side effects when *azathioprine* or *mercaptopurine* therapy is employed.

In general, physicians who treat IBD believe that the long-term risks of *azathioprine-mercaptopurine* are lower than those of steroids. Thus, these purines are used in glucocorticoid-unresponsive or glucocorticoid-dependent disease and in patients who have had recurrent flares of disease requiring repeated courses of steroids. In addition, patients who have not responded adequately to *mesalamine* but are not acutely ill may benefit by conversion from glucocorticoids to immunomodulatory drugs. Immunomodulators therefore may be viewed as steroid-sparing agents.

ADME. Favorable responses to *azathioprine-mercaptopurine* are seen in up to two-thirds of patients. *Mercaptopurine* has three metabolic fates (Figure 55–5):

- Conversion by XO to 6-thiouric acid
- Metabolism by TPMT to 6-MMP
- Conversion by HGPRT to 6-thioguanine nucleotides and other metabolites

The relative activities of these different pathways may explain, in part, individual variations in efficacy and adverse effects.

The plasma $t_{1/2}$ of *mercaptopurine* is limited by its relatively rapid (i.e., within 1–2 h) uptake into erythrocytes and other tissues. Following this uptake, differences in TPMT activity determine the drug's fate. Approximately 80% of the U.S. population has what is considered "normal" metabolism, whereas 1 in 300 individuals have minimal TPMT activity. In the latter setting, *mercaptopurine* metabolism is shifted away from 6-MMP and driven toward 6-thioguanine nucleotides, which can severely suppress the bone marrow. About 10% of people have intermediate TPMT activity; given a similar dose, these individuals will tend to have higher 6-thioguanine levels than the normal metabolizers. Finally, about 10% of the population are rapid metabolizers. In these individuals, *mercaptopurine* is shunted away from 6-thioguanine nucleotides toward 6-MMP, which has been associated with abnormal liver function tests. In addition, relative to normal metabolizers, the 6-thioguanine levels of these rapid metabolizers are lower for an equivalent oral dose, possibly reducing therapeutic response. Pharmacogenetic typing can guide therapy (see Chapter 7).

Xanthine oxidase in the small intestine and liver converts *mercaptopurine* to thiouric acid, which has no therapeutic activity. Inhibition of XO by *allopurinol* diverts *mercaptopurine* to more active metabolites, such as 6-thioguanine, increasing both immunomodulatory and potential toxic effects. Thus, patients on *mercaptopurine* should be warned about potentially serious interactions with medications used to treat gout or hyperuricemia, and the dose should be decreased to 25% of the standard dose in subjects who are already taking *allopurinol*.

Adverse Effects. Adverse effects of *azathioprine-mercaptopurine* can be either *idiosyncratic* or *dose related.* Adverse effects occur at any time after initiation of treatment and can affect up to 10% of patients. One of the most serious idiosyncratic reactions is pancreatitis, which affects about 5% of patients treated with these drugs. Fever, rash, and arthralgias are seen occasionally; nausea and vomiting are somewhat more frequent. The major dose-related adverse effect is bone marrow suppression, and blood counts should be monitored closely when therapy is initiated and at less-frequent intervals during maintenance therapy. Elevations in liver function tests also may be dose related. The serious adverse effect of cholestatic hepatitis is relatively rare. Thiopurines given in the setting of cancer chemotherapy or organ transplants have been associated with an increased incidence of malignancy, particularly non-Hodgkin lymphoma (4-fold increase).

Methotrexate

Methotrexate is a folic acid analogue that inhibits *dihydrofolate reductase*, thereby blocking DNA synthesis and causing cell death (Coskun et al., 2016). The anti-inflammatory effects of *methotrexate* may involve mechanisms in addition to inhibition of dihydrofolate reductase. These include inhibition of purine metabolism, inhibition of T-cell activation and production of cytokines and intercellular adhesion molecules, and inhibition of IL-1β receptor binding. For details of folate metabolism and the actions of *methotrexate*, see Chapters 45 and 70.

Therapeutic Uses. *Methotrexate* is reserved for patients whose IBD is either steroid resistant or steroid dependent. In Crohn's disease, it is used for maintenance of remission and as an adjunct to biologics to boost efficacy and reduce formation of antidrug antibodies (Patel et al., 2014). *Methotrexate* (15–25 mg/week) is generally administered subcutaneously. This choice of administration reflects the unpredictable intestinal absorption at higher doses of *methotrexate* and in the presence of intestinal disease. A dose of 12.5 to 25 mg weekly is often used for induction of the remission of the inflammatory response, with 15 to 25 mg given once weekly for maintenance of remission. *Methotrexate* is sometimes used in combination with anti–TNF-α antibody therapy (see discussion that follows).

ADME. *Methotrexate* is usually administered subcutaneously for induction and maintenance of remission of Crohn's disease. It can cause folate deficiency, so oral folic acid supplementation (1 mg/day) is often recommended. After administration, approximately 50% is bound to serum proteins. *Methotrexate* can cross the blood-brain barrier but is measured at much lower levels in cerebrospinal fluid than in serum. It has a $t_{1/2}$ of about 3 to 10 h at doses used for the treatment of Crohn's disease (see Appendix I). Approximately 90% of administered *methotrexate* is excreted unaltered in the urine, likely as a result of active tubular secretion.

Adverse Effects. Drugs that inhibit renal excretion of MTX may increase treatment-related toxicity. These include NSAIDs, *phenytoin*, *ciprofloxacin*, *penicillin*-type drugs, *probenecid*, *amiodarone*, and proton pump inhibitors. *Methotrexate*, at the doses used for the treatment of Crohn's disease, is generally well tolerated. When toxicity occurs, it manifests as nausea, loose stool, stomatitis, punctate cutaneous eruption, CNS symptoms (including headache, fatigue, and impaired ability to concentrate), alopecia, fever (drug related or due to infection), and hematologic abnormalities, particularly macrocytosis.

Cyclosporine

Cyclosporine is an inhibitor of calcineurin and a potent immunomodulator used most frequently after organ transplantation (see Chapter 39). It is effective in specific clinical settings in IBD, but the high frequency of significant adverse effects limits its use as a first-line medication.

Therapeutic Uses. Between 50% and 80% of severely ill patients with IBD improve significantly (generally within 7 days) in response to intravenous *cyclosporine* (2–4 mg/kg per day), sometimes avoiding emergent colectomy. Careful monitoring of *cyclosporine* levels is necessary to maintain a therapeutic level between 300 and 400 ng/mL of whole blood. Oral *cyclosporine* is less effective as maintenance therapy in Crohn's disease, perhaps because of its limited intestinal absorption. In this setting, long-term therapy with formulations of *cyclosporine* that have increased oral bioavailability may be more effective. *Cyclosporine* can be used to treat fistulous complications of Crohn's disease. A significant rapid response to intravenous *cyclosporine* has been observed; however, frequent relapses accompany oral *cyclosporine* therapy, and other medical strategies are required to maintain fistula closure. Thus, calcineurin inhibitors generally are used to treat specific problems over the short term while providing a bridge to longer-term therapy.

ADME. *Cyclosporine* given orally is erratically and incompletely absorbed by the intestine. Following absorption, 90% to 98% is bound to serum lipoproteins. Depending on the formulation, the $t_{1/2}$ is biphasic, with the terminal phase being about 9 to 18 h.

Adverse Effects. The significant adverse effects associated with the use of *cyclosporine* limit its use to specific types of severe IBD. These side effects most often include increased susceptibility to infections, renal insufficiency, hypertension, seizures, and peripheral neuropathy. Significant drug interactions have been reported.

Other immunosuppressants that are being evaluated in IBD include the calcineurin inhibitor *tacrolimus* (FK506) (Rodríguez-Lago et al., 2020) and the inhibitors of inosine monophosphate dehydrogenase, *mycophenolate mofetil* and *mycophenolate* (Macaluso et al., 2017), to which lymphocytes are especially susceptible (see Chapter 39). *Tacrolimus* has been used for severe, acute, steroid-resistant IBD with good efficacy and safety profile (Hoffmann et al., 2019). Similarly, *mycophenolate mofetil* has been used for the induction and maintenance of remission in difficult-to-treat IBD or patients who are intolerant of thiopurines or who are steroid dependent (Smith and Cooper, 2014). Paradoxically, *mycophenolate mofetil* has been reported to cause colitis in patients who are taking the drug as a treatment to prevent transplant rejection (Calmet et al., 2015).

Biological Therapies for IBD

Anti–TNF-α Monoclonal Antibodies

The biological activity of tumor necrosis factor (TNF), a proinflammatory cytokine, comprises a host of issues in IBD, including release of additional proinflammatory cytokines from macrophages, upregulation of collagen production, and enhanced expression of adhesion molecules that localize leukocytes. Antibodies to TNF can reduce the proinflammatory effects of TNF. *Infliximab, adalimumab, certolizumab pegol*, and *golimumab* are monoclonal immunoglobulins that have been developed for the treatment of chronic inflammatory diseases. *Infliximab* is a chimeric antibody (25% mouse, 75% human), whereas *adalimumab* and *golimumab* are fully humanized antibodies. *Certolizumab pegol* is a humanized fragment antigen binding that is "pegylated" (i.e., bound to a polyethylene glycol polymer to increase serum half-life of the parent compound). These drugs bind to and neutralize both soluble and membrane-bound TNF-α, one of the principal cytokines mediating the T_H1 immune response characteristic of Crohn's disease (see Figure 55–1), thereby preventing its binding to p55 and p75 receptors. Chapters 38 and 39 present a more detailed and mechanistic view of antibody production and the use of antibodies to regulate immune function. *Infliximab, adalimumab,* and *golimumab* are approved for acute and chronic treatment of moderate-to-severe ulcerative colitis. *Infliximab, adalimumab*, and *certolizumab* are for treatment of moderate-to-severe Crohn's disease in patients who have responded poorly to conventional therapies.

Therapeutic Uses and ADME—Crohn's Disease. Monoclonal anti–TNF-α antibodies are used for moderate-to-severe Crohn's disease, including fistulizing disease that is resistant to other therapies (Peyrin-Biroulet et al., 2008). The antibody preparations are used for both the induction and the maintenance of remission in adults and children and are given parenterally. For induction therapy for Crohn's disease, these agents produce a clinical response in approximately 14 days, with improvement of symptoms in approximately 60% and remission in approximately 30%

of patients. Responsive patients may progress to maintenance therapy; see dosing schedules below.

Infliximab is given intravenously at an initial dose of 5 mg/kg, with subsequent doses of 5 mg/kg at weeks 2 and 6, followed by maintenance doses of 5 mg/kg every 8 weeks. The $t_{1/2}$ is about 8 to 10 days, although the clearance rate increases in patients who develop anti-infliximab antibodies. *Adalimumab* is given subcutaneously at an initial dose of 160 mg, with subsequent doses of 80 mg at week 2 and maintenance doses of 40 mg every second week starting on week 4. Bioavailability following a 40-mg dose is about 64%; the $t_{1/2}$ is about 2 weeks. *Certolizumab pegol* is given at an induction dose of 400 mg subcutaneously at weeks 0, 2, and 4, and then every 4 weeks for maintenance of response. Bioavailability when given subcutaneously is about 80%; the $t_{1/2}$ is about 2 weeks. For induction to treat moderate-to-severe ulcerative colitis, *golimumab* is administered subcutaneously, 200 mg at starting, 100 mg at 2 weeks, and then a maintenance dose of 100 mg every 4 weeks. *Golimumab* has a half-life of approximately 14 days. In treating severe ulcerative colitis, *golimumab* and *adalimumab* appear less efficacious than *infliximab*. The clearance of monoclonal anti–TNF-α antibodies is not well understood but is likely due to proteolytic degradation. The polyethylene glycol moiety of *certolizumab pegol* is cleared by urinary excretion.

Adverse Effects. The major serious adverse effect of anti-TNF agents is infection resulting from suppression of the inflammatory response. Concomitant administration of steroids enhances the likelihood of infection. Both acute (fever, chills, urticaria, or even anaphylaxis) and subacute (serum sickness–like) reactions may develop after *infliximab* infusion. Antibodies to *infliximab* can decrease its clinical efficacy (Lichtenstein, 2013). Strategies to minimize the development of these antibodies (e.g., treatment with glucocorticoids or other immunosuppressives) may be critical to preserving *infliximab* efficacy but may increase the chance of infection. Because *adalimumab* and *certolizumab* are humanized antibodies, there is less chance for the development of an immune response against them. *Infliximab* therapy is associated with increased incidence of respiratory infections; of particular concern is potential reactivation of tuberculosis or development of opportunistic infections with subsequent dissemination. The FDA recommends that candidates for *infliximab* therapy be tested for latent tuberculosis with purified protein derivative; patients testing positive should be treated prophylactically with therapy for latent tuberculosis. *Infliximab* is contraindicated in patients with severe congestive heart failure. There is concern about a possible increased incidence of non-Hodgkin lymphoma, but a causal role has not been established. Other adverse events including drug-induced lupus, multiple sclerosis–like syndrome, and nonmelanoma skin cancer have been reported.

Although *infliximab* was designed specifically to target TNF-α, it may have more complex actions. *Infliximab* binds membrane-bound TNF-α and may cause lysis of these cells by antibody-dependent or cell-mediated cytotoxicity. Thus, *infliximab* may deplete specific populations of subepithelial inflammatory cells. These effects, together with its mean terminal plasma $t_{1/2}$ of 8 to 10 days, may explain the prolonged clinical effects of *infliximab*. *Infliximab* (5 mg/kg infused IV at intervals of every 6–8 weeks) decreases the frequency of acute flares in approximately two-thirds of patients with moderate-to-severe Crohn's disease and also facilitates the closing of enterocutaneous fistulas associated with Crohn's disease. Emerging evidence also supports its efficacy in maintaining remission and in preventing recurrence of fistulas. The combination of *infliximab* and *azathioprine* is more effective than *infliximab* alone in induction of remission and mucosal healing in steroid-resistant patients.

Other Monoclonal Antibodies for the Treatment of Crohn's Disease and Ulcerative Colitis

Antibodies have been derived that bind to subunits of the cell adhesion molecule integrin, thereby interfering with lymphocyte recruitment to areas of damage and inflammation. *Vedolizumab* is a humanized monoclonal antibody that binds to and inhibits the α_4-integrin subunit and therefore blocks binding of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ on lymphocytes to *addressin* (also known as mucosal vascular addressin cell adhesion molecule, MADCAM-1) on venular endothelial cells, thus preventing lymphocyte recruitment to the intestinal mucosa (Jovani and Danese, 2013). It is approved for use in the treatment of moderate-to-severe Crohn's disease and ulcerative colitis. *Vedolizumab* is generally given at a dose of 300 mg at 0, 2, and 6 weeks, with maintenance doses of 300 mg given every 8 weeks thereafter. The main side effects are headache, hypersensitivity reactions, arthralgia, nasopharyngitis, and fatigue. Anti–TNF-α drugs may enhance the adverse effects of *vedolizumab.*

Etrolizumab is another monoclonal antibody that binds the β_7 integrin subunit. It showed promise in phase III clinical trials for the treatment of moderate to severe Crohn's disease (Sandborn et al., 2020), whereas in trials with severe ulcerative colitis, *etrolizumab* had mixed results for induction of remission and no significant effect on maintenance of remission compared to control. Unlike *vedolizumab, etrolizumab* also blocks $\alpha E\beta_7$ heterodimers; thus, it has the potential to inhibit the interaction of $\alpha E\beta_7$-positive T cells with E-cadherin. Its effect is on the recruitment of T cells to both the vascular and epithelial compartments in the gut mucosa (Tang et al., 2018). Like many therapeutic monoclonal antibodies, *etrolizumab* has the potential to stimulate the production of antidrug antibodies, which have been observed in approximately 5% of patients.

Ustekinumab is a fully humanized monoclonal antibody that targets the p40 subunit common to the proinflammatory cytokines IL-12 and IL-23, thus preventing activation of IL-12β1 and IL-23 receptors on lymphocytes ($CD4^+$ T cells and natural killer cells) (Teng et al., 2015). Originally developed for the treatment of psoriasis, *ustekinumab* is also effective in the induction and maintenance of remission in Crohn's disease. Initial treatment is given intravenously at a dose of 260 to 520 mg depending on body weight, then 90 mg subcutaneously every 8 weeks thereafter for maintenance of remission. Time to peak C_p is 1 to 2 weeks. Side effects include upper respiratory tract infections, headache, arthralgia, infection, nausea, and nasopharyngitis. This agent has a variable and long elimination half-life (up to 120 days) and should be discontinued for 15 weeks prior to the administration of a live vaccine.

Therapeutic Uses and ADME—Ulcerative Colitis. Unlike Crohn's disease, ulcerative colitis can be cured with surgery (colectomy). Thus, the cost and potential adverse events associated with monoclonal antibody therapy need to be balanced with the effectiveness of the drug at preventing the need for colonic resection. Biologics are well established in their use in ulcerative colitis, particularly in those patients for whom primary therapy with glucocorticoids, 5-ASA, or immunomodulators has failed. *Infliximab, adalimumab,* and *golimumab* have become mainstays in the treatment of ulcerative colitis. *Vedolizumab* and *ustekinumab* are now indicated and approved for the treatment of moderate-to-severe ulcerative colitis. The administration, dosing, metabolism, and adverse events are similar for the use of these drugs in both ulcerative colitis and Crohn's disease.

Natalizumab is a chimeric (human-mouse) monoclonal antibody against $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins on leukocytes involved in inflammation. The drug effectively provides symptomatic relief from both multiple sclerosis and Crohn's disease and has been approved for induction and maintenance of remission in moderate-to-severe Crohn's disease. The drug must not be used in patients with immunodeficiency or in immunosuppressed patients due to possible increased risk of progressive multifocal leukoencephalopathy, an often lethal viral infection (human polyomavirus 2). As a result, *natalizumab* carries a boxed warning and is available only through TOUCH, a restricted distribution program.

Other Small-Molecule Drugs to Treat IBD

JAK Inhibitors

Janus kinases (JAKs) are signaling molecules that are activated following ligand binding and dimerization of many cytokine receptors. There are four members of the JAK family—JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2)—that act in various dimeric configurations. JAK1/JAK3 transduce signals from the receptors for a variety of interleukins that play roles in the activation of immune/inflammatory and autoimmune responses. IFN acts via JAK1/JAK2. By inhibiting JAK, *tofacitinib* inhibits immune/

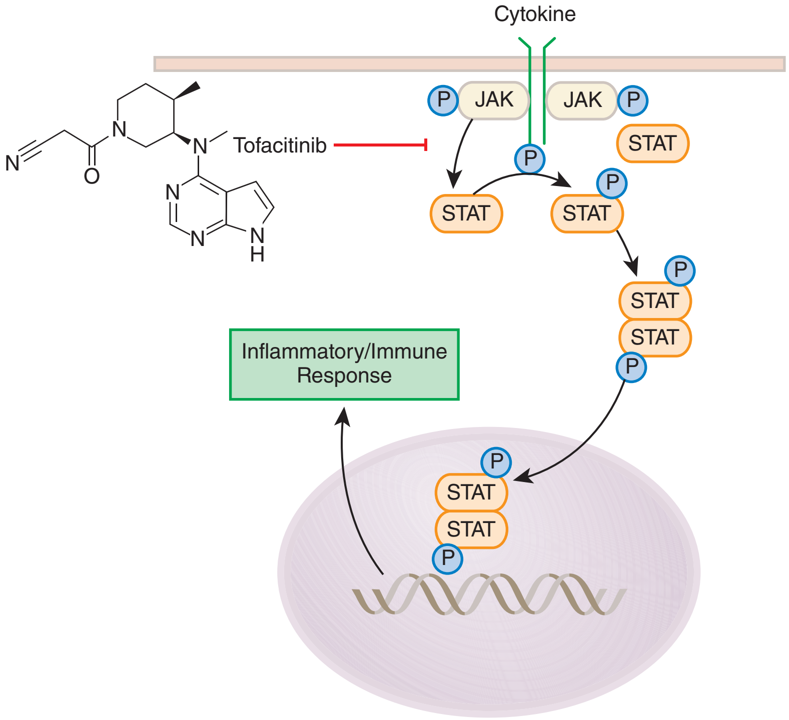

Figure 55–6 *Tofacitinib inhibits the JAK-STAT signaling pathway.* Binding of cytokines to their receptors triggers the phosphorylation of JAKs and receptor tyrosine kinases. The phosphorylation of JAKs leads to the phosphorylation and dimerization of STATs, which translocate to the nucleus to drive the expression of genes associated with numerous cellular processes including those associated with inflammation and immunity. *Tofacitinib* inhibits the activity of JAK and thereby prevents the phosphorylation of STAT and the resultant activation of inflammatory/immune responses. Unlike some biological therapies, *tofacitinib* does not elicit generation of neutralizing antibodies to itself.

inflammatory responses that are active in ulcerative colitis. Following activation, JAKs self-phosphorylate at tyrosine residues, and then phosphorylate members of the signal transducers and activators of transcription (STAT) family. Activated STAT proteins translocate to the nucleus where they stimulate the expression of genes related to the inflammatory response. The use of JAK inhibitors in IBD has recently been reviewed (Hernandez-Rocha and Vande Casteele, 2020).

Tofacitinib is a pyrrolopyrimidine that is pyrrolo[2,3-d]pyrimidine substituted at position 4 by an *N*-methyl,N-(1-cyanoacetyl-4-methylpiperidin-3-yl)amino moiety. Its citrated form is used therapeutically. *Tofacitinib* acts broadly, inhibiting JAK1, JAK2, JAK3, and TYK2, although its main effect is through inhibition of the JAK1/3 combination. Administration of *tofacitinib* also results in a decrease in circulating CD16/56$^+$ natural killer cells, which contributes to its immunosuppressive effects.

Several other so-called "jakinibs" have been developed to treat chronic inflammatory disease. For example, *ruxolitinib* is FDA-approved for the treatment of rheumatoid arthritis and psoriasis, but not for IBD at this point.

Therapeutic Uses and ADME. *Tofacitinib* is used to treat moderate-to-severe ulcerative colitis in patients who fail to respond to biological therapies. It is available in standard and extended-release formulations and is administered orally at a dose of 10 mg twice daily for 8 weeks. After this, it can be administered at a dose of 5 mg twice daily for the maintenance of remission of inflammation. The drug should be discontinued if a patient does not respond by 16 weeks. *Tofacitinib* is rapidly absorbed, with a time to peak plasma levels (T_{max}) of 0.5 to 1 h. Approximately 40% of the drug is bound to protein in the plasma, mostly albumin. It is active in the unmetabolized form, and the circulating drug is metabolized in the liver (~70%), primarily via CYP3A4, and also in the kidneys (~30%). Thus, *tofacitinib* is contraindicated in patients with hepatic insufficiency. Renal dysfunction or concomitant use of other substrates and inhibitors of CYP3A4 requires reduction of *tofacitinib* dosage (to 5 mg daily). The metabolized drug is excreted in the urine (80%) and the feces (14%).

Adverse Effects. The primary adverse effect of *tofacitinib* is immunosuppression, so patients can experience increased susceptibility to infection. The drug should not be taken with other immunosuppressants. Additional adverse effects include an increase in blood cholesterol, diarrhea, headaches, and stuffy or runny nose. Recently, *tofacitinib* has been associated with pulmonary embolism in a small number of patients. *Tofacitinib* is not recommended for use in children with *ulcerative colitis*, since its pharmacokinetics, safety, and effectiveness have not been established in the pediatric population. In September of 2021, the FDA modified the approved indications for *tofacitinib* and two JAK inhibitors, *baricitinib* and *upadacitinib,* that are used primarily in treating rheumatoid arthritis. Use of these agents is now limited to patients who have responded poorly to TNF antagonists. An updated boxed warning includes information about the risks of serious heart-related events, cancer, blood clots, and death (FDA, 2021). Two additional JAK inhibitors, *ruxolitinib* and *fedratinib*, are not indicated for the treatment of inflammatory conditions and are not included in the required modified labeling.

Sphingosine-1-Phosphate Receptor Agonists

Sphingosine-1-phosphate (S1P) is formed from ceramide through the action of ceramidase and the sphingosine kinase 1/2 enzymes. It is classed as a lysosphingolipid and signals through members of the S1P receptor family (S1PR$_{1-5}$) of GPCRs. Activation of S1PR triggers a number of signaling pathways to evoke numerous biological responses including lymphocyte trafficking, immune cell activation, and the synthesis of inflammatory mediators that contribute to gastrointestinal inflammatory disease (Sukocheva et al., 2020). The mechanism of action of S1PR agonists appears to relate to blocking the movement of lymphocytes from lymph nodes to sites of tissue injury (see Figure 39–3).

Therapeutic Use and ADME. *Ozanimod* is an S1PR$_1$ and S1PR$_5$ agonist (Scott et al., 2016) that is FDA-approved for the treatment of relapsing forms of multiple sclerosis (see Chapter 39). In clinical trials, *ozanimod* has shown significant benefit in cases of *ulcerative colitis* (Sandborn et al., 2016) and *Crohn's disease* (Feagan et al., 2020). *Ozanimod* is supplied as a monohydrochloride and is given orally in pill form. The dosage is titrated from 0.23 mg in the first 4 days to 0.46 mg in days 5 to 7 and 0.92 mg/day thereafter. Peak plasma levels occur in 6 to 8 h. Extensive metabolism of the parent compound produces a series of active metabolites with similar specificity for $S1P_1R$ and $S1P_5R$; thus, although the plasma $t_{1/2}$ of the parent drug is approximately 21 h, the $t_{1/2}$ of the effect is approximately 11 days.

Adverse Effects. Adverse effects (incidence ≥4%) include upper respiratory infection, elevation of hepatic transaminases, bradyarrhythmia/atrioventricular conduction delays, orthostatic hypotension, urinary tract infection, back pain, and hypertension. An active metabolite of *ozanimod* inhibits MAO-B, providing the potential of serious hypertensive interactions between *ozanimod* and agents that increase sympathetic tone, NE, or 5HT (opioids, selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, tricyclic antidepressants, MAO inhibitors, tyramine, and foods containing tyramine). Since the S1P receptors have roles in embryonic development, and based on animal studies, the FDA recommends that females of childbearing age should use contraception during therapy and for 3 months thereafter.

Manipulating the Intestinal Microbiome to Treat IBD

Antibiotics and Probiotics

A balance normally exists in the GI tract among the mucosal epithelium, the normal gut flora, and the immune response (Biteen et al., 2016; Shreiner et al., 2015). Dysbiosis of the intestinal microbiome is now considered a key factor in the development of IBD (Dalal and Chang, 2014). Thus, certain bacterial strains may be either pro- (e.g., *Bacteroides*) or anti-inflammatory (e.g., *Lactobacillus*), prompting attempts to manipulate the colonic flora in patients with IBD. Traditionally, antibiotics have been used most prominently in Crohn's disease.

Antibiotics can be used as:

- Adjunctive treatment along with other medications for active IBD in severe cases where there is concern about coexisting sepsis
- Treatment for perforating or fistulizing complications of Crohn's disease
- Prophylaxis for recurrence in postoperative Crohn's disease

Metronidazole, *ciprofloxacin*, *amoxicillin-clavulanate,* and *piperacillin-tazobactam* are the antibiotics used most frequently. Crohn's disease–related complications that may benefit from antibiotic therapy include intra-abdominal abscess and inflammatory masses, perianal disease (including fistulas and perirectal abscesses), small-bowel bacterial overgrowth secondary to partial small-bowel obstruction, secondary infections with organisms such as *Clostridium difficile*, and postoperative complications.

More recently, *probiotics* have been used to treat specific clinical situations in IBD. Probiotics are mixtures of putatively beneficial lyophilized bacteria given orally. Several studies have provided evidence for beneficial effects of probiotics in ulcerative colitis and pouchitis (Sokol, 2014). Jakubczyk and colleagues (2020) have suggested that species and strains of *Bifidobacterium* and *Lactobacillus* provide the best therapeutic effect. However, with conflicting reports in the literature, the utility of probiotics as a primary therapy for IBD remains unclear.

Fecal Transplant as Therapy in IBD

The recognition that the etiology of IBD involves dysbiosis of the intestinal microbiome has raised interest in methods to reestablish normal microflora in patients. Fecal transplant involves the instillation of a preparation of feces from a healthy donor into the colon, either by enema or during colonoscopy. This has proven to be an effective therapy for antibiotic-resistant *C. difficile* infection. Several clinical trials have assessed the efficacy of fecal transplant in Crohn's disease and ulcerative colitis, with varying results (Tan et al., 2020).

Supportive Therapy in IBD

Analgesic, anticholinergic, and antidiarrheal agents play supportive roles in reducing symptoms and improving quality of life. Oral iron, folate, and vitamin B_{12} should be administered as indicated. *Loperamide* or *diphenoxylate* (see Chapter 54) can be used to reduce the frequency of bowel movements and relieve rectal urgency in patients with mild disease in selected circumstances; these agents are contraindicated in patients with severe disease because they may predispose to the development of toxic megacolon. *Cholestyramine* can be used to treat bile salt–induced diarrhea in patients who have undergone limited ileocolic resections. Anticholinergic agents (*dicyclomine hydrochloride*, etc.; Chapter 11) are used to reduce abdominal cramps, pain, and rectal urgency. As with the antidiarrheal agents, they are contraindicated in severe disease or when obstruction is suspected.

With the legalization of cannabis in many jurisdictions, the use of cannabis and various formulations of cannabinoid compounds has garnered increasing attention as a supportive therapy for IBD. As recently reviewed, data suggest that cannabis may improve symptoms of IBD. However, studies to date have not involved properly designed randomly controlled trials, and the mechanisms of action are unclear (Nasser et al., 2020). Chapter 26 provides an overview of the pharmacological actions of cannabinoids.

Pediatric IBD

Children and adolescents remain the population of IBD patients with the fastest growing incidence. On average, children present with more severe disease than do adults. In addition, while many children present with the classical symptoms of IBD, approximately 22% of children present with additional symptoms, such as growth failure, perianal disease, or other extraintestinal manifestations, as the primary symptom, thus complicating diagnosis.

The drugs used for treating pediatric IBD are the same as those used for the treatment of these diseases in adults. Exclusive enteral nutrition is an effective alternative to 5-ASA compounds, glucocorticoids, immunosuppressants, and biologics. Indeed, 8 to 12 weeks of liquid formula as the sole source of calories is as effective as glucocorticoids in relieving symptoms and has the advantage of supporting growth (Rosen et al., 2015). In terms of drug therapy, a child's immunization status should be considered before commencing immunosuppressive therapy (glucocorticoids, *azathioprine-methotrexate*, anti–TNF-α drugs). Children should be tested for latent tuberculosis, particularly prior to treatment with anti–TNF-α drugs. During treatment, children may receive inactivated vaccines, but live vaccines are not recommended.

Antibiotics have recently been shown to have some utility in treating mild-to-moderate pediatric Crohn's disease. In particular, *ciprofloxacin*, *metronidazole,* and *rifaximin* were demonstrably effective in small clinical trials (Serban, 2015). Their role in the treatment of ulcerative colitis has yet to be established.

Therapy of IBD During Pregnancy

Inflammatory bowel disease is a chronic disease that affects women in their reproductive years. In general, decreased disease activity increases fertility and improves pregnancy outcomes (Kim et al., 2021). At the same time, limiting medication during pregnancy is always desired but sometimes conflicts with the goal of controlling the disease. The use of medical therapies to treat IBD during pregnancy and lactation has been reviewed (Laube et al., 2021), although studies that thoroughly investigated the use of medications to treat IBD in pregnancy are limited (Damas et al., 2015). *Mesalamine* and glucocorticoids are used frequently in pregnancy and generally are considered safe, whereas *methotrexate* is absolutely contraindicated in pregnant patients. There does not appear to be an increase in adverse outcomes in pregnant patients maintained on thiopurine-based immunosuppressives. Anti–TNF-α drugs, particularly *infliximab* and *adalimumab*, have been assessed for their safety for use during pregnancy and have been found to have low risk for adverse events (Laube et al., 2021). *Certolizumab pegol* is considered safe since it does not cross the placenta (Mariette et al., 2018). JAK inhibitors including *tofacitinib* are teratogenic in animal studies and have been associated with developmental abnormalities and pregnancy complications in some patients. Thus, JAK inhibitors should not be taken during pregnancy. Furthermore, since they are secreted in breast milk, they should not be taken by lactating mothers. While there have been data suggesting that the S1PR agonist *ozanimod* can cause developmental defects in experimental animals, there are limited data on the effects in pregnant patients. Thus, at present, *ozanimod* is contraindicated during pregnancy.

Drug Facts for Your Personal Formulary: *Drugs for the Treatment of Inflammatory Bowel Diseases*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Mesalamine-Based Drugs		
Mesalamine (5-ASA)	• Induction and maintenance of remission in mild-to-moderate ulcerative colitis • Used in combination with glucocorticoids for severe ulcerative colitis	• Effects are primarily topical with limited effects on deeper tissue inflammation • Following oral administration, jejunum is primary site of absorption, so utility in more distal disease is limited • Can be delivered as a suppository for rectal disease
Sulfasalazine	• Induction and maintenance of remission in mild-to-moderate ulcerative colitis • Used in combination with glucocorticoids for severe ulcerative colitis	• Prodrug, delivers 5-ASA to more distal GI regions following metabolism by colonic bacteria • Sulfapyridine is also released; may cause adverse effects in patients sensitive to sulfa drugs
Olsalazine	• Induction and maintenance of remission in mild-to-moderate ulcerative colitis • Used in combination with glucocorticoids for severe ulcerative colitis	• Prodrug with two azo-linked 5-ASA molecules • Eliminates the side effects associated with the sulfapyridine moiety of sulfasalazine
Balsalazide	• Induction and maintenance of remission in mild-to-moderate ulcerative colitis • Used in combination with glucocorticoids for severe ulcerative colitis	• Prodrug with a 5-ASA molecule linked to an inert, unabsorbable second moiety • Eliminates the side effects associated with the sulfapyridine moiety of sulfasalazine
Glucocorticoids: Minimize duration of use. Taper dose prior to stopping to minimize disease relapse and avoid adrenal insufficiency that follows rapid glucocorticoid withdrawal after prolonged therapy has suppressed the HPA axis.		
Prednisone	• Induction of remission in moderate-to-severe Crohn's disease and ulcerative colitis	• Hepatic metabolism to active moiety, prednisolone • Not used for maintenance therapy due to serious adverse effects
Methylprednisolone	• Induction of remission in moderate-to-severe Crohn's disease and ulcerative colitis	• Can be administered orally, intravenously, or intramuscularly to patients who respond poorly to oral prednisone • Preferred over hydrocortisone, which has higher incidence of Na^+ retention and K^+ wasting
Hydrocortisone	• Induction of remission in moderate-to-severe Crohn's disease and ulcerative colitis	• Administered intravenously to patients who respond poorly to oral prednisone
Budesonide	• Induction of remission in mild-to-moderate Crohn's disease and ulcerative colitis, particularly in distal disease • Not effective for long-term maintenance of clinical remission	• Prominent first-pass metabolism reduces side effects that can result from maintenance of higher systemic levels
Immunomodulatory Agents		
6-Mercaptopurine	• Used as an adjunct to glucocorticoids and biologics in the treatment of moderate-to-severe Crohn's disease and ulcerative colitis • Effective in maintenance of remission	• Slow-acting drug; maximum therapeutic benefit may take months to achieve • Other metabolites also have anti-inflammatory activity • Fourfold increased risk of lymphoma in patients with IBD treated with thiopurines
Azathioprine	• Used as an adjunct to glucocorticoids and biologics in the treatment of moderate-to-severe Crohn's disease and ulcerative colitis • Effective in maintenance of remission	• Prodrug metabolized nonenzymatically in blood to active form, 6-mercaptopurine • Other metabolites also have anti-inflammatory activity • Fourfold increased risk of lymphoma in patients with IBD treated with thiopurines
Methotrexate	• Maintenance of remission in Crohn's disease, particularly steroid-resistant or steroid-dependent disease • Often used in combination with biologic agents	• Folic acid analogue that has anti-inflammatory activity of unclear mechanism • Administered subcutaneously or orally • Cleared unaltered by the kidney, so inhibition of renal excretion mechanisms may lead to drug toxicity

Drug Facts for Your Personal Formulary: *Drugs for the Treatment of Inflammatory Bowel Diseases* (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Immunomodulatory Agents (cont.)		
Cyclosporine	• Used to treat specific cases of severe Crohn's disease, including fistulizing disease • Not useful for maintenance of remission	• Erratic and incomplete absorption means blood levels must be monitored • Significant adverse events profile
Tacrolimus (FK506)	• Useful for the treatment of refractory Crohn's disease	• Immunomodulator with similar mechanism as cyclosporine but with better oral absorption
Biologics: Anti–TNFα Antibodies		
Exacerbation of bacterial, viral, and fungal infections can be serious with these agents, especially in presence of other immunosuppressants. Prior to receiving antibodies to TNF, patients should be tested for TB and, if positive, receive prophylactic TB therapy prior to administration of anti-TNF antibodies.		
Infliximab	• Induction or maintenance of remission in moderate-to-severe Crohn's disease or ulcerative colitis in patients who have not responded well to other therapies	• Partly humanized, chimeric anti–TNF-α monoclonal antibody • Usually administered by intravenous infusion • Patients may develop antibodies against the drug
Adalimumab	• Induction or maintenance of remission in moderate-to-severe Crohn's disease or ulcerative colitis in patients who have not responded well to other therapies	• Fully humanized anti–TNF-α monoclonal antibody; reduced incidence of antidrug antibodies • Administered subcutaneously • Useful for patients for whom infliximab has lost efficacy or has caused adverse reactions
Certolizumab pegol	• Induction or maintenance of remission in moderate-to-severe Crohn's disease in patients who have not responded well to other therapies	• Humanized anti–TNF-α monoclonal antibody bound to PEG to increase plasma $t_{1/2}$ • Administered subcutaneously • Useful for patients for whom infliximab has lost efficacy or caused adverse reactions • May be a better option in pregnant women due to less drug crossing placental barrier
Golimumab	• Approved for moderate to severe ulcerative colitis in adults not responsive or intolerant of traditional therapy	
Biologics: Other		
Vedolizumab	• Induction or maintenance of remission in moderate-to-severe Crohn's disease or ulcerative colitis in patients who have not responded to other therapies	• Humanized anti-$\alpha_4\beta_7$ monoclonal antibody • Given by intravenous infusion • May cause hypersensitivity reactions
Etrolizumab	• Induction or maintenance of remission in moderate-to-severe Crohn's disease or ulcerative colitis in patients who have not responded to other therapies	• Humanized monoclonal antibody that blocks $\alpha_4\beta_7$ and $\alpha E\beta_7$ • Given by intravenous infusion • Good safety profile with few serious adverse events in clinical trials
Ustekinumab	• Induction or maintenance of remission in moderate-to-severe Crohn's disease in patients who have not responded to other therapies	• Humanized monoclonal antibody against p40 subunit of IL-12 and IL-23 • Administered subcutaneously or IV • Long-term safety profile has not yet been established
Natalizumab	• Induction and maintenance of remission in moderate-to-severe Crohn's disease in adults with inadequate response to TNF-α antagonists or other traditional therapies; also used in treating multiple sclerosis	• Available only under TOUCH, a restricted distribution program • Boxed warning: progressive multifocal leukoencephalopathy • IV administration; do not use with other immunosuppressants • Discontinue after 12 weeks if no benefit of induction therapy
Other Small-Molecule Drugs		
Tofacitinib	• Treatment of moderate-to-severe ulcerative colitis in patients who have failed biological therapies and for whom TNF antagonists have failed to provide sufficient response	• Blocks members of the Janus kinase (JAK) family of signaling molecules, thereby inhibiting actions of TNF-α • Administered orally • An immunosuppressant that can increase susceptibility to infection • Boxed warning issued in September 2021; see text
Ozanimod	• Treatment of moderate-to-severe ulcerative colitis in patients who have failed biological therapies; also used for multiple sclerosis	• Sphingosine-1-phosphate (S1P) receptor modulator • Metabolites inhibit MAO-B ⇒ possible hypertensive interactions with drugs that elevate adrenergic tone, NE, and 5HT, and with dietary tyramine • Main adverse effects are increased upper respiratory tract infections and elevated hepatic transaminases • Women of childbearing age should use birth control during and for 3 months after using ozanimod

Drug Facts for Your Personal Formulary: *Drugs for the Treatment of Inflammatory Bowel Diseases (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Antibiotics		
Metronidazole	• Used as adjunctive therapy in mild-to-moderate Crohn's disease • Sometimes used in conjunction with ciprofloxacin • Used in pediatric IBD	• Modest therapeutic benefit in Crohn's disease • Little to no benefit in ulcerative colitis
Ciprofloxacin	• Used as adjunctive therapy in mild-to-moderate Crohn's disease • Sometimes used in conjunction with metronidazole • Used in pediatric IBD	• Modest therapeutic benefit in Crohn's disease; little/no benefit in ulcerative colitis • Boxed warning: tendinopathy, tendon rupture, peripheral neuropathy, CNS effects • Fluoroquinolones exhibit neuromuscular blocking activity; avoid using in patients with myasthenia gravis
Rifaximin	• Used as adjunctive therapy in mild-to-moderate Crohn's disease • Used in pediatric Crohn's disease	• Less experience with this drug compared to metronidazole or ciprofloxacin
Probiotics		
Various types and formulations	• Some utility in ulcerative colitis and pouchitis but few clinical trials	• Effects are transient; long-term colonic colonization rarely occurs • Watch for progress on fecal transplant therapy

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Section VII

Chemotherapy of Infectious Diseases

Section Editor: Conan MacDougall

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Chapter 56

General Principles of Antimicrobial Therapy

Conan MacDougall

ANTIMICROBIAL CHEMOTHERAPY: CLASSES AND ACTIONS

TYPES AND GOALS OF ANTIMICROBIAL THERAPY

- Primary Prophylaxis
- Preemptive Therapy
- Empiric Therapy
- Definitive Therapy
- Posttreatment Suppressive Therapy and Secondary Prophylaxis

THE PHARMACOKINETIC BASIS OF ANTIMICROBIAL THERAPY

IMPACT OF SUSCEPTIBILITY TESTING ON SUCCESS OF ANTIMICROBIAL AGENTS

- Bacteria
- Fungi
- Viruses
- Parasites

BASIS FOR SELECTION OF DOSE AND DOSING SCHEDULE

MECHANISMS OF RESISTANCE TO ANTIMICROBIAL AGENTS

- Resistance Due to Reduced Concentration of Drug at Its Target Site
- Resistance Due to Alteration or Destruction of Antibiotic
- Resistance Due to Altered Target Structure
- Heteroresistance and Viral Quasi-Species

EVOLUTIONARY BASIS OF RESISTANCE EMERGENCE

- Development of Resistance via Mutation Selection
- Resistance by External Acquisition of Genetic Elements

Antimicrobial Chemotherapy: Classes and Actions

This chapter reviews the general classes of antimicrobial drugs, their mechanisms of action and mechanisms of resistance, and principles of drug selection. Chapters 57 through 68 present the pharmacological properties and uses of individual classes of antimicrobials.

Microorganisms of medical importance fall into four categories: *bacteria, viruses, fungi,* and *parasites.* The broad classification of *antibiotics*—a term we will use colloquially to encompass all manner of antimicrobial agents—follows this classification closely, so that we have antibacterial, antiviral, antifungal, and antiparasitic agents. However, there are many antibiotics that work against more than one category of microbes, especially those that target evolutionarily conserved pathways. Classification of an antibiotic can be performed along several dimensions, including the class and spectrum of microorganisms it kills, the biochemical pathway it interferes with, and the chemical structure of its pharmacophore.

Antimicrobial molecules should be viewed as ligands whose receptors are typically microbial proteins. The term *pharmacophore,* introduced by Ehrlich, defines that active chemical moiety of the drug that binds to the microbial receptor. The microbial proteins targeted by the antibiotic are essential components of biochemical reactions in the microbes, and interference with these physiological pathways inhibits the replication of or directly kills the microorganisms. The biochemical processes commonly inhibited include cell wall synthesis, cell membrane synthesis and function, ribosomal translation, nucleic acid metabolism, topoisomerase-mediated chromosomal conformational changes, viral proteases, viral integrases, viral envelope entry/fusion proteins, folate synthesis, and parasitic chemical detoxification processes. Recently, *antisense antibiotics* have been developed; these work by inhibiting gene expression in bacteria in a sequence-specific manner. Furthermore, *interferon*-based products work by inducing specific antiviral activities of the infected human cells.

Types and Goals of Antimicrobial Therapy

A useful way to organize the types and goals of antimicrobial therapy is to consider where antibiotics are initiated with respect to the disease progression timeline (Figure 56–1); therapy can be classified as *primary prophylaxis, preemptive, empirical, definitive,* or *suppressive/secondary prophylaxis.*

Primary Prophylaxis

Prophylaxis involves administering antibiotics to patients who are not yet infected or have not yet developed disease. The goal of *primary* prophylaxis is to prevent a first episode of infection in patients without evidence of infection. Primary prophylaxis can significantly reduce the likelihood of clinically significant infection but must be balanced against the risks of disruption of the microbiome, selection for antibiotic-resistant variants, toxicity, and cost. Thus, primary prophylaxis should be reserved for patients at significant risk, using antibiotics of the narrowest appropriate spectrum, for the shortest duration appropriate to provide adequate protection.

The most common use of antibiotics for primary prophylaxis is the administration of antibiotics in the perioperative period to prevent surgical site infections. Wound infection results when a critical number of bacteria are present in the wound at the time of closure, and chemoprophylaxis can be used to prevent wound infections after surgical procedures. Antibiotics directed against the invading microorganisms may reduce the number of viable bacteria below the critical level and thus prevent infection.

In some cases, primary prophylaxis may be initiated several days in advance of the surgical procedure, as with the use of intranasal *mupirocin* and topical *chlorhexidine gluconate* baths to reduce the burden of *Staphylococcus aureus* ahead of cardiac and orthopedic surgeries, among those found to be colonized with this organism on preprocedure screening (Schweizer et al., 2015). More commonly, for patients and procedures that carry significant risk of surgical site infection, antibiotics are administered in the perioperative period (Berrios-Torres et al., 2017).

Abbreviations

ABC: ATP binding cassette
AUC: area under the C_p-time curve
CCR5: chemokine receptor type 5
CD4: T-helper cells
CMV: cytomegalovirus
C_p: plasma concentration
C_{Pmax}: peak plasma concentration
CYP: cytochrome P450
DHFR: dihydrofolate reductase
DHPS: dihydropteroate synthase
E: effect
EC: effective concentration
ELF: epithelial lining fluid
E_{max}: maximal effect
HIV: human immunodeficiency virus
IC: inhibitory concentration
MALDI-TOF MS: matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MEC: minimum effective concentration
MIC: minimum inhibitory concentration
PAE: postantibiotic effect
PCR: polymerase chain reaction
PK/PD: pharmacokinetics-pharmacodynamics
PrEP: preexposure prophylaxis
***rpoB*:** RNA polymerase

The perioperative antimicrobial dose should be administered intravenously within 60 min prior to the surgical incision, so that local drug concentrations are above the *minimum inhibitory concentration* (MIC) of likely pathogens at the time of incision. The frequency of redosing during the procedure is based on the half-life of the drug to ensure adequate antibiotic concentrations above the MIC until closure of the surgical incision. This is especially important for those β-lactam antibiotics that have short half-lives; these should be redosed at intervals of two times the half-life.

For the majority of procedures, a single perioperative dose suffices to prevent infection, and administration of postoperative doses is associated with no significant increased benefit and increased risks of adverse effects and *Clostridioides difficile* superinfection (Branch-Elliman et al., 2019). The systemic antibiotic used is chosen based on the pathogen most likely to contaminate the incision, which in turn depends on the site where surgery is being performed. The most common pathogens infecting incision sites after clean surgery are staphylococci, specifically *S. aureus* and coagulase-negative staphylococci. In clean-contaminated surgery over the abdomen and pelvis, the same organisms remain important, but *Enterococcus* species and gram-negative rods are also common.

Primary prophylaxis may also be used in immunosuppressed patients such as those with HIV-AIDS or those status post solid organ transplantation on antirejection immunosuppressants. In these groups of patients, specific antiparasitic, antibacterial, antiviral, and antifungal therapy is administered based on the typical patterns of pathogens that are major causes of morbidity during immunosuppression. A risk-benefit analysis determines choice and duration of prophylaxis. Prophylaxis of opportunistic infections in patients with HIV-related immunosuppression is typically started when the CD4 count falls below 200 cells/mm^3 and may be discontinued with sustained increases in the CD4 count above this threshold in response to antiretroviral therapy. In posttransplant patients, use of prophylaxis depends on the type of transplantation, time since the transplant procedure, and type and dose intensity of immunosuppressive therapy. Prophylaxis may be discontinued in patients based on benchmarks either of time since transplantation or reduction of immunosuppression. Examples of pathogens against which primary prophylaxis may be used include *Pneumocystis jirovecii, Toxoplasma gondii, Candida* species, *Aspergillus* species, cytomegalovirus (CMV), and other Herpesviridae. Doses used for primary prophylaxis are often lower than when the same drug is used for acute treatment.

An emerging area of primary prophylaxis is termed *preexposure prophylaxis* (PrEP) and is employed in patients at increased risk of contracting HIV infection (Mayer and Allan-Blitz, 2019). PrEP involves taking oral antiretroviral drugs on a regular basis (a once-monthly injectable regimen is also under study) to prevent establishment of HIV infection upon exposure, typically through sex or injection drug use. PrEP may be used among patients who are part of a known HIV-serodiscordant couple or to reduce risk of transmission when the status of sexual partners is not known. PrEP has been shown to significantly reduce the risk of new diagnoses of HIV, although regular monitoring for drug-related adverse effects is necessary.

Other examples of primary prophylaxis include antiretroviral postexposure prophylaxis following needlestick exposures, administration of *rifampin* to contacts of patients with meningococcal meningitis, use of anti-influenza antivirals in household contacts of influenza cases, and administration of macrolides to close contacts of cases of pertussis.

Preemptive Therapy

Preemptive therapy is used as a substitute for primary prophylaxis and as early targeted therapy in high-risk patients in whom a laboratory or other test indicates infection despite a lack of symptoms. The principle is that delivery of therapy prior to development of symptoms aborts impending disease, and such therapy is used for a short and well-defined duration. Preemptive therapy may be particularly useful when there are concerns for drug toxicity with long-term use as prophylaxis. This strategy's most prominent application is in prevention of CMV disease after hematopoietic stem cell transplants and solid-organ transplantation, where detection of low-level viremia via PCR is possible and current antiviral therapies (e.g., *valganciclovir*) carry significant risks of cumulative toxicity (Razonable et al., 2019).

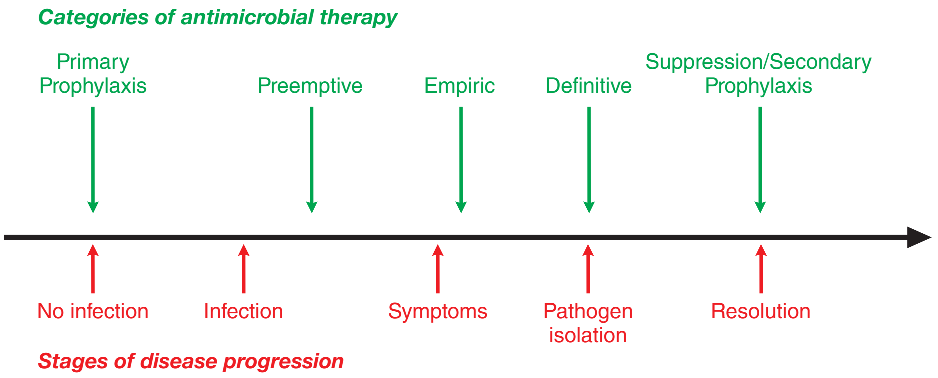


Figure 56–1 *Categories of antimicrobial therapy in relation to disease progression.*

Empiric Therapy

Empiric therapy is administered when an *infection is suspected but the specific causative organism and its susceptibility in that patient are not known*, with the antibiotics used based on the typical pathogens associated with the infectious syndrome(s). *Most use of antibiotics in clinical medicine is empiric use*. The rationale for this is 2-fold:

- Definitive identification and susceptibility of the causative microorganism(s) is typically delayed by at least 24 to 48 h from patient presentation (when microbiological results are able to be obtained), *and*
- For many infections, a delay in treatment until definitive identification of the infecting pathogen would be considered harmful to the patient.

For many subacute or chronic infections or acute infections of low severity, the risk in waiting a few days for definitive pathogen identification is low, and these patients can wait for more definitive microbiological evidence of infection without empirical treatment. If the risks of waiting are high, based on the nature of the infection, the patient's severity of illness, or the patient's immune status, then initiation of empiric antibiotic therapy should rely on the likely infectious syndrome, patient-specific risk factors (e.g., prior antibiotic use), and local epidemiology (e.g., prevalence of drug-resistant organisms). In some cases, it may be necessary to use a combination of antibiotics in order to achieve an adequate spectrum of activity for empiric coverage of the likely pathogens.

If the treating clinician wants to obtain samples for microbiological analysis to guide therapy, these samples are typically obtained during this period. It is optimal to obtain these samples before antimicrobials are administered, to improve the diagnostic yield; however, in some circumstances, it is not feasible to delay antimicrobial administration until diagnostic samples can be obtained.

Preliminary microbiology results may be available to allow tailoring of therapy before final microbiological data are available. The most valuable and time-tested method for early identification of bacteria is examination of the infected secretion or body fluid with Gram stain to identify the presence of gram-positive or gram-negative organisms. The predictive value of Gram staining varies by infection and specimen type but may be useful in reevaluating empirically selected regimens (e.g., if an empiric regimen has poor gram-positive coverage, the finding of gram-positive organisms in a sample may warrant expansion of the spectrum of activity). In malaria-endemic areas or in travelers returning from such an area, a simple thick-and-thin blood smear may mean the difference between a patient's survival on appropriate therapy or death while on the wrong therapy for a presumed bacterial infection. Rapid point-of-care diagnostic testing is increasingly available for a number of viral and bacterial infections. New technologies such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), nucleic amplification techniques, microarray detection, and morphokinetic cellular analysis may not be rapid enough to allow deferral of empiric therapy but can shorten the empiric therapy phase by providing microbiological information more rapidly than traditional approaches (Bauer et al., 2014).

Notably, in many situations, patients will receive empiric therapy for the entire duration of their treatment because the actual organism causing the patient's infection is never determined. This may be due to the high cost or invasiveness of microbiological sampling, short duration of antibiotic therapy, high predictability of the causative pathogen based on symptom presentation, or failure of microbiological samples to detect the pathogen. In these situations, monitoring for symptomatic response will determine whether more aggressive approaches to determine the microbiological etiology are required.

Definitive Therapy

If a pathogen has been identified and susceptibility results are available, the optimal antibiotic regimen for that patient should be selected—the *definitive* therapy. This may or may not require adjustment of the empiric regimen if one was initiated. Selecting an optimal definitive regimen requires balancing the need for potent activity against the pathogen, risks of untoward effects (e.g., toxicities or drug interactions), costs, practicality (e.g., number of doses administered per day), and the desire to minimize the contribution to patient- and population-level antimicrobial resistance. The latter consideration suggests that narrower-spectrum antibiotics are preferred over broader-spectrum agents when the other factors are more or less equivalent. When the initial empiric regimen is broader in spectrum than the definitive regimen, this consideration is often known as *streamlining* or *de-escalation*.

For definitive therapy, combination antibiotic therapy is an exception, rather than a rule. Once a pathogen has been isolated, monotherapy is preferred unless compelling data exist in favor of combination therapy. Using multiple antibiotics where a single agent should suffice can lead to increased toxicity and unnecessary damage to the patient's protective fungal and bacterial flora. There are, however, special circumstances where evidence favors combination therapy:

- Preventing emergence of resistance to monotherapy (e.g., combination antiretroviral therapy for HIV, multidrug regimens for treatment of active *Mycobacterium tuberculosis* infection)
- Accelerating the rapidity or extent of microbial kill (e.g., combining penicillins and aminoglycosides for treatment of severe enterococcal infections, combining *amphotericin B* and *flucytosine* in patients with cryptococcal meningitis)
- Reducing toxicity—when sufficient efficacy of a single antibacterial agent can be achieved only at doses that are toxic to the patient and a second drug is coadministered to permit lowering the dose of the first drug (e.g., the use of reduced-dose combinations of *ganciclovir* and *foscarnet* in treatment of some resistant CMV infections) (Mylonakis et al., 2002)

In some cases, the antibiotic combination is already incorporated into standard pharmaceutical preparations. For example, the combination of a sulfonamide and an inhibitor of DHFR, such as *trimethoprim*, is synergistic owing to the inhibition of sequential steps in microbial folate synthesis; the combination formulation of *sulfamethoxazole* and *trimethoprim* is more commonly used than either agent separately. Similarly, many combination anti-HIV regimens are now completely coformulated, often in a single daily pill.

Posttreatment Suppressive Therapy and Secondary Prophylaxis

In some patients, the infection is controlled but not completely eradicated by the initial round of antimicrobial treatment and/or the immunological or anatomical defect that led to the original infection is still present. In such patients, antibiotics may be continued as *suppressive therapy,* differentiated from definitive therapy by use of a lower dose, different route of administration, or different antibiotic. Examples include treatment of cryptococcal meningitis or treatment of infections of implanted prosthetic materials (e.g., a prosthetic hip) that cannot be removed and against which definitive therapy is unlikely to eradicate the infection. Among immunocompromised hosts, suppressive therapy may eventually be discontinued if the patient's immune system reconstitutes (e.g., with a sustained elevation in an HIV-infected patient's CD4 count). Some patients in whom pathogen eradication may be achieved may still be candidates for ongoing antibiotic use in the form of secondary prophylaxis if they are at high risk for a new infection. Risks of toxicity from prolonged use of suppressive therapy and secondary prophylaxis can be significant, and assessment for potential discontinuation should be performed regularly.

The Pharmacokinetic Basis of Antimicrobial Therapy

Typically, a pathogen causes disease not in the whole body but in specific organs. Within an infected organ, only specific pathological compartments may be infected. Antibiotics are often administered orally or parenterally, far away from these sites of infection. Therefore, in choosing an antimicrobial agent for therapy, a crucial consideration is whether the drug can penetrate to the site of infection. For example, the antibiotic *levofloxacin* achieves a ratio of peak concentrations in the skin tissue to plasma (C_{Pmax} ratio) of 1.4, a ratio of epithelial lining fluid (ELF) to plasma of 2.8, and a urine to plasma ratio of 67 (Chow et al., 2002; Conte et al., 2006; Wagenlehner et al., 2006). The two most important factors in predicting successful clinical and microbiological outcomes using *levofloxacin* are the site of infection and achieving a C_{Pmax} level of 12 times the MIC (C_{Pmax}/MIC ≥12). The failure rate of therapy is 0% in patients with urinary tract infections, 3% in patients with pulmonary infections, and 16% in patients with skin and soft-tissue infections (Preston et al., 1998). Clearly, the poorer the penetration into the anatomical compartment, the higher is the likelihood of failure.

The penetration of a drug into an anatomical compartment depends on the *physical barriers* that the molecule must traverse, the *chemical properties of the drug*, and the *presence of multidrug transporters*. Chapters 2 (pharmacokinetics) and 4 (membrane transporters) provide excellent discussions of these concepts. A unique consideration for drug penetration in treatment of infections is the presence of microorganism-produced *biofilms*. Examples of biofilms include endocardial vegetations on heart valves in endocarditis; biofilms formed by bacteria and fungi on prosthetic devices such as artificial heart valves, long-dwelling intravascular catheters, and artificial hips; and the biofilms formed within the lungs of patients suffering from cystic fibrosis. Bacterial and fungal biofilms are colonies of slowly growing cells enclosed within an exopolymer matrix. The exopolysaccharide is negatively charged and can bind positively charged antibiotics and restrict their access to the intended target. To be effective against infections in these compartments, antibiotics must penetrate the biofilm and endothelial barriers (Sun et al., 2013).

Impact of Susceptibility Testing on Success of Antimicrobial Agents

The microbiology laboratory plays a central role in the decision to choose a particular antibiotic agent over others. First, identification and isolation of the culprit organism take place when patient specimens are sent to the microbiology laboratory. Once the microbial species causing the disease has been identified, a more rational choice of the class of antibiotics likely to work in the patient can be made. The microbiology laboratory then plays a second role, which is to identify what antibiotics the organism isolated from that sample is susceptible to, allowing for definitive therapy.

Millions of individuals across the globe become infected by many different isolates of the same species of pathogen. Evolutionary processes cause each isolate to be slightly different from the next, so that each may have a unique susceptibility to antimicrobial agents. As the microorganisms divide within the patient, they may undergo further evolution between the time of infection and the time of diagnosis. Therefore, one observes a distribution of concentrations of antimicrobial agents that can kill the pathogens. Often, this distribution is Gaussian, with a skew that depends on local susceptibility patterns.

Because antimicrobial agents are ligands that bind to their targets to produce effects, the relationship between drug concentration and effect on a population of organisms is modeled using the standard Hill-type curve for receptor and agonist (see Chapters 2 and 3), characterized by three parameters:

- IC_{50} (also termed EC_{50}), the inhibitory concentration that is 50% effective, a measure of the antimicrobial agent's potency
- E_{max}, a measure of the maximal effect
- H, the slope of the curve, or Hill factor

With changes in susceptibility, the sigmoid E_{max} curve shifts in one of two basic ways. The first is a shift to the right, an increase in IC_{50} (Figure 56–2A), meaning that much higher concentrations of antimicrobials than before are now needed to show specific effect. *Susceptibility tests for bacteria, fungi, parasites, and viruses have been developed to determine whether these shifts have occurred at a sufficient magnitude to warrant higher doses of drug to achieve particular effect.* The change in IC_{50} may become so large that it is not possible to overcome the concentration deficit by increasing the antimicrobial dose without causing toxicity to the patient. At that stage, the organism is now "resistant" to the particular antibiotic.

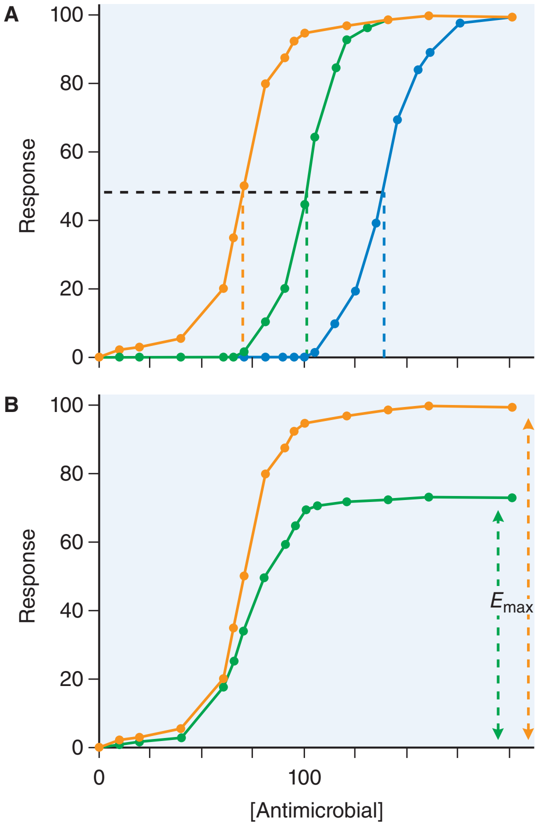


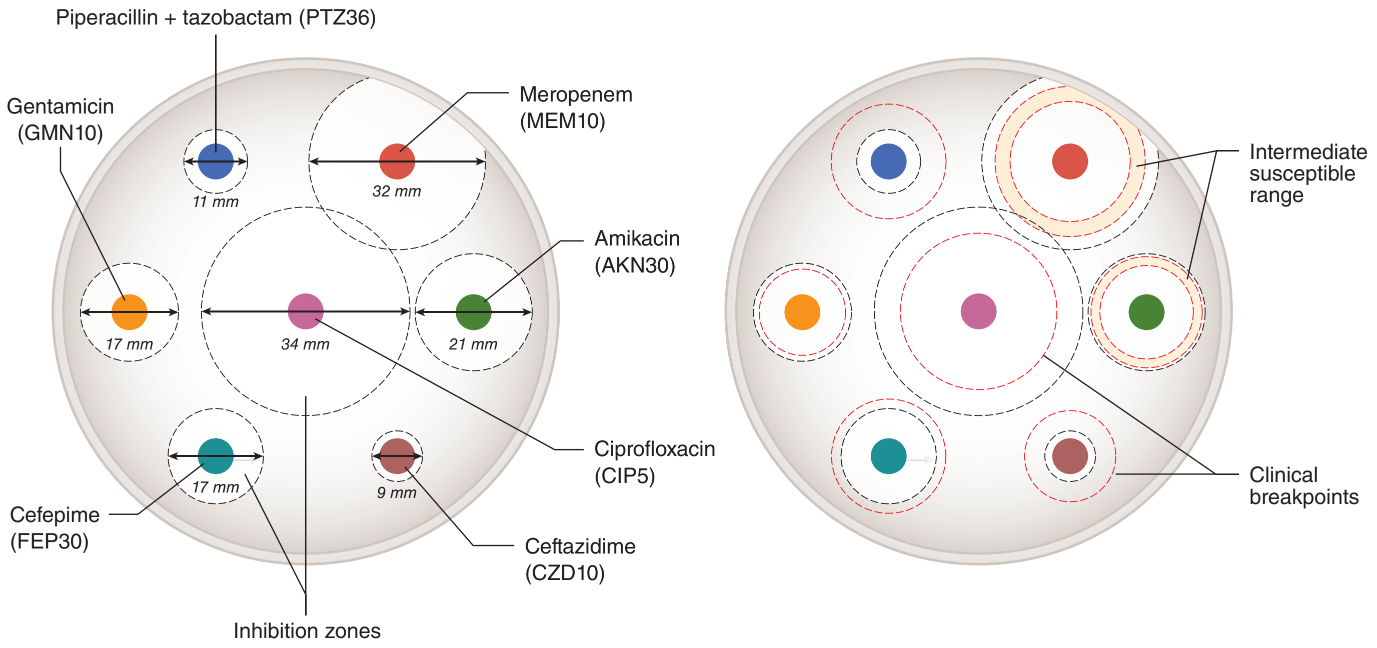
Figure 56–2 *Changes in sigmoid E_{max} model with increases in drug resistance.* An increase in resistance may show changes in IC_{50}: In **A**, the IC_{50} increases from 70 (orange line) to 100 (green line) to 140 (blue line). An increase in resistance may also show a decrease in E_{max}: In **B**, efficacy decreases from full response (orange line) to 70% (green line).

A second possible change in the curve is decrease in E_{max} (Figure 56–2B), such that increasing the dose of the antimicrobial agent beyond a certain point will achieve no further effect; that is, changes in the microbe are such that eradication of the microbe by the particular drug can never be achieved. This occurs because the available target proteins have been reduced or the microbe has developed an alternative pathway to overcome the biochemical inhibition. For example, *maraviroc* is an allosteric, noncompetitive antagonist that binds to the CCR5 receptor of a patient's CD4 cells to deny HIV entry into the cell. Viral resistance occurs by a mechanism that involves HIV adapting to use of the *maraviroc*-bound CCR5, which results in a decrease of E_{max} in phenotypic susceptibility assays (Hirsch et al., 2008).

Bacteria

For bacteria, *diffusion tests* use antimicrobial-impregnated disks placed on a solid growth medium upon which the bacterium of interest has been plated and allowed to incubate for 12 to 24 h (Figure 56–3A). The size of the zone of inhibition (area without bacterial growth) around each disk is given a categorical interpretation of susceptible, intermediate, or resistant. These interpretations are based on consensus *breakpoints* that have been established that relate the size of the zone of inhibition to the predicted clinical utility of the drug. In contrast, *dilution tests* employ antibiotics in serially diluted concentrations in a liquid broth medium that contains a culture of the test microorganism (Figure 56–3B).

A Disk diffusion test


Piperacillin + tazobactam (PTZ36)
Gentamicin (GMN10)
Meropenem (MEM10)
11 mm
32 mm
Amikacin (AKN30)
17 mm
34 mm
21 mm
Ciprofloxacin (CIP5)
17 mm
9 mm
Cefepime (FEP30)
Ceftazidime (CZD10)
Inhibition zones
Intermediate susceptible range
Clinical breakpoints

B Minimal Inhibitory Concentration (MIC)

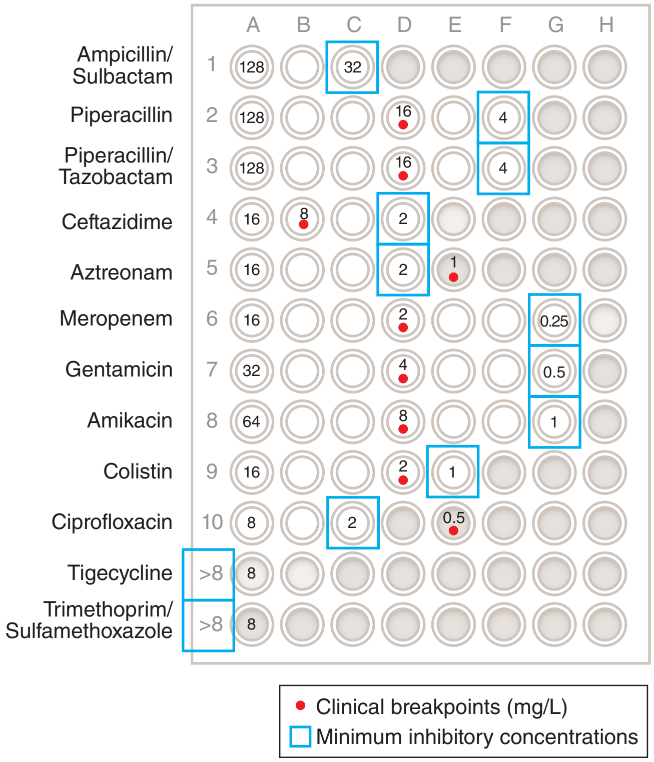

A B C D E F G H
1 Ampicillin/Sulbactam 128 32
2 Piperacillin 128 16 4
3 Piperacillin/Tazobactam 128 16 4
4 Ceftazidime 16 8 2
5 Aztreonam 16 2 1
6 Meropenem 16 2 0.25
7 Gentamicin 32 4 0.5
8 Amikacin 64 8 1
9 Colistin 16 2 1
10 Ciprofloxacin 8 2 0.5
Tigecycline >8 8
Trimethoprim/Sulfamethoxazole >8 8
● Clinical breakpoints (mg/L)
□ Minimum inhibitory concentrations

Figure 56–3 *Antibiotic susceptibility testing methods.* Once a bacterium is isolated from a clinical sample and grown in culture, it is incubated with antibiotics to determine if those drugs inhibit its growth. In **A**, *disk diffusion* testing is used. A solid agar plate is covered with a lawn of bacteria, and antibiotic-impregnated disks are placed on it. The area around the bacteria without visible growth is the zone of inhibition and used to determine susceptibility. In **B**, *dilution* testing is used. A sample of the bacterium is added to microtiter plates containing variable concentrations of antibiotics of interest. After incubation, the wells with the lowest concentration of an agent that has prevented visible growth are considered to represent the *minimum inhibitory concentration* (MIC) for the drug-organism pair. If the MIC is less than the clinical breakpoint, the isolate is considered to be susceptible to that antibiotic.

The lowest concentration of the agent that prevents visible growth after 18 to 24 h of incubation is the MIC, which is typically measured in doubling concentrations. In clinical laboratories, dilution tests are performed on commercial platforms that automate many of the steps in preparation, incubation, and interpretation. Interpretations are made by comparing the MIC obtained for each drug to the consensus breakpoints established for that drug-organism pair. When the MIC measured for an isolate is at or below the breakpoint for that drug-organism pair, the isolate is considered to be susceptible to that drug.

Recently, nucleic acid amplification–based reactions of specific bacterial genes have been used in the clinic for rapid diagnosis of drug resistance. The genes targeted are those encoding known drug resistance proteins or processes. For example, *rifampin* resistance in *M. tuberculosis* has been difficult to ascertain in a timely fashion: The bacteria take 2 to 3 weeks to grow in order to identify them as a cause of disease, and then a similar amount of time is needed to perform some version of the broth dilution tests. Small PCR reactors at points of care can purify and concentrate a patient's fluid sample, perform nucleic acid amplification of a target gene, identify mutations, and provide a result in less than 2 h. Similarly, PCR-based rapid identification of the *mecA* gene responsible for *methicillin* resistance in *S. aureus* in clinical or surveillance samples is frequently employed for infection control and clinical care in hospitals.

Fungi

For fungi that are yeasts (i.e., *Candida*), susceptibility testing methods are similar to those used for bacteria. However, the definitions of MIC differ based on drug and the type of yeast, so there are cutoff points of 50% decrease in turbidity compared to controls at 24 h, 80% at 48 h, or total clearance of the turbidity. Susceptibility tests and MICs for triazoles (e.g., *fluconazole*) have been extensively shown to correlate with clinical outcomes. Standardized tests for echinocandin antifungals and *amphotericin B*–based compounds are also available.

Susceptibility tests for molds have been developed, especially for *Aspergillus* species. Different terminology is required when evaluating echinocandins against molds because the fungal burden cannot be readily measured, given that hyphae will break up into unpredictable numbers of discrete fungi under antifungal pressure. Furthermore, echinocandins often do not completely inhibit mold growth, but instead cause damage reflected by morphological changes in hyphae. Thus, the *minimum effective concentration* (MEC) for echinocandins is the lowest drug concentration at which short, stubby, and highly branched hyphae are observed on microscopic examination.

Viruses

In HIV phenotypic assays, the patient's HIV-RNA is extracted from plasma, and genes for targets of antiretroviral drugs such as reverse transcriptase and protease are amplified. The genes are then inserted into a standard HIV vector that lacks analogous gene sequences to produce a recombinant virus, which is coincubated with a drug of interest in a mammalian cell viability assay (Hanna and D'Aquila, 2001; Petropoulos et al., 2000). Growth is compared to a standardized wild-type control virus. Phenotypic assays are laborious and time-consuming, and genotypic testing is more commonly employed. These tests aim to detect the presence of mutations that are predicted to result in reduced phenotypic susceptibility. Where they are accessible, genotypic assays are a standard of care for HIV management and are also used to detect resistance-associated mutations in pathogens such as CMV.

Parasites

Susceptibility testing for parasites, especially those that cause malaria, has been performed in the laboratory. *Plasmodium* species in the patient's blood are cultured *ex vivo* in the presence of different dilutions of antimalarial drug. A sigmoid E_{max} curve for effect versus drug concentration is used to identify IC_{50} and E_{max}. These susceptibility tests are usually field tests at sentinel sites that are used to determine if there is drug resistance in a particular area. In general, susceptibility tests for parasitic infections are not standardized. These tests are primarily used in the research setting and not for individualization of therapy.

Basis for Selection of Dose and Dosing Schedule

Although susceptibility testing in the laboratory is central to decision making, it does not completely predict patient response. In susceptibility tests, the drug concentration is constant; by contrast, in patients, the drug concentration is dynamic and ever changing. Antibiotics are prescribed at a certain schedule (e.g., three times a day) so that there is a periodicity in the fluctuations of drug at the site of infection, and the microbe is exposed to a particular shape of the concentration-time curve. Harry Eagle performed studies on *penicillin* and discovered that the shape of the concentration-time profile was an important determinant of the efficacy of the antibiotic. This important observation was forgotten until William Craig and colleagues rediscovered it and performed systematic studies on several classes of antibiotics, initiating the era of antimicrobial PK/PD (Ambrose et al., 2007; Craig, 2007). These findings have now been extended to combination therapy and to microbes that require long treatment durations, such as *M. tuberculosis* and HIV.

As an example, consider an antibiotic with a serum $t_{1/2}$ of 3 h that is being used to treat a bloodstream infection by a pathogen with an MIC of 0.5 mg/L; the antibiotic is administered with a dosing interval of 24 h (that is, a once-daily schedule). Figure 56–4A depicts the concentration-time curve of the antibiotic, with definitions of C_{Pmax}, AUC, and the fraction of the dosing interval for which the drug concentration remains above the MIC (T > MIC), as shown. The AUC is a measure of the total

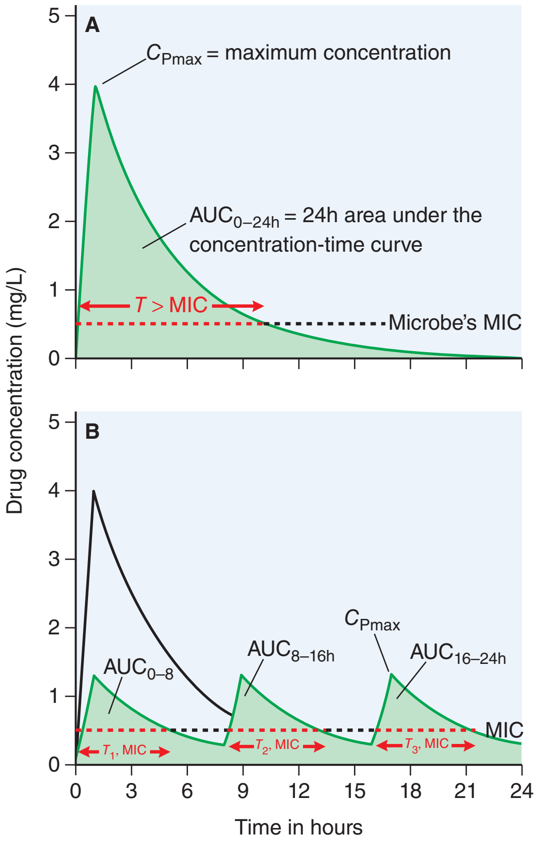


Figure 56–4 *Effect of different dose schedules on shape of the concentration-time curve.* The same total dose of a drug was administered as a single dose (panel **A**) and in three equal portions every 8 h (panel **B**). The total AUC for the fractionated dose in **B** is determined by adding AUC_{0-8h}, AUC_{8-16h}, and AUC_{16-24h}, which totals to the same AUC_{0-24h} in **A**. The time that the drug concentration exceeds MIC in **B** is also determined by adding T_1 > MIC, T_2 > MIC, and T_3 > MIC, which results in a fraction greater than that for **A**.

concentration of drug and is calculated by taking an integral between two time points, 0 to 24 h ($AUC_{0–24}$) in this case.

Now, if one were to change the dosing schedule of the same antibiotic amount by splitting it into three equal doses administered at 0, 8, and 16 h, the shape of the concentration-time curve changes to that shown in Figure 56–4B. Because the same cumulative dose has been given for the dosing interval of 24 h, the $AUC_{0–24}$ will be similar whether it was given once a day or three times a day. For the same pathogen, therefore, the change in dose schedule does not change the $AUC_{0–24}$/MIC. However, the C_{Pmax} will decrease by a third when the total dose is split into thirds and administered more frequently (Figure 56–4B). Thus, when a dose is fractionated and administered more frequently, the C_{Pmax}/MIC ratio *decreases*. In contrast, the time that the drug concentration persists above MIC (T > MIC) will *increase* with the more frequent dosing schedule, despite the same cumulative dose being administered.

Some classes of antimicrobial agents exert greater antimicrobial effects when their concentration persists above the MIC for longer durations of the dosing interval. Indeed, increasing the drug concentration beyond four to six times the MIC does not increase microbial kill for such antibiotics. Two good examples are β-lactam antibacterials (e.g., *penicillin*) and the antifungal agent 5-fluorocytosine (Ambrose et al., 2007; Andes and van Ogtrop, 2000). There are usually biochemical explanations for this pattern; the clinical implication, however, is that a drug optimized by T > MIC should be dosed more frequently; given as a prolonged, instead of rapid, infusion; or have its $t_{1/2}$ prolonged by other drugs (as with the coadministration of *probenecid* with *penicillin*), so that drug concentrations persist above MIC (or EC_{95}) as long as possible. Thus, the effectiveness of *penicillin* is enhanced when it is given as a continuous infusion. Some antibiotics, such as *ceftriaxone* ($t_{1/2}$ = 8 h), have long half-lives, such that infrequent dosing still allows maintenance of an adequate T > MIC. HIV protease inhibitors are often “boosted” with *ritonavir* or *cobicistat*. This “boosting” inhibits the metabolism of the protease inhibitors by CYPs 3A4 and 2D6, thereby prolonging time above EC_{95}.

Conversely, the peak concentration is most predictive of efficacy for other antimicrobial agents. Persistence of concentration above the MIC has less relevance for these drugs—described as having “time-independent killing,” meaning that these drugs can be dosed more intermittently. Aminoglycosides are a prime example of this class; aminoglycosides are highly effective when given once a day at sufficient dosage, despite their short half-lives. These C_{Pmax}/MIC–linked drugs can often be administered less frequently due to their long duration of PAE (post antibiotic effect), with effectiveness continuing long after antibiotic concentrations decline below the MIC.

Rifampin is such another such drug (Gumbo et al., 2007a). The entry of *rifampin* into *M. tuberculosis* increases with increased concentration in the bacillus microenvironment, likely because of a saturable transport process. Once inside the bacteria, the drug’s macrocyclic ring binds the β subunit of DNA-dependent RNA polymerase (*rpoB*) to form a stable drug-enzyme complex within 10 min, a process not enhanced by longer incubation of drug and enzyme and only slowly reversed. The PAE of the *rifampin* is long and concentration dependent (Gumbo et al., 2007a).

There is a third group of *drugs for which it is the cumulative dose that matters most and for which the daily dosing schedule has no effect on efficacy*. Thus, it is the ratio of the total concentration (AUC) to MIC that is most predictive of effect and not the time that concentration persists above a certain threshold. Antibacterial agents such as *daptomycin* fall into this class (Louie et al., 2001). These agents also have a long PAE.

The shape of the concentration-time curve that optimizes resistance suppression is often different from that which optimizes microbial kill. In many instances, the drug exposure required for resistance suppression is much higher than that for optimal kill. Ideally, this higher exposure should be achieved by each dose for optimal effect, rather than the EC_{80}, as discussed previously. However, this is often precluded by drug toxicity at higher dosages. Second, although the relationship between kill and exposure is based on the inhibitory sigmoid E_{max} model, experimental work with preclinical models demonstrated that this model does not apply to resistance suppression (Gumbo et al., 2007b; Tam et al., 2007).

Mechanisms of Resistance to Antimicrobial Agents

Antibiotics were viewed as miracle cures when first introduced into clinical practice. However, as became evident soon after the discovery of *penicillin*, resistance eventually develops and dims the luster of the miracle. Today, every major class of antibiotic is associated with the emergence of significant resistance. When a microbial species is subjected to an existential threat, chemical or otherwise, that pressure will select for random mutations in the species’ genome that permit survival. This evolution is greatly assisted by poor therapeutic practices by healthcare workers and the indiscriminant use of antibiotics in agriculture and animal husbandry.

Antimicrobial resistance can develop at any one or more of steps in the processes by which a drug reaches and combines with its target. Major mechanisms of antibiotic resistance include:

- Reduced concentration of the antibiotic at its target site
- Production of microbial enzymes that alter or destroy the antibiotic
- Alteration of antibiotic targets in ways that reduce antibiotic affinity

A host of less-common mechanisms have been discovered as well, including bypass of inhibited metabolic pathways, excision of antibiotic-target complexes, and overproduction of target enzymes. Organisms may also express resistance elements that interfere with the immune response; this can result in an effect similar to antibiotic resistance, as antibiotics typically work in concert with the immune system to clear infections (Sun et al., 2021). More than one mechanism may work in concert to confer resistance to an individual antibiotic.

Resistance Due to Reduced Concentration of Drug at Its Target Site

The outer membrane of gram-negative bacteria is a semipermeable barrier that excludes large polar molecules from entering the cell. Small polar molecules, including many antibiotics, enter the cell through protein channels called *porins*. Absence of, mutation in, or loss of a favored porin channel can slow the rate of drug entry into a cell or prevent entry altogether, effectively reducing drug concentration at the target site. If the target is intracellular and the drug requires active transport across the cell membrane, a mutation or phenotypic change that slows or abolishes this transport mechanism can confer resistance.

Once an antibiotic crosses the cell membrane, its concentration may be reduced below the effective concentration through the action of *efflux pumps*, energy-dependent transporters that expel antibiotics to which the microbes would otherwise be susceptible. There are five major systems of efflux pumps that are relevant to antimicrobial agents:

- The multidrug and toxin extruder
- The major facilitator superfamily transporters
- The small multidrug resistance system
- The resistance nodulation division exporters
- ABC transporters

Resistance due to reduced concentrations of drug at the site of infection is a prominent mechanism of resistance for parasites, bacteria, and fungi, and can function selectively or broadly. For example, in *Pseudomonas aeruginosa*, resistance to the antipseudomonal carbapenem *imipenem* is significantly mediated through mutational loss of the OprD porin, the primary means by which *imipenem* crosses the outer membrane (Fernandez and Hancock, 2012). In contrast, *meropenem* is only minimally affected by isolated loss of OprD, but its activity is significantly reduced by upregulation of efflux pump activity, such as that of the MexA-MexB-OprM system. Upregulation of these efflux systems has less impact on *imipenem* but tends to raise MICs for a broader array of antibiotics including cephalosporins and aminoglycosides.

Resistance Due to Alteration or Destruction of Antibiotic

Drug inactivation is a common mechanism of drug resistance. The most prominent example is the enzymatic inactivation of β-lactam antibiotics through the function of β-lactamase enzymes (Bush, 2018). Over a thousand distinct β-lactamases have been identified, some of which can confer resistance to nearly all β-lactams. In some cases, β-lactamase-mediated resistance can be averted by coadministration of β-lactamase inhibitors (e.g., *clavulanate*, *avibactam*). Other examples of enzymatic inactivation leading to resistance include production of aminoglycoside-modifying enzymes and esterification of macrolides.

Resistance Due to Altered Target Structure

A common consequence of either single or multiple point mutations is a change in amino acid composition and conformation of an antimicrobial's target protein. This change can lead to reduced affinity of drug for its target or of a prodrug for the enzyme that activates the prodrug. Such alterations may be due to mutation of the natural target (e.g., fluoroquinolone resistance), enzyme-mediated target modification (e.g., ribosomal protection type of resistance to macrolides and tetracyclines), or acquisition of a resistant form of the native, susceptible target (e.g., staphylococcal *methicillin* resistance caused by production of a low-affinity penicillin-binding protein) (Hooper, 2002; Lim and Strynadka, 2002; Nakajima, 1999). In HIV resistance, mutations associated with reduced affinity are encountered for protease inhibitors, integrase inhibitors, fusion inhibitors, and nonnucleoside reverse transcriptase inhibitors (Nijhuis et al., 2009). Similarly, benzimidazoles are used against myriad worms and protozoa and work by binding to the parasite's tubulin; point mutations in the β-tubulin gene lead to modification of the tubulin and drug resistance (Ouellette, 2001).

Heteroresistance and Viral Quasi-Species

Heteroresistance occurs when a subset of the total microbial population is resistant, despite the total population being considered susceptible on testing (Falagas et al., 2008; Rinder, 2001). A subclone that has alterations in genes associated with drug resistance is expected to reflect the normal mutation rates (occurrence in 1 in 10^6 to 10^5 colonies). In bacteria, heteroresistance has been described especially for *vancomycin* in *S. aureus* and *Enterococcus faecium*; *colistin* in *Acinetobacter baumannii-calcoaceticus*; *rifampin*, *isoniazid*, and *streptomycin* in *M. tuberculosis*; and *penicillin* in *S. pneumoniae* (Falagas et al., 2008; Rinder, 2001). Increased therapeutic failures and mortality have been reported in patients with heteroresistant staphylococci and *M. tuberculosis* (Falagas et al., 2008; Hofmann-Thiel et al., 2009). For fungi, heteroresistance leading to clinical failure has been described for *fluconazole* in *Cryptococcus neoformans* and *Candida albicans* (Marr et al., 2001; Mondon et al., 1999).

Viral replication is more error prone than replication in bacteria and fungi. Viral evolution under drug and immune pressure occurs relatively easily, commonly resulting in variants or quasi-species that may contain drug-resistant subpopulations. This is often not termed heteroresistance, but the principle is the same: A virus may be considered susceptible to a drug because either phenotypic or genotypic tests reveal "lack" of resistance, even though there is a resistant subpopulation just below the limit of assay detection. These minority quasi-species that are resistant to antiretroviral agents have been associated with failure of antiretroviral therapy (Metzner et al., 2009).

EVOLUTIONARY BASIS OF RESISTANCE EMERGENCE

Development of Resistance via Mutation Selection

Genetic mechanisms by which antibiotic resistance develops can include acquisition of genetic elements that code for the resistant mechanism, mutations that develop under antibiotic pressure, or constitutive induction. Mutations are random events that confer a survival advantage when drug is present. Mutation and antibiotic selection of resistant mutants are the molecular basis for resistance for many bacteria, viruses, and fungi. Mutations may occur in:

- A gene encoding the target protein, altering its structure so that it no longer binds the drug
- A gene encoding a protein involved in drug transport
- A gene encoding a protein important for drug activation or inactivation
- A regulatory gene or promoter affecting expression of the target, a transport protein, or an inactivating enzyme

In some instances, a single-step mutation results in a high degree of resistance. In *M. tuberculosis katG,* Ser315 mutations cause resistance to *isoniazid*; the M814V mutation in the reverse transcriptase gene of HIV-1 causes resistance to *lamivudine*; and *C. albicans fks1* Ser645 mutations cause resistance to echinocandins.

In other circumstances, however, it is the sequential acquisition of multiple mutations that leads to clinically significant resistance. For example, the combination of *pyrimethamine* (an inhibitor of DHFR) and *sulfadoxine* (an inhibitor of DHPS) blocks the folate biosynthetic pathway in *P. falciparum*. Clinically meaningful resistance occurs only when there is a single-point mutation in the *DHPS* gene accompanied by at least a double mutation in the *DHFR* gene.

Resistance by External Acquisition of Genetic Elements

As noted above, drug resistance may be acquired by mutation and selection, with passage of the trait *vertically* to daughter cells, provided the mutation is not lethal, does not appreciably alter virulence, and does not affect replication by the progeny. Drug resistance may also be acquired by *horizontal transfer* of resistance determinants from a donor cell, often of another bacterial species, by *transduction, transformation,* or *conjugation*. Resistance acquired by horizontal transfer can disseminate rapidly and widely either by clonal spread of the resistant strain or by subsequent transfers to other susceptible recipient strains. Horizontal transfer of resistance offers several advantages over mutation selection. Lethal mutation of an essential gene is avoided; the level of resistance often is higher than that produced by mutation, which tends to yield incremental changes. The gene, which still can be transmitted vertically, can be mobilized and rapidly amplified within a population by transfer to susceptible cells, and the resistance gene can be eliminated when it no longer offers a selective advantage. Horizontal transfer of resistance genes is greatly facilitated by mobile genetic elements. Mobile genetic elements include plasmids and transducing phages. Other mobile elements—*transposable elements, integrons,* and *gene cassettes*—also participate. *Transposable elements* are of three general types: *insertion sequences, transposons,* and *transposable phages*.

Resistance Transfer in Action

A startling example of how the transfer mechanisms spread resistance is the recent description of the plasmid-mediated colistin resistance gene (*mcr-1*), which confers resistance to one of the last-resort antibiotics for multidrug-resistant gram-negative bacteria (Liu et al., 2016). *Colistin* is used in agriculture and animal husbandry. *Escherichia coli* strains carrying the *mcr-1* gene were found in pigs, then in pork, and then in patients. The plasmid carrying *mcr-1* was mobilized by conjugation to *E. coli* at a frequency of 10^{-1} to 10^{-3} cells per recipient and could be spread and maintained in other gram-negative rods of clinical significance. The resistant bacteria were initially identified in China, but within months, isolates were also identified in North America, South America, Europe, East Asia, and Africa and in other organisms, such as *Salmonella typhimurium*. The gene has now been demonstrated in gut microbiota of healthy individuals, suggesting integration in the human gut and the capacity to spread to organisms in the human microbiome.

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Chapter 57

DNA Disruptors: Sulfonamides, Quinolones, and Nitroimidazoles

Conan MacDougall

SULFONAMIDES
- Mechanism of Action
- Synergists of Sulfonamides
- Antimicrobial Activity
- Bacterial Resistance
- ADME
- Pharmacological Properties of Individual Sulfonamides
- Therapeutic Uses
- Adverse Reactions
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TRIMETHOPRIM-SULFAMETHOXAZOLE
- Mechanism of Action
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THE QUINOLONES
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- Therapeutic Uses
- Adverse Effects
- Drug Interactions

NITROIMIDAZOLES
- Metronidazole

Sulfonamides

HISTORICAL PERSPECTIVE

The sulfonamide drugs were the first effective chemotherapeutic agents used systemically for the prevention and cure of bacterial infections in humans. Investigations in 1932 at the I. G. Farbenindustrie in Germany resulted in the patenting of *prontosil* and several other azo dyes containing a sulfonamide group. Because synthetic azo dyes had been studied for their action against streptococci, Domagk tested the new compounds and observed that mice with streptococcal and other infections could be protected by *prontosil*. In 1933, Foerster reported giving *prontosil* to a 10-month-old infant with staphylococcal septicemia and achieving a dramatic cure. Favorable clinical results with *prontosil* and its active metabolite, *sulfanilamide*, in puerperal sepsis and meningococcal infections awakened the medical profession to the new field of antibacterial chemotherapy, and experimental and clinical articles soon appeared in profusion. The development of the carbonic anhydrase inhibitor–type diuretics and the sulfonylurea hypoglycemic agents followed from observations made with the sulfonamide antibiotics. For discovering the chemotherapeutic value of *prontosil*, Domagk was awarded the Nobel Prize in Medicine for 1938 (Lesch, 2007). The advent of *penicillin* and other antibiotics diminished the usefulness of the sulfonamides, but the introduction of the combination of *trimethoprim* (TMP) and *sulfamethoxazole* (SMX) in the 1970s increased the use of sulfonamides for the prophylaxis and treatment of infections.

Sulfonamides are derivatives of *para*-aminobenzenesulfonamide (*sulfanilamide*; Figure 57–1) and are congeners of *para*-aminobenzoic acid (PABA). Most of them are relatively insoluble in water, but their sodium salts are readily soluble. The minimal structural prerequisites for antibacterial action are all embodied in *sulfanilamide* itself. The sulfur must be linked directly to the benzene ring. The *para*-NH_2 group (the N of which has been designated as N4) is essential and can be replaced only by moieties that can be converted *in vivo* to a free amino group. Substitutions made in the amide NH_2 group (position N1) have variable effects on antibacterial activity of the molecule; substitution of heterocyclic aromatic nuclei at N1 yields highly potent compounds. The sulfone agent *dapsone* is discussed in Chapter 65.

Mechanism of Action

Sulfonamides are competitive inhibitors of *dihydropteroate synthase*, the bacterial enzyme responsible for the incorporation of PABA into *dihydropteroic acid*, the immediate precursor of *folic acid* (Figure 57–2). Sensitive microorganisms are those that must synthesize their own folic acid; those that can use preformed folate are not affected. Sulfonamides administered as single agents are typically *bacteriostatic*; cellular and humoral defense mechanisms of the host are essential for final eradication of the infection. Toxicity is selective for nonmammalian cells because mammalian cells require preformed folic acid, cannot synthesize it, and are thus insensitive to drugs acting by this mechanism (Grayson, 2010).

Synergists of Sulfonamides

Trimethoprim (TMP) exerts a synergistic effect with sulfonamides. It is a potent and selective competitive inhibitor of microbial *dihydrofolate reductase*, the enzyme that reduces *dihydrofolate* to *tetrahydrofolate*, which is required for one-carbon transfer reactions. Coadministration of a sulfonamide and TMP (e.g., trimethoprim-sulfamethoxazole [TMP-SMX]) introduces sequential blocks in the biosynthetic pathway for tetrahydrofolate (see Figure 57–2); the combination is much more effective than either agent alone (Bushby and Hitchings, 1968). Similar complementary activity is seen with *pyrimethamine*, which is generally used in combination with agents such as *sulfadoxine*, *sulfadiazine*, or *dapsone*. The predominant systemic use of sulfonamides is now in such combinations.

Antimicrobial Activity

On their original introduction to therapeutic use, sulfonamides had a wide range of antimicrobial activity against both gram-positive and

Abbreviations

AIDS: acquired immunodeficiency syndrome
CSF: cerebrospinal fluid
DHFR: dihydrofolate reductase
GI: gastrointestinal
HIV: human immunodeficiency virus
PABA: *para*-aminobenzoic acid
TMP: trimethoprim
SMX: sulfamethoxazole
UTI: urinary tract infection

gram-negative bacteria; a high percentage of isolates of *Streptococcus pyogenes*, *Streptococcus pneumoniae, Staphylococcus aureus*, and *Haemophilus influenzae* were susceptible to systemically achievable concentrations of sulfonamides. However, the increase in sulfonamide resistance is such that sulfonamide activity against these pathogens in serious infections cannot be assumed, and they play little part in empiric therapy (Grayson, 2010). Potent activity remains against most isolates of *Haemophilus ducreyi, Nocardia* spp., and *Klebsiella granulomatis*. Isolates of *Neisseria meningitidis* and *Shigella* are generally resistant, as are many strains of *Escherichia coli* isolated from patients with urinary tract infections (UTIs) (Olson et al., 2009). Sulfonamides and derivatives also possess important activity against parasites and fungi, and those applications are further discussed in Chapters 61, 65, and 66.

Bacterial Resistance

Bacterial resistance to sulfonamides can originate by random mutation and selection or by transfer of resistance by plasmids; it usually does not involve cross-resistance to other classes of antibiotics except to the extent that other resistance elements may be carried on mobile elements such as plasmids. Resistance to folate antagonists can result from (1) a lower affinity of dihydropteroate synthase for sulfonamides, (2) decreased bacterial permeability or active efflux of the drug, (3) an alternative metabolic pathway for synthesis of an essential metabolite, or (4) increased production of an essential metabolite or drug antagonist (e.g., PABA) (Estrada et al., 2016). Plasmid-mediated resistance is due to plasmid-encoded, drug-resistant dihydropteroate synthetase.

ADME

Except for sulfonamides especially designed for their local effects in the bowel (see Chapter 55), this class of drugs is absorbed rapidly from the gastrointestinal (GI) tract. Typically, 70% to 100% of an oral dose is absorbed and can be found in the urine within 30 min of ingestion. Peak plasma levels are achieved in 2 to 6 h, depending on the drug. Peak plasma drug concentrations achievable *in vivo* are about 100 to 200 μg/mL. The small intestine is the major site of absorption, but some of the drug is absorbed from the stomach. Absorption from other sites, such as the vagina, respiratory tract, or abraded skin, is variable and unreliable, but a sufficient amount may enter the body to cause toxic reactions in susceptible persons or to produce sensitization.

All sulfonamides are bound in varying degree to plasma proteins, particularly to albumin. Sulfonamides are distributed throughout all tissues of the body. The sulfonamides readily enter pleural, peritoneal, synovial, ocular, and similar body fluids and may reach concentrations therein that are 50% to 80% of the simultaneously determined concentration in blood. Because the protein content of body fluids usually is low, the drug is present in the unbound active form. After systemic administration of adequate doses, *sulfadiazine* and *sulfisoxazole* attain concentrations in cerebrospinal fluid (CSF) that may be effective in meningitis. However, because of the emergence of sulfonamide-resistant microorganisms, these drugs are used rarely for the treatment of meningitis. Sulfonamides pass readily through the placenta and reach the fetal circulation. The concentrations attained in the fetal tissues may cause both antibacterial and toxic effects.

Sulfonamides are metabolized in the liver. The major metabolite is the N4-acetylated sulfonamide. Acetylation results in products that have no antibacterial activity but retain the toxic potential of the parent substance. Sulfonamides are eliminated from the body partly as the unchanged drug and partly as metabolic products. The largest fraction is excreted in the urine, and the $t_{1/2}$ depends on renal function. In acid urine, the older sulfonamides are insoluble, and crystalline deposits may form. Small amounts are eliminated in the feces, bile, milk, and other secretions.

Pharmacological Properties of Individual Sulfonamides

Sulfonamides for Systemic Use

Sulfisoxazole. *Sulfisoxazole* is a rapidly absorbed and excreted sulfonamide. It is bound extensively to plasma proteins. Following an oral dose of 2 to 4 g, peak concentrations in plasma of 110 to 250 μg/mL are found

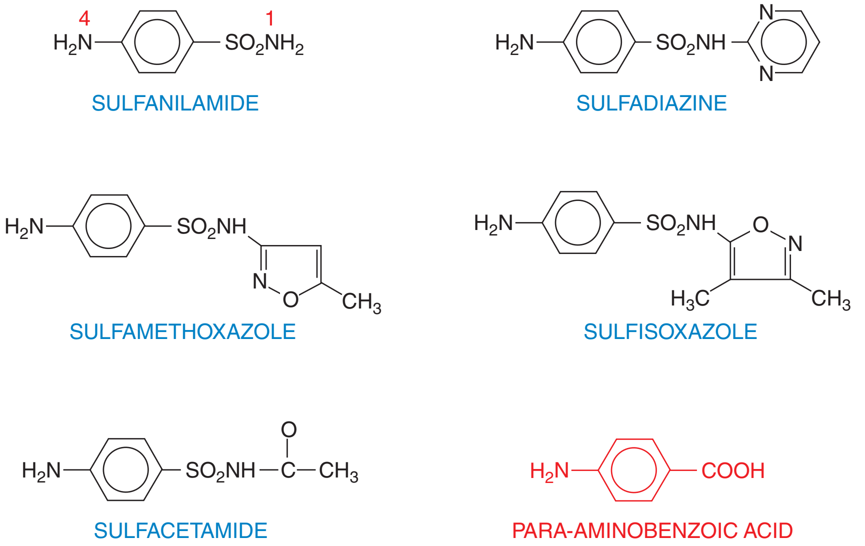


Figure 57–1 *Sulfanilamide and PABA.* Sulfonamides are derivatives of *sulfanilamide* and act by virtue of being congeners of PABA. The antimicrobial and dermatological anti-inflammatory agent *dapsone* (4,4′-diaminodiphenyl sulfone; see Chapters 65 and 75) also bears a resemblance to PABA and *sulfanilamide*.

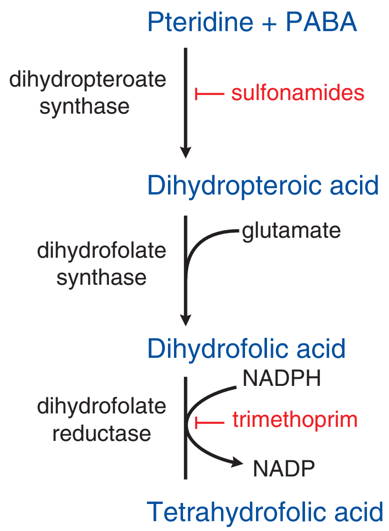


Figure 57–2 *Steps in folate metabolism blocked by sulfonamides and trimethoprim.* Coadministration of a sulfonamide and *trimethoprim* introduces sequential blocks in the biosynthetic pathway for tetrahydrofolate; the combination is much more effective than either agent alone.

in 2 to 4 h. Approximately 30% of *sulfisoxazole* in the blood and about 30% in the urine is in the acetylated form. The kidney excretes about 95% of a single dose in 24 h. Concentrations of the drug in urine thus greatly exceed those in blood and may be bactericidal. *Sulfisoxazole acetyl* is tasteless and hence preferred for oral use in children. *Sulfisoxazole acetyl* in combination with *erythromycin ethylsuccinate* is used in children with otitis media.

Sulfamethoxazole. *Sulfamethoxazole* is a close congener of *sulfisoxazole*, but its rates of enteric absorption and urinary excretion are slower ($t_{1/2}$ of 11 h). It is administered orally and employed for both systemic infections and UTIs. Precautions must be observed to avoid *sulfamethoxazole*-induced crystalluria because of the high percentage of the acetylated, relatively insoluble form of the drug in the urine. The clinical uses of *sulfamethoxazole* as a single agent are the same as those for *sulfisoxazole*. In the U.S., it is marketed only in fixed-dose combinations with TMP (discussed further in the following section Trimethoprim-Sulfamethoxazole).

Sulfadiazine. *Sulfadiazine* given orally is absorbed rapidly from the GI tract. Peak blood concentrations are reached within 3 to 6 h, with a $t_{1/2}$ of 10 h. About 55% of the drug is bound to plasma protein. Therapeutic concentrations are attained in CSF within 4 h of a single oral dose of 60 mg/kg. Both free and acetylated forms of *sulfadiazine* are readily excreted by the kidney; 15% to 40% of the excreted drug is in acetylated form. Alkalinization of the urine accelerates the renal clearance of both forms by diminishing their tubular reabsorption. Precaution must be taken to ensure fluid intake adequate to produce a daily urine output of at least 1200 mL in adults and a corresponding quantity in children. If this cannot be accomplished, sodium bicarbonate may be given to reduce the risk of crystalluria.

Sulfadoxine. This agent has a particularly long plasma $t_{1/2}$ of 7 to 9 days. Although no longer marketed in the U.S., its combination with *pyrimethamine* (500 mg *sulfadoxine* plus 25 mg *pyrimethamine*) is listed as a World Health Organization essential medicine and is used for the prophylaxis and treatment of malaria caused by *mefloquine*-resistant strains of *Plasmodium falciparum* (see Chapter 66). However, because of severe and sometimes fatal reactions, including the Stevens-Johnson syndrome, and the emergence of resistant strains, the drug has limited usefulness for the treatment of malaria.

Sulfonamides for Topical Use

Sulfacetamide is the N1-acetyl-substituted derivative of *sulfanilamide*. Its aqueous solubility is about 90 times that of *sulfadiazine*. Solutions of the sodium salt of the drug are employed extensively in the management of ophthalmic infections. Very high aqueous concentrations are not irritating to the eye and are effective against susceptible microorganisms. The drug penetrates into ocular fluids and tissues in high concentration. Sensitivity reactions to *sulfacetamide* are rare, but the drug should not be used in patients with known hypersensitivity to sulfonamides. A 30% solution of the sodium salt has a pH of 7.4, whereas the solutions of sodium salts of other sulfonamides are highly alkaline. See Chapters 74 and 75 for ocular and dermatological uses. *Silver sulfadiazine* and *mafenide* are sulfonamides used topically, primarily in the prevention of infection in burn patients. These agents are covered in Chapter 75.

Therapeutic Uses

Use of sulfonamides as single agents for treatment of systemic infections has become uncommon. Because a significant percentage of UTIs are caused by sulfonamide-resistant microorganisms, sulfonamides are no longer a therapy of first choice; TMP-SMX is preferred (although resistance to this agent is increasing as well). *Sulfisoxazole* may be used effectively for cystitis in areas where the prevalence of resistance is not high. The usual dosage is 2 to 4 g initially, followed by 1 to 2 g orally four times a day for 5 to 10 days. TMP-SMX is most commonly used for infections due to *Nocardia* spp., but *sulfisoxazole* and *sulfadiazine* are alternative agents, given in dosages of 6 to 8 g daily. For serious infections, addition of a second agent, such as *imipenem*, *amikacin*, or *linezolid*, is recommended. The combination of *pyrimethamine* and *sulfadiazine* is the treatment of choice for toxoplasmosis (see Chapter 67). *Pyrimethamine* is given as a loading dose of 2000 mg followed by 50 to 75 mg orally per day, with *sulfadiazine* 1 to 1.5 g orally every 6 h, plus folinic acid (*leucovorin*) 10 to 25 mg orally each day for at least 6 weeks (Panel on Opportunistic Infections, 2020). Patients should receive at least 2 L of fluid intake daily to prevent crystalluria.

Adverse Reactions

Hypersensitivity Reactions

Among the skin and mucous membrane manifestations attributed to sensitization to sulfonamide are morbilliform, scarlatinal, urticarial, erysipeloid, pemphigoid, purpuric, and petechial rashes, as well as erythema nodosum, erythema multiforme of the Stevens-Johnson type, Behçet syndrome, exfoliative dermatitis, and photosensitivity (Khan et al., 2019). Sulfonamide metabolites are hypothesized to be primarily responsible for dermatologic hypersensitivity reactions. These hypersensitivity reactions occur most often after the first week of therapy but may appear earlier in previously sensitized individuals. Fever, malaise, and pruritus frequently are present simultaneously. Patients living with human immunodeficiency virus (HIV) infection manifest a higher frequency of rashes with sulfonamide treatment than do other individuals. Patients who have allergic reactions are often advised to avoid other agents with sulfa moieties; however, only those with a sulfonamide (SO_2NH_2) moiety carry a risk of cross-allergenicity. Further, data suggest most patients who have a reaction to an antimicrobial sulfonamide can tolerate a non-antimicrobial agent with a sulfonamide moiety (e.g., *furosemide*), although avoidance may be preferred for severe reactions.

Disturbances of the Urinary Tract

Crystalluria has occurred in dehydrated patients with HIV who were receiving *sulfadiazine* for *Toxoplasma* encephalitis. Crystalluria can be prevented by maintaining daily urine volume of at least 1200 mL (in adults) or, if necessary, urine alkalinization because the solubility of *sulfadiazine* increases with elevations of pH.

Miscellaneous Reactions

Anorexia, nausea, and vomiting occur in 1% to 2% of persons receiving sulfonamides. Focal or diffuse necrosis of the liver owing to direct drug toxicity or sensitization occurs in less than 0.1% of patients. Headache, nausea, vomiting, fever, hepatomegaly, jaundice, and laboratory evidence of hepatocellular dysfunction usually appear 3 to 5 days after sulfonamide administration is started, and the syndrome may progress to acute yellow atrophy and death. Aplastic anemia involving complete suppression of bone marrow activity with profound anemia, granulocytopenia, and thrombocytopenia is an extremely rare occurrence with sulfonamide therapy. It probably results from a direct myelotoxic effect and may be fatal. Reversible suppression of the bone marrow is quite common in patients with limited bone marrow reserve (e.g., patients with acquired immunodeficiency syndrome [AIDS] or those receiving myelosuppressive chemotherapy). The administration of sulfonamides to newborn infants, especially if premature, may lead to the displacement of bilirubin from plasma albumin, potentially causing an encephalopathy called *kernicterus*. Sulfonamides should not be given to pregnant women near term because these drugs cross the placenta and are secreted in milk.

Drug Interactions

Drug interactions of the sulfonamides are seen mainly with *warfarin*, the sulfonylurea hypoglycemic agents, and the hydantoin anticonvulsants. In each case, sulfonamides can potentiate the effects of the other drug by inhibiting its metabolism or by displacing it from albumin. Frequent monitoring and dosage adjustment may be necessary when a sulfonamide is given concurrently.

Trimethoprim-Sulfamethoxazole

TMP inhibits bacterial dihydrofolate reductase (DHFR), an enzyme downstream from the one that sulfonamides inhibit in the same biosynthetic sequence (see Figure 57–2). The combination of TMP with *sulfamethoxazole* (SMX) was an important advance in the development of clinically effective and synergistic antimicrobial agents. In much of the world, the combination of TMP with SMX is known as *cotrimoxazole*. In addition to its combination with SMX, TMP is available in some countries as a single-entity preparation.

Mechanism of Action

The antimicrobial activity of the combination of TMP-SMX results from actions on sequential steps of the enzymatic pathway for the synthesis of tetrahydrofolic acid (see Figure 57–2). Tetrahydrofolate is essential for one-carbon transfer reactions (e.g., the synthesis of thymidylate from deoxyuridylate) in both bacteria and mammalian cells. However, TMP is a highly selective inhibitor of the DHFR of lower organisms relative to that of mammals: About 100,000 times more drug is required to inhibit human reductase than the bacterial enzyme. The most effective ratio of SMX to TMP across the greatest number of microorganisms is 20:1. The combination is thus formulated to achieve an SMX concentration *in vivo* that is 20 times greater than that of TMP; SMX has pharmacokinetic properties such that the concentrations of the two drugs will thus be relatively constant in the body over a long period. Although each agent alone usually exerts bacteriostatic activity, when the organism is sensitive to both agents, bactericidal activity may be achieved.

Antimicrobial Activity

The antibacterial spectrum of TMP is similar to that of SMX, although TMP is 20 to 100 times more potent.

Spectrum of TMP-SMX in Combination

Although most *S. pneumoniae* are susceptible to TMP-SMX, there has been a disturbing increase in resistance (paralleling the rise in *penicillin* resistance), and its value as empiric therapy for many respiratory tract infections is questionable. The vast majority (>90%) of strains of *S. aureus* remain susceptible, even among *methicillin*-resistant isolates, although geographic variation exists. Activity against *Staphylococcus epidermidis* is more variable. *S. pyogenes* is usually sensitive when proper testing procedures (media with low thymidine content) are followed (Bowen et al., 2012). The *viridans* group of streptococci is typically susceptible, although susceptibility among *penicillin*-resistant strains is low (Diekema et al., 2001). Susceptibility in *E. coli* varies significantly by geographic region, although it has been declining in general, and in many places, TMP-SMX is no longer considered adequate empiric therapy. *Proteus mirabilis, Klebsiella* spp., *Enterobacter* spp., *Salmonella, Shigella, Pseudomonas pseudomallei, Serratia* spp., *Stenotrophomonas maltophila*, and *Alcaligenes* spp. are typically susceptible. Also usually susceptible are *Brucella abortus, Pasteurella haemolytica, Yersinia pseudotuberculosis, Yersinia enterocolitica*, and *Nocardia asteroides*. *Pseudomonas aeruginosa, Bacteroides fragilis*, and enterococci are clinically resistant.

Bacterial Resistance

Bacterial resistance to TMP-SMX has eroded the efficacy of this agent, especially among pneumococci and *E. coli*, although resistance to the combination is lower than it is to either of the agents alone. In addition to the resistance mechanisms to sulfonamides described above, resistance specific to TMP may develop. Resistance is typically due either to point mutations in genes encoding for DHFR or to the acquisition of a plasmid that codes for an altered DHFR (Estrada et al., 2016), both of which are associated with reduced binding of TMP.

ADME

The pharmacokinetic profiles of SMX and TMP are closely, but not perfectly, matched to achieve a near-constant ratio of 20:1 in their concentrations in blood and tissues over the course of their distribution and elimination. After a single oral dose of the combined preparation, TMP is absorbed more rapidly than SMX. Peak blood concentrations of TMP usually occur by 2 h in most patients, whereas peak concentrations of SMX occur by 4 h after a single oral dose. The half-lives of TMP and SMX are 11 and 10 h, respectively.

When 800 mg SMX is given with 160 mg TMP (one "double-strength" tablet; "single-strength" is 400 mg to 80 mg, maintaining the same ratio) twice daily, the peak concentrations of the drugs in plasma are about 40 and 2 μg/mL. Peak concentrations are similar (46 and 3.4 μg/mL) after intravenous infusion of 800 mg SMX and 160 mg TMP over a period of 1 h.

TMP is distributed and concentrated rapidly in tissues; about 40% is bound to plasma protein in the presence of SMX. The volume of distribution of TMP is almost nine times that of SMX. The drug readily enters CSF and sputum. High concentrations of each component of the mixture also are found in bile. About 65% of SMX is bound to plasma protein. About 60% of administered TMP and from 25% to 50% of administered SMX are excreted in the urine in 24 h. Two-thirds of the sulfonamide is unconjugated. Metabolites of TMP also are excreted. The rates of excretion and the concentrations of both compounds in the urine are reduced significantly in patients with uremia.

Therapeutic Uses

Urinary Tract Infections

Treatment of UTIs with TMP-SMX is highly effective for sensitive bacteria. Use for empiric therapy of UTIs is complicated by the increase in resistance among *E. coli*; guidelines recommend avoiding empiric use for UTIs when local resistance among *E. coli* exceeds 20% or if patients have recently received TMP-SMX (Gupta et al., 2011). Most treatment guidelines recommend 160/800 mg administered twice daily for 3 days for uncomplicated cystitis and for 10 to 14 days for complicated disease or pyelonephritis. TMP also is found in therapeutic concentrations in prostatic secretions, and TMP-SMX is a common treatment for acute or chronic bacterial prostatitis.

Bacterial Respiratory Tract Infections

TMP-SMX is effective for outpatients with mild acute exacerbations of chronic bronchitis. TMP-SMX should not be used to treat streptococcal pharyngitis because it does not eradicate the microorganism from the pharynx. It is effective for acute otitis media in children and acute maxillary sinusitis in adults that are caused by susceptible strains of *H. influenzae* and *S. pneumoniae*.

GI Infections

The combination is an alternative to a fluoroquinolone for treatment of shigellosis, but increasing resistance limits its use unless susceptibility is confirmed. TMP and TMP-SMX are no longer recommended for prevention or treatment of traveler's diarrhea because of increasing resistance worldwide among likely pathogens.

Infection by Pneumocystis jirovecii

High-dose therapy (TMP 15–20 mg/kg per day plus SMX 75–100 mg/kg per day in three or four divided doses; typical maximum dose is 20 mg/kg per day of TMP) is effective for *Pneumocystis jirovecii* pneumonia (Panel on Opportunistic Infections, 2020). Adjunctive corticosteroids should be given at the onset of anti-*Pneumocystis* therapy in patients with a Po_2 less than 70 mmHg or an alveolar-arterial gradient less than 35 mmHg. Prophylaxis with TMP-SMX using a variety of dosing strategies (from daily to several times weekly) is effective in preventing pneumonia caused by this organism in patients with HIV as well as among patients with other immunocompromising conditions (e.g., neutropenia and solid-organ transplantation). Adverse reactions are less frequent with the lower prophylactic doses of TMP-SMX.

Methicillin-Resistant Staphylococcus aureus Infections

The increasing incidence of community-acquired infections due to *methicillin*-resistant *S. aureus* has provided a role for TMP-SMX as an adjunctive therapy to incision and drainage of complicated abscesses. However, it is less effective than standard therapy in the treatment of invasive *methicillin*-resistant *S. aureus* infections, including bacteremia (Paul et al., 2015).

Miscellaneous Infections

Nocardia infections have been treated successfully with the combination, but failures also have been reported. Although a combination of *doxycycline* and *streptomycin* or *gentamicin* is now considered the treatment of choice for brucellosis, TMP-SMX may be an effective substitute for the *doxycycline* combination. TMP-SMX also has been used successfully for infection by *Stenotrophomonas maltophilia* and infection by the intestinal parasites *Cyclospora* and *Isospora*. TMP-SMX is used as prophylaxis against infection due to *Toxoplasma gondii* in HIV-infected individuals and is an alternative for treatment of toxoplasmosis (see Chapter 67).

Adverse Effects

TMP-SMX extends the toxicity of the sulfonamides. Hematological reactions include various anemias, coagulation disorders, granulocytopenia, agranulocytosis, purpura, Henoch-Schönlein purpura, and sulfhemoglobinemia. TMP-SMX reportedly causes up to three times as many dermatological reactions as does *sulfisoxazole* (5.9% vs. 1.7%). Mild and transient jaundice has been noted and appears to have the histological features of allergic cholestatic hepatitis. Permanent impairment of renal function may follow the use of TMP-SMX in patients with renal disease due to SMX crystalluria; liberal fluid intake should be encouraged to dilute the urine during therapy. An increase in serum creatinine without decrement in glomerular filtration rate may be observed with high-dose therapy due to TMP's inhibition of creatinine secretion. Hyperkalemia can also be observed, as TMP has a similar structure to potassium-sparing diuretics such as *triamterene*. Patients with HIV frequently have hypersensitivity reactions to TMP-SMX (rash, neutropenia, Stevens-Johnson syndrome, Sweet syndrome, and pulmonary infiltrates). Both rapid and slow desensitization protocols have been established for patients intolerant to medically necessary therapy (Khan et al., 2019).

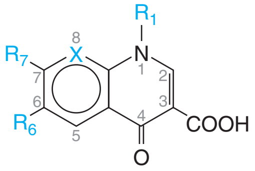
Drug Interactions

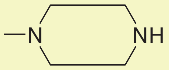
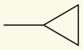
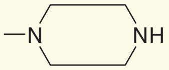
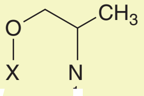
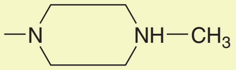
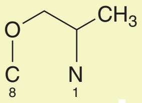
As with the sulfonamides alone, co-administration of TMP-SMX with *warfarin* can inhibit *warfarin* metabolism and lead to excessive anticoagulation with risks for bleeding. Caution is warranted with coadministration of agents that can increase potassium or suppress bone marrow when combined with high-dose TMP-SMX. Administration of TMP-SMX should be avoided in patients receiving high doses of *methotrexate* for treatment of malignancies, as TMP-SMX can increase *methotrexate* concentrations and lead to serious toxicity.

The Quinolones

The first quinolone, *nalidixic acid*, was isolated as a by-product of the synthesis of chloroquine and made available for the treatment of UTIs. The introduction of fluorinated 4-quinolones (fluoroquinolones), such as *norfloxacin*, *ciprofloxacin*, and *levofloxacin* (Table 57–1), represents a particularly important therapeutic advance. These agents have broad antimicrobial activity and are effective after oral administration for the treatment of a wide variety of infectious diseases. However, due to potentially fatal side effects, many quinolones had to be withdrawn from the U.S. market: *lomefloxacin* and *sparfloxacin* (phototoxicity, QTc prolongation); *gatifloxacin* (systemic forms only: hypoglycemia); *temafloxacin* (immune hemolytic anemia); *trovafloxacin* (hepatotoxicity); *grepafloxacin* (cardiotoxicity); and *clinafloxacin* (phototoxicity). In all cases, the side effects were discovered during postmarketing surveillance (Sheehan and Chew, 2003). The FDA has issued new warnings for fluoroquinolones still being marketed, calling attention to their toxicities and recommending against their routine use in uncomplicated infections (Food and Drug Administration, 2018).

TABLE 57–1 ■ STRUCTURAL FORMULAS OF SELECTED QUINOLONES AND FLUOROQUINOLONES



CONGENER	R_1	R_6	R_7	X
Nalidixic acid	$-C_2H_5$	–H	$-CH_3$	–N–
Norfloxacin	$-C_2H_5$	–F		–CH–
Ciprofloxacin		–F		–CH–
Levofloxacin		–F		

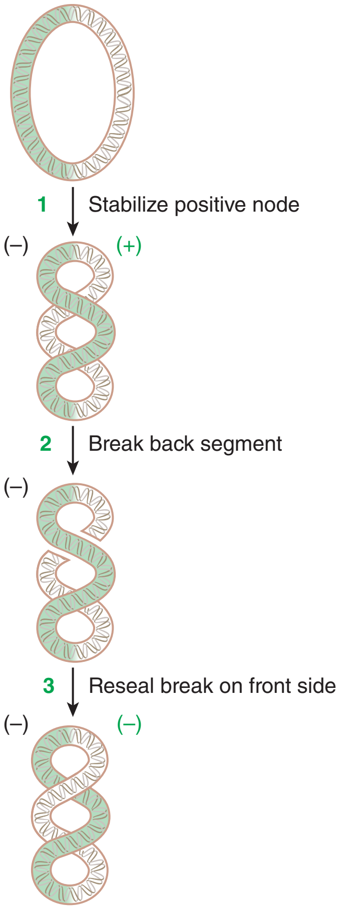


Figure 57–3 *Model of the formation of negative DNA supercoils by DNA gyrase.* DNA gyrase binds to two segments of DNA (1), creating a node of positive (+) superhelix. The enzyme then introduces a double-strand break in the DNA and passes the front segment through the break (2). The break is then resealed (3), creating a negative (–) supercoil. Quinolones inhibit the nicking and closing activity of the gyrase and, at higher concentrations, block the decatenating activity of topoisomerase IV.

Mechanism of Action

The quinolone antibiotics target bacterial *DNA gyrase* and *topoisomerase IV* (Mohammed et al., 2019). For many gram-positive bacteria, topoisomerase IV, which separates interlinked (catenated) daughter DNA molecules that are the product of DNA replication, is the primary target. In contrast, DNA gyrase is the primary quinolone target in many gram-negative microbes. The gyrase introduces negative supercoils into the DNA to combat excessive positive supercoiling that can occur during DNA replication (Figure 57–3) (Cozzarelli, 1980). The quinolones, as a drug-metal complex, inhibit gyrase-mediated DNA supercoiling at concentrations that correlate well with those required to inhibit bacterial growth (0.1–10 μg/mL).

Eukaryotic cells do not contain DNA gyrase. They do contain a mechanistically similar type II DNA topoisomerase, but quinolones inhibit it only at concentrations (100–1000 μg/mL) much higher than those needed to inhibit the bacterial enzymes.

Antimicrobial Activity

The fluoroquinolones were potent, bactericidal agents against most gram-negative pathogens when first introduced, including *Proteus, E. coli, Klebsiella,* and various species of *Salmonella, Shigella, Enterobacter,* and *Campylobacter*. As with TMP-SMX, resistance has continuously eroded the coverage that fluoroquinolones provide, especially among *E. coli* and *Proteus* spp., such that fluoroquinolones may not be reliable for empiric therapy due to the prevalence of these organisms in some regions (Olson et al., 2009). While once a standard therapy for *Neisseria gonorrhoeae* infections, resistance has increased to the point these agents are no longer recommended in most countries for empiric therapy of gonorrhea (Centers for Disease Control and Prevention, 2021). *Ciprofloxacin* and *levofloxacin* have sufficient activity against *Pseudomonas* spp. for use in systemic infections; a newly introduced agent, *delafloxacin*, also possesses *in vitro* activity, but to date, few patients with *Pseudomonas* infections have been treated with this agent. Fluoroquinolones have good *in vitro* activity against staphylococci, but they are less active against *methicillin*-resistant strains, and there is concern over development of resistance during therapy. Activity against streptococci is significantly greater with the newer agents, including *levofloxacin*, *gemifloxacin*, *moxifloxacin*, and *delafloxacin*. Several intracellular bacteria are inhibited by fluoroquinolones at concentrations that can be achieved in plasma; these include species of *Chlamydia, Mycoplasma, Legionella, Brucella*, and *Mycobacterium* (including *Mycobacterium tuberculosis*). *Ciprofloxacin*, *ofloxacin*, *levofloxacin*, and *moxifloxacin* have activity against *Mycobacterium fortuitum, Mycobacterium kansasii*, and *M. tuberculosis*. *Moxifloxacin* also has useful activity against intestinal anaerobes, such as *Bacteroides fragilis*.

Bacterial Resistance

Resistance to quinolones may develop during therapy via mutations in the bacterial chromosomal genes encoding DNA gyrase or topoisomerase IV, leading to reduced binding affinity for the fluoroquinolones (Correia et al., 2017). Chromosomal mutations leading to upregulation of efflux pump–mediated active transport of the drug out of the bacteria or the reduction in expression of porin channels allowing quinolones to transit the outer membrane also contribute to resistance. Less commonly, plasmids can transfer genes that encode proteins capable of binding to and protecting the topoisomerases from quinolone effects or that directly modify the quinolone itself. Resistance emerging during the course of therapy can occur, especially in *E. coli*, *Pseudomonas*, and staphylococci.

ADME

Most quinolones are well absorbed after oral administration. Peak serum levels of the fluoroquinolones are obtained within 1 to 3 h of an oral dose. The volume of distribution of quinolones is high, with concentrations in urine, kidney, lung, and prostate tissue and stool, bile, and macrophages and neutrophils higher than serum levels. Food may delay the time to peak serum concentrations. Many fluoroquinolones have been detected in human breast milk; because of their excellent bioavailability, the potential exists for substantial exposure of nursing infants. Except for *moxifloxacin*, quinolones are cleared predominantly by the kidney, and dosages must be adjusted for renal failure.

Pharmacological Properties of Individual Quinolones

Norfloxacin

The gram-negative activity of *norfloxacin* (not available in the United States) is similar to, but somewhat less potent than, that of *ciprofloxacin*. However, the relatively low serum levels reached with *norfloxacin* limit its usefulness in the treatment of UTIs and gastrointestinal infections. The serum $t_{1/2}$ is 3 to 5 h for *norfloxacin*; approximately 25% of the drug is eliminated unchanged in the urine, with hepatic metabolism also occurring.

Ciprofloxacin

Ciprofloxacin's bioavailability is approximately 70%. Typical oral doses are 250 to 750 mg and intravenous doses are 200 to 400 mg twice daily (maximum dose 1.5 g/day orally). The elimination $t_{1/2}$ is about 5 h, and the drug is typically dosed twice daily, with the exception of an extended-release formulation, which can be dosed once daily.

Ofloxacin/Levofloxacin

Ofloxacin has somewhat more potent gram-positive activity than *ciprofloxacin*; separation of the more active S- or levorotatory isomer yields *levofloxacin*, which has even better antistreptococcal activity. Bioavailability of both of these agents is excellent, such that intravenous and oral doses are the same; *levofloxacin* is dosed once daily (250–750 mg) as opposed to twice-daily dosing for *ofloxacin* (200–400 mg daily divided every 12 h).

Moxifloxacin

Moxifloxacin improves further on the gram-positive potency of *levofloxacin*, typically having minimal inhibitory concentrations one to two dilutions lower against *S. pneumoniae*. It also has expanded activity against anaerobic pathogens but is substantially less active than *ciprofloxacin* or *levofloxacin* against *P. aeruginosa*. *Moxifloxacin* is well absorbed, with equivalent intravenous and oral doses; the $t_{1/2}$ is about 12 h, allowing for daily dosing (usual dose 400 mg daily). *Moxifloxacin* undergoes hepatic sulfation and glucuronidation. Less than a quarter of systemic *moxifloxacin* is excreted unchanged via the kidneys, and because high concentrations are not achieved in the urine, it is not recommended for UTIs.

Gatifloxacin, Gemifloxacin

The agents *gatifloxacin* and *gemifloxacin* have a similar spectrum of activity to *moxifloxacin*, with enhanced potency against gram-positive organisms and poor activity versus *Pseudomonas*. They are less active than *moxifloxacin* against *B. fragilis*. Both have high bioavailability and renal elimination. *Gatifloxacin* is no longer available for systemic use in the U.S. due to toxicity concerns, but an ophthalmic preparation is licensed for the treatment of bacterial conjunctivitis.

Delafloxacin

Delafloxacin is a newly approved fluoroquinolone with potent activity against staphylococci; minimal inhibitory concentrations are at least 6-fold lower than *levofloxacin* for most isolates of *S. aureus*. Activity against gram-negatives, including *Pseudomonas*, is similar to that of *levofloxacin*. It is available for intravenous and oral administration and undergoes mixed renal and nonrenal elimination.

Therapeutic Uses

Urinary Tract Infections

The fluoroquinolones are a mainstay of treatment of upper and lower UTIs, being more efficacious than TMP-SMX or oral β-lactams. Because of their broad spectrum of activity and adverse effects, however, recent guidelines suggest reserving their use for complicated cystitis or pyelonephritis when possible (Gupta et al., 2011). *Moxifloxacin* does not accumulate in the urine and is not approved for treatment of UTIs. Typical treatment durations for quinolones are 3 days for uncomplicated cystitis and 5 to 7 days for uncomplicated pyelonephritis.

Prostatitis

Norfloxacin, *ciprofloxacin*, *ofloxacin*, and *levofloxacin* achieve good levels in prostatic secretions and are effective in the treatment of prostatitis caused by sensitive bacteria. Fluoroquinolones administered for 4 to 6 weeks appear to be effective in patients not responding to TMP-SMX.

Sexually Transmitted Diseases

Fluoroquinolones lack activity for *Treponema pallidum* but have activity *in vitro* against *Chlamydia trachomatis* and *H. ducreyi*. For chlamydial urethritis/cervicitis, a 7-day course of *ofloxacin* or *levofloxacin* is an alternative to a 7-day treatment with *doxycycline* or a single dose of *azithromycin*; other available quinolones have not been reliably effective. Previously, a single oral dose of a fluoroquinolone such as *ciprofloxacin* had been effective treatment of sensitive strains of *N. gonorrhoeae*, but increasing resistance to fluoroquinolones has led to *ceftriaxone* being the first-line agent for this infection. A study of *delafloxacin* for single-dose therapy failed to meet its endpoint for efficacy. Chancroid (infection by *H. ducreyi*) can be treated with 3 days of *ciprofloxacin*.

GI and Abdominal Infections

Norfloxacin, *ciprofloxacin*, *ofloxacin*, and *levofloxacin* given for 1 to 3 days all have been effective in the treatment of patients with traveler's diarrhea, reducing the duration of loose stools by 1 to 3 days. *Ciprofloxacin* in a single daily dose has been used for prophylaxis of traveler's diarrhea, but resistance among *Campylobacter* and *Shigella* and increasing recognition of fluoroquinolone adverse effects have led to authorities discouraging this use. *Ciprofloxacin* and *ofloxacin* can cure most patients with enteric fever caused by *Salmonella typhi*, as well as bacteremic nontyphoidal infections in patients with HIV, and clear chronic fecal carriage. Quinolones should be avoided in the treatment of diarrhea due to Shiga toxin–producing *E. coli*. *Ciprofloxacin* and *levofloxacin*, when combined with *metronidazole*, or *moxifloxacin* alone, may be useful in the management of intra-abdominal infections if local susceptibilities allow.

Respiratory Tract Infections

Many newer fluoroquinolones, including *levofloxacin*, *moxifloxacin*, *gemifloxacin*, and *delafloxacin*, have excellent activity against *S. pneumoniae, H. influenzae,* and the atypical respiratory pathogens. Thus, these agents are frequently used in the management of community-acquired pneumonia. *Ciprofloxacin* and *levofloxacin* also play a role in the treatment of respiratory exacerbations owing to *P. aeruginosa* in patients with cystic fibrosis and in combination with a β-lactam agent to provide broad gram-negative coverage for nosocomial pneumonia in patients at high risk for resistant isolates.

Bone, Joint, and Soft-Tissue Infections

The treatment of chronic osteomyelitis may require prolonged (weeks to months) antimicrobial therapy with agents active against *S. aureus* or gram-negative rods. Failures are often associated with the development of resistance, particularly in *S. aureus*. Combination therapy with a fluoroquinolone and *rifampin* is an option for the management of early-onset prosthetic joint infections. *Levofloxacin*, *moxifloxacin*, and *delafloxacin* are approved for the treatment of skin and soft-tissue infections; although, they should generally be reserved for situations where their expanded spectrum of activity can be leveraged, such as diabetic foot infections.

Other Infections

Ciprofloxacin and *levofloxacin* are used for the prophylaxis of anthrax and are effective for the treatment of tularemia and plague due to *Yersinia pestis* (Hendricks et al., 2014). *Levofloxacin* and *moxifloxacin* may be used as part of multiple-drug regimens for the treatment of multidrug-resistant tuberculosis and atypical mycobacterial infections as well as *Mycobacterium avium* complex infections in AIDS (see Chapter 65). Quinolones, when used as prophylaxis in neutropenic patients, have decreased the incidence of gram-negative rod bacteremias (Freifeld et al., 2011).

Adverse Effects

Gastrointestinal Adverse Effects

Common adverse reactions involve the GI tract, with 3% to 17% of patients reporting mild nausea, vomiting, and abdominal discomfort. Fluoroquinolones have emerged as a leading cause of *Clostridium difficile* colitis due to the spread of quinolone-resistant strains.

Neurologic Adverse Effects

Common side effects (1%–11%) involving the CNS include mild headaches, dizziness, insomnia, and anxiety. Rarely, hallucinations, delirium, and seizures have occurred, especially in patients who were also receiving *theophylline* or nonsteroidal anti-inflammatory drugs. Patients with a history of epilepsy are at higher risk for fluoroquinolone-induced convulsions. Recently, the fluoroquinolones have been recognized as rare causes of peripheral neuropathy and possibly optic neuritis, which in some cases has been irreversible.

Musculoskeletal Adverse Effects

Arthralgias and joint pain are occasionally reported with fluoroquinolones. Tendon rupture or tendinitis (usually of the Achilles tendon) is a recognized serious adverse effect, especially in those more than 60 years old, in patients taking corticosteroids, and in solid-organ transplant recipients. Early animal studies suggested an increased risk of cartilage damage and malformation among young animals (Burkhardt et al., 1997). While arthralgias and joint pain during therapy are more common among children receiving quinolones relative to comparators during the course of therapy, studies have not noted long-term joint abnormalities or growth inhibition among children exposed to fluoroquinolones. The American Academy of Pediatrics suggests that fluoroquinolone use in children is appropriate when limited treatment options exist or when oral administration offers a significant risk/benefit advantage (Jackson and Schutze, 2016). Similarly, limited data suggest that fluoroquinolone use may be appropriate in pregnant women in the absence of alternative therapies.

Other Adverse Effects

Among the quinolones available in the U.S., *moxifloxacin* carries the highest risk for QT interval prolongation and torsades de pointes arrhythmias; *gemifloxacin*, *levofloxacin*, and *ofloxacin* appear to have lower risk; and *ciprofloxacin* has the lowest risk. However, the overall risk of torsades de pointes is small with the use of fluoroquinolones. *Gatifloxacin*'s propensity to cause both hypo- and hyperglycemia, especially in older adults, led to its removal for systemic use in the U.S. (Park-Wyllie et al., 2006). Other agents such as *levofloxacin* may rarely be associated with dysglycemias among at-risk patients. Rashes, including photosensitivity reactions, also can occur; patients with frequent sun exposure should be advised to protect themselves with clothing or sunscreen.

Drug Interactions

All quinolones form complexes with divalent and trivalent cations (e.g., calcium, iron, aluminum). When coadministered orally with quinolones, these cations can chelate the quinolone and reduce systemic bioavailability. Thus, a separation of at least 2 h between oral administration of quinolones and these cations is recommended. *Ciprofloxacin* inhibits the metabolism of theophylline, and toxicity from elevated concentrations of the methylxanthine may occur. Nonsteroidal anti-inflammatory drugs may augment displacement of γ-aminobutyric acid (GABA) from its receptors by the quinolones, enhancing neurological adverse effects (Halliwell et al., 1993). Due to risk for QT prolongation, quinolones should be used with caution in patients on class III (*amiodarone*) and class IA (*quinidine*, *procainamide*) antiarrhythmics.

Nitroimidazoles

Metronidazole and *tinidazole* are nitroimidazoles with activity against anaerobic bacteria and parasites. Here, we will discuss the antibacterial activity of *metronidazole*; an in-depth discussion of *metronidazole* and *tinidazole*, including their pharmacokinetics, adverse effects, and antiparasitic uses, will be reserved for Chapter 67; applications for treatment of *Helicobacter pylori* disease are discussed in Chapter 53 and for inflammatory bowel disease in Chapter 55.

Metronidazole

Antibacterial Activity and Resistance

Metronidazole is essentially a prodrug: The nitro group of *metronidazole* is reduced in anaerobic bacteria, some microaerophilc bacteria, and protozoans, producing the active form of the drug. The activation leads to formation of reactive compounds that interact with DNA, possibly disrupting its structure and inhibiting replication (Dingsang and Hunter, 2018). *Metronidazole* displays excellent activity against most anaerobic bacteria, including *Bacteroides*, *Clostridium*, *Fusobacterium*, *Peptococcus*, *Peptostreptococcus*, and *Eubacterium*. It is less active against *Gardnerella* and *Helicobacter,* and the gram-positive anaerobes *Actinomyces, Propionibacterium*, and *Lactobacillus* are typically resistant. Acquired resistance among normally susceptible organisms is uncommon, and resistance mechanisms are complex and incompletely described. In the case of *Bacteroides* spp., *metronidazole* resistance has been linked to a family of nitroimidazole (*nim*) resistance genes, which can be encoded chromosomally or episomally. These *nim* genes appear to encode a nitroimidazole reductase capable of converting a 5-nitroimidazole to a 5-aminoimidazole, thus stopping the formation of the reactive nitroso group responsible for microbial killing.

Therapeutic Uses and Dosage

Metronidazole is a relatively inexpensive agent with efficacy against a broad spectrum of anaerobic bacteria. Typical doses are 250 to 500 mg twice or three times daily, via the intravenous or oral routes. The drug is frequently given in combination with other antimicrobial agents to treat polymicrobial infections with aerobic and anaerobic bacteria. *Metronidazole* is used as a component of prophylaxis for colorectal surgery and is employed as a single agent to treat bacterial vaginosis. It is used in combination with other antibiotics and a proton pump inhibitor in regimens to treat infection with *H. pylori* (see Chapter 53). *Metronidazole* has been used as therapy for nonsevere *C. difficile* infection, although *vancomycin* or *fidaxomicin* are now preferred agents. For patients with fulminant, life-threatening *C. difficile* infection, intravenous *metronidazole* is administered in combination with oral *vancomycin*.

Drug Facts for Your Personal Formulary: *DNA Disruptors: Sulfonamides, Quinolones, and Nitroimidazoles*

Drug	Therapeutic Uses	Clinical Pharmacology and Tips
Sulfonamides: Competitive inhibitors of bacterial dihydropteroate synthase, thereby disrupting folate synthesis **General: Bacteriostatic; limited efficacy as monotherapy, renal elimination, hypersensitivity reactions**		
Sulfadiazine (PO)	• Toxoplasmosis (with pyrimethamine)	• Good activity against *T. gondii* • Reasonable CSF penetration • Higher risk of crystalluria, requires hydration
Sulfadoxine (PO)	• Prophylaxis and treatment of malaria (with pyrimethamine)	• Some activity vs. *P. falciparum* • Long $t_{1/2}$
Sulfonamide and Dihydrofolate Reductase Inhibitor Combination: Sequential inhibition of folate synthesis, often bactericidal		
Trimethoprim-sulfamethoxazole (IV, PO)	• UTI • Upper respiratory tract infections • Shigellosis • *P. jirovecii* pneumonia • Skin/soft-tissue infections due to *S. aureus* • Infections due to *Nocardia*, *S. maltophila*, *Cyclospora*, *Isospora*	• Excellent activity vs. *S. aureus*, *S. epidermidis*, *S. pyogenes* • Good activity vs. *Proteus*, *E. coli*, *Klebsiella*, *Enterobacter*, *Serratia*, *Nocardia*, *Brucella* • Some activity vs. *S. pneumoniae* • Formulated in 5:1 (SMX:TMP) ratio, giving 20:1 serum levels • Well absorbed on oral administration • Good penetration into CSF • Metabolized and renally eliminated • Hypersensitivity reactions (i.e., rash) common • Dose-related bone marrow suppression, hyperkalemia
Quinolones: Bactericidal inhibitors of bacterial gyrase and topoisomerase, prevent DNA unwinding **General: Drug interactions with cations, neurological adverse effects, tendonitis/tendon rupture, photosensitivity, QT prolongation; typically avoided in children and pregnant women except for compelling indications**		
Norfloxacin (PO)	• UTI, prostatitis • Traveler's diarrhea	• Good activity vs. *E. coli, Klebsiella, Proteus, Serratia, Salmonella, Shigella* • Some activity vs. *Pseudomonas* • Effective concentrations only achieved in GI and urinary tracts
Ciprofloxacin (IV, PO)	• UTI, prostatitis • Traveler's diarrhea • Intra-abdominal infections (with metronidazole) • *Pseudomonas* infections • Anthrax, tularemia	• Excellent activity vs. *E. coli, Klebsiella, Proteus, Serratia, Salmonella, Shigella* • Good activity vs. *Pseudomonas* • Some activity vs. *S. aureus*, streptococci • Good bioavailability and tissue distribution • Renal and nonrenal elimination
Levofloxacin (IV, PO)	• Respiratory tract infections • UTI, prostatitis • *Chlamydia* • Traveler's diarrhea • Intra-abdominal infections (with metronidazole) • *Pseudomonas* infections	• Excellent activity vs. *E. coli, Klebsiella, Proteus, Serratia, Salmonella, Shigella,* streptococci, *H. influenzae, Legionella, Chlamydia* • Good activity vs. *Pseudomonas, S. aureus* • Good bioavailability and tissue distribution • Renal elimination • S-isomer of ofloxacin
Moxifloxacin (IV, PO)	• Respiratory tract infections • Intra-abdominal infections • Mycobacterial infections	• Excellent activity vs. *E. coli, Klebsiella, Proteus, Serratia,* streptococci, *H. influenzae, Legionella, Chlamydia* • Good activity vs. *S. aureus, B. fragilis* • Good bioavailability and tissue distribution • Renal and nonrenal elimination; not for UTI • QT prolongation
Nitroimidazoles: Bactericidal DNA-damaging agents; require activation by reductases present in anaerobes		
Metronidazole (IV, PO, topical)	• *C. difficile* colitis • Empiric coverage of anaerobic organisms, as in intra-abdominal and skin and soft-tissue infections • *H. pylori* gastritis (in combination with other agents) • Bacterial vaginosis	• Bacterial spectrum limited to anaerobic organisms, including *B. fragilis* and *Clostridium* • Excellent absorption • Wide distribution including CNS • Hepatic elimination • Inhibitor of CYP enzymes; drug interactions with warfarin • Peripheral neuropathy with prolonged use

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Chapter 58

Cell Envelope Disruptors: β-Lactam, Glycopeptide, and Lipopeptide Antibacterials

Conan MacDougall

β-LACTAMS: MECHANISMS OF ACTION

β-LACTAMS: MECHANISMS OF BACTERIAL RESISTANCE

β-LACTAMASE INHIBITORS

THE PENICILLINS

- Classification of the Penicillins and Summary of Their Pharmacological Properties
- Penicillin G and Penicillin V
- The Penicillinase-Resistant Penicillins
- The Aminopenicillins: Ampicillin and Amoxicillin
- Antipseudomonal Penicillins: The Carboxypenicillins and the Ureidopenicillins

THE CEPHALOSPORINS

- Mechanism of Action
- Mechanism of Bacterial Resistance
- Classification and General Pharmacology
- ADME and Agent-Specific Antibacterial Activity
- Adverse Reactions
- Therapeutic Uses

OTHER β-LACTAM ANTIBIOTICS

- Carbapenems
- Monobactams

OTHER CELL ENVELOPE DISRUPTORS

- Glycopeptides
- Lipopeptides
- Bacitracins

The bacterial cellular envelope typically consists of the inner membrane, the cell wall, and, in gram-negative organisms, the outer membrane. The cell envelope is a key target for antibacterial agents, including the β-lactam antibiotics, glycopeptides, and lipopeptides, as well as other minor classes (including bacitracin, discussed below, and polymyxins, discussed in Chapter 59). β-Lactam antibiotics—penicillins, cephalosporins, carbapenems, and monobactams—share a common structure (β-lactam ring) and mechanism of action (i.e., inhibition of the synthesis of the bacterial peptidoglycan cell wall). β-Lactams are the single most important antibacterial class given their broad and varied spectrum of activity, their potent antibacterial killing, and their generally favorable tolerability. Unfortunately, resistance to β-lactams has steadily increased, requiring development of new agents, which can evade (e.g., *ceftaroline*) or neutralize (e.g., β-lactamase inhibitors) these mechanisms. The glycopeptides, including *vancomycin*, and lipopeptides (*daptomycin*) provide important treatment alternatives for infections due to gram-positive organisms.

β-Lactams: Mechanisms of Action

The bacterial cell wall is comprised of heteropolymeric peptidoglycan that provides rigid mechanical stability. The β-lactam antibiotics inhibit the last step in peptidoglycan synthesis. In gram-positive microorganisms, the cell wall is 50 to 100 molecules thick; in gram-negative bacteria, it is only one or two molecules thick (Figure 58–1A). The peptidoglycan is composed of glycan chains, which are linear strands of two alternating amino sugars (*N*-acetylglucosamine and *N*-acetylmuramic acid), that are cross-linked by peptide chains. Peptidoglycan precursor formation takes place in the cytoplasm. The synthesis of UDP–acetylmuramylpentapeptide is completed with the addition of a dipeptide, D-alanyl-D-alanine, which is formed by racemization and condensation of L-alanine. UDP-acetylmuramyl-pentapeptide and UDP-acetylglucosamine are linked with the release of the uridine nucleotides to form a long polymer. The cross-link is completed by a transpeptidation reaction that occurs outside the plasma membrane (Figure 58–1B).

The β-lactam antibiotics inhibit this last step in peptidoglycan synthesis (Figure 58–2) by acylating the transpeptidase via cleavage of the –CO–N– bond of the β-lactam ring. The transpeptidase targets for the actions of β-lactam antibiotics are collectively termed penicillin-binding proteins (PBPs). Notably, bacteria may produce multiple functionally related but distinct PBPs, and each PBPs can have varying affinities for individual β-lactams. The lethality of penicillins for bacteria appears to involve both lytic and nonlytic mechanisms (Bayles, 2000), and inhibition of some PBPs may be more consequential than others for bacterial killing.

β-Lactams: Mechanisms of Bacterial Resistance

Bacterial resistance to β-lactam antibiotics typically occurs through one of three mechanisms: alterations in the PBP target, reduction of concentration at the target site, and/or enzymatic degradation of the β-lactam itself. A sensitive strain may acquire resistance via mutations that decrease the affinity of PBPs for the antibiotic or by acquiring the ability to express new, low-affinity PBPs (e.g., via plasmid transfer). Altered PBPs with decreased affinity for β-lactam antibiotics can also be acquired by homologous recombination between PBP genes of different bacterial species (Zapun et al., 2008). Four of the five high-molecular-weight PBPs of the most highly penicillin-resistant *Streptococcus pneumoniae* isolates have decreased affinity for β-lactam antibiotics as a result of interspecies homologous recombination events. In contrast, isolates with high-level resistance to third-generation cephalosporins contain alterations of only two of the five high-molecular-weight PBPs because the other PBPs have inherently low affinity for the third-generation cephalosporins. *Methicillin*-resistant *Staphylococcus aureus* (MRSA) is resistant via acquisition of an additional high-molecular-weight PBP (via a transposon) with a very low affinity for all β-lactam antibiotics; this mechanism is also responsible for *methicillin* resistance in the coagulase-negative staphylococci. In general,

Abbreviations

ESBL: extended-spectrum β-lactamase
GI: gastrointestinal
KPC: *Klebsiella pneumoniae* carbapenemase
MIC: minimum inhibitory concentration
MRSA: methicillin-resistant *Staphylococcus aureus*
MRSE: methicillin-resistant *Staphylococcus epidermidis*
PBP: penicillin-binding protein

resistance via alterations to the β-lactam target is more common among gram-positive versus gram-negative bacterial pathogens.

Bacterial resistance to β-lactam antibiotics also results from the inability of the agent to achieve sufficient concentrations at its site of action (Fernández and Hancock, 2012). In gram-positive bacteria, the peptidoglycan polymer is very near the cell surface (see Figure 58–1A), and small β-lactam antibiotic molecules can penetrate easily to the outer layer of the cytoplasmic membrane and the PBPs. In gram-negative bacteria, the inner membrane is internal to the outer membrane and capsule (see Figure 58–1A); the outer membrane functions as an impenetrable barrier for some antibiotics. Some small hydrophilic antibiotics, however, diffuse through aqueous channels in the outer membrane that are formed by proteins called *porins*. The number and size of pores in the outer membrane vary among different gram-negative bacteria, thereby providing greater or lesser access for antibiotics to the site of action. Active efflux pumps serve as another mechanism of resistance, removing the antibiotic from its site of action before it can act (Figure 58–3) (Fernández and Hancock, 2012).

Bacteria also can inactivate β-lactam antibiotics enzymatically via the action of β-lactamases (see Figure 58–1A). Thousands of different β-lactamases have been variously characterized by according to their molecular class or functional characteristics (Bush and Jacoby, 2010). Their substrate specificities can be relatively narrow or can extend to almost all β-lactams. In general, gram-positive bacteria produce and secrete a large amount of β-lactamase, typically narrow-spectrum penicillinases. The sequence for staphylococcal penicillinase is encoded in a plasmid; this may be transferred by bacteriophage to other bacteria and their expression is inducible by substrate antibiotics. In gram-negative bacteria, β-lactamases are found in lower quantities, but their location in the periplasmic space between the inner and outer membranes (see Figure 58–1A) provides maximal protection of the microbe. β-Lactamases of gram-negative bacteria are encoded either chromosomally or via transferable elements such as plasmids; their expression may be constitutive or inducible. Of particular concern are carbapenemases: β-lactamases that are capable of hydrolyzing carbapenems, as well as penicillins and cephalosporins. Microorganisms expressing such β-lactamases (along with other resistance mechanisms) may be resistant to all or almost all antibacterials in clinical use (Queenan and Bush, 2007).

More than one of the aforementioned resistance mechanisms may be present in a pathogen, and they can work in concert to confer resistance. The local environment can also contribute to resistance to β-lactam antibiotics. Microorganisms adhering to implanted prosthetic devices (e.g., catheters, artificial joints, prosthetic heart

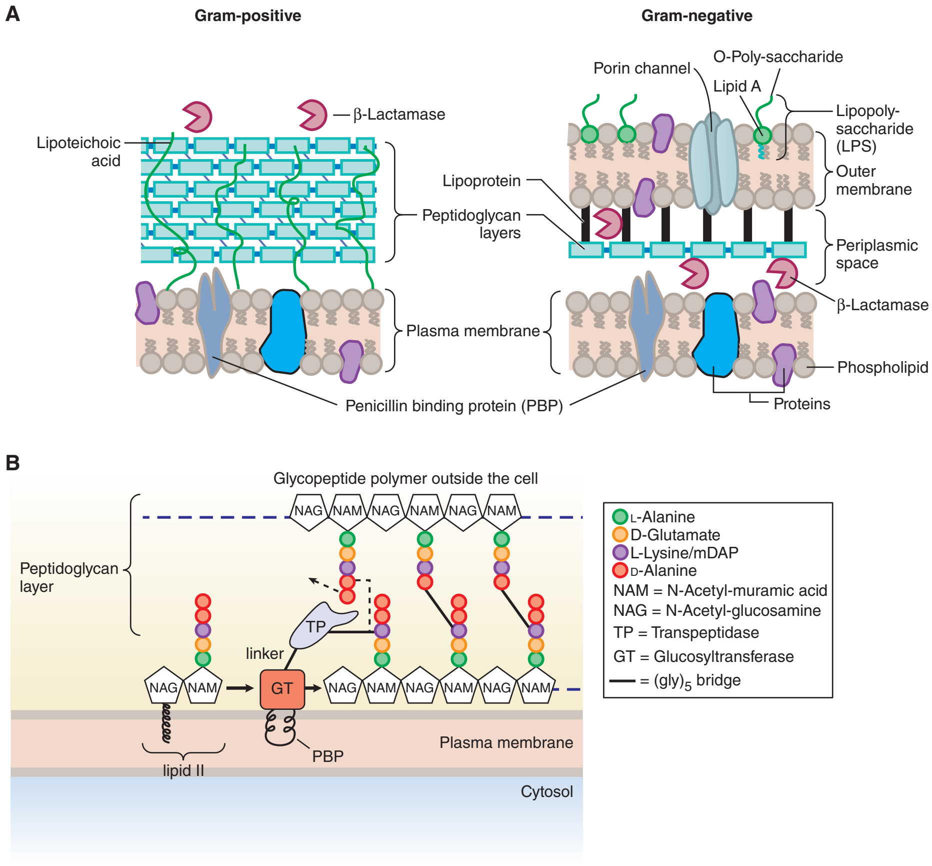

A
Gram-positive
Gram-negative
Lipoteichoic acid
β-Lactamase
Lipoprotein
Peptidoglycan layers
Plasma membrane
Penicillin binding protein (PBP)
Porin channel
O-Poly-saccharide
Lipid A
Lipopoly-saccharide (LPS)
Outer membrane
Periplasmic space
β-Lactamase
Phospholipid
Proteins
B
Glycopeptide polymer outside the cell
Peptidoglycan layer
NAG
NAM
TP
linker
GT
PBP
lipid II
Plasma membrane
Cytosol
L-Alanine
D-Glutamate
L-Lysine/mDAP
D-Alanine
NAM = N-Acetyl-muramic acid
NAG = N-Acetyl-glucosamine
TP = Transpeptidase
GT = Glucosyltransferase
= (gly)$_5$ bridge

Figure 58–1 **A.** *Structure and composition of gram-positive and gram-negative cell envelope.* **B.** *Penicillin binding protein (PBP) activity and inhibition.* PBPs have two enzymatic activities that are crucial to synthesis of the peptidoglycan layers of bacterial cell walls: a transpeptidase that cross-links amino acid side chains, as shown for gram-positives, and a glycosyltransferase that links subunits of the glycopeptide polymer. The transpeptidase and glycosyltransferase domains are separated by a linker region. The glycosyltransferase is thought to be partially embedded in the membrane.

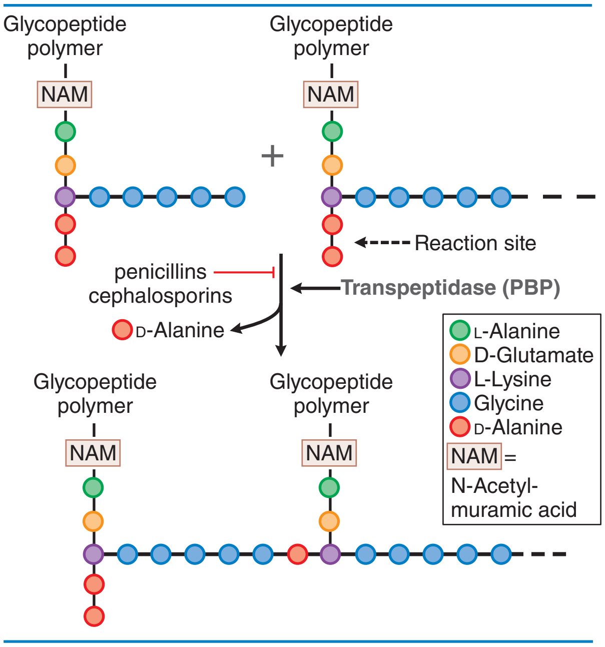


Figure 58–2 *Action of β-lactam antibiotics in S. aureus.* The bacterial cell wall consists of glycopeptide polymers (an NAM-NAG amino-hexose backbone) linked via bridges between amino acid side chains. In *S. aureus*, the bridge is $(Gly)_5$-D-Ala between lysines. The cross-linking is catalyzed by a transpeptidase, the enzyme that penicillins and cephalosporins inhibit.

valves) produce biofilms. Bacteria in biofilms secrete a protective extracellular matrix, which can consist of secreted exopolysaccharides, proteinaceous fibers, and DNA and, in part owing to decreased growth rates and drug penetrance, are much less sensitive to antibiotic therapy (Donlan, 2001). The β-lactam antibiotics are most active against bacteria in the logarithmic phase of growth and have little effect on microorganisms in the stationary phase. Similarly, bacteria that survive inside viable cells of the host generally are protected from the action of the β-lactam antibiotics, which limits the activity of these drugs against some important intracellular pathogens.

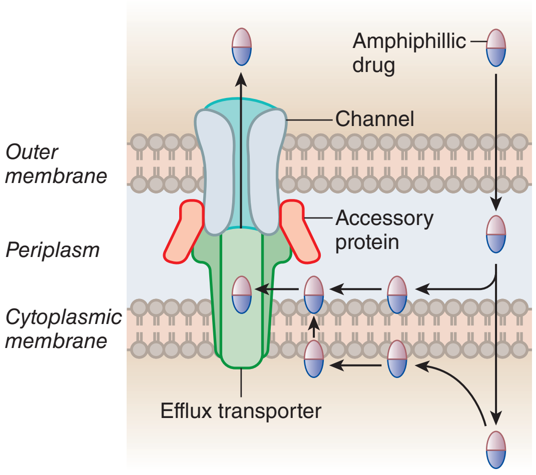
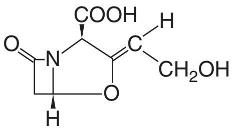


Figure 58–3 *Antibiotic efflux pumps of gram-negative bacteria.* Multidrug efflux pumps traverse both the inner and outer membranes of gram-negative bacteria. The pumps are composed of a minimum of three proteins and are energized by the proton motive force. Increased expression of these pumps is an important cause of antibiotic resistance. (Reprinted with permission from Oxford University Press. Nikaido H. Antibiotic resistance caused by gram-negative multidrug efflux pumps. *Clin Infect Dis*, **1998**, *27*(suppl 1): S32–S41. © 1998 by the Infectious Diseases Society of America. All rights reserved.)

β-Lactamase Inhibitors

Because of the key role that β-lactamases play in conferring resistance to β-lactams, an increasing number of β-lactams are coformulated with molecules whose role is to "protect" the β-lactam from the β-lactamase. These β-lactamase inhibitors bind to β-lactamases and prevent the enzymes from hydrolyzing β-lactam agents in the vicinity. Older-generation β-lactamase inhibitors (e.g., *clavulanate*, *sulbactam*, and *tazobactam*) inactivate many plasmid-encoded β-lactamases but fail to provide protection at clinically achievable concentrations against the AmpC β-lactamases encoded chromosomally in some gram-negative bacilli (e.g., *Enterobacter*, *Citrobacter*, and *Pseudomonas*), as well as carbapenemases of the *Klebsiella pneumoniae* carbapenemase (KPC)- and metallo-β-lactamase type. *Avibactam*, *vaborbactam*, and *relebactam* are new β-lactamase inhibitors that are structurally dissimilar from the older generation, with a broader spectrum of inhibition.

Clavulanic acid has poor intrinsic antimicrobial activity but is an irreversible mechanism-based inhibitor that binds β-lactamases produced by a wide range of gram-positive and gram-negative microorganisms. *Clavulanic acid* is well absorbed by mouth and also can be given parenterally. It is combined with *amoxicillin* as an oral preparation and with *ticarcillin* as a parenteral preparation (*ticarcillin/clavulanate*, not available in the U.S.).



CLAVULANIC ACID

Sulbactam is a β-lactamase inhibitor similar in structure to *clavulanic acid*. It is available for intravenous or intramuscular use combined with *ampicillin* and with *cefoperazone* (not available in the U.S.). *Sulbactam* also possesses intrinsic activity against *Acinetobacter* spp. and has been used in high dosages to treat multidrug-resistant *Acinetobacter* infections.

Tazobactam is a β-lactamase inhibitor with good activity against many of the plasmid-mediated β-lactamases, including some of the extended-spectrum class. It is available as parenteral combination products with *piperacillin* and with *ceftolozane*.

Avibactam and ***relebactam*** are novel, structurally similar non–β-lactam β-lactamase inhibitors that provide clinically useful inhibition against both narrow- and extended-spectrum β-lactamase (ESBL)-type, chromosomal AmpC, and KPC-type β-lactamases (although not metallo-β-lactamases). *Avibactam* is coformulated with *ceftazidime*, while *relebactam* is coformulated with *imipenem/cilastatin*.

Vaborbactam is a novel, boronic acid–based non–β-lactam β-lactamase inhibitor that provides broad inhibition of β-lactamases similar to *avibactam* and *relebactam*. *Vaborbactam* is coformulated with *meropenem*.

The Penicillins

Despite the emergence of resistance, the penicillins remain the drugs of choice for a significant number of infectious diseases. Penicillins (Figure 58–4) consist of a thiazolidine ring (A) connected to a β-lactam ring (B) to which is attached a side chain (R). The penicillin nucleus itself is the chief structural requirement for biological activity. Side chains can be added that alter the susceptibility of the resulting compounds to inactivating enzymes (β-lactamases), improve affinity for PBPs, enhance the ability of the drug to traverse the outer membrane of gram-negative bacteria, and change the pharmacokinetic properties of the drug (Table 58–1).

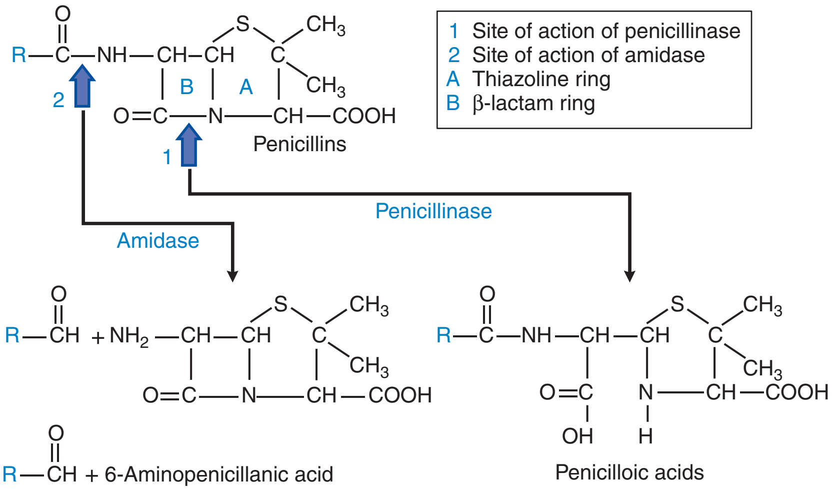

1 Site of action of penicillinase
2 Site of action of amidase
A Thiazoline ring
B β-lactam ring
Penicillins
Amidase
Penicillinase
R—CH + 6-Aminopenicillanic acid
Penicilloic acids

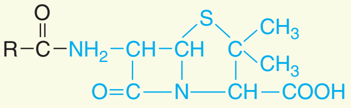
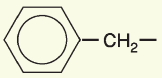
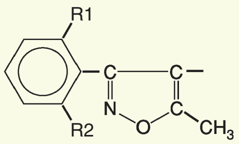
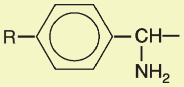
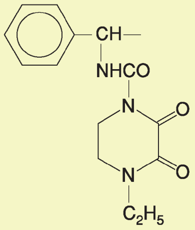
Figure 58–4 *Structure of penicillins and products of their enzymatic hydrolysis.*

Classification of the Penicillins and Summary of Their Pharmacological Properties

Penicillins are classified according to their spectra of antimicrobial activity.

- ***Penicillin G*** and its close congener ***penicillin V*** are highly active against sensitive strains of gram-positive cocci, but they are readily hydrolyzed by penicillinases. Thus, they are ineffective against most strains of *S. aureus*.
- **The penicillinase-resistant penicillins** *methicillin* (discontinued in the U.S.), *cloxacillin* and *flucloxacillin* (not currently marketed in the U.S.), *nafcillin, oxacillin*, and *dicloxacillin* have less-potent antimicrobial activity against microorganisms that are sensitive to *penicillin G*, but they are preferred agents for treatment of penicillinase-producing *S. aureus* and *Staphylococcus epidermidis* that are not *methicillin* resistant.
- *Ampicillin, amoxicillin,* and others such as *bacampicillin* and *pivampicillin* (not currently marketed in the U.S.) are the **aminopenicillins**, whose antimicrobial activity is similar to *penicillin G* but extends to include some gram-negative microorganisms (e.g., *Haemophilus influenzae*, *Escherichia coli*, and *Proteus mirabilis*) when those pathogens do not produce β-lactamases. These drugs are also available as coformulations with a β-lactamase inhibitor, such as *clavulanate* or *sulbactam*, which restores activity against some β-lactamase–producing organisms.
- Agents with extended antimicrobial activity against *Pseudomonas*, *Enterobacter*, and *Proteus* spp. include older agents largely out of use: *azlocillin, carbenicillin, mezlocillin, ticarcillin, ticarcillin/clavulanate,* and *carbenicillin indanyl sodium* (all discontinued in the U.S.). *Piperacillin* and the coformulation of *piperacillin/tazobactam* have antimicrobial activity against many isolates of *Pseudomonas*, *E. coli, Klebsiella*, and other gram-negative microorganisms. *Piperacillin* retains the activity of *ampicillin* against gram-positive cocci and *Listeria monocytogenes*.

TABLE 58–1 ■ CHEMICAL STRUCTURES OF SELECTED PENICILLINS

 Penicillins are substituted 6-aminopenicillanic acid	
Addition of the substituents (R groups) shown below to the parent structure produces penicillins with altered susceptibility to inactivating enzymes (β-lactamases), antibacterial activity, and pharmacological properties.	
 Penicillin G (Benzylpenicillin)	 Oxacillin (R1=R2=H)/Cloxacillin (R1=Cl, R2=H) /Dicloxacillin (R1=R2=Cl)
 Ampicillin (R=-H)/ Amoxicillin (R=-OH)	 Piperacillin

General Common Properties

Following absorption of an oral dose, penicillins are distributed widely throughout the body. Therapeutic concentrations of penicillins are achieved readily in tissues and in secretions such as joint fluid, pleural fluid, pericardial fluid, and bile. Penicillins do not penetrate living phagocytic cells to a significant extent, and only low concentrations of these drugs are found in prostatic secretions, brain tissue, and intraocular fluid. Concentrations of penicillins in CSF are variable but are less than 1% of those in plasma when the meninges are normal. When there is inflammation, concentrations in CSF may increase to as much as 5% of the plasma value. Penicillins are eliminated rapidly by glomerular filtration and renal tubular secretion, such that their half-lives in the body are short, typically 30 to 90 min. As a consequence, concentrations of these drugs in urine are high.

Penicillin G and Penicillin V

Antimicrobial Activity

The antimicrobial spectra of *penicillin G* (*benzylpenicillin*) and *penicillin V* (the phenoxymethyl derivative) are similar for aerobic gram-positive microorganisms. Most streptococci remain susceptible, but penicillin-resistant viridans streptococci and *S. pneumoniae* are becoming more common. Penicillin-resistant pneumococci are especially common in pediatric populations and may also be resistant to third-generation cephalosporins. Greater than 90% of strains of *S. aureus*, most strains of *S. epidermidis,* and many strains of gonococci are now resistant to *penicillin G*. With rare exceptions, the meningococcus (*Neisseria meningitidis*) remains quite sensitive to *penicillin G*.

Most anaerobic gram-positive microorganisms, including *Clostridium* spp., are highly sensitive. Activity against gram-negative anaerobes is more variable, with the intestinal anaerobe *Bacteroides fragilis* displaying resistance to penicillins and cephalosporins by virtue of expressing a broad-spectrum cephalosporinase. *Actinomyces israelii*, *Streptobacillus moniliformis*, *Pasteurella multocida*, and *L. monocytogenes* are inhibited by clinically achievable concentrations of *penicillin G*. Spirochetes, including *Leptospira* spp. (leptospirosis), *Treponema pallidum* (syphilis), and *Borrelia burgdorferi* (Lyme disease), are typically penicillin susceptible. Penicillins are not effective against amebae, plasmodia, rickettsiae, fungi, or viruses.

ADME

Oral Administration of Penicillin G and V. The virtue of *penicillin V* in comparison with *penicillin G* is that it is more stable in an acidic medium and therefore is better absorbed from the gastrointestinal (GI) tract, yielding plasma concentrations two to five times those provided by *penicillin G*. Thus, *penicillin V* is used for oral administration. Absorption is rapid, and maximal concentrations in blood are attained in 30 to 60 min. Ingestion of food may interfere with enteric absorption of all penicillins. Thus, oral penicillins should generally be administered at least 30 min before a meal or 2 h after.

Parenteral Administration of Penicillin G. After intramuscular injection, peak concentrations in plasma are reached within 15 to 30 min, declining rapidly thereafter ($t_{1/2}$ ~30 min). Repository preparations of *penicillin G* (*penicillin G benzathine*, *penicillin G procaine*) increase the duration of the effect. The repository compound favored for most indications is *penicillin G benzathine*, which releases *penicillin G* slowly from the injection site and produces relatively low but persistent concentrations in the blood. The average duration of demonstrable antimicrobial activity in the plasma is about 26 days for *benzathine penicillin G*. *Penicillin G procaine* has a prolonged $t_{1/2}$ compared to *penicillin G*, but shorter than that of *benzathine* formulations; it is typically dosed once daily. Neither depot formulation should be given intravenously as serious toxicity can result.

Distribution. *Penicillin G* is distributed extensively throughout the body, but the concentrations in various fluids and tissues differ widely. Its apparent volume of distribution is about 0.35 L/kg. Approximately 60% of the *penicillin G* in plasma is reversibly bound to albumin. Significant amounts appear in liver, bile, kidney, semen, joint fluid, lymph, and intestine. *Probenecid* markedly decreases the tubular secretion of the penicillins and also produces a significant decrease in the apparent volume of distribution of the penicillins (see Figure 42–2).

Penetration Into Cerebrospinal Fluid. *Penicillin* does not readily enter the CSF but penetrates more easily when the meninges are inflamed. The concentrations are usually in the range of 5% of the value in plasma and are therapeutically effective against susceptible microorganisms if the minimum inhibitory concentration (MIC) of the organism is sufficiently low. *Penicillin* and other organic acids are secreted rapidly from the CSF into the bloodstream by an active transport process. *Probenecid* competitively inhibits this transport and thus elevates the concentration of *penicillin* in CSF. In uremia, other organic acids accumulate in the CSF and compete with *penicillin* for secretion; the drug occasionally reaches toxic concentrations in the brain and can produce convulsions.

Excretion. Approximately 60% to 90% of an intramuscular dose of *penicillin G* in aqueous solution is eliminated in the urine, largely within the first hour after injection. The remainder is metabolized to penicilloic acid. The $t_{1/2}$ for elimination of *penicillin G* is about 30 min in normal adults. Approximately 10% of the drug is eliminated by glomerular filtration and 90% by tubular secretion. Renal clearance approximates the total renal plasma flow. Clearance values are considerably lower in neonates and infants; as a result, *penicillin* persists in the blood several times longer in premature infants than in children and adults. The $t_{1/2}$ of the antibiotic in children less than 1 week of age is 3 h; by 14 days of age, it is .4 h. After renal function is fully established in young children, the rate of renal excretion of *penicillin G* is considerably more rapid than in adults. Anuria increases the $t_{1/2}$ of *penicillin G* from 0.5 to about 10 h. When renal function is impaired, 7% to 10% of the antibiotic may be inactivated each hour by the liver. The dose of the drug must be adjusted in patients with renal insufficiency or receiving dialysis. If hepatic insufficiency also is present, the $t_{1/2}$ will be prolonged even further.

HISTORY

The history of the brilliant research that led to the discovery and development of *penicillin* is well chronicled (Lax, 2004). In 1928, while studying *Staphylococcus* variants in the laboratory at St. Mary's Hospital in London, Alexander Fleming observed that a mold contaminating one of his cultures caused the bacteria in its vicinity to undergo lysis. Broth in which the fungus was grown was markedly inhibitory for many microorganisms. Because the mold belonged to the genus *Penicillium*, Fleming named the antibacterial substance *penicillin*.

A decade later, *penicillin* was developed as a systemic therapeutic agent by the concerted research of a group of investigators at Oxford University headed by Florey, Chain, and Abraham. By May 1940, a crude preparation was found to produce dramatic therapeutic effects when administered parenterally to mice with streptococcal infections. Sufficient *penicillin* was accumulated by 1941 to conduct therapeutic trials in several patients desperately ill with staphylococcal and streptococcal infections refractory to all other therapy. At this stage, the crude, amorphous *penicillin* was only about 10% pure, and it required nearly 100 L of growth broth to obtain enough of the antibiotic to treat one patient for 24 h. Bedpans were used by the Oxford group for growing cultures of *Penicillium notatum*. Case 1 in the 1941 report from Oxford was that of a policeman, who was suffering from a severe mixed staphylococcal and streptococcal infection. He was treated with *penicillin*, some of which had been recovered from the urine of other patients who had been given the drug. It is said that an Oxford professor referred to *penicillin* as a remarkable substance grown in bedpans and purified by passage through the Oxford Police Force.

A vast research program soon was initiated in the U.S. There were 122 million units of *penicillin* made available during 1942, and the first clinical trials were conducted at Yale University and the Mayo Clinic, with dramatic results. By the spring of 1943, there were 200 patients who had been treated with the drug. The results were so impressive that the surgeon general of the U.S. Army authorized a trial of the antibiotic in a military hospital. Soon thereafter, *penicillin* was adopted throughout the medical services of the U.S. Armed Forces.

The deep-fermentation procedure for the biosynthesis of *penicillin* marked a crucial advance in the large-scale production of the antibiotic. From a total production of a few hundred million units a month in the early days, the quantity manufactured rose to over 200 trillion units (nearly 150 tons) by 1950. The first marketable *penicillin* cost several dollars per 100,000 units; today, the same dose costs only a few cents.

Therapeutic Uses

Pneumococcal Infections. *Penicillin G* remains the agent of choice for the management of infections caused by sensitive strains of *S. pneumoniae*, but resistance is an increasing concern. For parenteral therapy of sensitive isolates of pneumococci, *penicillin G* is favored. Because of concerns for β-lactam resistance, pneumococcal meningitis should be treated with a combination of *vancomycin* and a third-generation cephalosporin until it is established that the infecting pneumococcus is *penicillin* sensitive. *Dexamethasone* given prior to or at the same time as antibiotics is associated with an improved outcome in pneumococcal meningitis (de Gans et al., 2002). The recommended regimens for severe pneumococcal infections range from 12 to 24 million units of *penicillin G* per day by constant intravenous infusion or divided into boluses every 4 to 6 h for 7 to 14 days.

β-Hemolytic Streptococcal Infections. Streptococcal pharyngitis is a common respiratory manifestation of infection due to *Streptococcus pyogenes* (group A β-hemolytic *Streptococcus*). Penicillin-resistant isolates have yet to be observed. The preferred oral therapy is with *penicillin V*, 500 mg twice daily for 10 days. *Penicillin* therapy of streptococcal pharyngitis reduces the risk of subsequent acute rheumatic fever; however, current evidence suggests that the incidence of glomerulonephritis that follows streptococcal infections is not reduced to a significant degree by treatment with *penicillin* (Shulman et al., 2012). *S. pyogenes* is also a common cause of skin infections, ranging in severity from erysipelas and cellulitis to toxic shock and necrotizing fasciitis. The former two infections can be treated with oral *penicillin V*. The latter two are life-threatening infections associated with toxin production. Recommended treatment is with *penicillin* plus *clindamycin*, which may provide benefit by decreasing streptococcal toxin production (Stevens et al., 2014).

Infections Caused by Other Streptococci and Enterococci. The viridans group of streptococci is the most common cause of native valve infectious endocarditis. These are nongroupable α-hemolytic microorganisms that are increasingly resistant to *penicillin G*. In patients with endocarditis, it is important to determine quantitative microbial sensitivities to *penicillin G*, which guides drug selection, dosing, and use of combination therapy. Patients with highly penicillin-susceptible viridans group streptococcal native valve endocarditis can be treated successfully with daily doses of 12 to 20 million units of intravenous *penicillin G* for 4 weeks or for 2 weeks if given in combination with *gentamicin*. *Penicillin G* is a less-preferred alternative to *ampicillin* for the treatment of susceptible enterococcal infections.

Infections With Anaerobes. Pulmonary and periodontal infections usually respond well to *penicillin G*. Mild-to-moderate infections at these sites may be treated with oral medication (either *penicillin G* or *penicillin V* 250 mg four times daily). More severe infections should be treated with 12 to 24 million units of *penicillin G* IV. *Penicillin G* (12–24 million units per day given parenterally) plus *clindamycin* is recommended for clostridial gas gangrene. Adequate debridement of the infected areas is essential. Antibiotics probably have no effect on the outcome of tetanus due to *Clostridium tetani*. Debridement and administration of human tetanus immune globulin may be indicated.

Neisseria *spp.* Infections. *Penicillin G* is an alternative to third-generation cephalosporins for treatment of infections due to *N. meningitidis*. Patients should be treated with high doses of *penicillin* given IV. The occurrence of penicillin-resistant strains should be considered in patients who are slow to respond to treatment. *Penicillin G* does not eliminate the meningococcal carrier state, and its administration thus is ineffective as a prophylactic measure. Gonococci gradually have become more resistant to *penicillin G*, and penicillins are no longer the therapy of choice.

Syphilis. Therapy of syphilis with *penicillin G* is highly effective. Primary, secondary, and latent syphilis of less than 1 year in duration may be treated with one to three weekly intramuscular doses of 2.4 million units of *penicillin G benzathine*. Patients with neurosyphilis or cardiovascular syphilis typically receive intensive therapy with 18 to 24 million units of *penicillin G* daily for 10 to 14 days. There are no proven alternatives for treating syphilis in pregnant women, so penicillin-allergic individuals must be acutely desensitized to prevent anaphylaxis.

Patients with secondary syphilis may develop the Jarisch-Herxheimer reaction, including chills, fever, headache, myalgias, and arthralgias occurring several hours after the first dose of *penicillin*. This reaction is thought to be due to release of spirochetal antigens with subsequent host reactions to the products. Antipyretics give symptomatic relief, and therapy with *penicillin* should not be discontinued.

Actinomycosis. *Penicillin G* is the agent of choice for the treatment of all forms of actinomycosis (18–24 million units of *penicillin G* IV per day for 6 weeks). Surgical drainage or excision of the lesion may be necessary before cure is accomplished.

Listeria Infections. *Ampicillin* or *penicillin G*—with consideration for addition of *gentamicin* to both for immunosuppressed patients with meningitis—are the drugs of choice in the management of infections owing to *L. monocytogenes*. The recommended dose of *penicillin G* is 18 to 24 million units parenterally per day for at least 2 weeks. For endocarditis, the dose is the same, but the duration of treatment should be no less than 4 weeks.

Pasteurella multocida. *P. multocida* is a cause of wound infections after a cat or dog bite. It is susceptible to *penicillin G* and *ampicillin* and resistant to penicillinase-resistant penicillins and first-generation cephalosporins.

Prophylactic Uses of the Penicillins

Patients with anatomic or functional asplenia are at risk for infection with encapsulated bacteria including *S. pneumoniae* and *N. meningitidis*. In addition to vaccination, some asplenic patients receive antibacterial prophylaxis with *penicillin V*. The oral administration of 200,000 units of *penicillin G* or *penicillin V* every 12 h decreases the incidence of recurrences of rheumatic fever in susceptible individuals. The intramuscular injection of 1.2 million units of *penicillin G benzathine* once a month also yields excellent results. Prophylaxis must be continued throughout the year. Some suggest that prophylaxis should be continued for life because instances of acute rheumatic fever have been observed in the fifth and sixth decades, but the necessity of lifetime prophylaxis has not been established.

The Penicillinase-Resistant Penicillins

The penicillinase-resistant penicillins are resistant to hydrolysis by staphylococcal penicillinase. However, an increasing number of isolates of *S. aureus*, around half in most U.S. hospitals, and *S. epidermidis*, more than three-quarters, express a low-affinity PBP, giving them the MRSA or *methicillin*-resistant *S. epidermidis* (MRSE) phenotype. This term denotes resistance of these bacteria to all β-lactams, with the exception of *ceftaroline* and *ceftobiprole* (not available in the U.S.). Note that because *methicillin* was the first penicillinase-resistant penicillin in widespread use, the terms *MRSA* and *MRSE* are commonly used, despite the fact *methicillin* is currently rarely used. Alternative agents such as *vancomycin* or *daptomycin*, discussed below, may be used for infections due to organisms with this resistance mechanism.

The Isoxazolyl Penicillins: Oxacillin, Cloxacillin, and Dicloxacillin

Oxacillin, *cloxacillin* (not available in the U.S.), and *dicloxacillin* are semisynthetic isoxazolyl penicillin congeners that are markedly resistant to cleavage by penicillinase. *Nafcillin* is a similar congener of a slightly different structure. These drugs are not substitutes for *penicillin G* in the treatment of diseases amenable to it and are not active against enterococci, *Listeria*, or gram-negative organisms.

Pharmacological Properties. The isoxazolyl penicillins are potent inhibitors of the growth of most penicillinase-producing staphylococci. *Dicloxacillin* is the most active, and many strains of *S. aureus* are inhibited by concentrations of 0.05 to 0.8 μg/mL. *Nafcillin* is slightly more active than *oxacillin* against *penicillin G*–resistant *S. aureus* (most strains are inhibited by 0.06–2 μg/mL). Although it is the most active of the penicillinase-resistant penicillins against other microorganisms, it is not as potent as *penicillin G*.

Dicloxacillin and *cloxacillin* are available for oral administration; these agents are absorbed rapidly but incompletely (30%–80%) from the GI tract. Absorption increases when administered 1 h before or 2 h after meals. Peak concentrations in plasma are attained by 1 h. *Nafcillin* is only available for parenteral administration. All these congeners are bound to plasma albumin to a great extent (~90%–95%); none is removed from the circulation to a significant degree by hemodialysis. Concentrations of the drug in CSF appear to be adequate for therapy of staphylococcal meningitis. The isoxazolyl penicillins are excreted by the kidney; there is also significant hepatic degradation and elimination in the bile. The $t_{1/2}$ for all are between 30 and 60 min. No dosing adjustments are needed for patients with renal failure. *Nafcillin* is a known inducer of the cytochrome P450 enzyme system, and caution should be used during coadministration with drugs metabolized via this pathway.

Therapeutic Indications

For mild to moderate skin and soft-tissue infections, the penicillinase-resistant penicillins can be administered orally (e.g., *dicloxacillin* 500 mg every 6 h) or parenterally (e.g., *nafcillin* 1–2 g every 6 h). For treatment of serious methicillin-susceptible *S. aureus* infections such as endocarditis, higher doses (e.g., *oxacillin* 2 g IV every 4 h) are employed.

The Aminopenicillins: Ampicillin and Amoxicillin

Aminopenicillins expand the spectrum of activity of *penicillin G* in a different direction from the penicillinase-resistant penicillins—they allow for useful activity against more gram-negative organisms. They are hydrolyzed by β-lactamases (from both gram-positive and gram-negative bacteria); thus, further expansion of their activity is enabled through coformulation with β-lactamase inhibitors (see the end of the chapter for further discussion of the chemistry and activity of β-lactamase inhibitors).

Antimicrobial Activity

Ampicillin and *amoxicillin* are generally bactericidal for susceptible gram-positive and gram-negative bacteria. The antimicrobial spectrum of *amoxicillin* is essentially identical to that of *ampicillin*, except that *amoxicillin* is less active and less effective than *ampicillin* for shigellosis. Gram-positive activity is broadly similar to that of the natural penicillins. Pneumococcal and viridans group streptococci isolates have varying levels of resistance to *ampicillin*, and penicillin-resistant strains should be considered *ampicillin/amoxicillin* resistant. Enterococci are about twice as sensitive to *ampicillin* as they are to *penicillin G*. That fraction of *H. influenzae* isolates that do not produce β-lactamases (between 60% and 80%) are typically *aminopenicillin* susceptible. From 30% to 60% of *E. coli*, a significant number of *P. mirabilis*, and all *Klebsiella* are resistant. Most strains of *Shigella*, *Pseudomonas*, *Serratia*, *Acinetobacter*, *B. fragilis,* and indole-positive *Proteus* also are resistant to this group of penicillins. Resistant strains of *Salmonella* are recovered with increasing frequency. Concurrent administration of a β-lactamase inhibitor such as *clavulanate* or *sulbactam* expands their spectrum of activity, particularly against *S. aureus*, *H. influenzae*, *E. coli*, *Klebsiella, Proteus*, and *B. fragilis*.

ADME

Ampicillin. An oral dose of 0.5 g of *ampicillin* produces peak concentrations in plasma of about 3 μg/mL at 2 h. Intake of food prior to ingestion of *ampicillin* diminishes absorption. Intramuscular injection of 0.5 to 1 g of sodium *ampicillin* yields peak plasma concentrations of about 7 to 10 μg/mL, respectively, at 1 h. Plasma levels decline with a $t_{1/2}$ of about 80 min. Severe renal impairment markedly prolongs the $t_{1/2}$. Peritoneal dialysis is ineffective in removing the drug from the blood, but hemodialysis removes approximately 40% of the body store in about 7 h. Adjustment of the dose of *ampicillin* is required in the presence of renal dysfunction. *Ampicillin* appears in the bile, undergoes enterohepatic circulation, and is excreted in the feces.

Amoxicillin. *Amoxicillin* is a close chemical and pharmacological relative of *ampicillin*. *Amoxicillin* is stable in acid, designed for oral use, and absorbed more rapidly and completely from the GI tract than *ampicillin*. The absorption of *amoxicillin* appears to be partly saturable, with less fractional absorption at higher doses. Peak plasma concentrations of *amoxicillin* are 2 to 2.5 times greater than for *ampicillin* after oral administration of the same dose. Food does not interfere with absorption. Perhaps because of more complete absorption of this congener, the incidence of diarrhea with *amoxicillin* is less than that following administration of *ampicillin*. The incidence of other adverse effects appears to be similar. Although the $t_{1/2}$ of *amoxicillin* is similar to that for *ampicillin*, effective concentrations of orally administered *amoxicillin* are detectable in the plasma for twice as long as with *ampicillin* because of the more complete absorption. For all these reasons, *amoxicillin* is generally preferred over *ampicillin* for oral administration. About 20% of *amoxicillin* is protein bound in plasma, a value similar to that for *ampicillin*. Most of a dose of the antibiotic is excreted in an active form in the urine, and dose adjustment is required in renal dysfunction. *Probenecid* delays excretion of the drug.

Ampicillin/Sulbactam and Amoxicillin/Clavulanate. The pharmacokinetic properties of the coformulated agents (*ampicillin/sulbactam* IV, *amoxicillin/clavulanate* by mouth) are broadly similar to those of the single-agent formulations.

Therapeutic Indications

Respiratory Infections. *Ampicillin* and *amoxicillin* are active against *S. pyogenes* and many strains of *S. pneumoniae* and *H. influenzae*. The drugs constitute effective therapy for sinusitis, otitis media, acute exacerbations of chronic bronchitis, epiglottitis, and pneumonia caused by sensitive strains of these organisms. *Amoxicillin* is the most active of all the oral β-lactam antibiotics against both penicillin-susceptible and penicillin-nonsusceptible *S. pneumoniae*. Based on the increasing prevalence of pneumococcal resistance to *penicillin*, an increase in dose of oral *amoxicillin* (from 40 to 45 up to 80 to 90 mg/kg per day) for empirical treatment of acute otitis media in children is recommended. *Ampicillin*-resistant *H. influenzae* is a problem in many areas; use of *ampicillin/sulbactam* or *amoxicillin/clavulanate* can provide coverage for these organisms as well as for *Moraxella* (which universally produces a β-lactamase). *Amoxicillin* is also an option for empiric treatment of community-acquired pneumonia when patients are at low risk for drug-resistant pathogens or complications at a dose of 1 g every 8 h. *Amoxicillin* is an alternative treatment to *penicillin* for bacterial pharyngitis.

Urinary Tract Infections. Most uncomplicated urinary tract infections are caused by Enterobacterales, and *E. coli* is the most common species isolated from urinary tract infection patients. Aminopenicillins can be effective agents for urinary tract infections, but the high prevalence of resistance among *E. coli* and *Klebsiella* makes empiric use of these drugs for urinary tract infections challenging; using *amoxicillin/clavulanate* can provide broader coverage of these organisms. Cure rates generally with oral β-lactam agents for cystitis are lower than those with other drug classes such as fluoroquinolones or *trimethoprim-sulfamethoxazole*. Enterococcal urinary tract infections can be treated effectively with an aminopenicillin alone.

Enterococcal Bloodstream Infections and Endocarditis. *Ampicillin* at high doses (2 g IV every 4–6 h) is the drug of choice for treatment of serious enterococcal infections including endocarditis. The vast majority of isolates of *Enterococcus faecalis* are susceptible to aminopenicillins. Aminopenicillins as single agents may not provide bactericidal activity against enterococci; thus, for treatment of enterococcal endocarditis, synergistic combinations are recommended. Historically, this would be the combination of *ampicillin* plus *gentamicin*, but recent data suggest the combination of *ampicillin* and *ceftriaxone* provides similar therapeutic effect with less toxicity (Fernandez-Hidalgo et al., 2013). Addition of a β-lactamase inhibitor rarely adds to the activity an aminopenicillin alone against *Enterococcus*, as resistance in enterococci is almost exclusively mediated by PBP changes.

Meningitis. Acute bacterial meningitis in children is frequently due to *S. pneumoniae* or *N. meningitidis*. Because 20% to 30% of strains of *S. pneumoniae* now may be resistant to *ampicillin*, it is not indicated for empiric single-agent treatment of meningitis. *Ampicillin* has excellent activity against *L. monocytogenes*, a cause of meningitis in immunocompromised persons. The combination of high-dose *ampicillin* and *vancomycin* plus a third-generation cephalosporin is a recommended regimen for empirical treatment of suspected bacterial meningitis in patients at risk for *L. monocytogenes*.

Antipseudomonal Penicillins: The Carboxypenicillins and the Ureidopenicillins

Antimicrobial Activity

This class contains a number of agents no longer in widespread use, including *carbenicillin*, *ticarcillin*, and *mezlocillin* (all discontinued in the U.S.). These agents are active against some isolates of *Pseudomonas aeruginosa* and certain indole-positive *Proteus* spp. that are resistant to ampicillin and its congeners but are ineffective against most strains of *S. aureus*, *E. faecalis*, *Klebsiella*, and *L. monocytogenes.* The ureidopenicillin *piperacillin* is used most commonly as the combination product *piperacillin/tazobactam* and has broad activity against streptococci, enterococci, and enteric gram-negative rods and good activity against *P. aeruginosa.*

ADME

Carbenicillin Indanyl Sodium. This indanyl ester of *carbenicillin* is acid stable and is suitable for oral administration. After absorption, the ester is converted rapidly to *carbenicillin* by hydrolysis of the ester linkage. The active moiety is excreted rapidly in the urine, where it achieves effective concentrations. Thus, where available, the only use of this drug is for the management of urinary tract infections caused by *Proteus* spp. other than *P. mirabilis* and by *P. aeruginosa.*

Ticarcillin and Ticarcillin/Clavulanate. The semisynthetic penicillin *ticarcillin* is more active than *carbenicillin* versus *P. aeruginosa* but less active than *piperacillin*. The combination of *ticarcillin* and *clavulanate* has activity against other gram-negative aerobic and anaerobic organisms, such as *Stenotrophomonas maltophilia*, and has been used for intra-abdominal and urinary tract infections. In the U.S., the manufacture of *ticarcillin* alone and in combination with *clavulanate* has been discontinued.

Piperacillin and Piperacillin/Tazobactam. *Piperacillin* extends the spectrum of *ampicillin* to include most strains of *P. aeruginosa*, Enterobacterales (non–β-lactamase producing), many *Bacteroides* spp., and *E. faecalis*. Combined with a β-lactamase inhibitor (*piperacillin/tazobactam*), it has the broadest antibacterial spectrum of the penicillins, including activity against *methicillin*-susceptible *S. aureus*, *H. influenzae*, *B. fragilis*, and most *E. coli* and *Klebsiella*. The drug is only available for parenteral administration. High biliary concentrations are achieved. Distribution into the CNS by *piperacillin* is similar to that of other penicillins, but CSF concentrations of *tazobactam* may be inadequate to protect *piperacillin* against β-lactamase–producing organisms. The drug is eliminated renally and requires adjustment in renal dysfunction.

Therapeutic Uses

Piperacillin/tazobactam is an important agent for the treatment of patients with serious infections caused by gram-negative bacteria, including infections acquired in the hospital. This agent finds its greatest use in treating bacteremias, pneumonias, infections following burns, and urinary tract infections owing to microorganisms resistant to *ampicillin*; the bacteria especially responsible include *P. aeruginosa*, indole-positive strains of *Proteus*, and *Enterobacter* spp. While organisms producing extended-spectrum β-lactamases may test as susceptible to *piperacillin/tazobactam*, a randomized trial found increased mortality among patients with infections due to ESBL-producing organisms compared to *meropenem* (Harris et al., 2018). Because of *piperacillin/tazobactam*'s good activity against *E. faecalis* and *B. fragilis*, this drug also has utility in mixed intra-abdominal infections. *Piperacillin/tazobactam* is dosed every 6 to 8 h in patients with normal renal function, at a dose of 4 g of *piperacillin* and 0.5 g of *tazobactam*. The infusion can be extended over 4 h to improve its antibacterial activity.

Adverse Reactions

Hypersensitivity Reactions. Hypersensitivity reactions are the most clinically important adverse effects noted with the penicillins, and these agents are among the most common causes of drug allergy.

Manifestations of hypersensitivity to penicillins include maculopapular rash, urticarial rash, fever, bronchospasm, vasculitis, serum sickness, exfoliative dermatitis, Stevens-Johnson syndrome, and anaphylaxis (Romano et al., 2003). Hypersensitivity reactions may occur with any dosage form of penicillin. Hypersensitivity reactions may appear in the absence of a previous known exposure to the drug. This may be caused by unrecognized prior exposure to penicillin in the environment (e.g., in foods of animal origin or from the fungus-producing penicillin). Although elimination of the antibiotic usually results in rapid clearing of the allergic manifestations, they may persist for 1 to 2 weeks or longer after therapy has been stopped. In some cases, the reaction is mild and disappears even when the penicillin is continued; in others, immediate cessation of penicillin treatment is required. Penicillins and their breakdown products act as haptens after covalent reaction with proteins. The most abundant breakdown product is the penicilloyl moiety (known as the "major determinant"), which is formed when the β-lactam ring is opened. A large percentage of IgE-mediated reactions are to the major determinant, but a significant fraction are to other breakdown products. The terms *major* and *minor determinants* refer to the frequency with which antibodies to these haptens appear to be formed, not the severity of the reaction that may result. Antipenicillin antibodies are detectable in virtually all patients who have received the drug as well as some who have never knowingly been exposed to it. Immediate allergic reactions are mediated by skin-sensitizing or IgE antibodies, usually of minor-determinant specificities. Accelerated and late urticarial reactions usually are mediated by major determinant–specific skin-sensitizing antibodies.

The most serious hypersensitivity reactions produced by the penicillins are angioedema and anaphylaxis. Acute anaphylactic or anaphylactoid reactions induced by various preparations of penicillin constitute the most important immediate danger connected with their use. Anaphylactoid reactions may occur at any age. Their incidence is thought to be 0.004% to 0.04%. About 0.001% of patients treated with these agents die from anaphylaxis. Anaphylaxis most often has followed the injection of penicillin, although it also has been observed after oral or intradermal administration. The most dramatic reaction is sudden, severe hypotension and rapid death. In other instances, bronchoconstriction with severe asthma; abdominal pain, nausea, and vomiting; extreme weakness; or diarrhea and purpuric skin eruptions have characterized the anaphylactic episodes.

Skin rashes of all types may be caused by allergy to penicillin. The incidence of skin rashes appears to be highest following the use of *ampicillin*, at about 9%. Rashes follow the administration of *ampicillin* frequently in patients with infectious mononucleosis, but in such cases, patients can tolerate subsequent courses of *ampicillin* without experiencing a rash (Kerns et al., 1973). Serum sickness of variable intensity and severity, mediated by IgG antibodies, is rare; when it occurs, it appears after penicillin treatment has been continued for 1 week or more; it may be delayed until 1 or 2 weeks after the drug has been stopped and may persist for a week or longer. Vasculitis may be related to penicillin hypersensitivity. The Coombs reaction frequently becomes positive during prolonged therapy, but hemolytic anemia is rare. Reversible neutropenia has been noted, occurring in up to 30% of patients treated with 8 to 12 g of *nafcillin* for more than 21 days. The bone marrow shows an arrest of maturation. Eosinophilia is an occasional accompaniment of other allergic reactions to penicillin. Penicillins rarely cause interstitial nephritis; *methicillin* (no longer marketed in the U.S.) has been implicated most frequently, but other antistaphylococcal penicillins as well as *piperacillin* appear to be among the most common culprits among β-lactams.

Evaluation of the patient's history is the most practical way to avoid the use of penicillin in patients who are at the greatest risk of adverse reaction. Although many patients are labeled as penicillin allergic, studies suggested that 90% or more of patients with a history of penicillin allergy will not manifest immediate hypersensitivity reactions on immunologic testing. Such testing can be performed in the clinical setting through commercially available penicillin skin-testing kits that contain the major antigenic determinant (benzylpenicilloyl polylysine); the negative predictive value of a penicillin skin test exceeds 95% for immediate-type hypersensitivity reactions (Shenoy et al., 2019). Occasionally, *desensitization* is recommended for truly penicillin-allergic patients who must receive the drug. This procedure consists of administering gradually increasing doses of penicillin in the hope of avoiding a severe reaction and should be performed only in an intensive care setting. When full doses are reached, the penicillin should not be discontinued and then restarted during treatment of the infectious episode because immediate reactions may recur.

Other Adverse Reactions. The penicillins have minimal direct toxicity. Toxic effects include bone marrow depression, granulocytopenia, and hepatitis; the last effect is rare but is seen most commonly following the administration of *oxacillin* and *nafcillin*. The administration of *penicillin G* and *piperacillin*, and also of *carbenicillin* and *ticarcillin*, has been associated with impaired hemostasis due to defective platelet aggregation (Fass et al., 1987). Most common among the irritative responses to penicillins are pain and sterile inflammatory reactions at the sites of intramuscular injections. In some individuals who receive penicillins intravenously, phlebitis or thrombophlebitis develops. Adverse responses to oral penicillin preparations may include nausea, vomiting, and mild-to-severe diarrhea.

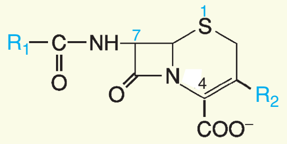
Intrathecal injection of *penicillin G* may produce arachnoiditis or severe and fatal encephalopathy. Because of this, intrathecal or intraventricular administration of penicillins or other β-lactams should be avoided. Similarly, high CSF concentrations of penicillins achieved through intravenous administration of excessive doses, including failure to adjust for reduced renal clearance, can lead to CNS dysfunction. Accidental intravenous instead of intramuscular injection of *penicillin G procaine* may result in an immediate reaction, characterized by dizziness, tinnitus, headache, hallucinations, and sometimes seizures. This is due to the rapid liberation of toxic concentrations of *procaine*. Intravenous injection of *benzathine penicillin G* has been associated with cardiorespiratory arrest and death.

Penicillin changes the composition of the microflora in the GI tract by eliminating sensitive microorganisms. Normal microflora typically reestablish shortly after therapy is stopped; however, in some patients, superinfection results. Intestinal disease, ranging from mild diarrhea to life-threatening pseudomembranous colitis, due to *Clostridium difficile* superinfection has followed oral and, less commonly, parenteral administration of penicillins.

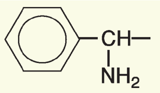
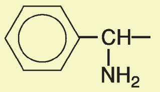
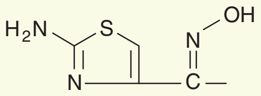
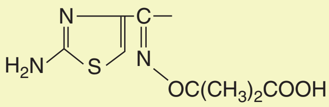
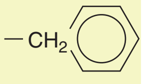
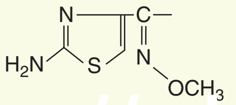
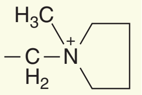
The Cephalosporins

Compounds containing 7-aminocephalosporanic acid are relatively stable in dilute acid and relatively resistant to penicillinase regardless of the nature of their side chains and their affinity for the enzyme. Modifications at position seven of the β-lactam ring are associated with alteration in antibacterial activity; substitutions at position three of the dihydrothiazine ring alter the metabolism and pharmacokinetic properties of the drugs. The cephamycins are similar to the cephalosporins but have a methoxy group at position seven of the β-lactam ring of the 7-aminocephalosporanic acid nucleus (Table 58–2).

TABLE 58–2 ■ CHEMICAL STRUCTURES FOR SELECTED CEPHALOSPORINS



Cephem Nucleus

COMPOUND	R_1	R_2
First generation Cephalexin		$—CH_3$
Second generation Cefaclor		—Cl
Third generation Cefdinir		$CH=CH_2$
Antipseudomonal Ceftazidime		
Anti-MRSA Ceftaroline		

HISTORY

Cephalosporium acremonium, the first source of the cephalosporins, was isolated in 1948 by Brotzu from the sea near a sewer outlet off the Sardinian coast (Grayson, 2010). Crude filtrates from cultures of this fungus were found to inhibit the *in vitro* growth of *S. aureus* and to cure staphylococcal infections and typhoid fever in humans. Culture fluids in which the Sardinian fungus was cultivated were found to contain three distinct antibiotics, which were named *cephalosporin P, N,* and *C*. With isolation of the active nucleus of cephalosporin C, 7-aminocephalosporanic acid, and with the addition of side chains, it became possible to produce semisynthetic compounds with antibacterial activity very much greater than that of the parent substance.

Mechanism of Action

Cephalosporins and cephamycins inhibit bacterial cell wall synthesis in a manner similar to that of penicillin. PBP binding profiles differ somewhat from penicillins; for example, the lack of binding of cephalosporins to essential PBPs in *Enterococcus* spp. means this class as a whole lacks clinically useful activity against these organisms.

Mechanisms of Bacterial Resistance

As with the penicillins, resistance to the cephalosporins may be related to the inability of the antibiotic to reach its sites of action or to alterations in the PBPs that are targets of the cephalosporins. Alterations in two PBPs (1A and 2X) that decrease their affinity for cephalosporins render pneumococci resistant to third-generation cephalosporins because the other three PBPs have inherently low affinity. With the exception of *ceftaroline* and *ceftobiprole*, cephalosporins lack activity against *methicillin*-resistant staphylococci due to their inability to bind to the low-affinity PBP expressed by these organisms.

The most prevalent mechanism of resistance to cephalosporins is inactivation by hydrolysis of the β-lactam ring. The cephalosporins have variable susceptibility to β-lactamases. *Cefoxitin*, *cefuroxime*, and the third-generation cephalosporins are more resistant to hydrolysis by the β-lactamases produced by gram-negative bacteria than first-generation cephalosporins. First- through third-generation cephalosporins, such as *ceftazidime* and *ceftriaxone*, are susceptible to hydrolysis by inducible, chromosomally encoded (*ampC*) β-lactamases present in gram-negative organisms such as *Citrobacter*, *Enterobacter*, and *Pseudomonas*. The inducible nature of these β-lactamases leads to a lower degree of susceptibility among wild-type isolates, whereas selection for mutants with high-level expression (stable derepression) can lead to clinical resistance. These class C enzymes are not substantially inactivated by classical β-lactamase inhibitors, such as *clavulanate* and *tazobactam*. *Cefepime*, *ceftolozane*, and *cefiderocol*, by virtue of their structures, may be less susceptible to hydrolysis by class C β-lactamases than are the third-generation agents. They are, however, susceptible to degradation by OXA- or KPC-type carbapenemases and metallo-β-lactamases. The β-lactamase inhibitor *avibactam* significantly inhibits the activity of AmpC-, OXA-, and KPC-type β-lactamases and enhances the activity of *ceftazidime* in the *ceftazidime/avibactam* coformulation.

Classification and General Pharmacology

Classification has traditionally been by unofficial *generations*, based on general features of antimicrobial activity (Table 58–3). Recent development of novel cephalosporins makes further use of this classification scheme problematic, as newer agents expand activity in different ways. In the absence of consensus on a new generations scheme to date, we will continue to employ this scheme for the first three generations and then differentiate agents after the third generation by their notable activity. None of the cephalosporins have reliable activity against the following bacteria: *Enterococcus*; *L. monocytogenes*; the atypical respiratory

TABLE 58–3 ■ CEPHALOSPORIN CLASSIFICATIONS ("GENERATIONS")

DRUG CLASS	USEFUL ANTIBACTERIAL SPECTRUM[a]
First Generation	
Cefazolin Cephalexin monohydrate Cefadroxil Cephradine*	Streptococci[b]; *S. aureus*[c]; some *Proteus, E. coli, Klebsiella*
Second Generation	
Cefuroxime Cefuroxime axetil Cefprozil	*E. coli*, *Klebsiella*, *Proteus*, *H. influenzae*, *Moraxella catarrhalis*. Not as active against gram-positive organisms as first-generation agents.
Cefoxitin Cefeotetan Cefmetazole*	Inferior activity against *S. aureus* compared to cefuroxime but with added activity against *Bacteroides fragilis* and other *Bacteroides* spp.
Third Generation	
Cefotaxime Ceftriaxone Cefdinir Cefditoren pivoxil Ceftibuten Cefpodoxime proxetil Ceftizoxime	*E. coli*, *Klebsiella*, *Proteus*, *H. influenzae*, *Moraxella catarrhalis, Citrobacter*[d], *Enterobacter*[d]; *Serratia*; *N. gonorrhoeae*; activity for *S. aureus*, *S. pneumoniae*, and *S. pyogenes* comparable to first-generation agents
Antipseudomonal Cephalosporins	
Ceftazidime	Gram-negative activity similar to third-generation with addition of activity against *Pseudomonas*[d]; poor activity vs gram-positive organisms
Ceftazidime/avibactam	Expands ceftazidime's activity against *Pseudomonas*[d] and multidrug-resistant Enterobacterales, but not against gram-positives
Ceftolozane/tazobactam	Similar to ceftazidime, with enhanced activity against *Pseudomonas*[d] and extended-spectrum beta-lactamase-producing Enterobacterales
Cefepime	Comparable to third generation but more resistant to some β-lactamases (especially those of *Pseudomonas*[d] and *Enterobacter*[d]); gram-positive activity similar to cefotaxime
Cefiderocol	Similar to ceftazidime with enhanced activity against *Pseudomonas*[d] and multidrug-resistant (including metallo-beta-lactamase-producing) Enterobacterales, and *Acinetobacter*
Anti-MRSA Cephalosporins	
Ceftaroline Ceftobiprole*	Similar activity to third generation but uniquely adds activity against methicillin-resistant *S. aureus*

[a] All cephalosporins lack clinically useful activity against enterococci, *Listeria monocytogenes*, and atypical respiratory pathogens (*Legionella, Mycoplasma, Chlamydophila* spp.).
[b] Except for penicillin-resistant strains. [c] Except for methicillin-resistant strains. [d] Resistance to cephalosporins may develop during therapy through selection of isolates with de-repression of bacterial chromosomal β-lactamases, which destroy the cephalosporins. *Not marketed in the U.S.

pathogens (*Legionella pneumophila*, *Mycoplasma pneumoniae, Chlamydophila pneumoniae*); *C. difficile*; and *Campylobacter jejuni*.

Many cephalosporins, such as *cephalexin*, *cephradine*, *cefaclor*, *cefadroxil*, *loracarbef*, *cefprozil*, *cefpodoxime proxetil*, *ceftibuten*, *cefuroxime axetil*, *cefdinir*, and *cefditoren* (not all of these agents are available in the U.S.), are absorbed readily after oral administration; others can be administered intramuscularly or intravenously. Cephalosporins are excreted primarily by the kidney; thus, in general, the dosage should be reduced in patients with renal insufficiency. Exceptions are *cefpiramide* (no longer marketed in the U.S.) and *cefoperazone*, which are excreted predominantly in the bile; and *ceftriaxone*, which has mixed renal/nonrenal elimination. Just as for penicillins, *probenecid* slows renal tubular secretion of most cephalosporins. *Cefotaxime* is deacetylated to a metabolite with less antimicrobial activity than the parent compound that is excreted by the kidneys. The other cephalosporins do not undergo appreciable metabolism. Several cephalosporins, most notably *ceftriaxone*, *cefotaxime*, *ceftazidime*, and *cefepime*, penetrate across inflamed meninges in sufficient concentrations relative to typical MICs of the usual pathogens to be useful for the treatment of meningitis. Cephalosporins also cross the placenta, and they are found in high concentrations in synovial and pericardial fluids. Penetration into the aqueous humor of the eye is relatively good after systemic administration of third-generation agents, but penetration into the vitreous humor is poor.

ADME and Agent-Specific Antibacterial Activity

First-Generation Cephalosporins

First-generation cephalosporins (e.g., *cefazolin*, *cephalexin*, and *cefadroxil*) have good activity against gram-positive bacteria and modest activity against gram-negative microorganisms. Most streptococci and *methicillin*-susceptible variants of *S. aureus* are susceptible; enterococci, MRSA, and *S. epidermidis* are resistant. Most oral cavity anaerobes are sensitive, but the *B. fragilis* group is resistant. These agents have modest activity against *Moraxella catarrhalis*, *E. coli*, and *K. pneumoniae*, sufficient for empiric use for mild but not severe infections. They lack activity against *H. influenzae*.

Cefazolin is relatively well tolerated after either intramuscular or intravenous administration; it is excreted by glomerular filtration and is about 85% bound to plasma proteins. *Cefazolin* is the only parenteral first-generation cephalosporin marketed in the U.S.

Cephalexin has the same antibacterial spectrum as the other first-generation cephalosporins. It is somewhat less active against penicillinase-producing staphylococci. Oral therapy with *cephalexin* (usually 0.5 g twice to four times daily) results in peak concentrations in plasma adequate for the inhibition of many gram-positive and gram-negative pathogens. The drug is not metabolized, and 70% to 100% is excreted in the urine.

Cephradine (not available in the U.S.) and ***cefadroxil*** are oral agents similar in activity and pharmacokinetics to cephalexin.

Second-Generation Cephalosporins

The **second-generation cephalosporins** have somewhat increased activity against gram-negative microorganisms (including activity against *H. influenzae*) but less than the third-generation agents. A subset of second-generation agents (*cefoxitin* and *cefotetan*) also has modest activity against *B. fragilis*.

Cefoxitin and ***cefotetan*** are technically **cephamycins** and are resistant to some β-lactamases produced by gram-negative rods. Typical of second-generation cephalosporins, they have broader gram-negative activity, including most strains of *Haemophilus* spp., indole-positive *Proteus* spp., and *Klebsiella* spp. These antibiotics are less active than the first-generation cephalosporins against gram-positive bacteria but are more active against anaerobes, especially *B. fragilis*. ***Cefmetazole*** is a similar agent only marketed outside the U.S.

Cefuroxime has good activity against *H. influenzae* (including strains resistant to *ampicillin*), *N. meningitidis*, and *S. pneumoniae*. Activity against *E. coli* and *Klebsiella* is modest. Antistaphylococcal activity is inferior to first-generation cephalosporins. Unlike *cefoxitin*, *cefotetan*, and *cefmetazole*, *cefuroxime* lacks activity against *B. fragilis*. The drug can be given orally, intravenously, or intramuscularly every 8 to 12 h. Concentrations in CSF are about 10% of those in plasma, and the drug is effective but inferior to *ceftriaxone* for treatment of meningitis due to susceptible organisms.

Cefuroxime axetil is the 1-acetyloxyethyl ester of *cefuroxime*. Between 30% and 50% of an oral dose is absorbed, and the drug then is hydrolyzed to *cefuroxime*; resulting concentrations in plasma are variable.

Cefprozil, ***cefaclor***, and ***loracarbef*** (the latter not available in the U.S.) are orally administered agents generally similar to *cefuroxime axetil*.

Third-Generation Cephalosporins

Third-generation cephalosporins generally are less active than first-generation agents against gram-positive cocci, although *ceftriaxone* and *cefotaxime* in particular have excellent antistreptococcal activity. These agents are much more active than prior generations against the Enterobacterales, although resistance is dramatically increasing due to β-lactamase–producing strains.

Cefotaxime is resistant to many narrow-spectrum β-lactamases and has good activity against most gram-positive and gram-negative aerobic bacteria. However, activity against *B. fragilis* is poor, and the increasingly prevalent ESBLs and KPCs confer resistance to *cefotaxime*. *Cefotaxime* has a $t_{1/2}$ in plasma of about 1 h and should be administered every 4 to 8 h for serious infections. The drug is metabolized *in vivo* to desacetylcefotaxime, which is less active than is the parent compound. Concentrations achieved in the CSF are adequate for treatment of meningitis caused by *H. influenzae*, penicillin-sensitive *S. pneumoniae*, and *N. meningitidis*.

Ceftriaxone has activity very similar to that of *cefotaxime* but a longer $t_{1/2}$ (~8 h), allowing for once-daily dosing for most indications. Administration of the drug twice daily has been effective for patients with meningitis. About half the drug can be recovered from the urine; the remainder is eliminated by biliary secretion. Single doses of intramuscular *ceftriaxone* have long been used in the management of urethral, cervical, rectal, or pharyngeal gonorrhea; increasing resistance has necessitated the use of higher doses (recently increased to 500 mg) (Centers for Disease Control and Prevention, 2021).

Ceftizoxime (not marketed in the U.S.) has a spectrum of activity *in vitro* that is similar to that of *cefotaxime*, except that it is less active against *S. pneumoniae* and more active against *B. fragilis*. The $t_{1/2}$ is 1.8 h, and the drug thus can be administered every 8 to 12 h for serious infections. *Ceftizoxime* is not metabolized; 90% is recovered in urine.

Cefpodoxime proxetil and ***cefditoren pivoxil*** (the latter not available in the U.S.) are orally administered prodrugs that are hydrolyzed by esterases during absorption to the active forms (*cefpodoxime* and *cefditoren*, respectively). These drugs provide similar, but less potent, activity as *cefotaxime* against *methicillin*-susceptible strains of *S. aureus* and *penicillin*-susceptible strains of *S. pneumoniae*, *S. pyogenes*, *H. influenzae*, *H. parainfluenzae*, *M. catarrhalis,* and some enteric gram-negative rods. They are eliminated unchanged in the urine.

Cefixime is orally effective against urinary tract infections caused by *E. coli* and *P. mirabilis*; otitis media caused by *H. influenzae* and *S. pyogenes*; pharyngitis due to *S. pyogenes*; and uncomplicated gonorrhea (although intramuscular *ceftriaxone* is preferred for gonorrhea). It is available as an oral suspension. *Cefixime* has a plasma $t_{1/2}$ of 3 to 4 h and is both excreted in the urine and eliminated in the bile. The standard dose for adults is 400 mg/day for 5 to 7 days and for a longer interval in patients with *S. pyogenes*. Doses must be reduced in patients with renal impairment.

Ceftibuten (not available in the U.S.) and ***cefdinir*** are orally administered cephalosporins similar in spectrum and pharmacokinetics to *cefixime*.

Antipseudomonal Cephalosporins

Antipseudomonal cephalosporins include *ceftazidime* (often classified as a third-generation cephalosporin), *ceftolozane/tazobactam*, *cefepime* (sometimes called a fourth-generation cephalosporin), *ceftazidime/avibactam*, and *cefiderocol*. *Ceftazidime* (with and without *avibactam*), *ceftolozane/tazobactam*, and *cefiderocol* have weaker gram-positive activity than third-generation agents, while *cefepime*'s activity is similar to that of *ceftriaxone*. The primary value of these agents is their expanded gram-negative activity, including activity against *P. aeruginosa* and, in the case of *ceftazidime/avibactam* and *cefiderocol*, extensively drug-resistant gram-negative pathogens such as carbapenemase-producing carbapenem-resistant Enterobacterales.

Ceftazidime is one-quarter to one-half as active against gram-positive microorganisms as is *cefotaxime*; activity against staphylococci is particularly poor. Its activity against the *Enterobacterales* is similar to *ceftriaxone*, but its major distinguishing feature is excellent activity against *Pseudomonas*. *Ceftazidime* has poor activity against *B. fragilis*. It only achieves therapeutic levels through parenteral administration, with a $t_{1/2}$ in plasma of about 1.5 h; the drug is renally eliminated and requires adjustment in renal dysfunction. The activity of *ceftazidime* against ESBL- and KPC-producing *Enterobacterales* and AmpC β-lactamase–overexpressing *Pseudomonas* is enhanced when it is combined with the β-lactamase inhibitor *avibactam* in ***ceftazidime/avibactam***.

Ceftolozane is a structural analogue of *ceftazidime* that has enhanced activity against *Pseudomonas*, including activity against strains resistant to *ceftazidime* through β-lactamase overexpression. It has similarly weak activity to *ceftazidime* against gram-positive organisms. It is commercially available as the coformulation ***ceftolozane/tazobactam***, which improves its activity against ESBL-producing *Enterobacterales*. Its pharmacokinetics are similar to *ceftazidime*, with a $t_{1/2}$ after intravenous administration of approximately 2.5 h and renal elimination.

Cefiderocol is a novel parenteral antipseudomonal cephalosporin. It has potent antipseudomonal activity and poor gram-positive activity similar to *ceftazidime* and *ceftolozane*. However, it is more stable against β-lactamase–mediated hydrolysis, including against broad-spectrum KPC-type and metallo-β-lactamases. In addition, it acts as a siderophore, binding to free extracellular iron and taking advantage of active transport mechanisms for iron that gram-negative bacteria employ. In this manner, it is able to achieve high concentrations in the periplasmic space. In combination, these attributes confer *in vitro* activity against most gram-negative aerobes, including those with multiple resistance mechanisms such as efflux pumps, porin channel loss, and β-lactamase production. The pharmacokinetics of this agent are similar to *ceftazidime* and *ceftolozane*, although penetration into the CSF is currently not well characterized.

Cefepime and ***cefpirome*** (not available in the U.S.) are parenteral antipseudomonal cephalosporins sometimes also classified as "fourth-generation" agents. They provide similarly excellent activity to *ceftriaxone* against Enterobacterales and are relatively resistant to AmpC chromosomally encoded β-lactamases. Thus, they are active against many organisms such as *Enterobacter* and *Pseudomonas* that are resistant to other cephalosporins via overexpression of chromosomally encoded AmpC β-lactamases. However, other mechanisms (such as active efflux) in *Pseudomonas* may still confer *cefepime* resistance. *Cefepime* is susceptible to varying degrees to hydrolysis by ESBLs and to a significant extent to KPCs. *Cefepime* has higher activity than *ceftazidime* and comparable activity to *cefotaxime* for streptococci and methicillin-sensitive *S. aureus*. *Cefepime* is excreted renally; doses should be adjusted for renal failure. The serum $t_{1/2}$ is 2 h. *Cefepime* has excellent penetration into the CSF in animal models of meningitis.

Anti-MRSA cephalosporins have structural modifications allowing for binding to and inactivation of the altered PBPs expressed by MRSA, MRSE, and penicillin-resistant *S. pneumoniae*. *Ceftaroline* and *ceftobiprole* (not available in the U.S.) are the currently used agents in this class.

Ceftaroline fosamil is a cephalosporin with gram-negative activity comparable to *cefotaxime*. Its distinguishing feature is its enhanced gram-positive activity, especially its ability to bind to the low-affinity PBPs of MRSA and penicillin-resistant *S. pneumoniae*. Over 95% of MRSA and penicillin-resistant *S. pneumoniae* isolates are inhibited by *ceftaroline*. The parenteral preparation is a prodrug that is rapidly converted to active *ceftaroline* on intravenous administration. It is primarily eliminated by the kidneys with a $t_{1/2}$ of approximately 2 h. *Ceftaroline* has minimal protein binding (~20%) and appears to distribute well into most tissues, although penetration into the CSF has not yet been well characterized.

Ceftobiprole medocaril (not available in the U.S.) has similar activity to *ceftaroline* against gram-positive organisms. Its gram-negative spectrum includes activity similar to *ceftazidime* against *Pseudomonas* spp. and other gram-negative bacilli. As with *ceftaroline*, its intravenous formulation is a prodrug that is rapidly cleaved to the active moiety. Its pharmacokinetics are similar to those of *ceftaroline*.

Adverse Reactions

Hypersensitivity reactions to the cephalosporins are the side effects of greatest concern; they are similar in manifestation to those caused by the penicillins but generally less frequent than reactions to that class. Immediate reactions such as anaphylaxis, bronchospasm, and urticaria are observed. More commonly, maculopapular rash develops, usually after several days of therapy; this may or may not be accompanied by fever and eosinophilia. Allergic cross-reactivity (i.e., the likelihood of a patient with a hypersensitivity reaction to penicillin having a reaction to a cephalosporin) appears to be primarily determined by reactions to β-lactam side chains, which may be similar between agents in different classes, rather than the β-lactam core ring structures. Thus, estimating the likelihood of cross-reactivity between a penicillin and cephalosporin depends on the agents involved (Chaudhry et al., 2019). Patients with a history of a mild or a temporally distant reaction to penicillin appear to be at low risk of allergic reaction following the administration of a cephalosporin. However, patients who have a history of a severe, immediate reaction to a penicillin should be skin tested to confirm penicillin allergy before cephalosporin administration or initiated on cephalosporins via a test dose protocol, depending on the particular cephalosporin used.

A positive Coombs reaction appears frequently in patients who receive large doses of a cephalosporin, but hemolysis is rare. Cephalosporins have produced rare instances of bone marrow depression, characterized by granulocytopenia. Cephalosporins, when used by themselves in recommended doses, rarely produce significant renal toxicity. Diarrhea can result from the administration of cephalosporins and may be more frequent with *cefoperazone*, perhaps because of its greater biliary excretion. The high binding affinity of *ceftriaxone* for serum albumin may displace bilirubin, potentially causing jaundice in neonates; for this reason, *cefotaxime* is the preferred agent in this patient population. *Ceftriaxone*'s high biliary concentrations combined with its affinity for calcium can lead to biliary pseudolithiasis. Cephalosporins containing a thiotetrazole group (*cefazolin*, *cefamandole* [not available in the U.S.], *cefotetan*, and *cefoperazone* [not available in the U.S.]) can prolong the prothrombin time, an effect that may be associated with clinically significant bleeding among patients receiving anticoagulation or with vitamin K deficiency. Encephalopathy and nonconvulsive status epilepticus have been reported with *cefepime*, especially when administered at high doses or among patients with renal dysfunction or preexisting brain injury.

Therapeutic Uses

The **first-generation cephalosporins** are excellent agents for skin and soft-tissue infections owing to their activity against *S. pyogenes* and *methicillin*-susceptible *S. aureus* (Stevens et al., 2014). A single 1- to 2-g dose of *cefazolin* just before surgery is the preferred prophylaxis for procedures in which skin flora are the likely pathogens (Bratzler et al., 2013). Parenteral *cefazolin* (2 g IV every 8 h) is a drug of choice for serious infections, including endocarditis, due to *methicillin*-susceptible *S. aureus* (Baddour et al., 2015). Oral agents in this generation have utility for mild-to-moderate upper respiratory (e.g., pharyngitis) and urinary tract infections as well (e.g., *cephalexin* 250–500 mg orally every 6 h).

Second-generation cephalosporins generally have been replaced by third-generation agents. The oral second-generation cephalosporins can be used to treat respiratory tract infections, although they are suboptimal (compared with oral *amoxicillin*) for treatment of penicillin-nonsusceptible *S. pneumoniae* pneumonia and otitis media. *Cefoxitin* and *cefotetan* play a useful role in perioperative prophylaxis for patients undergoing intra-abdominal and gynecological surgical procedures. They may also be used for treatment of certain anaerobic and mixed aerobic-anaerobic infections, such as peritonitis and pelvic inflammatory disease, although because of increasing resistance among *B. fragilis*, these agents are best used for mild-to-moderate infections.

The **third-generation cephalosporins** are drugs of choice for serious infections caused by *E. coli*, *Klebsiella*, *Proteus*, *Providencia*, *Serratia*, and *Haemophilus* spp. *Ceftriaxone* is the therapy of choice for all forms of gonorrhea and for severe forms of Lyme disease. *Cefotaxime* and *ceftriaxone* at higher doses (2 g every 12 h in adults) are used for the empiric treatment of meningitis in non-immunocompromised adults and children (in combination with *vancomycin* and *ampicillin* pending identification of the causative agent), owing to their excellent activity against *H. influenzae*, sensitive *S. pneumoniae*, *N. meningitidis*, and gram-negative enteric bacteria (Tunkel et al., 2004). Third-generation cephalosporins lack activity against *L. monocytogenes* and penicillin-resistant pneumococci, pathogens that may cause community-acquired meningitis. The antimicrobial spectra of *cefotaxime* and *ceftriaxone* are excellent for the treatment of community-acquired pneumonia (with addition of a non–β-lactam agent with activity against atypical respiratory pathogens for empiric coverage).

The **antipseudomonal cephalosporins** are indicated for the empirical treatment of nosocomial infections where *Pseudomonas* and other resistant gram-negative bacilli are likely to be pathogens, including pneumonia, urinary tract infections, and intra-abdominal infections (in the latter instance, combined with an agent with activity against anaerobic organisms). *Ceftolozane/tazobactam* may have superior activity against some *ceftazidime*-resistant *Pseudomonas*, whereas *cefepime* has superior activity against nosocomial isolates of *Enterobacter*, *Citrobacter*, and *Serratia* spp. Activity of *ceftazidime*, *ceftolozane/tazobactam*, and *cefepime* is variable against ESBL-producing isolates and absent against KPC-expressing strains; *ceftazidime/avibactam* is more likely to be active against these highly resistant organisms. *Cefiderocol* is usually reserved for treatment of extensively drug-resistant gram-negative pathogens against which few or no alternative antibiotics are active.

The **anti-MRSA cephalosporins** are typically used for patients with documented or suspected severe infections due to staphylococci or streptococci. *Ceftaroline* is dosed at 600 mg every 12 h for treatment of community-acquired pneumonia or every 8 h if MRSA is suspected.

Other β-Lactam Antibiotics

Carbapenems

Carbapenems are β-lactams that contain a fused β-lactam ring and a five-member ring system that differs from the penicillins because it is unsaturated and contains a carbon atom instead of the sulfur atom. This class of antibiotics has a broader spectrum of activity than most other β-lactam antibiotics largely due to their greater resistance to β-lactamase–mediated hydrolysis.

Imipenem/Cilastatin and Imipenem/Cilastatin/Relebactam

Imipenem is formulated in combination with *cilastatin* (*imipenem/cilastatin*), a drug that inhibits the degradation of *imipenem* by a renal tubular dipeptidase and extends its $t_{1/2}$. Now, *imipenem* is available as a coformulation with the β-lactamase inhibitor *relebactam* (*imipenem/cilastatin/relebactam*), which extends its spectrum against organisms producing carbapenemases. For brevity, we will omit *cilastatin* when referring to these agents below.

Antimicrobial Activity. *Imipenem*, like other β-lactam antibiotics, binds to PBPs, disrupts bacterial cell wall synthesis, and causes death of susceptible microorganisms. It is highly resistant to hydrolysis by most β-lactamases. The *in vitro* activity of *imipenem* is excellent across a wide variety of aerobic and anaerobic microorganisms. Streptococci, including penicillin-resistant *S. pneumoniae*; *Enterococcus faecalis*; staphylococci (including penicillinase-producing strains but not MRSA); and *Listeria* (although *ampicillin* is more active) all are typically susceptible. Activity is excellent against the Enterobacterales with the exception of emerging carbapenemase-producing strains. Most strains of *Pseudomonas* and *Acinetobacter* are inhibited, but resistance to carbapenems among these organisms is increasing and can emerge during therapy. Anaerobes, including *B. fragilis*, are highly susceptible. *Imipenem* also displays activity against *Nocardia* spp. and some species of rapidly growing mycobacteria. Addition of *relebactam* restores the activity of *imipenem* against most carbapenemase-producing Enterobacterales but not metallo-β-lactamase producers; activity of the combination against *imipenem*-resistant *Pseudomonas* is variable.

ADME and Adverse Reactions. *Imipenem* is not absorbed orally. The drug is hydrolyzed rapidly by a dipeptidase found in the brush border of the proximal tubule. Both *imipenem* and *cilastatin* have a $t_{1/2}$ of about 1 h. When administered concurrently with *cilastatin*, about 70% of administered *imipenem* is recovered in the urine as the active drug. Dosage should be modified for patients with renal insufficiency. The $t_{1/2}$ of *relebactam* is also approximately 1 h, and it is excreted in the urine. Nausea and vomiting are the most common adverse reactions (1%–20%) across *imipenem* and *imipenem/relebactam*. Seizures have been noted in up to 1.5% of patients, especially when high doses are given to patients with CNS lesions and to those with renal insufficiency. Most patients who are allergic to other β-lactam antibiotics can safely receive carbapenems, although those with severe immediate-type reactions should consider an initial carbapenem challenge following a test dose protocol.

Therapeutic Uses. *Imipenem*, dosed at 500 to 1000 mg IV every 6 to 8 h in normal renal function, is effective for a wide variety of infections, including urinary tract and lower respiratory infections; intra-abdominal and gynecological infections; and skin, soft-tissue, bone, and joint infections. Its primary role is for empirical treatment of serious infections in hospitalized patients who are at risk for resistant pathogens, such as those who have recently received other β-lactam antibiotics. When *imipenem* is used for treatment of severe *P. aeruginosa* infections, resistance may develop during therapy. *Imipenem/relebactam* is reserved for treatment of gram-negative pathogens resistant to all or almost all other alternative antibiotics.

Meropenem and Meropenem/Vaborbactam

Meropenem is a derivative of *thienamycin*. It does not require coadministration with *cilastatin* because it is not sensitive to renal dipeptidase. It may be coformulated with the β-lactamase inhibitor *vaborbactam*.

Antimicrobial Activity. The spectra of activity of *imipenem* and *meropenem* are broadly similar, with *meropenem* being somewhat less active against gram-positive organisms—particularly *Enterococcus*—and more active against gram-negative organisms. As with *imipenem*, carbapenemases that gram-negative organisms may produce can render *meropenem* ineffective; *vaborbactam* restores the susceptibility of *meropenem* against a large subset of these.

ADME and Adverse Reactions. *Meropenem* and *meropenem/vaborbactam* are available for intravenous administration and are renally cleared with half-lives on the order of 1 h. Although typically infused over 30 min, extending the infusion over 3 h can increase the time that *meropenem* concentrations spend above the organism's MIC and allow for treatment of low-level resistant pathogens. *Meropenem*'s toxicity is similar to that of *imipenem* except that it may be less likely to cause seizures; thus, it is preferred for treatment of meningitis when carbapenem therapy is required. Notably, *meropenem*, and other carbapenems to a lesser extent, significantly lowers serum concentrations of the antiepileptic agent *valproic acid* and should not be coadministered with this drug.

Therapeutic Uses. As with *imipenem*, *meropenem* is typically employed for hospital-onset infections of the respiratory, gastrointestinal, and urinary tracts when cephalosporin- or penicillin-resistant organisms are suspected (dosed at 1–2 g every 8 h in patients with normal renal function). *Meropenem/vaborbactam* is reserved for multidrug-resistant gram-negative pathogens (2 g of *meropenem* and 2 g of *vaborbactam* administered every 8 h in normal renal function).

Ertapenem

Ertapenem differs from *imipenem* and *meropenem* by having a longer $t_{1/2}$ that allows once-daily dosing and by lacking clinically useful activity against *Enterococcus*, *P. aeruginosa*, and *Acinetobacter* spp. Its activity against Enterobacterales, including ESBL-producing isolates, and anaerobes makes it useful in intra-abdominal or urinary tract infections. An advantage of this agent is its once-daily dose (at 1 g IV), which facilitates outpatient therapy.

Doripenem

Doripenem (not available in the U.S.) has a spectrum of activity that is similar to that of *meropenem*, with greater activity against some resistant isolates of *Pseudomonas*.

Monobactams

Monobactams are β-lactams that contain only a fused β-lactam ring, not a thiazolidine or dihydrothiazidine ring. Currently, *aztreonam* is the only member of this class in therapeutic use.

Aztreonam

Aztreonam is resistant to narrow-spectrum β-lactamases elaborated by most gram-negative bacteria as well as metallo-β-lactamases, but not most extended-spectrum or KPC-type β-lactamases. *Aztreonam* has activity only against gram-negative bacteria; it has no activity against gram-positive bacteria and anaerobic organisms. Activity against Enterobacterales and *P. aeruginosa* is similar to that of *ceftazidime*. It is also highly active *in vitro* against *H. influenzae*. *Aztreonam* is administered intramuscularly, intravenously, or as an inhaled formulation. The $t_{1/2}$ for elimination of intravenously administered *aztreonam* is 1.7 h; most of the drug is recovered unaltered in the urine. The $t_{1/2}$ is prolonged to about 6 h in anephric patients. A notable feature is a lack of allergic cross-reactivity with other β-lactam antibiotics, with the possible exception of *ceftazidime*, *ceftolozane*, and *cefiderocol* with which it shares similar or identical side chains. *Aztreonam* is therefore useful for treating gram-negative infections among patients with severe hypersensitivity reactions to other β-lactam classes. *Aztreonam* has a growing role in the treatment of metallo-β-lactamase–producing organisms, since it is generally stable to these enzymes; however, as co-production of *aztreonam*-hydrolyzing enzymes may occur, *aztreonam* is often coadministered with *ceftazidime/avibactam* in these cases (a specific *aztreonam/avibactam* combination agent is in development). The inhalation formulation is used to reduce the frequency of *Pseudomonas*-associated pulmonary exacerbations in patients with cystic fibrosis. *Aztreonam* generally is well tolerated, although hepatotoxicity, especially in infants and young children, can occur.

Other Cell Envelope Disruptors

Glycopeptides

Glycopeptides offer another mechanism by which the cell wall synthesis pathway in bacteria can be targeted. The class originator for glycopeptides is *vancomycin*, a tricyclic glycopeptide antibiotic produced by *Streptococcus orientalis*. *Teicoplanin* is a mixture of related glycopeptides available as an antibiotic in Europe. *Teicoplanin* is similar to *vancomycin* in chemical structure, mechanism of action, spectrum of activity, and route of elimination (i.e., primarily renal). A new generation of glycopeptide congeners, the lipoglycopeptides, has been recently introduced into clinical practice. These agents include *telavancin*, *dalbavancin*, and *oritavancin*.

Antimicrobial Activity

Vancomycin possesses activity against the vast majority of gram-positive bacteria, including MRSA, penicillin-resistant streptococci, and *ampicillin*-resistant enterococci. Gram-positive organisms intrinsically resistant to *vancomycin* include *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Erysipelothrix*; *vancomycin* susceptibility across species of *Enterococcus* is variable. Essentially all species of gram-negative bacteria and mycobacteria are resistant to glycopeptides. The activity of *teicoplanin*, *telavancin*, *dalbavancin*, and *oritavancin* is generally similar to that of *vancomycin*; these agents are also active against some *vancomycin*-resistant enterococci (Goldstein et al., 2004).

Mechanism of Action

Glycopeptides inhibit the synthesis of the cell wall in sensitive bacteria by noncovalent, high-affinity binding to the D-alanyl-D-alanine terminus of cell wall precursor units. This directly blocks through steric hindrance both transglycosidase-mediated polymerization and the PBP-mediated cross-linking of cell wall units (similarly to β-lactams) (Figure 58–5). Because of their large molecular size, they are unable to penetrate the outer membrane of gram-negative bacteria. The lipoglycopeptides are able to dimerize and anchor their lipid moieties into the bacterial membrane, allowing for increased binding to the D-Ala-D-Ala target site and improved potency. *Telavancin* and *oritavancin* possess a second mechanism of action: direct disruption of the bacterial membrane. This effect leads to more rapid bactericidal activity than that of *vancomycin*.

Resistance to Glycopeptides and Lipoglycopeptides

Glycopeptide-resistant strains of enterococci, primarily *E. faecium*, have emerged as major nosocomial pathogens in hospitals in the U.S. Determinants of *vancomycin* resistance are located on a transposon that is readily transferable among enterococci and, potentially, other gram-positive bacteria. These strains are typically resistant to multiple antibiotics, including *streptomycin*, *gentamicin*, and *ampicillin*.

Enterococcal resistance to glycopeptides is the result of alteration of the D-alanyl-D-alanine target to D-alanyl-D-lactate or D-alanyl D-serine, both of which bind glycopeptides poorly (Zeng et al., 2016). Several enzymes within the *van* gene cluster are required for this target alteration to occur. The *vanA* genotype confers inducible resistance to *teicoplanin* and *vancomycin* in *E. faecium* and *E. faecalis*. Consistent with their dual mode of action, while MICs to *telavancin* and *oritavancin* may increase in isolates expressing *vanA*, they often remain in the susceptible range. In contrast, *vanA*-expressing isolates are frequently *dalbavancin* resistant. The *vanB* genotype, which tends to confer a lower level of resistance, also has been identified in *E. faecium* and *E. faecalis*. The trait is inducible

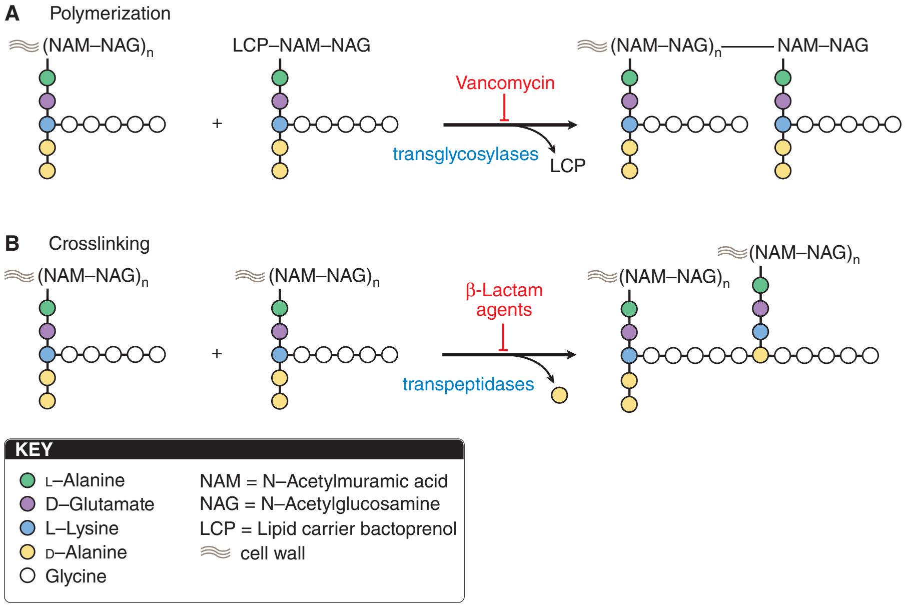


Figure 58–5 *Inhibition of bacterial cell wall synthesis in gram-positive bacteria: vancomycin and β-lactam agents.* *Vancomycin* and other glycopeptides inhibit the polymerization or transglycosylase reaction (**A**) by binding to the D-alanyl-D-alanine terminus of the cell wall precursor unit attached to its lipid carrier and blocking linkage to the glycopeptide polymer (indicated by the subscript n). These (NAM–NAG)$_n$ peptidoglycan polymers are located within the cell wall. VanA-type resistance is due to expression of enzymes that modify cell wall precursor by substituting a terminal D-lactate for D-alanine, reducing affinity for vancomycin by 1000-fold. β-Lactam antibiotics inhibit the cross-linking or transpeptidase reaction (**B**) that links glycopeptide polymer chains by formation of a cross-bridge with the stem peptide (the five glycines in this example) of one chain, displacing the terminal D-alanine of an adjacent chain. Glycopeptides may also act through this mechanism.

by *vancomycin* but not *teicoplanin*; consequently, many strains remain susceptible to *teicoplanin*. *Telavancin*, *dalbavancin*, and *oritavancin* are usually active as well. The *vanC* genotype that confers resistance only to *vancomycin* is the least important clinically and least characterized.

S. aureus and coagulase-negative staphylococci may express reduced or "intermediate" susceptibility to *vancomycin* (MIC 4–8 μg/mL) or, very rarely, high-level resistance (MIC ≥16 μg/mL) (Howden et al., 2010). Intermediate resistance is associated with a heterogeneous phenotype in which a small proportion of cells within the population (1 in 10^5 to 1 in 10^6 colony-forming units) will grow in the presence of *vancomycin* concentrations greater than 4 μg/mL. Prior treatment courses and low *vancomycin* levels may predispose patients to infection and treatment failure with *vancomycin*-intermediate strains. These strains typically are resistant to *methicillin* and multiple other antibiotics; their emergence is a major concern because of *vancomycin*'s prominent role in the treatment of MRSA infection. High-level *vancomycin*-resistant *S. aureus* strains (MIC ≥32 μg/mL) harbor a conjugative plasmid into which the *vanA* transposon is integrated by an interspecies horizontal gene transfer from *E. faecalis* to an MRSA (Limbago et al., 2014). These isolates have been variably susceptible to *teicoplanin* and the lipoglycopeptides.

ADME

All glycopeptides are poorly absorbed after oral administration; the oral formulation of *vancomycin* is exclusively used in patients with *C. difficile* colitis. *Vancomycin* should be only administered intravenously, not intramuscularly, due to pain with intramuscular injection. Approximately 30% of *vancomycin* is bound to plasma protein. *Vancomycin* appears in various body fluids, including the CSF when the meninges are inflamed (7%–300%), bile, and pleural, pericardial, synovial, and ascitic fluids. About 90% of an injected dose of *vancomycin* is excreted by glomerular filtration; elimination $t_{1/2}$ is about 6 h in normal renal function. The drug accumulates if renal function is impaired, and dosage adjustments must be made. The drug can be cleared from plasma with hemodialysis.

Teicoplanin can be administered by intramuscular injection as well as intravenous administration. An IV dose of 1 g in adults produces plasma concentrations of 15 to 30 μg/mL 1 h after a 1- to 2-h infusion. *Teicoplanin* is highly bound by plasma proteins (90%–95%) and has an extremely long serum elimination $t_{1/2}$ (up to 100 h), allowing for once-daily dosing. Excretion is through glomerular filtration.

Telavancin achieves peak concentrations of approximately 90 μg/mL when administered at a dose of 10 mg/kg once daily. *Telavancin* is highly protein bound (>90%), with a $t_{1/2}$ of about 7 h. Studies of penetration into epithelial lining fluid and skin blister fluid demonstrated adequate tissue concentrations to provide effective therapy. *Telavancin* is eliminated primarily (70%–80%) by renal excretion, with a small component of metabolism. Dosage adjustment is required in renal dysfunction.

Dalbavancin and *oritavancin* have unique pharmacokinetic properties that allow for intermittent (weekly or less) dosing. Both are characterized by extremely long plasma $t_{1/2}$ (on the order of 10 days for terminal elimination) and are highly (>90%) protein bound. Penetration of *dalbavancin* into skin blister fluid and bone appears to be adequate, but penetration into CSF is very low. Between 33% and 50% of *dalbavancin* is eliminated unchanged in the urine, and dosage adjustment is recommended for renal dysfunction. *Oritavancin* has a large volume of distribution (~1 L/kg). Renal excretion is very slow, and dosage adjustment is not required in mild-to-moderate renal dysfunction.

Therapeutic Uses

Vancomycin should be infused at a rate of no more than 1 g/h to avoid infusion-related adverse reactions; recommended initial doses for adults are typically in the range of 30- to 45-mg/kg per day in two or three divided doses. Alteration of dosage is required for patients with impaired renal function. In functionally anephric patients and patients receiving dialysis with non–high-flux membranes, administration of 1 g (~15 mg/kg) every 5 to 7 days typically achieves adequate serum levels. In patients receiving intermittent high-efficiency or high-flux dialysis, maintenance doses administered after each dialysis session are typically required. For treatment of *C. difficile* colitis, *vancomycin* is available as capsules for oral administration or as a commercially prepared oral solution; alternatively, the intravenous formulation may be compounded into a solution for oral administration. The recommended oral dose of *vancomycin* is 125 mg four times daily, with escalation up to 500 mg four times daily in patients with life-threatening disease.

Monitoring recommendations and target drug exposures for *vancomycin* are evolving. Previously, recommendations called for monitoring serum trough concentrations, within 30 min prior to a dose, at steady state, typically before the fourth dose of a given dosage regimen. A trough serum concentration of at least 10 μg/mL is recommended; for patients with more serious infections, including endocarditis, osteomyelitis, meningitis, and MRSA pneumonia, trough levels of 15 to 20 μg/mL are recommended. However, more recent data suggest the area under the curve as a more appropriate target for *vancomycin* exposures. Thus, new guidelines recommend targeting an area under the curve between 400 and 600 mg*h/L for serious MRSA infections (Rybak et al., 2020). Area under the curve can be estimated through sampling one or more levels during a dosing interval and applying standard population-based or Bayesian procedures.

Telavancin is administered intravenously at a dose of 10 mg/kg daily, with dosage adjustment required for patients with renal dysfunction. The approved dosage of intravenous *dalbavancin* for treatment of skin and soft-tissue infection is 1000 mg at the initiation of treatment, followed by a 500-mg dose 7 days later. *Dalbavancin* can be administered as a single 1500-mg IV dose for skin and soft-tissue infections. *Oritavancin* has been studied for skin and soft-tissue infections as a single 1200-mg IV dose. A variety of multiple-dose regimens for *dalbavancin* and *oritavancin* for treatment of complicated infections, such as osteomyelitis, are under investigation.

Skin/Soft-Tissue and Bone/Joint Infections. *Vancomycin* has long been a mainstay in the treatment of skin/soft-tissue and bone/joint infections, where gram-positive organisms, including MRSA, are the leading pathogens (Stevens et al., 2014). *Telavancin*, *dalbavancin*, and *oritavancin* offer alternatives for treatment of this condition, with *dalbavancin* and *oritavancin* offering the option for single or infrequent dosing.

Respiratory Tract Infections. *Vancomycin* is employed for the treatment of pneumonia when MRSA is suspected. Because *vancomycin* penetration into lung tissue is relatively low, aggressive dosing is generally recommended. *Telavancin* displayed similar efficacy to *vancomycin* in studies of nosocomial pneumonia due to gram-positive pathogens.

CNS Infections. *Vancomycin* is a key component in the initial empirical treatment of community-acquired bacterial meningitis in locations where penicillin-resistant *S. pneumoniae* is common (Tunkel et al., 2004). Penetration of *vancomycin* across meninges is poor, especially with steroid coadministration; thus, aggressive dosing is typically warranted. *Vancomycin* is also used to treat nosocomial meningitis, often caused by staphylococci. Intraventricular *vancomycin* (at doses of 10–20 mg once daily) has been used in ventricular shunt infections.

Endocarditis and Vascular Catheter Infections. *Vancomycin* is standard therapy for staphylococcal endocarditis when the isolate is *methicillin* resistant or patients have a severe penicillin allergy (Baddour et al., 2015). However, β-lactams such as *nafcillin* or *cefazolin* are more effective than *vancomycin* for treatment of *methicillin*-susceptible *S. aureus* bloodstream infections; thus, patients should only receive *vancomycin* for *methicillin*-susceptible *S. aureus* infections if they have a documented, life-threatening allergy. *Vancomycin* is an effective alternative for the treatment of endocarditis caused by viridans streptococci in patients who are allergic to penicillin. In combination with an aminoglycoside, it may be used for enterococcal endocarditis in patients with serious penicillin allergy or for penicillin-resistant isolates. *Vancomycin* is used for the treatment of vascular catheter infections due to gram-positive organisms.

Other Infections. Orally administered *vancomycin* is a drug of choice for patients with *C. difficile*–associated diarrhea. *Vancomycin* is frequently employed as a component of empiric therapy for patients with fever and neutropenia. It is also used in surgical prophylaxis in patients with β-lactam allergies or if there is a high risk of MRSA infection.

Adverse Effects

Infusion-Related Reactions. Rapid intravenous infusion of *vancomycin* may cause erythematous or urticarial reactions, flushing, tachycardia, and rarely hypotension. The extreme flushing that can occur is not an allergic reaction but a direct effect of *vancomycin* on mast cells, causing them to release histamine. Typically, this reaction can be ameliorated by administering *vancomycin* more slowly, sometimes with premedication with histamine blockers. This reaction is generally not observed with *teicoplanin* but has been reported with lipoglycopeptides (i.e., *telavancin*).

Nephrotoxicity. Initial formulations of *vancomycin* contained impurities that were associated with a high incidence of nephrotoxicity. With the availability of impurity-free formulations, there was a question regarding whether *vancomycin* was intrinsically nephrotoxic. However, as the recommended dosage range for *vancomycin* dosages has increased, it seems clear there is indeed a degree of dose-related nephrotoxicity (Lodise et al., 2009). Coadministration with some penicillins might further increase the risk of nephrotoxicity. Results of clinical trials suggest *telavancin*'s nephrotoxicity may exceed that of *vancomycin*.

Other Toxic and Irritative Effects. True hypersensitivity reactions produced by glycopeptides are less common than the pseudoallergic infusion-related reactions and include macular skin rashes and anaphylaxis. Because of the long half-lives of *dalbavancin* and *oritavancin*, there is concern over prolonged effects if patients were to experience a severe hypersensitivity reaction, although, to date, few prolonged or delayed reactions have been described. *Telavancin* can cause QT interval prolongation and is contraindicated in pregnancy due to teratogenic effects observed in animal studies. Auditory impairment, sometimes permanent, has been described in association with *vancomycin* use; some investigators believe ototoxicity is associated with excessive concentrations of *vancomycin* in plasma (60–100 μg/mL or greater).

Drug Interactions. *Oritavancin* has a minor effect on CYP-mediated metabolism; it should be used with *warfarin* only with careful monitoring.

Lipopeptides

Daptomycin, the only member of its class, is a cyclic lipopeptide antibiotic derived from *Streptomyces roseosporus* with bactericidal activity against gram-positive bacteria, including *vancomycin*-resistant isolates.

Antimicrobial Activity

Daptomycin is a bactericidal antibiotic selectively active against aerobic, facultative, and anaerobic gram-positive bacteria. *Daptomycin* may be active against *vancomycin*-resistant strains, although MICs tend to be higher for these organisms than for their *vancomycin*-susceptible counterparts (Critchley et al., 2003). *Daptomycin* lacks clinically useful activity against gram-negative organisms.

Mechanism of Action

Daptomycin binds to the bacterial inner membrane, resulting in depolarization, loss of membrane potential, and cell death. It has concentration-dependent bactericidal activity.

Resistance to Lipopeptides

Daptomycin resistance has been reported to emerge while on therapy. Resistance occurs most commonly in treatment of high-inoculum infections, such as endocarditis, and among enterococci. The mechanisms of resistance to *daptomycin* have not been fully characterized but appear to be related to changes in cell surface charge that impede *daptomycin* binding (Stefani et al., 2015). Interestingly, coadministration of β-lactams with *daptomycin* (even when the pathogen is resistant to the β-lactam) can reverse this resistance; some early data suggest these combinations may be effective in treatment of severe staphylococcal and enterococcal infections (Bartash and Nori, 2017).

ADME

Daptomycin is poorly absorbed orally and should be administered intravenously. Direct toxicity to muscle precludes intramuscular injection. The serum $t_{1/2}$ is 8 to 9 h, permitting once-daily dosing. Approximately 80% of the administered dose is recovered in urine; a small amount is excreted in feces. Although the drug penetrates adequately into the lung, the drug is inactivated by pulmonary surfactant and thus is not useful in the treatment of pneumonia (Silverman et al., 2005). If the creatinine clearance is less than 30 mL/min, the dose is administered only every 48 h. For patients on hemodialysis, the dose should be given immediately after dialysis.

Therapeutic Uses

Daptomycin is indicated for treatment of complicated skin and soft-tissue infections and complicated bacteremia and right-sided endocarditis, where its efficacy is comparable to that of *vancomycin* or antistaphylococcal β-lactams (Fowler et al., 2006). FDA-approved doses are 4 to 6 mg/kg, but data suggest higher doses (8–12 mg/kg) are well tolerated and thus are recommended for invasive staphylococcal and enterococcal infections (Figueroa et al., 2009).

Adverse Effects

Musculoskeletal Toxicity. Elevations of creatine kinase may occur; this does not require discontinuation unless levels are greater than 10 times the upper limit of normal or findings suggest an otherwise-unexplained myopathy. Rhabdomyolysis has been reported to occur rarely. Eosinophilic pneumonia has been rarely described.

Drug Interactions. *Daptomycin* does not affect CYPs and has no important drug-drug interactions. Caution is recommended when *daptomycin* is coadministered with aminoglycosides or statins because of potential risks of nephrotoxicity and myopathy, respectively (Bland et al., 2014).

Bacitracins

Bacitracin is an antibiotic produced by the Tracy-I strain of *Bacillus subtilis*. The bacitracins are a group of polypeptide antibiotics. The commercial products have multiple components; the major constituent is *bacitracin A*. A unit of the antibiotic is equivalent to 26 μg of the U.S. Pharmacopeia standard.

Antimicrobial Activity, Mechanism of Action, and Resistance

Bacitracin inhibits the synthesis of the cell wall; a variety of gram-positive cocci and bacilli, *Neisseria*, *H. influenzae*, and *T. pallidum* are sensitive to the drug at 0.1 unit/mL or less. *Actinomyces* and *Fusobacterium* are inhibited by concentrations of 0.5 to 5 units/mL. *Enterobacteriaceae*, *Pseudomonas*, *Candida* spp., and *Nocardia* are resistant to the drug. Few data are available on *bacitracin* resistance.

ADME and Therapeutic Uses

Current use is largely restricted to topical application. *Bacitracin* is available in ophthalmic and dermatologic ointments and creams. A number of topical preparations of *bacitracin*, to which *neomycin* or *polymyxin B* or both (with or without *pramoxine* or *lidocaine*) have been added, are available. For open infections, such as infected eczema and infected dermal ulcers, the local application of the antibiotic may be of some help in eradicating sensitive bacteria. *Bacitracin* rarely produces hypersensitivity. Suppurative conjunctivitis and infected corneal ulcer, when caused by susceptible bacteria, respond well to the topical use of *bacitracin*. *Bacitracin* has been used with limited success for eradication of nasal carriage of staphylococci.

Adverse Effects

Nephrotoxicity results from the parenteral use of *bacitracin*.

Drug Facts for Your Personal Formulary: *Cell Envelope Disruptors: β-Lactam, Glycopeptide, and Lipopeptide Antibacterials*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Penicillins—Inhibitors of Bacterial Cell Wall Synthesis		
General: Bactericidal, renal elimination, hypersensitivity reactions (rash, anaphylaxis)		
Penicillin G (IV), penicillin V (PO); IM depot formulations (benzathine, procaine)	• Penicillin-susceptible *Streptococcus pneumoniae* infections: pneumonia, meningitis • Streptococcal pharyngitis, endocarditis, skin and soft-tissue infection • *Neisseria meningitidis* infections • Syphilis	• Excellent activity vs. *Treponema pallidum*, β-hemolytic streptococci, *N. meningitidis,* gram-positive anaerobes • Good activity vs. *S. pneumoniae*, viridans streptococci • CSF penetration with inflammation
Penicillinase-resistant penicillins Oxacillin (IV), nafcillin (IV), dicloxacillin (PO)	• Skin and soft-tissue infections • Serious infections due to MSSA	• Excellent activity vs. MSSA • Good activity vs. streptococci • Nafcillin nonrenal elimination • CSF penetration with inflammation
Aminopenicillins and aminopenicillin/β-lactamase inhibitor combinations Amoxicillin (PO), ampicillin (PO/IV), amoxicillin/clavulanate (PO), ampicillin/sulbactam (IV)	• Respiratory tract infections (sinusitis, pharyngitis, otitis media, community-acquired pneumonia) • *Enterococcus faecalis* infections • *Listeria* infections • Intra-abdominal infections (amoxicillin/clavulanate, ampicillin/sulbactam)	• Ampicillin, amoxicillin: excellent activity vs. β-hemolytic streptococci, *E. faecalis*; good activity vs. *S. pneumoniae*, viridans streptococci, *Haemophilus influenzae;* some activity vs. *Proteus, Escherichia coli* • Ampicillin/sulbactam, amoxicillin/clavulanate: excellent activity vs. *H. influenzae, Bacteroides fragilis, Proteus*; good activity vs. *E. coli, Klebsiella*, MSSA • CSF penetration with inflammation • Rash more common than other penicillins; amoxicillin/clavulanate has more GI adverse effects than amoxicillin alone
Antipseudomonal penicillins Piperacillin/tazobactam (IV)	• Nosocomial infections: pneumonia, intra-abdominal infections, urinary tract infections	• Activity: ampicillin/sulbactam plus excellent activity vs. *E. coli, Klebsiella*; good activity vs. *Pseudomonas, Citrobacter, Enterobacter* • Poor CSF penetration of tazobactam component
Cephalosporins—Inhibitors of Bacterial Cell Wall Synthesis		
General: Bactericidal, renal elimination, hypersensitivity reactions (rash, anaphylaxis)		
First-generation cephalosporins Cefazolin (IV), cephalexin (PO), cefadroxil (PO)	• Skin and soft-tissue infections • Serious infections due to MSSA • Perioperative surgical prophylaxis	• Excellent activity vs. MSSA, streptococci • Some activity vs. *Proteus, E. coli, Klebsiella* • Poor CSF penetration
Second-generation cephalosporins Cefuroxime (IV/PO), cefoxitin (IV), cefotetan (IV), cefaclor (PO), cefprozil (PO)	• Upper respiratory tract infections (sinusitis, otitis media) • Cefoxitin/cefotetan: gynecologic infections, perioperative surgical prophylaxis	• Good activity vs. MSSA, streptococci, *H. influenzae*, *Proteus*, *E. coli, Klebsiella* • Cefoxitin/cefotetan: some activity vs. *B. fragilis*
Third-generation cephalosporins Cefotaxime (IV), ceftriaxone (IV), cefpodoxime (PO), cefixime (PO), cefdinir (PO), cefditoren (PO), ceftibuten (PO)	• Community-acquired pneumonia, meningitis, urinary tract infections • Streptococcal endocarditis • Gonorrhea • Severe Lyme disease	• Excellent activity against streptococci, *H. influenzae*, *Proteus*, *E. coli*, *Klebsiella*, *Serratia, Neisseria* • Good activity vs. MSSA • Ceftriaxone renal and nonrenal elimination • Good CSF penetration • Ceftriaxone: neonatal kernicterus (use cefotaxime), biliary pseudolithiasis
Antipseudomonal cephalosporins Ceftazidime (IV), ceftolozane/tazobactam (IV), ceftazidime/avibactam (IV), cefepime (IV), cefiderocol (IV)	• Nosocomial infections: pneumonia, meningitis, urinary tract infections, intra-abdominal infections (with metronidazole)	• Excellent activity against *H. influenzae*, *Proteus*, *E. coli*, *Klebsiella*, *Serratia, Neisseria,* streptococci, MSSA[a] • Good activity vs. *Pseudomonas* (ceftazidime/avibactam, cefiderocol, ceftolozane/tazobactam >> ceftazidime, cefepime) • Some activity vs. *Enterobacter* (cefepime, ceftazidime/avibactam, cefiderocol >> ceftazidime, ceftolozane/tazobactam) • Ceftazidime/avibactam, cefiderocol active vs. ESBL- and KPC-producing Enterobacterales • Good CSF penetration • Cefepime: encephalopathy at high doses
Anti-MRSA cephalosporins Ceftaroline (IV)	• Community-acquired pneumonia • Skin and soft-tissue infections	• Excellent activity against streptococci, MSSA, MRSA[b] *H. influenzae*, *Proteus*, *E. coli, Klebsiella, Serratia*

Drug Facts for Your Personal Formulary: *Cell Envelope Disruptors: β-Lactam, Glycopeptide, and Lipopeptide Antibacterials* (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Carbapenems—Inhibitors of Bacterial Cell Wall Synthesis **General: Bactericidal, renal elimination, hypersensitivity reactions (rash, anaphylaxis), seizure risk**		
Imipenem/cilastatin (IV), imipenem/cilastatin/relebactam (IV), meropenem (IV), meropenem/vaborbactam (IV)	• Nosocomial infections: pneumonia, intra-abdominal infections, urinary tract infections • Meningitis (meropenem)	• Excellent activity against streptococci, MSSA, *H. influenzae*, *Proteus*, *E. coli*, *Klebsiella*, *Serratia*, *Enterobacter*, *B. fragilis* • Good activity vs. *Pseudomonas Acinetobacter, Enterococcus faecalis*[c] • Good activity vs. carbapenemase-producing gram-negatives (imipenem/cilastatin/relebactam, meropenem/vaborbactam only) • Good CSF penetration • Imipenem coformulated with renal dihydropeptidase inhibitor cilastatin • Seizures at high doses in patients with prior seizure history (imipenem > meropenem)
Ertapenem (IV)	• Community-acquired infections and nosocomial infections without *Pseudomonas* risk	• Excellent activity against streptococci, MSSA, *H. influenzae*, *Proteus*, *E. coli*, *Klebsiella*, *Serratia*, *Enterobacter*, *B. fragilis* • Lacks activity against *Pseudomonas, Acinetobacter, Enterococcus* • Lower seizure risk than imipenem
Monobactam—Inhibitor of Bacterial Cell Wall Synthesis		
Aztreonam (IV, inhaled)	• Nosocomial infections: pneumonia, urinary tract infections	• Excellent activity against *H. influenzae*, *Proteus*, *E. coli, Klebsiella*, *Serratia* • Good activity vs. *Pseudomonas* • Lacks any gram-positive activity • Lacks cross-allergenicity with other β-lactams (except ceftazidime, ceftolozane, cefiderocol) • Good CSF penetration, renal elimination
Glycopeptides and Lipoglycopeptides—Inhibitors of Cell Wall Synthesis		
Vancomycin (IV, PO)	• Skin and soft-tissue infections • Bacteremia and endocarditis due to gram-positive bacteria • Pneumonia • Meningitis • *Clostridium difficile* colitis (oral formulation) • Surgical prophylaxis for procedures with high risk of MRSA	• Good activity vs. vast majority of gram-positive bacteria, *Staphylococcus* (including MRSA), streptococci, *E. faecalis* • Oral formulation not well absorbed and used only for treatment of *C. difficile* colitis • Modest CNS penetration in presence of inflammation • Renal elimination • Infusion-related reactions associated with rapid infusion • Nephrotoxicity with greater doses
Telavancin (IV)	• Skin and soft-tissue infections • Pneumonia	• Similar activity to vancomycin with activity against some vancomycin-resistant strains of *Enterococcus* • Renal elimination • Higher nephrotoxicity relative to vancomycin • QT prolongation • Avoid in pregnancy
Dalbavancin (IV)	• Skin and soft-tissue infections	• Similar activity to vancomycin • Highly protein bound • Extremely long $t_{1/2}$; once-weekly dosing for skin infections
Oritavancin (IV)	• Skin and soft-tissue infections	• Similar activity to telavancin • Highly protein bound • Extremely long $t_{1/2}$; single-dose therapy for skin infections
Lipopeptides—Disruptors of Bacterial Cell Membranes		
Daptomycin (IV)	• Skin and soft-tissue infections • Staphylococcal and streptococcal bacteremia • Vancomycin-resistant enterococcal infections	• Lipopeptide, similar spectrum of activity as vancomycin • Active against many vancomycin-resistant strains of *Enterococcus* • Highly protein bound; limited CNS penetration • Inactivated by pulmonary surfactant; not effective for pneumonia • Renal elimination • Rare myositis and rhabdomyolysis

MSSA, methicillin-sensitive *S. aureus*.

[a] Cefepime only.

[b] Only β-lactam with significant activity versus MRSA.

[c] Imipenem only.

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Chapter 59

Miscellaneous Antibacterials: Aminoglycosides, Polymyxins, Urinary Antiseptics, Bacteriophages

Conan MacDougall and Robert T. Schooley

AMINOGLYCOSIDES
- Mechanism of Action
- Antimicrobial Activity
- Resistance to the Aminoglycosides
- ADME
- Dosing and Monitoring
- Therapeutic Uses
- Adverse Effects

POLYMYXINS
- Mechanism of Action
- Antimicrobial Activity
- Resistance to Polymyxins
- ADME
- Therapeutic Uses
- Adverse Effects

ANTISEPTIC AGENTS FOR URINARY TRACT INFECTIONS
- Nitrofurantoin
- Fosfomycin
- Methenamine

BACTERIOPHAGES
- Phage Biology
- Phage Selection and Production
- Pharmacology
- Clinical Indications
- Future Prospects

The small-molecule agents discussed in this chapter—aminoglycosides, polymyxins, and urinary antiseptics—primarily target gram-negative bacteria and have a limited set of clinical applications due to their toxicities and pharmacokinetic properties. Additionally, we discuss the (re-) emerging application of phages in infectious diseases therapeutics.

Aminoglycosides

ORIGINS

Aminoglycosides are natural products or semisynthetic derivatives of compounds produced by a variety of soil actinomycetes. *Streptomycin* was first isolated from a strain of *Streptomyces griseus*. *Gentamicin* and *netilmicin* are derived from species of the actinomycete *Micromonospora*. The difference in spelling (*-micin*) compared with the other aminoglycoside antibiotics (*-mycin*) reflects this difference in origin. *Tobramycin* is one of several components of an aminoglycoside complex known as "nebramycin" that is produced by *Streptomyces tenebrarius*. It is most similar in antimicrobial activity and toxicity to *gentamicin*. In contrast to the other aminoglycosides, *amikacin* (a derivative of kanamycin) and *netilmicin* and *plazomicin* (derivatives of sisomicin) are semisynthetic products.

Aminoglycosides (*gentamicin, tobramycin, amikacin, netilmicin, plazomicin, kanamycin, streptomycin, paromomycin,* and *neomycin*) are used primarily to treat infections caused by aerobic gram-negative bacteria. *Streptomycin* and *amikacin* are important agents for the treatment of mycobacterial infections, and *paromomycin* is used orally for intestinal amebiasis. Aminoglycosides are *bactericidal inhibitors* of protein synthesis. Most commonly, resistance is due to aminoglycoside-modifying enzymes or impaired accumulation of drug at the target site; these mechanisms may confer resistance to all aminoglycosides or only select agents. Resistance genes are frequently acquired via plasmids or transposons.

Aminoglycosides contain amino sugars linked to an aminocyclitol ring by glycosidic bonds (Figure 59–1). They are polycations, and their polarity is responsible in part for pharmacokinetic properties shared by all members of the group. For example, none is absorbed adequately after oral administration, inadequate concentrations are found in CSF, and all are excreted relatively rapidly by the normal kidney. All members of the group share the same spectrum of toxicity, most notably nephrotoxicity and ototoxicity, which can involve the auditory and vestibular functions of the eighth cranial nerve, although the relative propensities for toxicity vary somewhat among the agents.

Mechanism of Action

The aminoglycoside antibiotics are rapidly bactericidal against susceptible gram-negative organisms. Bacterial killing is concentration dependent: The higher the concentration, the greater is the rate of bacterial killing. The ratio of the peak concentration to the organism's *MIC* is thus a driver of aminoglycoside efficacy, although total drug exposure (AUC:MIC) is also an important predictor of antibacterial effect (Bland et al., 2018). The inhibitory activity of aminoglycosides persists after the serum concentration has fallen below the MIC, a phenomenon known as the *postantibiotic effect*. These properties help to explain the efficacy of high-dose, extended-interval dosing regimens.

Aminoglycosides diffuse through aqueous channels formed by *porin* proteins in the outer membrane of gram-negative bacteria to enter the periplasmic space. Transport of aminoglycosides across the cytoplasmic (inner) membrane depends on a transmembrane electrical gradient coupled to electron transport to drive permeation of these antibiotics. This energy-dependent phase is rate limiting and can be blocked or inhibited by divalent cations (e.g., Ca^{2+} and Mg^{2+}), hyperosmolarity, a reduction in pH, and anaerobic conditions. Thus, the antimicrobial activity of aminoglycosides is reduced markedly in the anaerobic environment of an abscess and in hyperosmolar acidic urine.

Once inside the cell, aminoglycosides bind to polysomes and interfere with protein synthesis by causing misreading and premature termination of mRNA translation (Figure 59–2). The primary intracellular site of action of the aminoglycosides is the 30S ribosomal subunit. At least three

Abbreviations

AC: acetylase
AD: adenylase
AUC: area under the curve
CMS: colistin methanesulfonate
CNS: central nervous system
CsCl: cesium chloride
CSF: cerebrospinal fluid
FDA: Food and Drug Administration
G6PD: glucose-6-phosphate dehydrogenase
GI: gastrointestinal
IM: intramuscular
IV: intravenous
MIC: minimal inhibitory concentration
mRNA: messenger RNA
PFU: plaque-forming units
PO: by mouth
UTI: urinary tract infection

of these ribosomal proteins, and perhaps the 16S ribosomal RNA as well, contribute to the *streptomycin*-binding site. *Aminoglycosides interfere with the initiation of protein synthesis, leading to the accumulation of abnormal initiation complexes; the drugs also can cause misreading of the mRNA template and incorporation of incorrect amino acids into the growing polypeptide chains* (Davis, 1988). The resulting aberrant proteins may be inserted into the cell membrane, leading to altered permeability and further stimulation of aminoglycoside transport (Busse et al., 1992).

Antimicrobial Activity

The antibacterial activity of *gentamicin, tobramycin, amikacin*, and *plazomicin* is directed primarily against aerobic gram-negative bacilli (Mingeot-Leclercq et al., 1999). *Kanamycin*, like *streptomycin*, has a more limited spectrum. The aerobic gram-negative bacilli vary in their susceptibility to the aminoglycosides (Table 59–1), although most Enterobacterales are susceptible. The superior activity of *tobramycin* against *Pseudomonas aeruginosa* makes it the preferred aminoglycoside for treatment of serious infections known or suspected to be caused by this organism. Among other nonfermenting gram-negative rods such as *Acinetobacter*, *Stenotrophomonas*, and *Burkholderia,* aminoglycosides are uncommonly to rarely active. Gram-negative aerobic cocci such as *Neisseria*, *Moraxella*, and *Haemophilus* have varying susceptibilities. An increasing number of gram-negative bacilli encountered in health-care settings (especially *Klebsiella* and *Pseudomonas*) display extensive resistance to multiple classes of antibacterials; in these isolates, aminoglycosides may be the only class of commonly used agents with *in vitro* activity. *Amikacin* and *plazomicin* are typically the most active aminoglycosides against multidrug-resistant gram-negative bacilli.

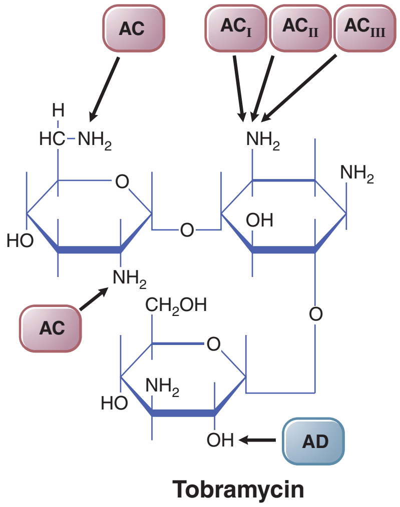

Figure 59–1 *Aminoglycoside structure and sites of activity of plasmid-mediated enzymes capable of inactivating aminoglycosides.* *Tobramycin* is shown as a representative; structural characteristics protect some aminoglycosides from the actions of some of these enzymes, explaining differences in spectrum of activity. AC, acetylase; AD, adenylase.

Aminoglycosides have little activity against anaerobic microorganisms or facultative bacteria under anaerobic conditions. Their action against most gram-positive bacteria is limited, and they should not be used as single agents to treat infections caused by gram-positive bacteria. However, in combination with a cell wall–active agent, such as a *penicillin* or *vancomycin*, an aminoglycoside may produce a synergistic bactericidal effect *in vitro*. This effect has been most commonly employed for treatment of infections due to staphylococci, enterococci, viridans group streptococci, and *Listeria*. *Gentamicin* (or in some cases *streptomycin*) is the drug of choice for use in combination therapies against gram-positive organisms, since other agents such as *tobramycin* and *amikacin* are minimally active. Clinically, the superiority of aminoglycoside combination regimens over cell wall agents alone is not proven except in relatively few infections (discussed later in the chapter).

Paromomycin (also known as *aminosidine*) is an aminoglycoside that is structurally related to *neomycin*. It has antibacterial activity similar to other aminoglycosides but has particularly notable antiparasitic activity. Parasites that are usually susceptible to *paromomycin* include *Leishmania* spp., *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum.*

Resistance to the Aminoglycosides

Bacteria may be resistant to aminoglycosides through

- inactivation of the drug by microbial enzymes;
- failure of the antibiotic to penetrate intracellularly; and
- low affinity of the drug for the bacterial ribosome.

Clinically, drug inactivation is the most common mechanism for acquired microbial resistance. The genes encoding aminoglycoside-modifying enzymes are acquired primarily by conjugation and transfer of resistance plasmids. These enzymes phosphorylate, adenylate, or acetylate specific hydroxyl or amino groups (see Figure 59–1). The ability of these enzymes to attack these groups in differing aminoglycosides explains some of the variability in antimicrobial activity across the class. *Amikacin* is a suitable substrate for only a few of these inactivating enzymes; thus, strains that are resistant to multiple other aminoglycosides tend to be susceptible to *amikacin*, particularly among gram-negative bacilli. *Plazomicin* likewise has structural modifications that make it a poor substrate for most aminoglycoside-modifying enzymes, expanding its activity against resistant gram-negatives, including among carbapenem-resistant isolates. A significant percentage of clinical isolates of *Enterococcus faecalis* and *Enterococcus faecium* are highly resistant to all aminoglycosides (Eliopoulos et al., 1984). Resistance to *gentamicin* in these organisms indicates cross-resistance to *tobramycin*, *amikacin*, *kanamycin*, and *netilmicin* because the inactivating enzyme is bifunctional and can modify all these aminoglycosides. Owing to differences in the chemical structures between *streptomycin* and other aminoglycosides, the most common enzyme seen in enterococci does not modify *streptomycin*, which is inactivated by another enzyme. Consequently, *gentamicin*-resistant strains of enterococci may be susceptible to *streptomycin*. Intrinsic resistance to aminoglycosides may be caused by failure of the drug to penetrate the cytoplasmic (inner) membrane. Transport of aminoglycosides across the cytoplasmic membrane is an active process that depends on oxidative metabolism. Strictly anaerobic bacteria thus are resistant to these drugs because they lack the necessary transport system.

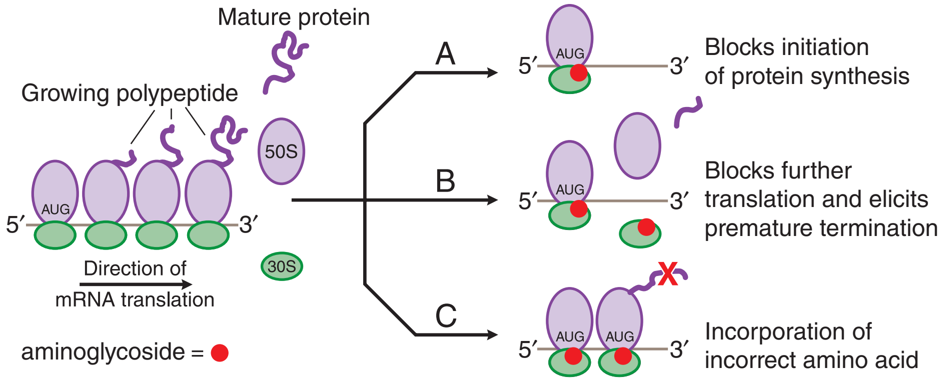
Mature protein
Growing polypeptide
50S
30S
AUG
5′
3′
Direction of mRNA translation
aminoglycoside = ●
A
B
C
Blocks initiation of protein synthesis
Blocks further translation and elicits premature termination
X
Incorporation of incorrect amino acid

Figure 59–2 *Effects of aminoglycosides on protein synthesis.* **A.** Aminoglycoside (represented by red circles) binds to the 30S ribosomal subunit and interferes with initiation of protein synthesis by fixing the 30S-50S ribosomal complex at the start codon (AUG) of mRNA. As 30S-50S complexes downstream complete translation of mRNA and detach; the abnormal initiation complexes, so-called streptomycin monosomes, accumulate, blocking further translation of the message. Aminoglycoside binding to the 30S subunit also causes misreading of mRNA, leading to **B**, premature termination of translation with detachment of the ribosomal complex and incompletely synthesized protein or **C**, incorporation of incorrect amino acids (indicated by the red X), resulting in the production of abnormal or nonfunctional proteins.

ADME

Absorption

The aminoglycosides are polar cations and therefore are poorly absorbed from the GI tract. Less than 1% of a dose is absorbed after either oral or rectal administration. Nonetheless, long-term oral or rectal administration of aminoglycosides may result in accumulation to toxic concentrations in patients with renal impairment. Absorption of *gentamicin* from the GI tract may be increased by GI disease (e.g., ulcers or inflammatory bowel disease). Instillation of these drugs into body cavities with serosal surfaces also may result in rapid absorption and unexpected toxicity (i.e., neuromuscular blockade). Intoxication may occur when aminoglycosides are applied topically for long periods to large wounds, burns, or cutaneous ulcers, particularly if there is renal insufficiency.

All the aminoglycosides are absorbed rapidly from intramuscular sites of injection. Peak concentrations in plasma occur after 30 to 90 min. These concentrations range from 4 to 12 μg/mL following a 1.5- to 2-mg/kg dose of *gentamicin*, *tobramycin*, or *netilmicin*; from 20 to 35 μg/mL following a 7.5-mg/kg dose of *amikacin* or *kanamycin*; and from 100 to 150 μg/mL following a 15-mg/kg dose of *plazomicin*. There is increasing use of aminoglycosides administered via inhalation, primarily for the management of patients with cystic fibrosis who have chronic *P. aeruginosa* or mycobacterial pulmonary infections (Geller et al., 2002). *Amikacin* and *tobramycin* solutions for injection have been used, and commercially available preparations of these agents designed specifically for inhalation are now available. *Neomycin* is not used for parenteral administration; it is currently available in many brands of creams, ointments, and other products alone and in combination with *polymyxin*, *bacitracin*, other antibiotics, a variety of corticosteroids, and *lidocaine*. *Paromomycin* is available for parenteral use outside of the United States but is primarily used for its intraluminal activity as a poorly absorbed antiparasitic agent.

Distribution

Because of their polar nature, the aminoglycosides do not penetrate well into many tissues. Except for *streptomycin*, there is negligible binding of aminoglycosides to plasma albumin. The apparent volume of distribution of these drugs is 25% of lean body weight and approximates the volume of extracellular fluid. The aminoglycosides distribute poorly into adipose tissue, which must be considered when using weight-based dosing regimens in obese patients.

Concentrations of aminoglycosides in secretions and tissues are low (Panidis et al., 2005). High concentrations are found only in the renal cortex and the endolymph and perilymph of the inner ear; the high concentration in these sites likely contributes to the nephrotoxicity and ototoxicity caused by these drugs. As a result of active hepatic secretion, concentrations in bile approach 30% of those found in plasma, but this represents a very minor excretory route for the aminoglycosides. Inflammation increases the penetration of aminoglycosides into peritoneal and pericardial cavities. Concentrations of aminoglycosides achieved in CSF with parenteral administration usually are subtherapeutic (Kearney and

TABLE 59–1 ■ SUSCEPTIBILITIES AND TYPICAL MINIMAL CONCENTRATIONS THAT WILL INHIBIT 90% (MIC_{90}) OF CLINICAL ISOLATES FOR GRAM-NEGATIVE ORGANISMS

SPECIES	% SUSCEPTIBLE (MIC_{90} μg/mL)					
	GENTAMICIN	TOBRAMYCIN	AMIKACIN	POLYMYXIN B	NITROFURANTOIN	FOSFOMYCIN
Escherichia coli	88.2% (8)	86.3% (8)	99.0% (4)	N/A (<=0.5)	90.3% (32)	99.6% (4)
Klebsiella spp	89.2% (8)	82.4% (32)	88.2% (32)	N/A (<=0.5)	9.0% (128)	N/A (32)
Enterobacter spp.	97.0% (1)	96.0% (1)	100.0% (2)	N/T	11.4% (128)	N/A (32)
Pseudomonas aeruginosa	88.0% (16)	90.0% (4)	98.0% (16)	N/A (1)	N/T	N/A (128)
Serratia spp.	97.0% (1)	94.0% (4)	99.0% (4)	N/T	N/T	N/T
Acinetobacter baumanii	37.0% (>128)	51.0% (>128)	58.0% (>128)	N/A (<=0.5)	N/T	N/T

N/A: not applicable; N/T: not tested. *Source*: Data from Sader HS, et al. Arbekacin activity against contemporary clinical bacteria isolated from patients hospitalized with pneumonia. *Antimicrob Agents Chemother*, **2015**, *59*:3263–3270; Gales AC, et al. Contemporary activity of colistin and polymyxin B against a worldwide collection of Gram-negative pathogens: results from the SENTRY Antimicrobial Surveillance Program (2006-2009). *J Antimicrob Chemother*, **2011**, *66*:2070-2074; Keepers TR, et al. Fosfomycin and comparator activity against select Enterobacteriaceae, *Pseudomonas*, and *Enterococcus* urinary tract infection isolates from the United States in 2012. *Infect Dis Ther*, **2017**, *6*:233-243.

Aweeka, 1999), and owing to their dose-related toxicity, dose increases to provide greater concentrations are not feasible. Treatment of meningitis with intravenous administration is generally suboptimal. Intrathecal or intraventricular administration of aminoglycosides has been used to achieve therapeutic levels in the CNS, but the availability of extended-spectrum cephalosporins has generally made this unnecessary.

Administration of aminoglycosides to women late in pregnancy may result in accumulation of drug in fetal plasma and amniotic fluid. *Streptomycin* and *tobramycin* can cause hearing loss in children born to women who receive the drug during pregnancy. Insufficient data are available regarding the other aminoglycosides; therefore, these agents should be used with caution during pregnancy and only for strong clinical indications in the absence of suitable alternatives.

Metabolism and Excretion

The aminoglycosides undergo minimal metabolism and are excreted almost entirely by glomerular filtration, achieving urine concentrations of 50 to 200 μg/mL. The half-lives of the aminoglycosides in plasma are 2 to 3 h in patients with normal renal function. Because the elimination of aminoglycosides depends almost entirely on the kidney, a linear relationship exists between the concentration of creatinine in plasma and the $t_{1/2}$ of all aminoglycosides in patients with moderately compromised renal function. In anephric patients, the $t_{1/2}$ varies from 20 to 40 times that determined in normal individuals. *Because the incidence of nephrotoxicity and ototoxicity is likely related to the overall exposure to aminoglycosides, it is critical to reduce the maintenance dose and/or extend the dosing interval of these drugs in patients with impaired renal function.*

Although excretion of aminoglycosides is similar in adults and children older than 6 months, half-lives of aminoglycosides may be prolonged significantly in the newborn: 8 to 11 h in the first week of life in newborns weighing less than 2 kg and about 5 h in those weighing more than 2 kg. Thus, it is critically important to monitor plasma concentrations of aminoglycosides during treatment of neonates. Aminoglycoside clearances are increased and half-lives are reduced in patients with cystic fibrosis (Young et al., 2013). Larger doses of aminoglycosides may likewise be required in burn patients because of more rapid drug clearance, possibly because of drug loss through burn tissue. Aminoglycosides can be removed from the body by either hemodialysis or peritoneal dialysis.

Aminoglycosides can be inactivated by various penicillins *in vitro* and thus should not be admixed in solution (Blair et al., 1982). Some reports indicate that this inactivation may occur *in vivo* in patients with end-stage renal failure, making monitoring of aminoglycoside plasma concentrations even more necessary in such patients. *Amikacin* appears to be the aminoglycoside least affected by this interaction; penicillins with more nonrenal elimination (e.g., *piperacillin*) may be less prone to cause this interaction.

Dosing and Monitoring

High-dose, extended-interval administration of aminoglycosides is the preferred means of administering aminoglycosides for most indications and patient populations. Administering higher doses at extended intervals (i.e., once daily) is likely to be at least equally efficacious and potentially less toxic than administration of divided doses. This dosing strategy takes advantage of the concentration-dependent activity of aminoglycosides to achieve maximal initial bacterial killing, and because of the post-antibiotic effect of aminoglycosides, good therapeutic response can be attained even when concentrations fall below inhibitory concentrations for a substantial fraction of the dosing interval. High-dose, extended-interval dosing schemes for aminoglycosides may also reduce the characteristic oto- and nephrotoxicity of these drugs. This diminished toxicity is probably due to a threshold effect from accumulation of drug in the inner ear or in the kidney. High-dose, extended-interval regimens, despite the higher peak concentration, provide a longer period when concentrations fall below the threshold for toxicity than does a multiple-dose regimen (compare the two dosage regimens shown in Figure 59–3). Typical doses for high-dose, extended-interval strategies are 5 to 7 mg/kg for *gentamicin* and *tobramycin*, 15 to 25 mg/kg for *amikacin*, and 15 mg/kg for *plazomicin*; among patients with normal renal function, these doses are administered every 24 h.

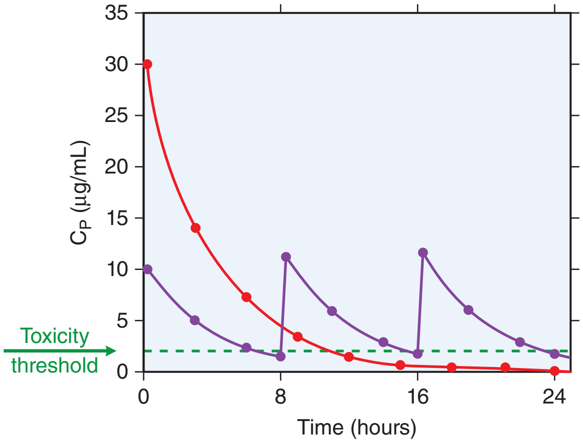

Figure 59–3 *Comparison of single-dose and divided-dose regimens for gentamicin.* In a hypothetical patient, a dose of *gentamicin* (5.1 mg/kg) is administered intravenously as a single bolus (red line) or in three portions, a third of the dose every 8 h (purple line), such that the total drug administered is the same in the two cases. The threshold for toxicity (green dashed line) is the plasma concentration of 2 μg/mL, the maximum recommended for prolonged exposure. The single-dose regimen produces a higher plasma concentration than the regimen given every 8 h; this higher peak provides efficacy that otherwise might be compromised due to prolonged subthreshold concentrations later in the dosing interval or that is provided by the lower peak levels achieved with the regimen every 8 h. The once-daily regimen also provides a 13-h period during which plasma concentrations are below the threshold for toxicity. The every-8-h regimen, by contrast, provides only three short (~1 h) periods in 24 h during which plasma concentrations are below the threshold for toxicity. The single high-dose, extended interval is generally preferred for aminoglycosides, with a few exceptions (during pregnancy, in neonates, etc.), as noted in the text.

Populations in which use of the high-dose/extended-interval dosing strategy is more controversial include pregnant patients, neonates, and pediatric patients and as combination therapy for endocarditis (Contopoulos-Ioannidis et al., 2004; Nestaas et al., 2005; Ward and Theiler, 2008). In these infections, multiple daily doses (with a lower total daily dose) are preferred by some clinicians since data documenting equivalent safety and efficacy of extended-interval dosing are more limited than in other adult populations. Extended-interval dosing is sometimes avoided in patients with significant renal dysfunction (i.e., creatinine clearance <25 mL/min), since every-36-h or every-48-h dosing schedules are often required. Typical doses for divided-dosing strategies are 1.7 to 2 mg/kg every 8 h for *gentamicin* and *tobramycin*. The dose range of *streptomycin* for most indications is 15 to 25 mg/kg daily or in divided doses twice daily. For long-term therapy of mycobacterial infections, three-times-weekly dosing of *amikacin* or *streptomycin* may be employed.

Concentrations of aminoglycosides achieved in plasma after a given dose vary widely among patients, and therapeutic drug monitoring is standard practice, especially among seriously ill patients (Abdul-Aziz et al., 2020). Traditionally, the peak concentration has been used to document that the dose produces therapeutic concentrations, while the trough concentration is used to avoid toxicity. For high-dose, extended-interval dosing regimens, fully characterizing the pharmacokinetics can be done through drawing peak and "midpoint" (6–18 h after the end of infusion) concentrations and calculating the predicted AUC (Bland et al., 2018). Alternatively, use of "random" concentrations obtained 6 to 18 h after infusion with comparison to a published nomogram is a less-intensive monitoring approach. For *plazomicin*, the manufacturer recommends obtaining trough concentrations, with dosage adjustment as necessary to maintain a trough below 3 μg/mL. For twice-daily and three-times-daily dosing strategies, steady-state trough concentrations should be less than 1 to 2 μg/mL for *gentamicin*, *netilmicin*, and *tobramycin* and less than

10 μg/mL for *amikacin* and *streptomycin*. Peak level goals vary by indication and infection severity but range from 4 to 8 μg/mL for *gentamicin*, *netilmicin*, and *tobramycin* and from 20 to 35 μg/mL for *amikacin* under twice-daily and three-times-daily strategies. When *gentamicin* is used at lower doses for synergistic effects with cell wall–acting agents to treat gram-positive infections, peak levels of 3 to 4 μg/mL and less than 1 μg/mL are recommended.

Therapeutic Uses

Several different types of infections can be treated successfully with aminoglycosides; however, owing to their toxicities, prolonged use should be restricted to the therapy of life-threatening infections and those for which a less-toxic agent is contraindicated or less effective. When multiple aminoglycosides are appropriate for an indication, *gentamicin* is typically preferred because of long experience with its use and its lower cost.

Aminoglycosides may be used in combination with a cell wall–active agent (*β-lactam or glycopeptide*) for the therapy of serious proven or suspected bacterial infections. The three rationales for this approach are as follows:

- To expand the empiric spectrum of activity of the antimicrobial regimen
- To provide synergistic bacterial killing
- To prevent the emergence of resistance to the individual agents

Empiric combination therapy is used in infections such as healthcare-associated pneumonia or sepsis, where multidrug-resistant gram-negative organisms such as *P. aeruginosa*, *Enterobacter*, *Klebsiella*, and *Serratia* may be causative and the consequences of failing to provide initially active therapy are dire. The use of aminoglycosides to achieve synergistic bacterial killing and improve clinical response is most well established for the treatment of endocarditis due to gram-positive organisms, most importantly *Enterococcus* (Le and Bayer, 2003). Clinical data do not support the routine use of combination therapy for synergistic killing or suppression of emergent resistance of gram-negative organisms, with the possible exceptions of serious *P. aeruginosa* infections (Bliziotis et al., 2005). Aminoglycosides (primarily *streptomycin* and *amikacin*) may be a component of combination regimens for treatment of mycobacterial infections, usually due to the presence of multidrug resistance (see Chapter 65).

Urinary Tract Infections

Although the spectrum of activity and concentration in the urinary tract of aminoglycosides make them well suited for treatment of urinary tract infections, less-toxic alternatives are preferred for uncomplicated infections. However, as strains of *Escherichia coli* have acquired resistance to β-lactams, *trimethoprim-sulfamethoxazole*, and fluoroquinolones, use of aminoglycosides for urinary tract infections may increase. A single intramuscular dose of *gentamicin* (5 mg/kg) has been effective in uncomplicated infections of the lower urinary tract. A 10- to 14-day course of *gentamicin* or *tobramycin* is an alternative for treatment of pyelonephritis if other agents cannot be used. *Plazomicin* is FDA-approved for treatment of complicated urinary tract infections including pyelonephritis when patients have limited or no treatment options, at a dose of 15 mg/kg daily.

Pneumonia

Aminoglycosides are ineffective for the treatment of pneumonia due to *Streptococcus pneumoniae*, which is a common cause of community-acquired pneumonia. In hospital-acquired pneumonia where aerobic multidrug-resistant gram-negative bacilli are frequently causative pathogens, an aminoglycoside in combination with a β-lactam antibiotic is recommended as standard empiric therapy to increase the likelihood that at least one agent is active against the infecting pathogen (Kalil et al., 2016). Once it is established that the β-lactam is active against the causative agent, there is generally no benefit from continuing the aminoglycoside.

Meningitis

Availability of third-generation cephalosporins, especially *cefotaxime* and *ceftriaxone*, has reduced the need for treatment with aminoglycosides in most cases of meningitis, except for infections caused by gram-negative organisms resistant to β-lactam antibiotics (e.g., species of *Pseudomonas* and *Acinetobacter*) and for *Listeria* meningitis (where a combination of *ampicillin* and *gentamicin* is recommended). If an aminoglycoside is necessary, direct instillation into the CNS is more likely to achieve therapeutic levels than intravenous administration. In adults, this can be achieved with 5 mg of a preservative-free formulation of *gentamicin* (or equivalent dose of another aminoglycoside) administered intrathecally or intraventricularly once daily.

Bacterial Endocarditis

"Synergistic" or low-dose *gentamicin* (3 mg/kg per day) in combination with a *penicillin* or *vancomycin* has been recommended in certain circumstances for treatment of bacterial endocarditis due to certain gram-positive organisms. *Penicillin* and *gentamicin* in combination are effective as a short-course (i.e., 2-week) regimen for uncomplicated native-valve streptococcal endocarditis. For this indication, *gentamicin* may be given as a consolidated once-daily dose. In cases of enterococcal endocarditis, concomitant administration of *penicillin* (or *ampicillin*) and *gentamicin* (given as divided doses) for 4 to 6 weeks is recommended as standard therapy. However, safer alternatives, such as *ampicillin/ceftriaxone* combinations or use of the aminoglycoside for only the first 2 to 3 weeks, are gaining favor to limit the risk of toxicity due to prolonged aminoglycoside administration (Baddour et al., 2015). A 2-week regimen of *gentamicin* in combination with *nafcillin* is effective for the treatment of selected cases of staphylococcal tricuspid native-valve endocarditis. For patients with native mitral or aortic valve staphylococcal endocarditis, the risks of aminoglycoside administration likely outweigh the benefits (Cosgrove et al., 2009).

Tularemia

Streptomycin (or *gentamicin*) is the drug of choice for the treatment of tularemia. Most cases respond to the administration of 1 to 2 g (15–25 mg/kg) *streptomycin* per day (in divided doses) for 10 to 14 days.

Plague

A 10-day treatment course of *streptomycin* or *gentamicin* is recommended for severe forms of plague.

Mycobacterial Infections

Amikacin and *streptomycin* are second-line agents for the treatment of active tuberculosis as part of a combination regimen, usually employed if the patient's organism is resistant to standard therapy. *Amikacin* is also used as a component of combination regimens for nontuberculous mycobacteria (e.g., *M. avium, M. abscessus, M. chelonae*). See Chapter 65 for more about the use of these agents in treating tuberculosis and *M. avium* complex.

Parasitic Infections

Paromomycin's antiparasitic activity is leveraged to treat intestinal protozoal infections due to *E. histolytica*, *G. lamblia*, and *C. parvum*. It is available as oral capsules and indicated for treatment of intestinal amebiasis at a dose of 25 to 35 mg/kg per day in three divided doses. The drug also has activity against *Leishmania* spp., and is used parenterally for visceral leishmaniasis and topically for cutaneous leishmaniasis, although these formulations are not available in the United States (Ben Salah et al., 2013).

Cystic Fibrosis

Recurrent infections due to multidrug-resistant gram-negative bacilli, especially *Pseudomonas* species, are a hallmark of cystic fibrosis. Aminoglycosides are frequently used as therapy during acute exacerbations of cystic fibrosis, for which higher-than-standard doses (e.g., 10 mg/kg of *tobramycin*) are frequently employed due to the unusual pharmacokinetics observed in patients with cystic fibrosis. These agents may also be administered via inhalation between exacerbations to improve lung function and reduce exacerbation frequency.

Topical Applications

Neomycin is used widely for topical application in a variety of infections of the skin and mucous membranes. The oral administration of *neomycin* (usually in combination with *erythromycin* base) has been employed primarily for "preparation" of the bowel for surgery. Orally administered *neomycin* is poorly absorbed from the GI tract—about 97% of an oral dose of *neomycin* is not absorbed and is eliminated unchanged in the feces. The portion that is absorbed is excreted by the kidney; a total daily intake of 10 g for 3 days yields a blood concentration below that associated with systemic toxicity if renal function is normal. *Neomycin* and *polymyxin B* have been used for irrigation of the bladder to prevent bacteriuria and bacteremia associated with indwelling catheters. For this purpose, 1 mL of a preparation containing 40 mg *neomycin* and 200,000 units *polymyxin B* per milliliter is diluted in 1 L of 0.9% sodium chloride solution and is used for continuous irrigation of the urinary bladder through appropriate catheter systems. The bladder is irrigated at the rate of 1 L every 24 h.

Neomycin is frequently employed as a topical agent for treatment of skin and mucous membrane infections, including as a component of over-the-counter therapies. Oral administration of aminoglycosides may be employed as "bowel prep" prior to surgical procedures or as "selective digestive decontamination" to reduce the risk of ventilator-associated pneumonia.

Adverse Effects

All aminoglycosides have the potential to produce reversible and irreversible vestibular, cochlear, and renal toxicity and neuromuscular blockade.

Ototoxicity

Vestibular and auditory dysfunction can follow the administration of any of the aminoglycosides (Guthrie, 2008). Aminoglycoside-induced ototoxicity may result in irreversible, bilateral, high-frequency hearing loss or vestibular hypofunction. Degeneration of hair cells and neurons in the cochlea correlates with the loss of hearing. Accumulation within the perilymph and endolymph occurs predominantly when aminoglycoside concentrations in plasma are high. Diffusion back into the bloodstream is slow; the half-lives of the aminoglycosides are five to six times longer in the otic fluids than in plasma. Drugs such as *ethacrynic acid* and *furosemide* potentiate the ototoxic effects of the aminoglycosides in animals, but data from humans implicating *furosemide* are less convincing (Smith and Lietman, 1983).

Streptomycin and *gentamicin* produce predominantly vestibular effects, whereas *amikacin*, *kanamycin*, and *neomycin* primarily affect auditory function; *tobramycin* affects both equally. The incidence of ototoxicity is difficult to determine. Audiometric data suggest that the incidence could be as high as 25% (Brummett and Morrison, 1990). The incidence of vestibular toxicity is particularly high in patients receiving *streptomycin*; nearly 20% of individuals who received 500 mg twice daily for 4 weeks for enterococcal endocarditis developed clinically detectable irreversible vestibular damage. Because the initial symptoms may be reversible, patients receiving high doses or prolonged courses of aminoglycosides should be monitored carefully for ototoxicity; however, deafness may occur several weeks after therapy is discontinued.

A high-pitched tinnitus often is the first symptom of cochlear toxicity. If the drug is not discontinued, auditory impairment may develop after a few days. The tinnitus may persist for several days to 2 weeks after therapy is stopped. Because perception of sound in the high-frequency range (outside the conversational range) is lost first, the affected individual is not always aware of the difficulty, and it will not be detected except by careful audiometric examination. If the hearing loss progresses, the lower sound ranges are affected.

Among patients experiencing vestibular toxicity, moderately intense headache lasting 1 to 2 days may precede the onset of labyrinthine dysfunction. This is followed immediately by an acute stage in which nausea, vomiting, and difficulty with equilibrium develop and persist for 1 to 2 weeks. Prominent symptoms include vertigo in the upright position, inability to perceive termination of movement ("mental past-pointing"), and difficulty in sitting or standing without visual cues. The acute stage ends suddenly and is followed by chronic labyrinthitis, in which the patient has difficulty when attempting to walk or make sudden movements; ataxia is the most prominent feature. The chronic phase persists for about 2 months. Recovery from this phase may require 12 to 18 months, and most patients have some permanent residual damage. Early discontinuation of the drug may permit recovery before irreversible damage of the hair cells.

Nephrotoxicity

Approximately 8% to 26% of patients who receive an aminoglycoside for several days develop mild renal impairment that is almost always reversible. The toxicity results from accumulation and retention of aminoglycoside in the proximal tubular cells. The initial manifestation of damage at this site is excretion of enzymes of the renal tubular brush border followed by mild proteinuria and the appearance of hyaline and granular casts. The glomerular filtration rate is reduced after several additional days. The nonoliguric phase of renal insufficiency is thought to be due to the effects of aminoglycosides on the distal portion of the nephron with a reduced sensitivity of the collecting duct epithelium to vasopressin. Although severe acute tubular necrosis may occur rarely, the most common significant finding is a mild rise in plasma creatinine. The impairment in renal function is almost always reversible because the proximal tubular cells have the capacity to regenerate (Lietman and Smith, 1983). Toxicity correlates with the total amount of drug administered and with longer courses of therapy (de Jager and van Altena, 2002). High-dose, extended-interval dosing approaches lead to less nephrotoxicity at the same level of total drug exposure (as measured by the area under the curve) than divided-dose approaches (see Figure 59–3). *Neomycin*, which concentrates to the greatest degree, is highly nephrotoxic in human beings and should not be administered systemically. *Streptomycin* does not concentrate in the renal cortex and is the least nephrotoxic. Drugs such as *amphotericin B, vancomycin*, angiotensin-converting enzyme inhibitors, *cisplatin*, and *cyclosporine* may potentiate aminoglycoside-induced nephrotoxicity.

Neuromuscular Blockade

Acute neuromuscular blockade and apnea have been attributed to the aminoglycosides; patients with myasthenia gravis are particularly susceptible. In humans, neuromuscular blockade generally has occurred after intrapleural or intraperitoneal instillation of large doses of an aminoglycoside; however, the reaction can follow intravenous, intramuscular, and even oral administration of these agents. Most episodes have occurred in association with anesthesia or the administration of other neuromuscular blocking agents. Neuromuscular blockade may be reversed by intravenous administration of a Ca^{2+} salt.

Other Adverse Effects

In general, the aminoglycosides have little allergenic potential. Rare hypersensitivity reactions—including skin rashes, eosinophilia, fever, blood dyscrasias, angioedema, exfoliative dermatitis, stomatitis, and anaphylactic shock—have been reported as cross-hypersensitivity among drugs in this class. Aminoglycosides appear to be less commonly associated with superinfection due to *Clostridium difficile* than other classes of antibacterials. Individuals treated with 4 to 6 g/day of *neomycin* by mouth sometimes develop a sprue-like syndrome with diarrhea, steatorrhea, and azotorrhea; overgrowth of yeasts in the intestine also may occur. Orally administered *paromomycin* is associated with dose-related gastrointestinal toxicity, including nausea, abdominal pain, and diarrhea.

Polymyxins

The polymyxins are a group of closely related antibiotics elaborated by strains of *Bacillus polymyxa*. *Polymyxin B* is a mixture of polymyxins B_1 and B_2. *Colistin*, also known as *polymyxin E,* produced by *Bacillus colistinus,* is marketed either as *colistimethate* for intravenous administration or *colistin* base for topical use. These agents were initially developed more than 50 years ago but quickly fell out of favor for systemic use due to their toxicities. With the rise of resistant gram-negative organisms in the past decade, the use of polymyxins has increased.

Mechanism of Action

Polymyxins, simple basic peptides with molecular masses of approximately 1000 Da, are surface-active amphipathic agents that act as cationic detergents. They interact strongly with phospholipids and disrupt the structure of cell membranes; sensitivity to *polymyxin B* appears related to the phospholipid content of the cell wall–membrane complex. *Polymyxin B* binds to the lipid A portion of endotoxin (the lipopolysaccharide of the outer membrane of gram-negative bacteria) and inactivates this molecule.

Antimicrobial Activity

The antimicrobial activities of *polymyxin B* and *colistin* are similar and restricted to gram-negative bacteria, primarily aerobes. Most *Pseudomonas*, *Acinetobacter*, and Enterobacterales are susceptible, except for *Proteus* and *Serratia* spp. *Stenotrophomonas* and *Burkholderia* are usually resistant.

Resistance to Polymyxins

Although resistance among normally susceptible isolates to polymyxins is uncommon, emergence of resistance while on treatment has been documented and has become problematic among extensively drug-resistant *Acinetobacter* and *Klebsiella* (Rojas et al., 2017). Emergent resistance may occur over the course of a single treatment course.

ADME

Polymyxin B and *colistin* are not absorbed when given orally and are poorly absorbed from mucous membranes and surfaces of large burns. CMS (*colistimethate*) is the prodrug formulation for parenteral administration; it is hydrolyzed relatively slowly in the bloodstream to the active colistin sulfate moiety. There is significant interpatient variability in active colistin sulfate levels due to the competing effects of mostly renal elimination of the CMS parent drug, conversion of CMS to active *colistin*, and mostly nonrenal elimination of active *colistin*. CMS may be administered via inhalation for prevention and adjunctive treatment of lung infections. Intraventricular and intrathecal administration of CMS has been used for treatment of CNS infections. Patients with renal dysfunction require dose modification for CMS. *Polymyxin B* does not require conversion to its active moiety and primarily undergoes nonrenal clearance.

Therapeutic Uses

Because dosing of these agents varies by drug (*polymyxin B* or *colistin*), by the particular commercial preparation marketed in a specific country, and by the patient's degree of renal dysfunction, expert consultation is recommended (Tsuji et al., 2019).

Systemic Uses

Polymyxins are used systemically only for serious infections due to pathogens resistant to other effective therapies. Studies suggest polymyxins are less effective than newly introduced agents (e.g., *ceftazidime/avibactam*) for treatment of serious multidrug-resistant infections and should be reserved for infections due to pathogens resistant to these agents. Polymyxins have been used for treatment of a variety of infections when more effective and less toxic alternatives are not available, including bacteremia, pneumonia, bone/joint infections, burns, cellulitis, cystic fibrosis, endocarditis, gynecologic infections, meningitis, and ventriculitis. Polymyxins are often used in combination with other antimicrobials, including carbapenems, tetracyclines, and aminoglycosides. *Polymyxin B* is preferred for treatment of most systemic infections, while CMS is recommended for treatment of infections originating in the urinary tract (Tsuji et al., 2019). Inhaled *colistin* is used for prophylaxis against infection in some patients at risk for infection (e.g., lung transplant) and as an adjunct in the treatment of pneumonia.

Topical Uses

Polymyxin B sulfate is available for ophthalmic, otic, and topical use in combination with a variety of other compounds. *Colistin* is available as otic drops. Infections of the skin, mucous membranes, eye, and ear due to *polymyxin B*–sensitive microorganisms respond to local application of the antibiotic in solution or ointment. External otitis, frequently due to *Pseudomonas*, may be cured by the topical use of the drug. *P. aeruginosa* is a common cause of infection of corneal ulcers; local application or subconjunctival injection of *polymyxin B* often is curative.

Adverse Effects

The primary toxicity of polymyxins is dose-related nephrotoxicity via damage to renal tubular cells; the therapeutic window between effective and toxic exposures is narrow. Depending on the definitions of nephrotoxicity and patient populations, acute kidney injury may occur in up to 50% to 60% of patients. Although data are limited, nephrotoxicity may be modestly lower with *polymyxin B* relative to CMS (Zavascki and Nation, 2017). Patients who experience nephrotoxicity may require dose reduction or drug discontinuation.

Neurological reactions include muscle weakness, apnea, paresthesia, vertigo, and slurred speech. *Polymyxin B* applied to intact or denuded skin or mucous membranes produces no systemic reactions because of its almost complete lack of absorption from these sites. Hypersensitivity reactions are uncommon.

Antiseptic Agents for Urinary Tract Infections

Urinary tract antiseptics are concentrated in the lower urinary tract, where they inhibit the growth of many species of bacteria. These agents cannot be used to treat systemic infections at the concentrations achievable with oral administration because effective concentrations are not achieved in plasma with safe doses; however, they can achieve concentrations adequate to treat and/or prevent UTIs.

Nitrofurantoin

Nitrofurantoin is a synthetic nitrofuran that is used for the prevention and treatment of UTIs.

Mechanism of Action and Antimicrobial Activity

Nitrofurantoin is activated by enzymatic reduction, with the formation of highly reactive intermediates that seem to be responsible for the observed capacity of the drug to damage DNA. Bacteria reduce *nitrofurantoin* more rapidly than do mammalian cells, and this is thought to account for the selective antimicrobial activity of the compound. *Nitrofurantoin* is active against many strains of *E. coli* and enterococci. However, most *Proteus* and *Pseudomonas* spp. and many species of *Enterobacter* and *Klebsiella* are resistant. *Nitrofurantoin* is bacteriostatic for most susceptible microorganisms at concentrations of 32 μg/mL or less and is bactericidal at concentrations of 100 μg/mL or more. The antibacterial activity is higher in acidic urine.

Pharmacology, Toxicity, and Therapeutic Uses

Nitrofurantoin is absorbed rapidly and nearly completely from the GI tract. Antibacterial concentrations are not achieved in plasma following ingestion of recommended doses because the drug is eliminated rapidly: The plasma $t_{1/2}$ is 0.3 to 1 h; about 40% is excreted unchanged into the urine. The average dose of *nitrofurantoin* yields a concentration in urine of about 200 μg/mL. This concentration is soluble at pH greater than 5, but the urine should not be alkalinized because this reduces antimicrobial activity. The rate of excretion is linearly related to the creatinine clearance; thus, in patients with impaired glomerular function, the efficacy of the drug to treat UTIs may be decreased and the systemic toxicity increased (ten Doesschate et al., 2020).

The oral dosage of *nitrofurantoin* for adults is 50 to 100 mg four times a day with meals and at bedtime, and less for the macrocrystalline formulation (100 mg every 12 h for 7 days). A single 50- to 100-mg dose at bedtime may be sufficient to prevent recurrences. The daily dose for children is 5 to 7 mg/kg but may be as low as 1 mg/kg for long-term therapy. A course of therapy should not exceed 14 days; repeated courses should be separated by rest periods. Pregnant women and children younger than 1 month should not receive *nitrofurantoin*, and it should be used cautiously in patients with impaired renal function.

Nitrofurantoin is approved for the treatment of lower urinary tract infections and is recommended as first-line therapy for uncomplicated cystitis. It is not recommended for treatment of pyelonephritis or prostatitis.

Adverse Effects

The most common untoward effects are nausea, vomiting, and diarrhea; the macrocrystalline preparation is better tolerated than traditional formulations. Various other reactions occur occasionally, including chills, fever, leukopenia, granulocytopenia, hemolytic anemia (associated with G6PD deficiency and in newborns exhibiting low levels of reduced glutathione in their red blood cells), cholestatic jaundice, and hepatocellular damage. Acute pneumonitis with fever, chills, cough, dyspnea, chest pain, pulmonary infiltration, and eosinophilia may occur within hours to days of the initiation of therapy; these symptoms usually resolve quickly after discontinuation of the drug. Interstitial pulmonary fibrosis can occur in patients (especially the elderly) taking the drug chronically. Headache, vertigo, drowsiness, muscular aches, and nystagmus occur occasionally but are readily reversible. Severe polyneuropathies with demyelination and degeneration of both sensory and motor nerves have been reported; neuropathies are most likely to occur in patients with impaired renal function and in persons on long-continued treatment. *Nitrofurantoin* colors the urine brown.

Fosfomycin

Fosfomycin is a phosphonic acid derivative that is used primarily for the prevention and treatment of UTIs in the U.S. where only an oral formulation is currently available. In other countries, the intravenous formulation is often used as an adjunctive agent for treatment of serious infections.

Mechanism of Action and Antimicrobial Activity

Fosfomycin inhibits MurA, an enolpyruvyl transferase that catalyzes the initial step in bacterial cell wall synthesis. This mechanism is unique among antibacterials; thus, cross-resistance to other agents is rarely seen. Optimal testing of *fosfomycin* activity requires supplementation of the media with glucose-6-phosphate. *Fosfomycin*'s usual spectrum of activity includes the uropathogens *E. coli*, *Proteus*, *Enterococcus*, and *Staphylococcus saprophyticus*. Activity against *Klebsiella*, *Enterobacter*, and *Serratia* spp. is variable; *Pseudomonas* and *Acinetobacter* are resistant. *Staphylococcus aureus* is frequently susceptible, although emergence of resistance during therapy has been reported.

Pharmacology, Toxicity, and Therapeutic Uses

Outside the U.S., *fosfomycin* is available as an intravenous formulation that can achieve adequate levels to treat some systemic infections, often as a combination with other antibacterials. In the U.S., *fosfomycin* is available only as a powder (*fosfomycin tromethamine*) that is dissolved in water and taken orally. Bioavailability of the oral formulation is approximately 40%, with a $t_{1/2}$ of 5 to 8 h. With oral administration of 3 g, systemic concentrations are low, but urinary concentrations are as high as 1000 to 4000 μg/mL. The FDA-approved dosing regimen is a single 3-g dose for uncomplicated UTI; some investigators have administered 3 g every other day for three doses for complicated UTI or 3 g every 10 days for UTI prophylaxis.

Adverse Effects

Overall, *fosfomycin* is well tolerated. Adverse effects are uncommon and usually consist of GI distress, vaginitis, headache, or dizziness.

Methenamine

Mechanism of Action and Antimicrobial Activity

Methenamine (hexamethylenamine) is a urinary tract antiseptic and prodrug that acts by generating formaldehyde via the following reaction:

$$N_4(CH_2)_6 + 6H_2O + 4H^+ \rightarrow 6HCHO + 4NH^+_4$$

At pH 7.4, almost no decomposition occurs; the yield of formaldehyde is 6% of the theoretical amount at pH 6 and 20% at pH 5. Thus, acidification of the urine promotes formaldehyde formation and formaldehyde-dependent antibacterial action. The decomposition reaction is fairly slow, requiring 3 h to reach 90% completion. Nearly all bacteria are sensitive to free formaldehyde at concentrations of about 20 μg/mL. Microorganisms do not develop resistance to formaldehyde. Urea-splitting microorganisms (e.g., *Proteus* spp.) tend to raise the pH of the urine and thus inhibit the release of formaldehyde.

Pharmacology, Toxicology, and Therapeutic Uses

Methenamine is absorbed orally, but 10% to 30% decomposes in the gastric juice unless the drug is protected by an enteric coating. Because of the ammonia produced, *methenamine* is contraindicated in hepatic insufficiency. Excretion in the urine is nearly quantitative. When the urine pH is 6 and the daily urine volume is 1000 to 1500 mL, a daily dose of 2 g will yield a urine concentration of 18 to 60 μg/mL of formaldehyde; this is more than the MIC for most urinary tract pathogens. Low pH in and of itself is bacteriostatic, so acidification serves a double function; the acids commonly used are mandelic acid and hippuric acid. GI distress frequently is caused by doses more than 500 mg four times a day, even with enteric-coated tablets. Painful and frequent micturition, albuminuria, hematuria, and rashes may result from doses of 4 to 8 g/day given for longer than 3 to 4 weeks. Renal insufficiency is not a contraindication to the use of *methenamine* alone, but the acids given concurrently may be detrimental; *methenamine mandelate* is contraindicated in renal insufficiency. *Methenamine* combines with sulfamethizole and perhaps other sulfonamides in the urine, resulting in mutual antagonism; therefore, these drugs should not be used in combination.

Methenamine is not a primary drug for the treatment of acute UTIs but is FDA-approved for chronic suppressive treatment of UTIs. The agent is most useful when the causative organism is *E. coli*, but it usually can suppress the common gram-negative offenders and often *S. aureus* and *Staphylococcus epidermidis* as well. *Enterobacter aerogenes* and *Proteus vulgaris* are usually resistant. A urinary pH less than 5 is typically necessary for *methenamine* to be active; some clinicians recommend monitoring of the urinary pH and even urinary acidification with ammonium chloride or ascorbic acid.

Bacteriophages

Bacteriophages (or phages), literally "eaters of bacteria," are viruses that infect bacteria. Phages were initially described just over a century ago when it was noted that agents from environmental sources that could pass through small-pore filters were capable of destroying specific subsets of bacteria (Twort, 1915). Subsequently, phages were shown to be viruses capable of infecting a specific range of bacteria. The host range of infectible bacteria may be narrow for an individual phage, but with an estimated 10^{31} distinct phages in the planet's biosphere, every bacterium has a wide collection of phages to which it is vulnerable (Wommack et al., 1999).

From the perspective of antimicrobial therapy, phages are best conceptualized as living antibiotics (Schooley and Strathdee, 2020). They were promoted as antimicrobials globally until the 1930s, at which time interest was lost in the West as optimism about the utility of traditional antibiotics grew. Phage therapy continued unabated throughout the 20th century in Russia and the former Soviet Republics where antibiotics were less available because of cost. Over the past 5 years, however, as it has become apparent that the traditional antibacterial pipeline is failing to adequately address the antimicrobial drug resistance crisis and as a number of well-studied (albeit anecdotal) successful uses of phages in the treatment of serious bacterial infections have been described, interest in phage therapeutics has reemerged in the U.S. and Western Europe (Aslam et al., 2020; Hatfull et al., 2021).

Phage Biology

Rational use and clinical development of phages requires a fundamental understanding of their biological properties. Phages are small structures, 50 to 200 nm, found in abundance in the biosphere. Most environmental phages are composed of a head that encapsidates a segment of double-stranded DNA encoding the phage's genetic program and a tail that provides binding specificity to the phage's bacterial prey. Phages cannot reproduce on their own; rather, they replicate within a bacterial host. Bacteriophages have two lifestyles: *lytic* and *temperate* (Figure 59–4). Lytic phage infection produces a single outcome, host lysis. Temperate phage

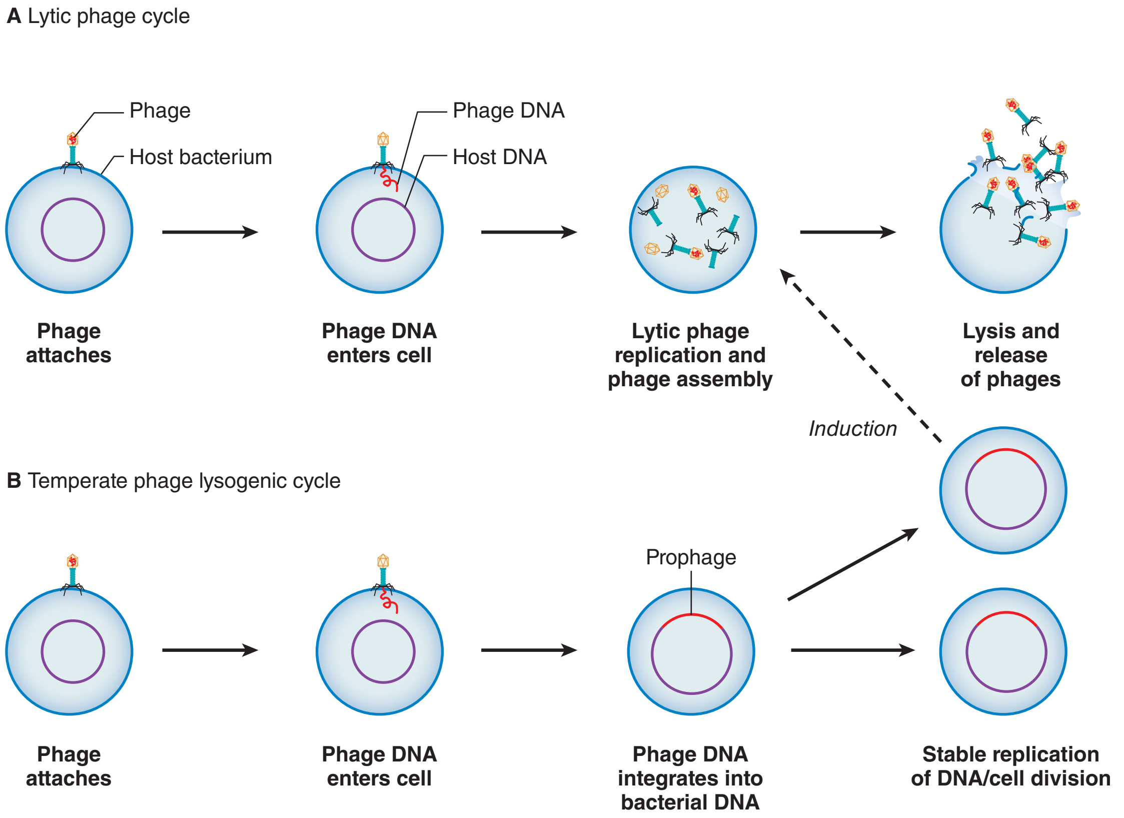
A Lytic phage cycle
Phage
Host bacterium
Phage DNA
Host DNA
Phage attaches
Phage DNA enters cell
Lytic phage replication and phage assembly
Lysis and release of phages
Induction
B Temperate phage lysogenic cycle
Prophage
Phage attaches
Phage DNA enters cell
Phage DNA integrates into bacterial DNA
Stable replication of DNA/cell division

Figure 59–4 *Bacteriophage life cycles* **A.** *Lytic phage cycle.* Lytic phages infect bacteria via attachment, penetration, and injection of their DNA into the bacterial host, leading to inactivation of the host's genome and hijacking of the host replicative machinery for replication of the phage genome and the synthesis and assembly of new phage particles. The result is lysis of the host cell and release of a new crop of bacteriophage progeny. **B.** *Temperate phage lysogenic cycle.* Temperate phages attach and inject their DNA as described for lytic phages. However, rather than phage replication leading to lysis of the host cell, the temperate phage cycle involves incorporation of the phage DNA into the bacterial DNA and subsequent cell division with daughter cells containing the expanded complement of DNA. Environmental factors can induce these prophage cells to enter a lytic cycle, as indicated by the arrow marked "Induction."

infection produces a stable incorporation of the phage genome into the host's DNA, forming a prophage, with the lysogen reproduced as the host bacterium divides, possibly to be activated to a lytic phase by environmental factors.

Lytic Phages

Lytic phages infect their bacterial hosts, disrupting the host's DNA, taking over the host's synthetic machinery to make hundreds to thousands of progeny, producing lysins, and then lysing and destroying their hosts before moving on to repeat the cycle with other host bacteria. Since the timeline for this process may be on the order of 20 min, the expansion of a phage population and the collapse of the population's susceptible prey can occur on an explosive timeline. As this process proceeds, populations of phage-resistant bacteria emerge that are, in turn, chased by evolving phage populations that are capable of growth in these emerging phage-resistant populations.

Temperate Phages

In the temperate lifestyle, phages enter the host bacterium but do not hijack the host's replicative machinery for their own purposes; rather, the phage DNA encode genes for integrases that enable them to integrate their genetic code into the DNA of host bacteria and for additional genes (termed *repressors*) that repress the ability of incoming phages to attack the bacterial host. The DNA of temperate phages is replicated with that of their host and passed down to progeny bacteria. Periodically, either spontaneously or in response to environmental stimuli, these integrated phages are "induced." Under these conditions, phage proteins are synthesized and the phages enter a lytic life cycle. Temperate phages are poor candidates for therapeutics because they do not immediately lyse their hosts; furthermore, when they are induced, they may take bits and pieces of the host DNA with them and pass them on to other bacteria that they infect. These genes can include genes encoding antibiotic resistance or enhance bacterial pathogenesis. Thus, it is generally accepted that temperate phages should not be administered with therapeutic intent. Recent advances in molecular biology have made it possible to genetically engineer phages to eliminate their lysogenic properties or to produce them synthetically with specifically designed host ranges and lytic capabilities.

Phage Selection and Production

Each lytic phage has a relatively narrow range (termed *host range*) of bacteria that are susceptible to attack; indeed, the host range of most phages is usually restricted to a single species. The host range of a given phage is determined by the presence or absence of receptors on the bacterial surface and on whether a given bacterium encodes enzymes (endonucleases) that can degrade the phage DNA after internalization. Some bacteria (e.g., *S. aureus*) are susceptible to populations of phages that can lyse a relatively large proportion of bacteria in the species. In these cases, a library of four to six phages might be able to lyse up to 85% of the bacteria that might be encountered clinically. Phages that attack other species (e.g., *Acinetobacter baumannii*) have a very narrow host range, and such a library would need to include 200 to 300 phages.

When a patient is deemed a candidate for phage therapy, it is first necessary to identify and to characterize phages to which the patient's pathogen is susceptible (Philipson et al., 2018). At present, this determination must be made empirically by pairing a patient's specific bacterium with a library of phages that have previously been identified as being active against bacteria of the same species and identifying those that specifically lyse the patient's isolate. If a laboratory is in possession of a collection of previously characterized phages with activity against the species of bacterium with which the patient is infected, such a screening process can be conducted in 24 to 48 h. Ideally, these phages have already been shown to have an exclusively lytic lifestyle and to be free of genetic material that encodes bacterial resistance or factors that may enhance bacterial pathogenesis. If active phages are not identified in prescreened phage libraries, environmental searches can be undertaken (typically involving sewage, soil, or other sources in which the target bacterium exists in nature). Such a process may take weeks and is further complicated by the need to fully characterize any phages that emerge from the environmental search. When phages (at least one, and preferably more) are identified that have the needed bactericidal activity, they must be propagated in feeder bacteria to produce a sufficient number for a therapeutic course. Once produced, the phages must be purified to remove endotoxin and other impurities that may cause toxicity and then suspended in a buffer in which they are stable.

Pharmacology

Phages may be administered by intravenous, oral, topical, or inhaled routes. Historically, most phage therapy was administered by topical or oral routes because the technology required to fully separate highly purified phage populations from the remnants of the bacteria in which they were propagated had not been developed. With the development of efficient techniques (e.g., CsCl gradient centrifugation, organic extraction, affinity column separation), highly purified populations of phages can be obtained and safely administered parenterally. When phages have been properly prepared and sufficiently depleted of endotoxin and other impurities, clinically apparent toxicity is extremely rare.

Optimal frequency and dosing of phages is an area that remains under development. When administered intravenously, phages are cleared from the circulation by the reticuloendothelial system within 30 to 90 min. Unlike antibiotics, however, once phages arrive at the site of infection, phages can replicate in their bacterial hosts, depending on the multiplicity of infection and the size and contiguity of the bacterial population. Thus, rather than focusing on routes of clearance and systemic half-life, pharmacologic considerations center on the persistence and activity of these self-replicating antibiotics at the site of infection. From the practical perspective, well-purified phages should be administered at doses of 10^9 to 10^{10} plaque-forming units (PFU) per dose every several hours. Most published parenteral regimens have included one or more phages that are administered at approximately 10^9 PFU per dose every 8 to 12 h. Less has been done to delineate proper dosing of topical or orally administered phage preparations. Because of the uncertain stability and distribution associated with topical administration and the added complexity of predictable absorption from the GI tract, parenteral administration is preferred for most patients with significant systemic infections. Since phages (like antibiotics) select for resistant bacterial populations, it is generally preferable to administer them as "cocktails" consisting of several phages with activity against the pathogen at hand and that do not have overlapping resistance pathways. Principles regarding optimal valency are under development but will likely be dependent on the population size and the bacterial species under treatment.

Clinical Indications

Phages are not currently FDA-approved in the U.S. and must be administered as investigational agents in clinical trials or for individual patients under investigator-initiated Investigational New Drug applications. Indications for which phages may be considered include patients with serious or life-threatening bacterial or mycobacterial infections for whom antibiotic therapy has failed or is unlikely to be successful. This may include patients with multidrug-resistant bacterial infections. In addition, because of the capacity of phages to disrupt biofilms, patients with persistent infections on implanted devices for which removal and replacement are difficult or impossible are also candidates for therapy. Investigational efforts are under way to explore the ability of phages to influence the microbiome and/or to prophylactically reduce populations of specific bacteria.

Future Prospects

The full range of clinical applications for phage therapeutics is an area under active investigation. The delineation of their role in clinical medicine will require rigorous clinical and translational research that is designed on the principles under which traditional antibiotics are evaluated. Although the field is over a century old, the modern era of phage therapy began less than decade ago; a full understanding of their role in clinical medicine, as stand-alone agents or in combination with traditional antibiotics, is still in its infancy.

Drug Facts for Your Personal Formulary: *Aminoglycosides*

Drug	Therapeutic Uses	Clinical Pharmacology and Tips
Aminoglycosides—Inhibitors of Bacterial Protein Synthesis **General: Bactericidal, no GI absorption (<1%), oral administration used only for bowel decontamination or intestinal parasites, poor CSF penetration, renal elimination, nephrotoxicity, ototoxicity (cochlear and vestibular), neuromuscular blockade**		
Gentamicin (IV)	• UTI • Peritonitis • Endocarditis in combination with a cell wall–active agent • Plague • Tularemia	• Good activity vs. Enterobacterales, *Pseudomonas* • Some activity vs. *Neisseria*, *Haemophilus, Moraxella* • Synergistic activity when combined with a cell wall agent against many organisms • Vestibular > cochlear toxicity • Toxicity primarily renal and reversible
Tobramycin (IV, inhalation)	• UTI • Lung infections, including cystic fibrosis exacerbations • Nosocomial sepsis of unknown origin	• Similar to gentamicin, with better activity against *Pseudomonas aeruginosa* • Cochlear ≈ vestibular toxicity
Amikacin (IV)	• UTI • Lung infections, including cystic fibrosis exacerbations • Nosocomial sepsis of unknown origin • Mycobacterial infections	• Similar to tobramycin, with activity against some gram-negative bacilli resistant to other aminoglycosides • Activity against a variety of mycobacteria • Cochlear > vestibular toxicity
Plazomicin (IV)	• UTI	• Similar to amikacin, with activity against some gram-negative bacilli resistant to other aminoglycosides

Drug Facts for Your Personal Formulary: *Aminoglycosides* (*continued*)

Drug	Therapeutic Uses	Clinical Pharmacology and Tips
Aminoglycosides—Inhibitors of Bacterial Protein Synthesis **General: Bactericidal, no GI absorption (<1%), oral administration used only for bowel decontamination or intestinal parasites, poor CSF penetration, renal elimination, nephrotoxicity, ototoxicity (cochlear and vestibular), neuromuscular blockade (cont.)**		
Streptomycin (IV)	• Endocarditis in combination with a cell wall–active agent • Tuberculosis • Plague • Tularemia	• Similar to gentamicin, with activity against some gentamicin-resistant enterococci • Activity against *Mycobacterium tuberculosis* • Vestibular > cochlear toxicity • Vestibular toxicity is irreversible
Neomycin (PO, topical; urological irrigation)	• Minor skin infections • Bowel preparation prior to intra-abdominal surgery • Bladder irrigation	• Similar activity to gentamicin but only used topically, not systemically • Can cause skin rash
Paromomycin (PO, IM, topical)	• *Cryptosporidium* infection • Intestinal amebiasis • Leishmaniasis	• Diarrhea, nausea, vomiting • IM use for visceral leishmaniasis • Topical use for cutaneous leishmaniasis
Polymyxins—Bactericidal Cell Membrane–Disrupting Agents **General: Substantial nephrotoxicity and neurotoxicity**		
Colistin (polymyxin E) (IV, inhaled)	• Serious infections due to multidrug-resistant gram-negative organisms • Prevention of cystic fibrosis exacerbations (inhaled)	• Good activity vs. *Acinetobacter*, *E. coli*, *Klebsiella*, *Pseudomonas*, including multidrug-resistant strains • Prodrug; complex pharmacokinetics with renal and nonrenal elimination
Polymyxin B (IV, topical)	• Serious infections due to multidrug-resistant gram-negative organisms • Topical treatment/prevention of skin and soft-tissue infections	• Similar activity and toxicity as colistin • Nonrenally eliminated; does not achieve high urinary levels
Urinary Agents: Diverse mechanisms, effective concentrations reached only in urine with oral formulations		
Nitrofurantoin (PO)	• Cystitis treatment • Cystitis prophylaxis	• DNA damage through reactive intermediates • Excellent activity vs. *E. coli, Enterococcus* • Some activity vs. *Klebsiella, Enterobacter* • Rapid absorption and elimination • Colors urine brown • Acute pneumonitis and chronic interstitial pulmonary fibrosis
Fosfomycin (PO)	• Cystitis treatment	• Inhibits early cell wall synthesis • Excellent activity vs. *E. coli, Proteus, Enterococcus* • Some activity vs. *Klebsiella, Enterobacter* • Single-dose treatment of acute uncomplicated cystitis
Methenamine (PO)	• Chronic suppression of cystitis	• Forms formaldehyde in urine • Requires acidic urine for activity • Excellent activity against most uropathogens except for *Proteus* and *Enterobacter* • GI distress at high doses

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Chapter 60

Protein Synthesis Inhibitors

Conan MacDougall

TETRACYCLINES AND DERIVATIVES
- Mechanism of Action
- Antimicrobial Activity
- Resistance to Tetracyclines and Derivatives
- ADME
- Therapeutic Uses
- Adverse Effects

MACROLIDES AND KETOLIDES
- Mechanism of Action
- Antimicrobial Activity
- Resistance to Macrolides and Ketolides
- ADME
- Therapeutic Uses
- Adverse Effects

LINCOSAMIDES
- Mechanism of Action
- Antimicrobial Activity
- Resistance to Lincosamides
- ADME
- Therapeutic Uses
- Adverse Effects

OXAZOLIDINONES
- Mechanism of Action
- Antimicrobial Activity
- Resistance to Oxazolidinones
- ADME
- Therapeutic Uses
- Adverse Effects

PLEUROMUTILINS
- Mechanism of Action
- Antimicrobial Activity
- Resistance to Pleuromutilins
- ADME
- Therapeutic Uses
- Adverse Effects

STREPTOGRAMINS
- Mechanism of Action
- Antimicrobial Activity
- Resistance to Streptogramins
- ADME
- Therapeutic Uses
- Adverse Effects

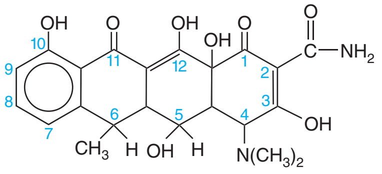
PHENICOLS
- Mechanism of Action
- Antimicrobial Activity
- Resistance to Chloramphenicol
- ADME
- Therapeutic Uses
- Adverse Effects

MUPIROCIN
- Mechanism of Action, Antimicrobial Activity, and Resistance
- Therapeutic Uses
- Adverse Effects

The agents discussed in this chapter are *bacteriostatic protein synthesis inhibitors that target the ribosome,* including tetracyclines and their modern derivatives; macrolides; lincosamides; streptogramins (*quinupristin/dalfopristin*); oxazolidinones; and pleuromutilins.

Tetracyclines and Derivatives

The tetracyclines are a series of derivatives of a basic four-ring structure (shown for *doxycycline*). *Demeclocycline, tetracycline, minocycline*, and *doxycycline* are available in the U.S. for systemic use. In the last two decades, tetracycline derivatives engineered to overcome resistance mechanisms have been introduced. Glycylcyclines (*tigecycline*), fluorocyclines (*eravacycline*), and aminomethylcyclines (*omadacycline*) are tetracycline congeners with substituents that confer broad-spectrum activity including against tetracycline-resistant bacteria.



DOXYCYCLINE

Mechanism of Action

Tetracyclines and their derivatives inhibit bacterial protein synthesis by binding to the 30S bacterial ribosome and preventing access of aminoacyl tRNA to the acceptor (A) site on the mRNA-ribosome complex (Figure 60–1). These drugs enter gram-negative bacteria by passive diffusion through channels formed by porins in the outer cell membrane and by active transport that pumps tetracyclines across the cytoplasmic membrane.

Antimicrobial Activity

Tetracyclines are typically bacteriostatic antibiotics with a spectrum of activity that encompasses a wide array of bacteria. Tetracyclines are intrinsically more active against gram-positive than gram-negative microorganisms, largely due to the ability of gram-negatives to efflux tetracyclines. Recent data from the U.S. on the activity of tetracyclines and other agents are displayed in Table 60–1. Activity against *Streptococcus pyogenes* and *penicillin*-susceptible *Streptococcus pneumoniae* is good, but resistance is common among group B streptococci and *penicillin*-resistant *S. pneumoniae*. Good activity is maintained against both *methicillin*-sensitive *Staphylococcus aureus* (MSSA) and *methicillin*-resistant *Staphylococcus aureus* (MRSA). Activity against enterococci improves with later-generation agents; *tigecycline* and

Abbreviations

CYP: cytochrome P450
HIV: human immunodeficiency virus
MAI: *Mycobacterium avium-intracellulare*
MRSA: methicillin-resistant *Staphylococcus aureus*
MSSA: methicillin-sensitive *Staphylococcus aureus*

eravacycline have good activity against multidrug-resistant enterococcal isolates. *Bacillus anthracis* is typically susceptible.

Activity of tetracyclines against *Haemophilus influenzae* has been largely retained since their introduction, but many Enterobacterales have acquired resistance to earlier-generation agents. Among almost 2000 isolates of *Escherichia coli*, only 59% were susceptible to *tetracycline*, with 84% susceptible to *minocycline* and 99% susceptible to *tigecycline* and *eravacycline* (Morrissey et al., 2020). Activity against *Klebsiella* spp. is typically more favorable, although even advanced-generation agents have poor activity versus *Proteus* spp. Among nonfermenting gram-negatives, *Pseudomonas aeruginosa* is intrinsically resistant, but *Burkholderia* spp., *Acinetobacter* spp., and *Stenotrophomonas maltophila* are often susceptible, with *minocycline*, *tigecycline*, and *eravacycline* having greater activity against these pathogens. Recent tetracycline derivatives are also typically more active against anaerobic gram-negatives such as *Bacteroides fragilis*. Most strains of *Brucella* also are susceptible to tetracyclines. Tetracyclines remain useful for infections caused by *Haemophilus ducreyi* (chancroid), *Vibrio cholerae*, and *Vibrio vulnificus* and inhibit the growth of *Campylobacter jejuni*, *Helicobacter pylori*, *Yersinia pestis*, *Yersinia enterocolitica*, *Francisella tularensis*, and *Pasteurella multocida*. Tetracyclines are alternative agents for treatment of actinomycosis.

Tetracyclines are effective against many microorganisms that are intrinsically resistant to cell-wall-active antimicrobial agents, such as *Rickettsia*, *Coxiella burnetii*, *Mycoplasma pneumoniae*, *Chlamydia* spp., *Legionella* spp., *Ureaplasma*, some atypical mycobacteria, and *Plasmodium* spp. The tetracyclines are active against many spirochetes, including *Borrelia recurrentis*, *Borrelia burgdorferi* (Lyme disease), *Treponema pallidum* (syphilis), and *Treponema pertenue.*

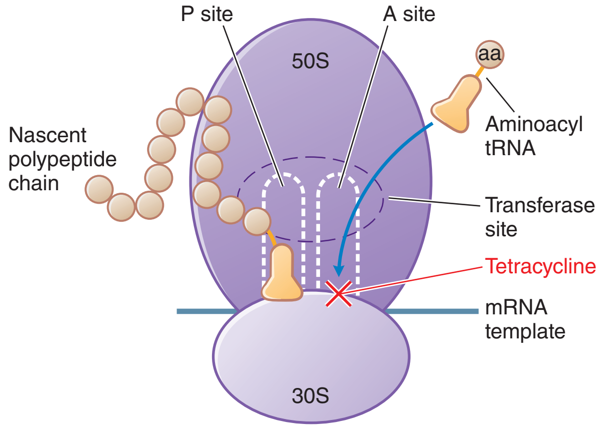


Figure 60–1 *Inhibition of bacterial protein synthesis by tetracyclines.* mRNA attaches to the 30S subunit of bacterial ribosomal RNA. The P (peptidyl) site of the 50S ribosomal RNA subunit contains the nascent polypeptide chain; normally, the aminoacyl tRNA charged with the next amino acid (aa) to be added moves into the A (acceptor) site, with complementary base pairing between the anticodon sequence of tRNA and the codon sequence of mRNA. Tetracyclines bind to the 30S subunit, block tRNA binding to the A site, and thereby inhibit protein synthesis.

Resistance to Tetracyclines and Derivatives

Resistance is frequently plasmid-mediated and often inducible. The three primary resistance mechanisms are as follows:

- Decreased accumulation of tetracyclines as a result of either decreased antibiotic influx or expression of an energy-dependent efflux pathway
- Production of a ribosomal protection protein that displaces tetracyclines from their targets
- Enzymatic inactivation of tetracyclines

Cross-resistance, or lack thereof, among tetracyclines depends on which mechanism is operative (Grossman, 2016). Tetracycline resistance due to a ribosomal protection mechanism (e.g., *tetM*) produces cross-resistance to *doxycycline* and *minocycline*. *Minocycline* and, to a

TABLE 60–1 ■ ACTIVITY OF SELECTED ANTIMICROBIALS AGAINST KEY GRAM-POSITIVE PATHOGENS

	PERCENTAGE OF ISOLATES SUSCEPTIBLE TO SELECTED AGENTS AND TYPICAL MINIMAL INHIBITORY CONCENTRATIONS OF AMINOGLYCOSIDES THAT WILL INHIBIT 90% OF CLINICAL ISOLATES (MIC_{90}, μg/mL)						
	Streptococcus pyogenes	***Streptococcus pneumoniae***		***Staphylococcus aureus***		***Enterococcus faecalis***	***Enterococcus faecium***
		PCN-S	**PCN-R**	**MSSA**	**MRSA**		
Tetracycline	89.7% (4)	94.6% (≤2)	36.7% (>8)	95.7% (≤2)	93.4% (≤2)	24.6% (>8)	58.7% (>8)
Tigecycline	100% (≤0.03)	NR (≤0.03)	NR (≤0.03)	100% (0.25)	99.9% (0.25)	99.9% (0.25)	NR (0.12)
Eravacycline	98.0% (0.06)	NR (0.015)		88.3% (0.12)	80.8% (0.12)	94.5% (0.06)	95.0% (0.06)
Omadacycline	98.4% (0.12)	99.9% (0.06)	100% (0.12)	99.9% (0.25)	96.1% (0.25)	97.2% (0.25)	96.8% (0.12)
Erythromycin	89.7% (1)	87.3% (>2)	17.2% (>2)	70.8%(>2)	6.1% (>2)	9.1% (>2)	3.0% (>2)
Clindamycin	97.7% (≤0.25)	97.1% (≤0.25)	44.4% (>2)	94.6% (≤0.25)	57.9% (>2)	NA	NA
Linezolid	100% (1)	100% (1)	100% (1)	99.9% (2)	99.9% (2)	99.9% (2)	98.0% (2)
Quinupristin/ dalfopristin	100% (≤0.12)	99% (0.5)	100% (0.5)	100% (0.25)	100% (0.5)	3.9% (8)	92.6% (2)

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; NA, not applicable; NR, not reported; PCN-R, penicillin-resistant; PCN-S, penicillin-susceptible.

Entries are percentage of isolates inhibited at established or proposed susceptibility breakpoints. In parentheses are drug concentrations, in μg/mL, required to inhibit growth of 90% of isolates of that organism.

Sources: Data from: Gales AC, et al. *Diagn Microbiol Infect Dis*, **2008**, *60*:421–427; Critchley IA, et al. *Antimicrob Agents Chemother*, **2003**, *47*:1689–1693; Mendes RE, et al. *Antimicrob Agents Chemother,* **2015**, *59*:702–706; Jones RN, et al. *Diagn Microbiol Infect Dis,* **2013**, *75*:304–307; Mendes RE, et al. *Clin Infect Dis*, **2012**, *54*(S3):S203–S213; Morrissey I, et al. *Antimicrob Agents Chemother*, **2020**, *64*:e01715–01719; Pfaller MA, et al. *Antimicrob Agents Chemother*, **2018**, *62*:e02327; Sader HS, et al. *Antimicrob Agents Chemother*, **2012**, *56*:1619–1623.

lesser extent, *doxycycline* are more resistant to efflux and typically display lower minimum inhibitory concentrations against organisms expressing efflux pumps, although cross-resistance may still exist. Structural modifications to the glycylcyclines, fluorocyclines, and aminomethylcyclines reduce affinity for most efflux pumps, restoring activity against many organisms displaying tetracycline resistance due to this mechanism. Binding to ribosomes is also enhanced, improving activity against organisms that harbor ribosomal protection proteins that confer resistance to other tetracyclines. Less common emergent resistance mechanisms include enzymatic modification of the tetracyclines through "destructases" or mutation at the ribosomal target site.

ADME

Oral absorption of tetracyclines varies by the individual agent, ranging from approximately 33% for *omadacycline* to around 90% for *doxycycline* and *minocycline*. *Tigecycline* and *eravacycline* are available only for parenteral administration. Concurrent ingestion of divalent and trivalent cations (e.g., Ca^{2+}, Mg^{2+}, Al^{3+}, $Fe^{2+/3+}$, and Zn^{2+}) impairs absorption. Thus, dairy products, antacids, aluminum hydroxide gels; calcium, magnesium, and iron or zinc salts; bismuth subsalicylate; and dietary iron and zinc supplements can interfere with absorption of tetracyclines. After a single oral dose, the peak plasma concentration is attained in 2 to 4 h. These drugs have half-lives in the range of 6 to 12 h and frequently are administered two to four times daily. Food, including dairy products, does not interfere with absorption of *doxycycline* and *minocycline* but significantly impairs the absorption of *omadacycline*, which should be administered at least 4 h after and 2 h before a meal.

Tetracyclines distribute widely throughout the body, including urine and prostate. They accumulate in reticuloendothelial cells of the liver, spleen, and bone marrow and in bone, dentine, and enamel of unerupted teeth. *Tigecycline* and *eravacycline* distribute rapidly and extensively into tissues, resulting in low serum levels that may be inadequate to treat bacteremias with an endovascular source. Inflammation of the meninges is not required for the passage of tetracyclines into the CSF. Tetracyclines cross the placenta and enter the fetal circulation and amniotic fluid. Relatively high concentrations are found in breast milk.

Tetracycline and *demeclocycline* are eliminated primarily by the kidney, although they also are concentrated in the liver, excreted in bile, and partially reabsorbed via enterohepatic recirculation. *Doxycycline* and *omadacycline* are largely excreted unchanged in both the bile and urine, *tigecycline* and *eravacycline* are mostly excreted unchanged along with a small amount of metabolites, and *minocycline* is extensively metabolized by the liver before excretion. Hence, no dose adjustment is needed in patients with renal dysfunction. Specific dosage adjustment recommendations in hepatic disease are available for *tigecycline* and *eravacycline*.

Therapeutic Uses

The early-generation tetracyclines remain useful as first-line therapy for infections caused by rickettsiae, mycoplasmas, and chlamydiae. *Doxycycline*, the most frequently used tetracycline, is a drug of choice for many sexually transmitted diseases, rickettsial infections, plague, brucellosis, tularemia, and spirochetal infections and is also used for treatment of respiratory tract infections and for skin and soft-tissue infections caused by MRSA. *Minocycline* has utility for *doxycycline*-resistant strains of MRSA, *Acinetobacter* spp., and some nontuberculous mycobacteria. The glycylcyclines, fluorocyclines, and aminomethylcyclines have restored much of the antibacterial activity lost to the tetracyclines due to resistance and offer *in vitro* activity against a number of gram-positive and gram-negative organisms (De Rosa et al., 2015). However, some clinical data for these agents warrants caution. A pooled analysis of *tigecycline* clinical trials found a small but statistically significant increased risk of death with *tigecycline* versus comparators (Food and Drug Administration, 2016). While the activity of *eravacycline* and *omadacycline* is promising against multidrug-resistant gram-negatives commonly seen in urinary tract infections, both agents did not meet criteria for noninferiority in randomized trials for treatment of complicated urinary tract infections, possibly due to lack of accumulation in the urine.

The oral dose of *tetracycline* ranges from 1 to 2 g/day in adults. The typical oral or intravenous dose of *doxycycline* for adults is 100 mg every 12 h; for children more than 8 years of age, the dose is 4.4 mg/kg per day in two divided doses the first day, then 2.2 mg/kg given once or twice daily. The typical dose of *minocycline* for adults is 200 mg orally or intravenously initially, followed by 100 mg every 12 h; for children, it is 4 mg/kg initially followed by 2 mg/kg every 12 h. *Tigecycline* is administered intravenously to adults as a 100-mg loading dose, followed by 50 mg every 12 h. For patients with significant hepatic impairment, the loading dose should be followed by a reduced maintenance dose of 25 mg every 12 h. *Eravacycline*'s dose is 1 mg/kg intravenously every 12 h; in patients with severe hepatic impairment, the frequency is reduced to every 24 h. *Omadacycline* is administered with a loading dose (200 mg for 1 day intravenously or 450 mg for 2 days orally) followed by a maintenance dose of 100 mg intravenously or 300 mg orally. Dosages for *tigecycline*, *eravacycline*, or *omadacycline* in pediatrics have not been established.

Respiratory Tract Infections

Tetracyclines have good activity against *S. pneumoniae*, *H. influenzae,* and *Moraxella catarrhalis* and excellent activity against atypical pathogens such as *Mycoplasma* and *Chlamydophila pneumoniae*. *Doxycycline* is recommended as a single agent for treatment of uncomplicated community-acquired pneumonia in the outpatient setting or as an adjunctive agent with β-lactams for patients with complicated community -acquired pneumonia. *Doxycycline* is also used for treatment of acute exacerbations of chronic bronchitis and for sinusitis. *Tigecycline* and *omadacycline* are approved for the treatment of adults with community -acquired bacterial pneumonia.

Skin and Soft-Tissue Infections

Doxycycline and *minocycline* have good activity against staphylococci and may be useful in treatment of cutaneous infections especially if MRSA is suspected. *Tigecycline* and *omadacycline* are approved for the treatment of complicated skin and soft-tissue infections. Low doses of *tetracycline* have been used to treat acne (25 mg orally twice a day).

Intra-abdominal Infections

Resistance among Enterobacterales and gram-negative anaerobes limits the utility of older tetracyclines for intra-abdominal infections. However, *tigecycline* and *eravacycline* possess excellent activity against these pathogens as well as *Enterococcus* and have FDA indications for treatment of complicated intra-abdominal infections.

Sexually Transmitted Diseases

Doxycycline no longer is recommended for gonococcal infections because of the spread of resistance (Centers for Disease Control and Prevention, 2021). A 7-day treatment course of *doxycycline* is more effective than single-dose *azithromycin* in the treatment of uncomplicated genital infections due to *Chlamydia trachomatis. C. trachomatis* is often a coexistent pathogen in acute pelvic inflammatory disease, and *doxycycline* is a component of combination therapy regimens for this condition. Acute epididymitis is caused by infection with *C. trachomatis* or *Neisseria gonorrhoeae* in men less than 35 years of age. Effective regimens include a single injection of *ceftriaxone* (250 mg) plus *doxycycline* for 10 days. Sexual partners also should be treated. *Doxycycline* for 21 days is first-line therapy for treatment of lymphogranuloma venereum. Nonpregnant penicillin-allergic patients who have primary, secondary, or latent syphilis can be treated with a tetracycline regimen, such as *doxycycline* for 2 weeks. Tetracyclines should not be used for treatment of neurosyphilis.

Zoonoses

Tetracyclines can be lifesaving in rickettsial infections, including Rocky Mountain spotted fever, recrudescent epidemic typhus (Brill disease), murine typhus, scrub typhus, rickettsialpox, and Q fever. Tetracyclines are also effective in treatment of ehrlichiosis and anaplasmosis. *Doxycycline* is the drug of choice for treatment of Rocky Mountain spotted fever, ehrlichiosis, and anaplasmosis in adults and in children, including those less than 9 years of age, in whom the risk of staining of permanent teeth is outweighed by the seriousness of this potentially fatal infection (Woods, 2013). *Doxycycline* is a first-line treatment for adults with infections due to *Borrelia* spp., including *B. burgdorferi* (Lyme disease). Tetracyclines in combination with *rifampin* or *streptomycin* are effective for acute and chronic infections caused by *Brucella melitensis*, *Brucella suis*, and *Brucella abortus.* Although *streptomycin* is preferable, tetracyclines also are effective in tularemia. *Doxycycline* is indicated for prevention or treatment of anthrax. It should be used in combination with another agent when treating inhalational or GI infection. The recommended duration of therapy is 60 days for bioterrorism exposures.

Other Infections

Actinomycosis, although most responsive to *penicillin G*, may also be successfully treated with a tetracycline. *Minocycline* is an alternative for the treatment of nocardiosis, but a sulfonamide should be used concurrently. Tetracyclines are useful in the acute treatment and for prophylaxis of leptospirosis (*Leptospira* spp.). The tetracyclines have been used to treat susceptible atypical mycobacterial pathogens, including *Mycobacterium marinum.* Tetracyclines in combination with *bismuth*, *metronidazole*, and a proton pump inhibitor are a first-line therapy for *H. pylori* infections (Chey et al., 2017; see also Table 53–4).

Adverse Effects

Gastrointestinal

All tetracyclines can produce GI irritation, most commonly after oral administration. Epigastric burning and distress, abdominal discomfort, nausea, vomiting, and diarrhea may occur. Tolerability can be improved by administering these drugs with food, but tetracyclines should not be taken with dairy products or antacids. *Omadacycline* specifically requires administration on an empty stomach, at least 4 h after and 2 h before food consumption. Tetracyclines have been associated with esophagitis and esophageal ulcers; patients should take oral formulations with a full glass of water while standing. *Tigecycline* administered intravenously has also been associated with nausea and vomiting that can be treatment-limiting.

Photosensitivity

Tetracyclines and derivatives may produce photosensitivity reactions in treated individuals exposed to sunlight. For ambulatory patients, reduction in sun exposure and use of sunscreen during therapy are recommended. Onycholysis and pigmentation of the nails may develop with or without accompanying photosensitivity (Vassileva et al., 1998).

Hepatic Toxicity

Hepatic toxicity has developed in patients with renal failure receiving 2 g or more of *tetracycline* per day parenterally, but this effect also may occur when large quantities are administered orally. Pregnant women are particularly susceptible.

Renal Toxicity

Tetracyclines may aggravate azotemia in patients with renal disease because of the catabolic effects of the drugs. *Doxycycline*, *minocycline*, and *tigecycline* have fewer renal side effects than other tetracyclines. Nephrogenic diabetes insipidus has been observed in some patients receiving *demeclocycline*, and this phenomenon has been exploited for the treatment of the syndrome of inappropriate secretion of antidiuretic hormone (see Chapter 29). Fanconi syndrome (characterized by nausea, vomiting, polyuria, polydipsia, proteinuria, acidosis, glycosuria, and aminoaciduria) has been observed in patients ingesting outdated *tetracycline*, presumably due to toxic effects of one or more breakdown products on the proximal renal tubules.

Effects on Bone

Children treated with tetracyclines may develop permanent brown discoloration of the teeth. The duration of therapy appears to be less important than the total quantity of antibiotic administered. The risk is highest when a tetracycline is given to infants before the first dentition but may develop if the drug is given between the ages of 2 months and 5 years when these teeth are being calcified. Treatment of pregnant patients with tetracyclines may produce discoloration of the teeth in their children. Concern also exists over the potential for depressed bone growth due to deposition of tetracyclines in the skeleton during gestation and throughout childhood. Thus, tetracyclines are typically avoided in pregnant women and in children less than 8 years old unless there is a compelling indication (e.g., Rocky Mountain spotted fever).

Other Toxic and Irritative Effects

Thrombophlebitis may follow intravenous administration. This irritative effect of tetracyclines has been used therapeutically in patients with malignant pleural effusions. Long-term tetracycline therapy may produce leukocytosis, atypical lymphocytes, toxic granulation of granulocytes, and thrombocytopenic purpura. Tetracyclines may cause increased intracranial pressure (pseudotumor cerebri) in young infants, even when given in the usual therapeutic doses. Patients receiving *minocycline* may experience vestibular toxicity, manifested by dizziness, ataxia, nausea, and vomiting. The symptoms occur soon after the initial dose and generally disappear within 24 to 48 h after drug cessation. Skin reactions rarely may follow the use of any of the tetracyclines. Among the more severe allergic responses are angioedema and anaphylaxis; anaphylactoid reactions can occur even after oral use.

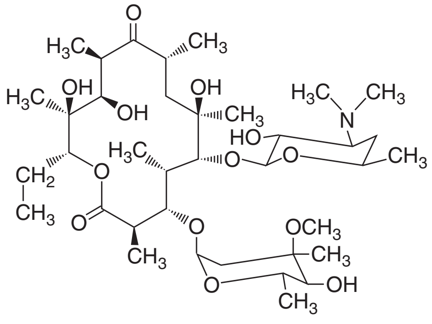
Drug Interactions

As mentioned, oral coadministration of tetracyclines and divalent and trivalent cations can lead to chelation of the tetracycline, with resultant poor absorption. There is some evidence for drug interactions between *doxycycline* and hepatic enzyme-inducing agents such as *phenytoin* and *rifampin*, but not for *minocycline* or *tigecycline*. *Eravacycline* is a substrate of CYP3A4 and its concentrations are reduced by inducers (e.g., *rifampin*) and increased by inhibitors (e.g., *itraconazole*) of CYP3A4.

Macrolides and Ketolides

Macrolide antibiotics are widely used agents for treatment of respiratory tract infections caused by the common pathogens of community-acquired pneumonia. Four macrolides are available for clinical use in the United States: *erythromycin*, *clarithromycin*, *azithromycin*, and *fidaxomicin*. *Erythromycin* is the original agent in the class, discovered in 1952 by McGuire and coworkers in the metabolic products of a strain of *Streptomyces erythraeus*. *Azithromycin* and *clarithromycin* are semisynthetic derivatives of *erythromycin* that have largely replaced it in clinical use. *Fidaxomicin* is a non–systemically absorbed macrolide used only for the treatment of *Clostridium difficile* colitis. Ketolides (*telithromycin, cethromycin, solithromycin*) are semisynthetic derivatives of *erythromycin* with activity against some macrolide-resistant strains.

Macrolide antibiotics contain a multimembered lactone ring (14-membered rings for *erythromycin* and *clarithromycin* and a 15-membered ring for *azithromycin*) to which are attached one or more deoxy sugars. *Clarithromycin* differs from *erythromycin* only by methylation of the hydroxyl group at the 6 position, and *azithromycin* differs by the addition of a methyl-substituted nitrogen atom into the lactone ring. These structural modifications improve acid stability and tissue penetration and broaden the spectrum of activity.



ERYTHROMYCIN

Ketolides are structurally similar multimembered ring systems but with different substituents; a 3-keto group replaces the α-L-cladinose of the 14-member macrolide ring, and there is a substituted carbamate at C11–C12. These modifications render ketolides less susceptible to methylase-mediated (*erm*) and efflux-mediated (*mef* or *msr*) mechanisms of resistance. Ketolides therefore are active against many macrolide-resistant gram-positive strains; however, concerns about the safety of *telithromycin* limited its use, and the manufacturer ceased marketing it in the U.S. (Brinker et al., 2009). The development of *cethromycin* and *solithromycin* has been stopped or stalled after unfavorable FDA reviews; whether their development will proceed is unclear.

Mechanism of Action

Macrolide and ketolide antibiotics are bacteriostatic agents that inhibit protein synthesis by binding reversibly to 50S ribosomal subunits of sensitive microorganisms (Figure 60–2). *Erythromycin* does not inhibit peptide bond formation per se but rather inhibits the translocation step wherein a newly synthesized peptidyl tRNA molecule moves from the acceptor site on the ribosome to the peptidyl donor site. Gram-positive bacteria accumulate about 100 times more *erythromycin* than do gram-negative bacteria.

Antimicrobial Activity

Erythromycin usually is bacteriostatic but may be bactericidal in high concentrations against susceptible organisms. *Erythromycin* has reasonably good activity against streptococci (see Table 60–1), but macrolide resistance among *S. pneumoniae* often coexists with *penicillin* resistance. Staphylococci are not reliably sensitive to *erythromycin*, and macrolide-resistant strains of *S. aureus* are potentially cross-resistant to *clindamycin* and *streptogramin B* (*quinupristin*). Gram-positive bacilli also are frequently sensitive to *erythromycin*, including *Clostridium perfringens*, *Corynebacterium diphtheriae*, and *Listeria monocytogenes*. *Erythromycin* is inactive against most aerobic enteric gram-negative bacilli. It has modest activity *in vitro* against *H. influenzae* and *Neisseria meningitidis* and good activity against most strains of *N. gonorrhoeae*. Useful antibacterial activity also is observed against *P. multocida*, *Borrelia* spp., and *Bordetella pertussis*. Macrolides are usually active against *C. jejuni*. *Erythromycin* is active against *M. pneumoniae* and *Legionella pneumophila*. Most strains of *C. trachomatis* are inhibited by *erythromycin*.

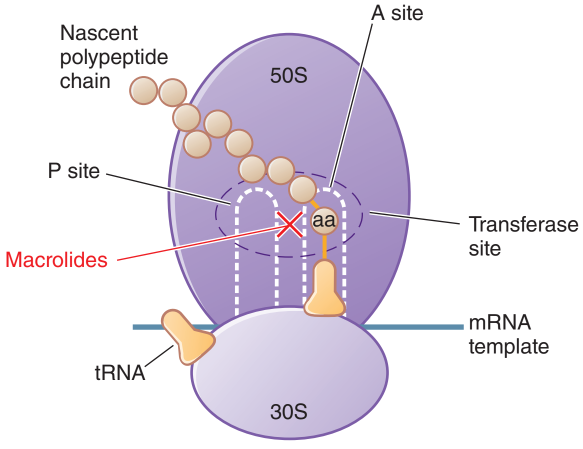


Figure 60–2 *Inhibition of bacterial protein synthesis by erythromycin, clarithromycin, and azithromycin.* Macrolide antibiotics are bacteriostatic agents that inhibit protein synthesis by binding reversibly to the 50S ribosomal subunits of sensitive organisms. *Erythromycin* appears to inhibit the translocation step such that the nascent peptide chain temporarily residing at the A site fails to move to the P, or donor, site. Alternatively, macrolides may bind and cause a conformational change that terminates protein synthesis by indirectly interfering with transpeptidation and translocation. See Figure 60–1 for additional information.

Azithromycin has similar activity as *erythromycin* against sensitive strains of streptococci and staphylococci, while *clarithromycin* is slightly more potent. *Clarithromycin* is somewhat less active than *erythromycin* against *H. influenzae*, whereas *azithromycin* is the most active macrolide against this organism. *Clarithromycin* and *azithromycin* have good activity against *M. catarrhalis*, *Chlamydia* spp., *L. pneumophila*, *B. burgdorferi*, *M. pneumoniae*, and *H. pylori*. *Azithromycin* and *clarithromycin* have enhanced activity against some nontuberculous mycobacteria, as well as against some protozoa (e.g., *Toxoplasma gondii*, *Cryptosporidium*, and *Plasmodium* spp.). *Clarithromycin* has good activity against *Mycobacterium leprae*. The spectrum of activity of the ketolides is similar to those of *clarithromycin* and *azithromycin*, but their capacity to withstand many macrolide resistance mechanisms increases their *in vitro* activity against macrolide-resistant *S. pneumoniae* and *S. aureus*.

Fidaxomicin is designed to have potent activity against *C. difficile* with minimal inhibition of other gastrointestinal flora. It does not have clinical utility for infections besides *C. difficile*.

Resistance to Macrolides and Ketolides

Resistance to macrolides usually results from one of four mechanisms (Nakajima, 1999):

- Drug efflux by an active pump mechanism
- Ribosomal protection by inducible or constitutive production of methylase enzymes, which modify the ribosomal target and decrease drug binding
- Macrolide hydrolysis by esterases produced by Enterobacterales
- Chromosomal mutations that alter a 50S ribosomal protein (in *Bacillus subtilis, Campylobacter* spp., mycobacteria, and gram-positive cocci)

ADME

Erythromycin base is incompletely absorbed from the upper small intestine. Because it is inactivated by gastric acid, it is administered as enteric-coated tablets or as capsules containing enteric-coated pellets that dissolve in the duodenum; food may delay absorption. Esters of *erythromycin* base (e.g., stearate, estolate, and ethylsuccinate) have improved acid stability, and their absorption is less altered by food. Protein binding is about 70% to 80% for *erythromycin* base and even higher for the estolate. *Erythromycin* traverses the placenta, and drug concentrations in fetal plasma are about 5% to 20% of those in the maternal circulation. Concentrations in breast milk are 50% of those in serum. *Erythromycin* is concentrated in the liver and excreted in the bile. The serum $t_{1/2}$ of *erythromycin* is about 1.6 h. Although the $t_{1/2}$ may be prolonged in patients with anuria, dosage reduction is not routinely recommended in patients in renal failure. The drug is not removed significantly by either peritoneal dialysis or hemodialysis.

Clarithromycin is absorbed rapidly from the GI tract after oral administration, but hepatic first-pass metabolism reduces its bioavailability to 50% to 55%. Peak concentrations occur about 2 h after drug administration. *Clarithromycin* may be given with or without food, but the extended-release form should be administered with food to improve bioavailability. *Clarithromycin* and its active metabolite, 14-hydroxyclarithromycin, achieve high intracellular concentrations throughout the body, including the middle ear. *Clarithromycin* is metabolized in the liver to several metabolites; the active 14-hydroxy metabolite is the most significant. The elimination $t_{1/2}$ are 3 to 7 h for *clarithromycin* and 5 to 9 h for 14-hydroxyclarithromycin. Metabolism is saturable, resulting in nonlinear pharmacokinetics and longer half-lives with higher dosages. The amount of *clarithromycin* excreted unchanged in the urine ranges from 20% to 40%, depending on the dose administered and the formulation (tablet vs. oral suspension). An additional 10% to 15% of a dose is excreted in the urine as 14-hydroxyclarithromycin. Dose adjustment is not recommended unless the creatinine clearance is less than 30 mL/min.

Azithromycin administered orally is absorbed rapidly (although incompletely; bioavailability for the immediate-release formulation is on the order of 30%–40%) and distributes widely throughout the body, except to the brain and CSF. *Azithromycin* also can be administered intravenously, producing plasma concentrations of 3 to 4 μg/mL after a 1-h infusion of 500 mg. *Azithromycin*'s unique pharmacokinetic properties include extensive tissue distribution and high drug concentrations within cells (including phagocytes), resulting in much greater concentrations of drugs in tissue or secretions compared to simultaneous serum concentrations. *Azithromycin* undergoes some hepatic metabolism to inactive metabolites, but biliary excretion is the major route of elimination. Only 12% of drug is excreted unchanged in the urine. The elimination $t_{1/2}$, 40 to 68 h, is prolonged because of extensive tissue sequestration and binding.

Fidaxomicin achieves high levels in the gut lumen and stool but is minimally absorbed, with peak plasma levels of *fidaxomicin* and its active metabolite in the nanogram per milliliter range; greater than 90% of the dose is recovered in stool as the parent drug or metabolite.

Therapeutic Uses

The usual oral dose of *erythromycin* (*erythromycin* base) for adults ranges from 1 to 2 g/day, in divided doses, usually given every 6 h. Food should not be taken concurrently, if possible, with *erythromycin* base or the stearate formulations, but this is not necessary with *erythromycin* estolate. The oral dose of *erythromycin* for children is 30 to 50 mg/kg per day, divided into four portions; this dose may be doubled for severe infections. Intravenous administration is generally reserved for the therapy of severe infections and is now used uncommonly; the usual dose is 0.5 to 1 g every 6 h.

Clarithromycin usually is given twice daily at a dose of 250 mg for adults with mild-to-moderate infections and 500 mg twice daily for more severe infections. The 500-mg extended-release formulation of *clarithromycin* is given as 2 tablets once daily.

Standard doses of *azithromycin* for treatment of infection are 250 to 500 mg orally or intravenously once daily. The extended-release *azithromycin* suspension should be given 1 h before or 2 h after meals when administered orally; there are no food requirements for the tablet or immediate-release suspension. Treatment or prophylaxis of nontuberculous mycobacterial infection can vary by indication and patient: 250 to 500 mg daily in combination with one or more other agents for treatment or 1200 mg once weekly for primary prevention of *Mycobacterium avium-intracellulare* (MAI).

Respiratory Tract Infections

Macrolides are suitable drugs for the treatment of a number of respiratory tract infections. *Azithromycin* and *clarithromycin* are suitable choices for treatment of community-acquired pneumonia among low-risk ambulatory patients in areas where resistance to macrolides among *S. pneumoniae* is low (e.g., <25%) (Metlay et al., 2019). For outpatient therapy of community-acquired pneumonia, pharyngitis, or sinusitis, a loading dose of 500 mg is given on the first day, and then 250 mg per day is given for days 2 through 5. In hospitalized patients, a macrolide is commonly added to an antipneumococcal β-lactam for coverage of atypical respiratory pathogens. Macrolides are also appropriate alternative agents for the treatment of acute exacerbations of chronic bronchitis, acute otitis media, acute streptococcal pharyngitis, and acute bacterial sinusitis. In children, the recommended dose of *azithromycin* oral suspension for acute otitis media and pneumonia is 10 mg/kg on the first day (maximum 500 mg) and 5 mg/kg (maximum 250 mg/day) on days 2 through 5. A single 30-mg/kg dose is approved as an alternative for otitis media. *Azithromycin* and *clarithromycin* are generally preferred to *erythromycin* due to their broader spectrum and superior tolerability.

Skin and Soft-Tissue Infections

Macrolides are alternatives for treatment of erysipelas and cellulitis among patients who have a serious allergy to *penicillin* (Stevens et al., 2014). *Erythromycin* has been an alternative agent for the treatment of relatively minor skin and soft-tissue infections caused by either *penicillin*-sensitive or *penicillin*-resistant *S. aureus*. However, many strains of *S. aureus* are resistant to macrolides.

Sexually Transmitted Infections

A single 1-g dose of *azithromycin* can be used for treatment of uncomplicated nongonococcal urethritis presumed to be caused by *C. trachomatis*, but is less effective than 7 days of doxycycline. (Centers for Disease Control and Prevention, 2021). This dose also is effective for chancroid. Chlamydial infections can be treated effectively with any of the macrolides. *Erythromycin* base is preferred for chlamydial pneumonia of infancy and ophthalmia neonatorum (50 mg/kg per day in four divided doses for 14 days). *Azithromycin*, 1 g per week for 3 weeks, may be effective for lymphogranuloma venereum.

Diphtheria

Erythromycin for 7 days is very effective for acute infections or for eradicating the diphtheria carrier state. Other macrolides are not FDA-approved for this indication. Antibiotics do not alter the course of an acute infection with diphtheria or decrease the risk of complications. Antitoxin is indicated in the treatment of acute infection.

Pertussis

Erythromycin is the drug of choice for treating persons with *B. pertussis* disease and for postexposure prophylaxis of household members and close contacts. *Clarithromycin* and *azithromycin* also are effective. If administered early in the course of whooping cough, *erythromycin* may shorten the duration of illness; it has little influence on the disease once the paroxysmal stage is reached. Nasopharyngeal cultures should be obtained from people with pertussis who do not improve with *erythromycin* therapy because resistance has been reported.

Helicobacter pylori *Infection*

Clarithromycin 500 mg, in combination with *omeprazole* 20 mg (or *lansoprazole* 30 mg), and *amoxicillin* 1 g, each administered twice daily for 10 to 14 days, are effective for treatment of peptic ulcer disease caused by *H. pylori* (see Table 53–4).

Mycobacterial Infections

Clarithromycin or *azithromycin* is recommended as first-line therapy for prophylaxis and treatment of disseminated infection caused by MAI in patients infected with human immunodeficiency virus (HIV) and for treatment of pulmonary disease in patients not infected with HIV (Masur et al., 2014). *Clarithromycin* (500 mg twice daily) plus *ethambutol* (15 mg/kg once daily) with or without *rifabutin* is an effective combination regimen. Either *clarithromycin* or *azithromycin* can be a component of combination therapy regimens for other nontuberculous mycobacterial infections such as those due to *Mycobacterium abscessus*. *Clarithromycin* also has been used with *minocycline* for the treatment of *M. leprae* in lepromatous leprosy.

Clostridium difficile Infection

Fidaxomicin administered as a dose of 200 mg orally twice daily is as effective as oral *vancomycin* for treatment of *C. difficile* colitis and is associated with a lower risk of relapse. Other macrolides are not effective in the treatment of *C. difficile* infection, and administration of these agents may predispose patients to *C. difficile* infection (although with a relatively lower risk than many other antimicrobial classes).

Adverse Effects

GI Toxicity

Oral or intravenous administration of *erythromycin* frequently is accompanied by moderate-to-severe epigastric distress. *Erythromycin* stimulates GI motility by acting on motilin receptors; indeed, *erythromycin* is used off-label as a prokinetic agent in the intensive care setting and in patients with diabetic gastroparesis. *Clarithromycin* and *azithromycin* may also cause GI distress, but typically to a lesser degree than *erythromycin*.

Cardiac Toxicity

Erythromycin, *clarithromycin*, *azithromycin*, and *telithromycin* have been reported to cause cardiac arrhythmias, including QT prolongation with ventricular tachycardia. A large cohort study found a small but statistically significant increase in the risk of sudden cardiac death with *azithromycin* compared to no antibiotic treatment or to *amoxicillin*. Risk factors for clinically significant cardiac toxicity include receipt of concomitant antiarrhythmic drugs or other agents that prolong QTc.

Hepatotoxicity

Cholestatic hepatitis is associated with long-term treatment with *erythromycin*. The illness starts after 10 to 20 days of treatment and is characterized initially by nausea, vomiting, and abdominal cramps. These symptoms are followed shortly thereafter by jaundice, which may be accompanied by fever, leukocytosis, eosinophilia, and elevated transaminases in plasma. Findings usually resolve within a few days after cessation of drug therapy. Hepatotoxicity has also been observed with *clarithromycin* and *azithromycin*, although at a lower rate than with *erythromycin* and *telithromycin*.

Other Toxic and Irritative Effects

Allergic reactions observed are fever, eosinophilia, and skin eruptions, which disappear shortly after therapy is stopped. Auditory impairment and tinnitus have been observed with macrolides, especially at higher doses.

Drug Interactions

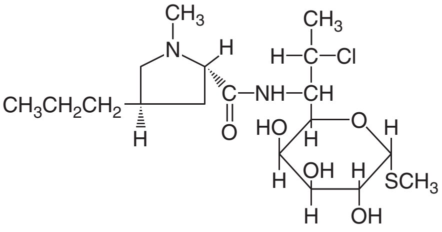
Erythromycin, *clarithromycin*, and *telithromycin* strongly inhibit CYP3A4 and cause significant drug interactions (Periti et al., 1992). *Erythromycin* and *clarithromycin* potentiate the effects of *carbamazepine*, corticosteroids, *cyclosporine*, *digoxin*, ergot alkaloids, *theophylline*, *triazolam*, *valproate*, and *warfarin*, probably by interfering with CYP-mediated metabolism of these drugs. Coadministration of *rifampin*, a potent inducer of CYP, may decrease serum concentrations of *clarithromycin* and *telithromycin*. CYP3A4 inhibitors (e.g., *itraconazole*) increase peak serum concentrations of *clarithromycin* and telithromycin. *Azithromycin* is much less likely to be involved in these drug interactions; however, caution is advised when the consequences of interaction are severe.

Lincosamides

The class originator *lincomycin* and its congener *clindamycin* are approved in the U.S. *Clindamycin* has largely replaced *lincomycin* in clinical practice and is principally used to treat gram-positive aerobic and anaerobic infections, as well as some parasitic infections.

Mechanism of Action

Clindamycin binds exclusively to the 50S subunit of bacterial ribosomes and suppresses protein synthesis. Although *clindamycin*, *erythromycin*, and *chloramphenicol* are not structurally related, they act at sites in close proximity, and binding by one of these antibiotics to the ribosome may inhibit the interaction of the others.



CLINDAMYCIN

Antimicrobial Activity

Clindamycin has good *in vitro* activity against *penicillin*-susceptible strains of pneumococci (but less against *penicillin*-resistant strains), *S. pyogenes*, and viridans streptococci (see Table 60–1). MSSAs usually are susceptible to *clindamycin*, but MRSA and coagulase-negative staphylococci are more likely to be resistant. *Clindamycin* is more active than macrolides against anaerobic bacteria, especially *B. fragilis*, but resistance to *clindamycin* in *Bacteroides* spp. increasingly is encountered. From 10% to 20% of clostridial species other than *C. perfringens* are resistant. Strains of *Actinomyces israelii* and *Nocardia asteroides* are sensitive. Essentially all aerobic gram-negative bacilli are resistant. *Clindamycin* plus *primaquine* and *clindamycin* plus *pyrimethamine* are second-line regimens for *Pneumocystis jirovecii* pneumonia and *T. gondii* encephalitis, respectively.

Resistance to Lincosamides

Macrolide resistance due to ribosomal methylation also may produce resistance to *clindamycin*. Because *clindamycin* does not induce the methylase, there is cross-resistance only if the enzyme is produced constitutively. However, selection for a subpopulation of constitutive methylase producers may occur among staphylococci and streptococci with a macrolide-inducible phenotype (Lewis and Jorgensen, 2005). *Clindamycin* is not a substrate for macrolide efflux pumps; thus, strains that are resistant to macrolides by this mechanism are susceptible to *clindamycin*.

ADME

Clindamycin is nearly completely absorbed following oral administration. Peak concentrations of 2 to 3 μg/mL are attained within 1 h after the ingestion of 150 mg. Food in the stomach does not reduce absorption significantly. The $t_{1/2}$ of the antibiotic is about 3 h. *Clindamycin palmitate*, an oral preparation for pediatric use, is an inactive prodrug that is hydrolyzed rapidly *in vivo*. The phosphate ester of *clindamycin*, which is given parenterally, also is rapidly hydrolyzed *in vivo* to the active parent compound.

Clindamycin is widely distributed in many fluids and tissues, including good concentrations in bone. CSF concentrations are limited, even when the meninges are inflamed, but concentrations sufficient to treat cerebral toxoplasmosis are achievable. The drug readily crosses the placental barrier. Ninety percent or more of *clindamycin* is bound to plasma proteins. *Clindamycin* accumulates in polymorphonuclear leukocytes and alveolar macrophages and in abscesses.

Clindamycin is inactivated by metabolism to *N*-demethylclindamycin and clindamycin sulfoxide, which are excreted in the urine and bile. Dosage adjustments may be required in patients with severe hepatic failure. Only about 10% of the *clindamycin* administered is excreted unaltered in the urine, and small quantities are found in the feces.

Therapeutic Uses

The oral dose of *clindamycin* for adults is 150 to 300 mg every 6 h; for severe infections, it is 300 to 600 mg every 6 h. Children should receive 8 to 12 mg/kg per day of *clindamycin palmitate hydrochloride* in three or four divided doses or, for severe infections, 13 to 25 mg/kg per day. *Clindamycin phosphate* is available for intramuscular or intravenous use. For serious infections, intravenous or intramuscular administration is recommended in dosages of 1200 to 2700 mg/day, divided into three or four equal doses for adults. Children should receive 15 to 40 mg/kg per day in three or four divided doses.

Skin and Soft-Tissue Infections

Clindamycin is an alternative agent for the treatment of skin and soft-tissue infections, especially in patients with β-lactam allergies (Stevens et al., 2014). It is also useful for oral treatment of skin infections when MRSA and streptococci are potential pathogens. Because *clindamycin* inhibits toxin production, it is recommended as an adjunctive agent in necrotizing fasciitis or gas gangrene when toxin-producing bacteria (e.g., streptococci, staphylococci, clostridia) are suspected. Topical *clindamycin* is used for treatment of acne.

Respiratory Tract Infections

Clindamycin is effective for treatment of lung abscess and anaerobic lung and pleural space infections due to susceptible organisms (Levison et al., 1983). It has been used as an alternative agent for treatment of sinusitis, pharyngitis, and otitis media. *Clindamycin* in combination with *primaquine* is useful as an alternative agent for the treatment of *P. jirovecii* pneumonia in patients with HIV.

Other Infections

Owing to its good activity against staphylococci and excellent bone penetration, *clindamycin* is an alternative agent for treatment of osteomyelitis. *Clindamycin* in combination with *pyrimethamine* and *leucovorin* (folinic acid) is an effective alternative for acute treatment of encephalitis caused by *T. gondii* in patients with AIDS. *Clindamycin* plus *quinine* is an alternative regimen for nonsevere malaria. *Clindamycin* is also administered vaginally for bacterial vaginosis.

Adverse Effects

GI Effects

The reported incidence of diarrhea associated with the administration of *clindamycin* ranges from 2% to 20%. In most cases, this is mild to moderate in severity and resolves on drug discontinuation. However, *clindamycin* carries a relatively high risk of superinfection with *C. difficile*, with an odds ratio versus no antibiotic exposure of 16 in one meta-analysis, compared to 5.5 for cephalosporins and 5.5 for fluoroquinolones (Brown et al., 2013). This colitis is characterized by watery diarrhea, fever, and elevated peripheral white blood cell counts. *Severe forms of* C. difficile *infection may be fatal.* The prescribing information for *clindamycin* in the U.S. includes a boxed warning about the risk of *C. difficile* infection. Discontinuation of the drug, combined with administration of oral *vancomycin* or *fidaxomicin*, is usually effective for treatment, but relapses occur. Agents that inhibit peristalsis (e.g., opioids) may prolong and worsen the condition.

Other Toxic and Irritative Effects

Skin rashes occur in about 10% of patients treated with *clindamycin* and may be more common in patients with HIV infection. Other uncommon reactions include exudative erythema multiforme (Stevens-Johnson syndrome), reversible elevation of aspartate aminotransferase and alanine aminotransferase, granulocytopenia, thrombocytopenia, and anaphylactic reactions. Local thrombophlebitis may follow intravenous administration of the drug. *Clindamycin* may potentiate the effect of concomitant neuromuscular blocking agents.

Oxazolidinones

Oxazolidinones are a new class of synthetic protein synthesis inhibitors with activity primarily against gram-positive organisms, including multidrug-resistant pathogens. *Linezolid* is the class originator; a second agent, *tedizolid*, was FDA approved in 2014.

Mechanism of Action

Oxazolidinones inhibit protein synthesis by binding to the P site of the 50S ribosomal subunit and preventing formation of the larger ribosomal-fMet-tRNA complex that initiates protein synthesis. Because of their unique mechanism of action, these agents are active against strains that are resistant to multiple other agents, including *penicillin*-resistant strains of *S. pneumoniae*; *methicillin*-resistant, *vancomycin*-intermediate, and *vancomycin*-resistant strains of staphylococci; and *vancomycin*-resistant strains of enterococci.

Antimicrobial Activity

Linezolid is active against the vast majority of gram-positive organisms, including staphylococci, streptococci, enterococci, gram-positive anaerobic cocci, and gram-positive rods such as *Corynebacterium* spp., *Nocardia* spp., and *L. monocytogenes* (see Table 60–1). It has poor activity against most gram-negative aerobic bacteria. It is bacteriostatic against enterococci and staphylococci but may be bactericidal against streptococci. *Mycobacterium tuberculosis* is moderately susceptible, as are most rapidly growing mycobacteria, but MAI is frequently resistant. The available data to date suggest *tedizolid* has similar activity to *linezolid* (Rybak et al., 2014).

Resistance to Oxazolidinones

Resistance in enterococci and staphylococci is most commonly due to point mutations of the 23S rRNA. Because bacteria have multiple copies of 23S rRNA genes, significant resistance generally requires mutations in two or more copies. Recently, a transferable methyltransferase that confers resistance through ribosomal modification has been described. *Linezolid* resistance remains relatively low among normally susceptible organisms, although some sites report increasing frequency in enterococci, including cases of nosocomial transfer. Limited data suggest *tedizolid* may be active against some *linezolid*-resistant isolates, although reports of clinical use for *linezolid*-resistant isolates are lacking.

ADME

Linezolid is well absorbed after oral administration, with a bioavailability of 100%, and may be administered without regard to food. Dosing for oral and intravenous preparations is the same. The $t_{1/2}$ is about 4 to 6 h. *Linezolid* is 30% protein bound and distributes widely to well-perfused tissues, including favorable distribution into the central nervous system. *Linezolid* is nonenzymatically oxidized to aminoethoxyacetic acid and hydroxyethyl glycine derivatives. Approximately 80% of a dose of *linezolid* appears in the urine, 30% as active compound and 50% as the two primary oxidation products. Ten percent of the administered dose appears as oxidation products in feces. No dose adjustment in renal insufficiency is currently recommended by the manufacturer, although some studies have suggested accumulation in patients with renal dysfunction (Crass et al., 2019). *Linezolid* and its breakdown products are eliminated by dialysis; therefore, the drug should be administered after hemodialysis.

Tedizolid is administered orally and parenterally as a prodrug (*tedizolid phosphate*) that is rapidly and completely hydrolyzed to *tedizolid*. *Tedizolid* is well absorbed after oral administration (bioavailability >80%). *Tedizolid* displays greater protein binding (70%–90%) and a longer $t_{1/2}$ of about 12 h. There is minimal elimination of unchanged drug in the urine; the drug undergoes sulfation in the liver and is excreted primarily in the feces.

Therapeutic Uses

Linezolid is most commonly administered at a dose of 600 mg twice daily orally or intravenously. Lower doses and/or reduced frequency of dosing (e.g., 300 mg twice daily or 600 mg once daily) may be used for long-term therapy of mycobacterial infections. *Tedizolid* is given as a 200-mg IV or oral daily dose.

Skin and Soft-Tissue Infections

Linezolid and *tedizolid* are FDA-approved for treatment of skin and skin-structure infections caused by streptococci and *S. aureus* (MSSA and MRSA). A 6-day regimen of *tedizolid* provided similar outcomes to 10 days of *linezolid*.

Respiratory Tract Infections

Linezolid is approved for treatment of community-acquired pneumonia due to *S. pneumoniae* and nosocomial pneumonia due to *S. aureus*. A randomized clinical trial in patients with MRSA pneumonia demonstrated similar or better outcomes to *vancomycin* (Wunderink et al., 2012). Studies of *tedizolid* for pneumonia are under way.

Other Infections

Linezolid is commonly used for a variety of infections due to *vancomycin*-resistant *E. faecium*. *Linezolid* has been used in combination therapy for extensively drug-resistant tuberculosis, nontuberculous mycobacterial infections, and infections due to *Nocardia*.

Adverse Effects

Myelosuppression

Myelosuppression, including anemia, leukopenia, pancytopenia, and thrombocytopenia, has been reported in patients receiving *linezolid*. Thrombocytopenia tends to be the most common effect, with an onset between 7 and 10 days. Effects are reversible on drug discontinuation. Platelet counts should be monitored in patients with risk of bleeding, preexisting thrombocytopenia, or intrinsic or acquired disorders of platelet function and in patients receiving courses of therapy lasting beyond 2 weeks. Treatment durations with *tedizolid* in clinical trials have been limited; based on early clinical and *in vitro* data, *tedizolid* may have a lower propensity for causing myelosuppression.

Mitochondrial Toxicities

Patients receiving treatment with *linezolid* have developed peripheral neuropathy, optic neuritis, and lactic acidosis (Narita et al., 2007). These effects typically manifest after prolonged treatment durations (at least 6 weeks), although some cases of lactic acidosis have been described after only a few days of therapy. The underlying mechanism of these toxicities is believed to be inhibition of mitochondrial protein synthesis. In a study of patients receiving long-term *linezolid* for treatment of drug-resistant tuberculosis, 18% experienced optic neuritis and 64% experienced peripheral neuropathy during the first year of treatment, although only three patients discontinued *linezolid* due to adverse effects (Lee et al., 2012). *Linezolid* should generally not be used for long-term therapy if there are alternative agents. There are currently insufficient data to judge the risk of mitochondrial toxicities with *tedizolid*.

Drug Interactions

Linezolid is a weak nonspecific inhibitor of monoamine oxidase. Patients receiving concomitant therapy with an adrenergic or serotonergic agent (including selective serotonin reuptake inhibitors) or consuming more than 100 mg of tyramine a day may experience serotonin syndrome (e.g., palpitations, headache, hypertensive crisis). Coadministration of these agents is best avoided. However, in patients receiving selective serotonin reuptake inhibitors who acutely require *linezolid* therapy for short-term (10- to 14-day) treatment, coadministration with careful monitoring is reasonable. The relative potential for this interaction with *tedizolid* may be lower based on preclinical data. Neither *linezolid* nor *tedizolid* is a substrate or an inhibitor of CYPs.

Pleuromutilins

Pleuromutilins are derivatives of natural products of the *Pleurotus* spp. of fungi and were discovered in the 1950s. Two pleuromutilin agents are available for clinical use in humans, the topical agent *retapamulin* and the orally and intravenously administered agent *lefamulin*.

Mechanism of Action

Pleuromutilins are inhibitors of bacterial protein synthesis by targeting the 50S ribosomal subunit. The site of binding is near the A and P sites and results in inhibition of peptide bond formation (Paukner et al., 2017). There is some overlap with binding sites of oxazolidinones, lincosamides, phenicols, and streptogramins, although the mechanism of the pleuromutilins is unique and cross-resistance so far is uncommon. *Lefamulin*'s activity against gram-positive pathogens is typically bactericidal.

Antimicrobial Activity

The pleuromutilins as a class have potent activity against gram-positive pathogens, with the exception of enterococci. Minimum inhibitory concentrations are very low against staphylococci, including MRSA, and streptococci, including *penicillin*-resistant *S. pneumoniae*. They do not exhibit useful activity against enteric gram-negatives but have some activity against gram-negatives seen in community-acquired respiratory pathogens such as *H. influenzae* and *M. catarrhalis*. Activity against atypical respiratory pathogens such as *Chlamydophila*, *Mycoplasma*, and *Legionella* spp. is good. There is strong interest in use of *lefamulin* for treatment of sexually transmitted infections based on good *in vitro* data against *N. gonorrhoeae* and *C. trachomatis*.

Resistance to Pleuromutilins

Resistance to pleuromutilins among typically susceptible organisms is currently rare. Identified mechanisms of resistance are generally similar to those seen with other protein synthesis inhibitors, with point mutations leading to reduced drug binding to the ribosome appearing to be most common. Upregulated efflux and modification of the target through ribosomal protection proteins have also been described.

ADME

Retapamulin is available for topical use; absorption is generally low, although it may be increased in children or when applied to broken skin. Following intravenous administration, *lefamulin* achieves peak plasma concentrations of approximately 1.5 to 2 mg/L. The oral formulation has a relatively low bioavailability of approximately 25%, which is modestly reduced by food; however, at the higher oral doses recommended, peak plasma concentrations approximating those of IV administration can be achieved. The drug is highly protein bound (~95%), with a half-life of around 12 h, allowing for twice-daily dosing. Elimination is largely unchanged in the feces, with no need for dose adjustment in renal or hepatic dysfunction.

Therapeutic Uses

Retapamulin is indicated for the treatment of impetigo in adults and children, applied as a thin layer of a 1% cream twice daily. *Lefamulin* is indicated for the treatment of community-acquired bacterial pneumonia at a dose of 150 mg IV or 600 mg orally every 12 h and is under study for the treatment of skin and soft-tissue infections.

Adverse Effects

Lefamulin is generally well tolerated, with gastrointestinal adverse effects such as nausea and diarrhea being most common. Prolongation of the QT interval has been observed and may warrant monitoring in patients with risk factors for arrhythmia.

Drug Interactions

Retapamulin and *lefamulin* are substrates of CYP3A4. Concomitant use of *lefamulin* and strong inducers or inhibitors of these enzymes should be avoided or undertaken with caution.

Streptogramins

Streptogramins are semisynthetic derivatives of naturally occurring agents produced by *Streptomyces pristinaespiralis*. The only streptogramin in clinical use for humans is a fixed combination of *quinupristin* (a streptogramin B) with *dalfopristin* (a streptogramin A) in a 30:70 ratio. *Quinupristin* and *dalfopristin* are more soluble derivatives of the congeners pristinamycin IA and IIA and therefore are suitable for intravenous administration.

Mechanism of Action

Quinupristin and *dalfopristin* are protein synthesis inhibitors that bind the 50S ribosomal subunit. *Quinupristin* binds at the same site as macrolides and has a similar effect, with inhibition of polypeptide elongation and early termination of protein synthesis. *Dalfopristin* binds at a site nearby, resulting in a conformational change in the 50S ribosome, synergistically enhancing the binding of *quinupristin* at its target site. *Dalfopristin* directly interferes with polypeptide chain formation. The net result of the cooperative and synergistic binding of these two molecules to the ribosome is enhanced and often bactericidal activity.

Antimicrobial Activity

Quinupristin/dalfopristin is active against most gram-positive cocci and organisms responsible for atypical pneumonia (e.g., *M. pneumoniae*, *Legionella* spp., and *C. pneumoniae*) but is inactive against gram-negative organisms (see Table 60–1). The combination is bactericidal against streptococci and many strains of staphylococci but bacteriostatic against *Enterococcus faecium*.

Resistance to Streptogramins

Resistance to *quinupristin* is mediated by genes encoding a ribosomal methylase that prevents binding of drug to its target or genes encoding lactonases that inactivate type B streptogramins. Resistance to *dalfopristin* is mediated by genes that encode acetyltransferases that inactivate type A streptogramins or staphylococcal genes that encode ATP-binding efflux proteins that pump type A streptogramins out of the cell. These resistance determinants are located on plasmids. Resistance to *quinupristin/dalfopristin* always is associated with a resistance gene for type A streptogramins. Methylase-encoding genes can render the combination bacteriostatic instead of bactericidal, making it ineffective in certain infections in which bactericidal activity is preferred (e.g., endocarditis).

ADME

Quinupristin/dalfopristin is administered by intravenous infusion over at least 1 h. The $t_{1/2}$ is 0.85 h for *quinupristin* and 0.7 h for *dalfopristin*. The volume of distribution is 0.87 L/kg for *quinupristin* and 0.71 L/kg for *dalfopristin*. Hepatic metabolism by conjugation is the principal means of clearance for both compounds, with 80% of an administered dose eliminated by biliary excretion. Renal elimination of active compound accounts for most of the remainder. No dosage adjustment is necessary for renal insufficiency. Pharmacokinetics are not significantly altered by peritoneal dialysis or hemodialysis. Hepatic insufficiency increases the plasma concentrations of active component and metabolites by 180% for *quinupristin* and 50% for *dalfopristin*.

Therapeutic Uses

Quinupristin/dalfopristin is approved in the U.S. for complicated skin and skin-structure infections caused by *methicillin*-susceptible strains of *S. aureus* or *S. pyogenes*. It is also used for treatment of infections caused by *vancomycin*-resistant strains of *E. faecium* (dose of 7.5 mg/kg every 8–12 h), and in Europe, it also is approved for treatment of nosocomial pneumonia and infections caused by MRSA. *Quinupristin/dalfopristin* should be reserved for treatment of serious infections caused by multidrug-resistant gram-positive organisms such as *vancomycin*-resistant *E. faecium*; it has largely been supplanted by newer agents with activity against resistant gram-positive organisms.

Adverse Effects

The most common side effects are infusion-related events, such as pain and phlebitis at the infusion site and arthralgias and myalgias. Phlebitis and pain can be minimized by infusion of drug through a central venous catheter. Arthralgias and myalgias, more likely to be problematic in patients with hepatic insufficiency, are managed by reducing the infusion frequency.

Drug Interactions

Quinupristin/dalfopristin inhibits CYP3A4. The concomitant administration of other CYP3A4 substrates with *quinupristin/dalfopristin* may result in significant toxicity. Caution and monitoring are recommended for drugs in which the toxic-therapeutic window is narrow or for drugs that prolong the QTc interval.

Phenicols

Chloramphenicol, an antibiotic produced by *Streptomyces venezuelae*, was introduced into clinical practice in 1948. *Chloramphenicol* can cause serious and fatal blood dyscrasias; consequently, the drug is now reserved for treatment of life-threatening infections (e.g., meningitis, rickettsial infections) in patients who cannot take safer alternatives because of resistance or allergies (Wareham et al., 2002). Other agents of similar structure (*thiamphenicol*, *florfenicol*) are used in veterinary medicine.

Mechanism of Action

Chloramphenicol inhibits protein synthesis in bacteria and, to a lesser extent, in eukaryotic cells. The drug readily penetrates bacterial cells, probably by facilitated diffusion. *Chloramphenicol* acts primarily by binding reversibly to the 50S ribosomal subunit (near the binding site for the macrolide antibiotics and *clindamycin*). The drug prevents the binding of the amino acid–containing end of the aminoacyl tRNA to the acceptor site on the 50S ribosomal subunit. The interaction between peptidyltransferase and its amino acid substrate cannot occur, and peptide bond formation is inhibited (Figure 60–3).

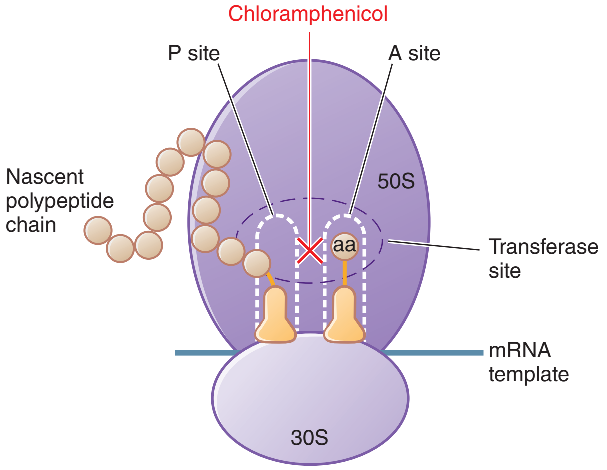


Figure 60–3 *Inhibition of bacterial protein synthesis by chloramphenicol. Chloramphenicol* binds to the 50S ribosomal subunit at the peptidyltransferase site, inhibiting transpeptidation. *Chloramphenicol* binds near the site of action of *clindamycin* and the macrolide antibiotics. These agents interfere with the binding of *chloramphenicol* and thus may interfere with each other's actions if given concurrently.

Chloramphenicol also can inhibit mitochondrial protein synthesis in mammalian cells, perhaps because mitochondrial ribosomes resemble bacterial ribosomes (both are 70S); erythropoietic cells are particularly sensitive.

Antimicrobial Activity

Chloramphenicol possesses a broad spectrum of antimicrobial activity. *Chloramphenicol* is bacteriostatic against most species, although it may be bactericidal against *H. influenzae*, *N. meningitidis*, and *S. pneumoniae*. Strains of *S. aureus* tend to be less susceptible, but some isolates of highly resistant MRSA have been susceptible. *Chloramphenicol* is active against enterococci, including multidrug-resistant *E. faecium*. *Chloramphenicol* is active against *Mycoplasma*, *Chlamydia*, and *Rickettsia*. Enterobacterales are variably sensitive to *chloramphenicol*, but *P. aeruginosa* is resistant to even very high concentrations of *chloramphenicol*. Strains of *V. cholerae* have remained largely susceptible to *chloramphenicol*.

Resistance to Chloramphenicol

Resistance to *chloramphenicol* usually is caused by a plasmid-encoded acetyltransferase that inactivates the drug. Resistance also can result from decreased permeability and from ribosomal mutation. Acetylated derivatives of *chloramphenicol* fail to bind to bacterial ribosomes.

ADME

Chloramphenicol has been available in oral, intravenous, and topical (e.g., ophthalmic) preparations. The oral and ophthalmic formulations are no longer available in the U.S., although they can be found in other parts of the world. *Chloramphenicol* administered in oral capsule form is absorbed rapidly from the GI tract. For parenteral use, *chloramphenicol succinate* is a prodrug that is hydrolyzed by esterases to *chloramphenicol in vivo*. *Chloramphenicol succinate* is rapidly cleared from plasma by the kidneys; this may reduce overall bioavailability of the drug because as much as 30% of the dose may be excreted before hydrolysis. Poor renal function in the neonate and other states of renal insufficiency result in increased plasma concentrations of *chloramphenicol succinate*. Decreased esterase activity has been observed in the plasma of neonates and infants, prolonging time to peak concentrations of active *chloramphenicol* (up to 4 h) and extending the period over which renal clearance of *chloramphenicol succinate* can occur.

Chloramphenicol is widely distributed in body fluids and readily reaches therapeutic concentrations in CSF. Hepatic metabolism to the inactive glucuronide is the major route of elimination. This metabolite and *chloramphenicol* are excreted in the urine. Patients with impaired hepatic function have decreased metabolic clearance, and dosage should be adjusted. Half-life is not altered significantly by renal insufficiency or hemodialysis, and dosage adjustment usually is not required. Variability in the metabolism and pharmacokinetics of *chloramphenicol* in neonates, infants, and children necessitates monitoring of drug concentrations in plasma.

Therapeutic Uses

Therapy with chloramphenicol must be limited to infections for which the benefits of the drug outweigh the risks of the potential toxicities. When other antimicrobial drugs that are equally effective and less toxic are available, they should be used instead of *chloramphenicol*.

Bacterial Meningitis

Chloramphenicol remains an alternative drug for the treatment of meningitis caused by *H. influenzae*, *N. meningitidis*, and *S. pneumoniae* in patients who have severe allergy to β-lactams and in developing countries. The total daily dose for children should be 50 mg/kg of body weight, divided into four equal doses given intravenously every 6 h.

Rickettsial Diseases

The tetracyclines usually are the preferred agents for the treatment of rickettsial diseases. However, in patients allergic to these drugs, in pregnant women, and in children less than 8 years of age who require prolonged or repeated courses of therapy, *chloramphenicol* is an alternative therapy. Rocky Mountain spotted fever; epidemic, murine, scrub, and recrudescent typhus; and Q fever respond well to *chloramphenicol*. For adults and children with these diseases, a dosage of 50 mg/kg per day divided into 6-h intervals is recommended. For severe or resistant infections, doses up to 100 mg/kg per day may be used for short intervals, but the dose must be reduced to 50 mg/kg per day as soon as possible. Therapy should be continued until the general condition has improved and the patient is afebrile for 24 to 48 h.

Adverse Effects

Chloramphenicol inhibits the synthesis of proteins of the inner mitochondrial membrane, probably by inhibiting the ribosomal peptidyltransferase. These include subunits of cytochrome *c* oxidase, ubiquinone–cytochrome *c* reductase, and the proton-translocating ATPase critical for aerobic metabolism. Much of the toxicity observed with this drug can be attributed to these effects.

Hematological Toxicity

Chloramphenicol affects the hematopoietic system in two ways: a dose-related toxicity that presents as anemia, leukopenia, or thrombocytopenia and an idiosyncratic response manifested by aplastic anemia, leading in many cases to fatal pancytopenia. Dose-related, reversible erythroid suppression probably reflects an inhibitory action of *chloramphenicol* on mitochondrial protein synthesis in erythroid precursors, which in turn impairs iron incorporation into heme. Bone marrow suppression occurs regularly when plasma concentrations are 25 μg/mL or greater and is observed with the use of large doses of *chloramphenicol*, prolonged treatment, or both. Dose-related suppression of the bone marrow may progress to fatal aplasia if treatment is continued, but most cases of bone marrow aplasia develop without prior dose-related marrow suppression.

Pancytopenia occurs more commonly in individuals who undergo prolonged therapy and especially in those who are exposed to the drug on more than one occasion. Although the incidence of the reaction is low, about 1 in 30,000 courses of therapy or more, the fatality rate is high when bone marrow aplasia is complete, and there is an increased incidence of acute leukemia in those who recover. Aplastic anemia accounts for about 70% of cases of blood dyscrasias due to *chloramphenicol*; hypoplastic anemia, agranulocytosis, and thrombocytopenia make up the remainder. The proposed mechanism involves conversion of the nitro group to a toxic intermediate by intestinal bacteria.

Effects on Neonates

Neonates, especially if premature, may develop a serious illness termed *gray baby syndrome* after exposure to *chloramphenicol*. This syndrome usually begins 2 to 9 days after treatment is started. Within the first 24 h, vomiting, refusal to suck, irregular and rapid respiration, abdominal distention, periods of cyanosis, and passage of loose green stools occur. Over the next 24 h, neonates turn an ashen-gray color and become flaccid and hypothermic. A similar "gray syndrome" has been reported in adults who were accidentally overdosed with the drug. Death occurs in about 40% of patients within 2 days of initial symptoms. Those who recover usually exhibit no sequelae. Two mechanisms apparently are responsible for *chloramphenicol* toxicity in neonates: (1) a developmental deficiency of glucuronyl transferase, the hepatic enzyme that metabolizes *chloramphenicol*; and (2) inadequate renal excretion of unconjugated drug. At the onset of the clinical syndrome, *chloramphenicol* concentrations in plasma usually exceed 100 μg/mL and may be as low as 75 μg/mL.

Drug Interactions

Chloramphenicol inhibits hepatic CYPs and thereby prolongs the half-lives of drugs that are metabolized by this system. Severe toxicity and death have occurred because of failure to recognize such effects. Concurrent administration of *phenobarbital* or *rifampin*, which potently induce CYPs, shortens the $t_{1/2}$ of the antibiotic and may result in subtherapeutic drug concentrations.

Mupirocin

Mupirocin is an antibiotic first isolated from *Pseudomonas fluorescens*. It is a mixture of several pseudomonic acids and is effective against gram-positive bacteria.

Mechanism of Action, Antimicrobial Activity, and Resistance

Mupirocin inhibits bacterial protein synthesis by reversible binding and inhibition of isoleucyl tRNA synthase, leading to depletion of factors required for protein synthesis (Khoshnood et al., 2019). There is no cross-resistance with other classes of antibiotics. Resistance may occur through a variety of mechanisms and to various degrees. *Mupirocin* is for topical use only. The drug's activity ranges from bacteriostatic to bactericidal dependent on the dose. It is active primarily against gram-positive bacteria, including *S. pyogenes*, MSSA, and MRSA. Low-level resistance typically occurs through point mutations in the target synthetase enzyme. High-level resistance is mediated by a plasmid, which encodes a "bypass" Ile tRNA synthase that binds *mupirocin* poorly.

Therapeutic Uses

Mupirocin is available as a 2% cream and a 2% ointment for dermatologic use and as a 2% ointment for intranasal use. The dermatologic preparations are indicated for treatment of traumatic skin lesions and impetigo secondarily infected with *S. aureus* or *S. pyogenes*. The nasal ointment is approved for eradication of *S. aureus* nasal carriage. The consensus is that patients who stand to benefit from *mupirocin* prophylaxis are those with proven *S. aureus* nasal colonization plus risk factors for distant infection or a history of skin or soft-tissue infections.

Adverse Effects

Mupirocin may cause irritation and sensitization at the site of application. Contact with the eyes causes irritation that may take several days to resolve. Polyethylene glycol present in the ointment can be absorbed from damaged skin. Application of the ointment to large surface areas should be avoided in patients with moderate-to-severe renal failure to avoid accumulation of polyethylene glycol.

Drug Facts for Your Personal Formulary: *Protein Synthesis Inhibitors*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Tetracyclines and Derivatives **General: Bacteriostatic; oral formulations interact with orally administered cations (calcium, iron, aluminum); avoid in pregnancy and children <8 years old due to permanent tooth discoloration; photosensitivity**		
Tetracycline (PO, topical)	• Inflammatory acne • *Helicobacter pylori* infections (in combination) • Topical first aid • Use for other indications has largely been replaced by doxycycline	• Good activity vs. rickettseae, *Chlamydia, Mycoplasma, Legionella, Ureaplasma, Borrelia, Francisella tularensis, Pasteurella multocida, Bacillus anthracis, Helicobacter pylori* • Some activity vs. *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Haemophilus influenzae* • Good CSF penetration • Renal excretion • Renal toxicity, hepatotoxicity at high doses
Doxycycline (IV, PO)	• Community-acquired pneumonia • Skin/soft-tissue infection • Urogenital chlamydia • Lymphogranuloma venereum • Syphilis (penicillin alternative) • Rocky Mountain spotted fever • Anthrax, tularemia • Lyme disease, leptospirosis • Periodontitis	• Similar to tetracycline, with improved activity vs. streptococci and staphylococci • Good CSF penetration • Dual renal/biliary elimination • Preferred tetracycline for most indications due to more favorable activity, tolerability, and frequency of administration
Minocycline (IV, PO, topical)	• Skin/soft-tissue infections • Mycobacterial infections • Nocardiosis • Acne	• Similar to doxycycline, with improved activity vs. staphylococci, *Acinetobacter*, and *Stenotrophomonas maltophilia* • Renal elimination • Vestibular toxicity
Tigecycline (IV) Eravacycline (IV) Omadacycline (IV, PO)	• Intra-abdominal infection • Skin and soft-tissue infection • Pneumonia • Tigecycline: increased risk of death in pooled analysis; reserve as alternative therapy	• Similar to minocycline, with improved activity vs. *Escherichia coli*, *Klebsiella*, enterococci, *Bacteroides fragilis* • Activity vs. nontuberculous mycobacteria • Wide distribution with low serum levels • Hepatic elimination
Macrolides and Ketolides **General: Bacteriostatic; widely distributed but with limited CSF penetration, gastrointestinal distress, QT prolongation, major (erythromycin, clarithromycin, telithromycin) to minor (azithromycin) inhibitor of drug-metabolizing CYPs**		
Erythromycin (IV, PO, topical)	• Erysipelas and cellulitis • Ophthalmia neonatorum • Diphtheria • Pertussis	• Good activity against *Mycoplasma, Chlamydia, Legionella, Campylobacter, Bordetella pertussis, Corynebacterium diphtheriae* • Some activity against *S. pneumoniae*, *S. pyogenes*, *H. influenzae* • Oral formulations have variable absorption • Stimulates motilin receptors; gastrointestinal prokinetic properties • Cholestatic hepatitis with long-term use
Clarithromycin (PO)	• Erysipelas and cellulitis • Community-acquired pneumonia • Acute exacerbations of chronic bronchitis • *Helicobacter pylori* gastritis (in combination with other agents) • *Mycobacterium avium* treatment and prophylaxis	• Similar to erythromycin, with improved activity vs. streptococci and staphylococci • Good activity vs. *Moraxella catarrhalis*, *H. pylori*, and nontuberculous mycobacteria • Active metabolite • Some drug accumulation in severe renal impairment • Tinnitus at high doses
Azithromycin (IV, PO, topical)	• Community-acquired pneumonia • Acute exacerbations of chronic bronchitis • Otitis media • Bacterial pharyngitis • Chlamydia • *Mycobacterium avium* treatment and prophylaxis	• Similar to clarithromycin, improved activity vs. *H. influenzae* • Extensive tissue distribution and concentration in tissues • Anti-inflammatory properties • Long $t_{1/2}$, ~48 h
Lincosamides **General: Bacteroiostatic**		
Clindamycin (IV, PO, topical)	• Skin and soft-tissue infection • Inflammatory acne • Lung abscess • Streptococcal pharyngitis • *Pneumocystis* pneumonia • Toxoplasma encephalitis • Nonsevere malaria • Bacterial vaginosis	• Good activity vs. *S. pneumoniae*, *S. pyogenes*, viridans streptococci, *Actinomyces*, *Nocardi*[a] • Some activity vs. *S. aureus*, *Bacteroides* spp., *Toxoplasma, Pneumocystis, Plasmodium* • Wide tissue distribution, especially into bone; modest CSF penetration • Metabolized in liver, excreted in urine and bile • Diarrhea, rarely *Clostridium difficile* colitis

Drug Facts for Your Personal Formulary: *Protein Synthesis Inhibitors (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Oxazolidinones **General: Bacteriostatic; excellent oral absorption; wide distribution, including to CNS; myelosuppression; peripheral neuropathy with long-term use; risk of serotonin syndrome with concomitant antidepressant use**		
Linezolid (IV, PO)	• Skin and soft-tissue infections • Pneumonia • Vancomycin-resistant enterococcal infections • Nocardiosis • Drug-resistant tuberculosis	• Good activity against streptococci, staphylococci, enterococci, *Nocardia, Listeria* • Some activity against mycobacteria • Nonenzymatic degradation with elimination in urine
Tedizolid (IV, PO)	• Skin and soft-tissue infections	• Similar activity to linezolid but lower risk of myelosuppression and drug interactions • Hepatic metabolism and fecal excretion • Longer $t_{1/2}$ than linezolid
Pleuromutilins **General: Bactericidal**		
Retapamulin (topical) Lefamulin (IV, PO)	• Skin and soft-tissue infections • Pneumonia	• Excellent activity against streptococci, staphylococci, *E. faecium, Mycoplasma, Legionella, Chlamydophila* • Good activity vs. *Haemophilus influenzae, Moraxella catarrhalis, Neisseria gonorrhoeae, Chlamydia trachomatis* • Hepatic metabolism and fecal excretion • CYP substrate; drug interaction potential • GI adverse effects, QT prolongation
Streptogramins **General: Bacteriostatic to bactericidal depending on species and resistance**		
Quinupristin/ dalfopristin (IV)	• Skin and soft-tissue infections • Vancomycin-resistant *Enterococcus faecium* infections	• Good activity against streptococci, staphylococci, *E. faecium, Mycoplasma, Legionella, Chlamydophila* • Hepatic metabolism with biliary excretion • Infusion site phlebitis • Arthralgias, myalgias • CYP inhibitor
Phenicols **General: Bacteriostatic**		
Chloramphenicol (IV, PO—not in the U.S.)	• Rickettsial infections • Bacterial meningitis • Because of risk of fatal toxicities, reserve as alternative therapy	• Good activity vs. *S. pneumoniae, H. influenzae, Neisseria meningitidis*, rickettsiae, *Vibrio, Enterococcus* • Variable serum levels due to clearance of prodrug before hydrolysis • Excellent CSF penetration • Hepatic clearance • Dose-dependent bone marrow suppression, idiosyncratic fatal aplastic anemia, fatal "gray baby syndrome" in neonates receiving high doses

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Chapter 61

Antifungal Agents

P. David Rogers and Damian J. Krysan

KINGDOM FUNGI AND ITS IMPACT ON HUMANS

SYSTEMIC ANTIFUNGAL AGENTS: DRUGS FOR DEEPLY INVASIVE FUNGAL INFECTIONS

- Amphotericin B
- Flucytosine
- Imidazoles and Triazoles
- Echinocandins
- Other Systemic Antifungal Agents
- Agents Active Against Microsporidia and *Pneumocystis*

TOPICAL ANTIFUNGAL AGENTS

- Topical Imidazoles and Triazoles
- Individual Agents
- Structurally Diverse Antifungal Agents

Kingdom Fungi and Its Impact on Humans

There are 200,000 known species of fungi, and estimates of the total size of the kingdom Fungi range to well over a million. Residents of the kingdom are quite diverse and include yeasts, molds, mushrooms, and smuts. About 400 fungal species cause disease in animals, and even fewer cause human disease. Nonetheless, fungal infections are associated with significant morbidity and mortality. The incidence of life-threatening fungal infections has increased in recent decades owing to an increase in immunocompromised patient populations, such as those receiving hematologic or solid-organ transplantation, cancer chemotherapy, and immunosuppressive medications, as well as those with human immunodeficiency virus–acquired immunodeficiency syndrome (HIV-AIDS). This has made antifungal agents increasingly important in the practice of modern medicine. With the currently available antifungal pharmacopeia, mortality rates for invasive fungal disease remain unacceptably high (Brown et al., 2012; Thornton, 2020).

Fungi are eukaryotes, making the discovery and development of drugs that target the pathogen without posing significant toxicity to the host a challenging undertaking. Differences in the biosynthesis of membrane sterols, the ability of fungi to deaminate cytosine, and the unique fungal cell wall that contains glucans and chitin have all been exploited to produce relatively safe and effective antifungal agents for the treatment of fungal infections (Roemer and Krysan, 2014). Since the advent of amphotericin B-deoxycholate in the late 1950s, research has sought safer and more effective alternatives for the treatment of systemic fungal infections. While amphotericin B remains the gold standard of systemic antifungal pharmacotherapy for a wide range of infections, alternative therapies have emerged for many clinically important fungal pathogens (Wiederhold, 2018).

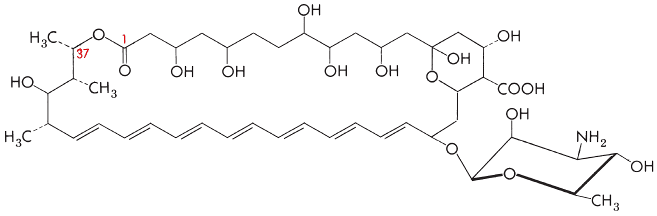
This chapter provides a comprehensive overview of currently available therapeutic options for the management of invasive, mucosal, and superficial fungal infections. With only a few exceptions, the antifungals in common clinical use act mainly at sites involving the cell wall and cell membrane (Figure 61–1). Table 61–1 summarizes common fungal infections and their pharmacotherapy. Recommended adult dosages are briefly discussed for each agent. Dosing recommendations for antifungal agents in children have been recently reviewed elsewhere (Downes et al., 2020).

Systemic Antifungal Agents: Drugs for Deeply Invasive Fungal Infections

Amphotericin B

Chemistry

Amphotericin B is an amphipathic or amphoteric polyene macrolide molecule with the broadest spectrum of activity of any of the currently available antifungal drugs. Polyene macrolide compounds share the characteristics of four to seven conjugated double bonds, an internal cyclic ester, poor aqueous solubility, substantial toxicity when administered systemically, and a common mechanism of antifungal action. *Amphotericin B*, a heptaene macrolide, contains seven conjugated trans-double bonds and a 3-amino-3,6-dideoxymannose (mycosamine) connected to the macrolide ring through a glycosidic bond (Figure 61–2). The amphoteric properties of the drug, from which it derives its name, are due to the presence of a carboxyl group on the main ring and a primary amino group on mycosamine; these groups confer aqueous solubility at extremes of pH.



AMPHOTERICIN B

Mechanism of Action

The antifungal activity of *amphotericin B* depends principally on its ability to bind *ergosterol* in the membrane of sensitive fungi. *Amphotericin B* has long been thought to form pores or channels that increase the permeability of the membrane and allow leakage of cytosolic molecules and ions, leading to loss of membrane integrity. However, recent evidence suggests *amphotericin B* forms aggregates that sequester ergosterol from lipid bilayers much like a sponge, resulting in fungal cell death (Anderson et al., 2014) (see Figure 61–2).

Formulations

Four formulations of *amphotericin B* are commercially available: C-AMB (conventional *amphotericin B*), ABCD (*amphotericin B* colloidal dispersion), L-AMB, (liposomal *amphotericin B*), and ABLC (*amphotericin B* lipid complex). Table 61–2 summarizes the pharmacokinetic properties of the available *amphotericin B* preparations, which have recently been extensively reviewed (see Hamill, 2013).

C-AMB. *Amphotericin B* is insoluble in water but, when formulated with the bile salt deoxycholate, becomes suitable for intravenous infusion. The complex is marketed as a lyophilized powder for injection. C-AMB forms a colloid in water, with particles largely less than 0.4 µm in diameter. As a result, filters in intravenous infusion lines that trap particles larger than 0.22 µm in diameter will remove significant amounts of drug. Furthermore, the addition of electrolytes to infusion solutions will cause the colloid to aggregate and complicate administration.

Abbreviations

ABCD: amphotericin B colloidal dispersion
ABLC: amphotericin B lipid complex
AIDS: acquired immunodeficiency syndrome
AUC: area under the C_P-time curve
C-AMB: conventional amphotericin B
CGD: chronic granulomatous disease
CDC: U.S. Centers for Diseases Control and Prevention
C_P: plasma concentration
CSF: cerebrospinal fluid
CYP: cytochrome P450
5FdUMP: 5-fluoro-2′-deoxyuridine-5′-monophosphate
5FU: 5-fluorouracil
5FUMP: 5-fluorouracil-ribose monophosphate
GI: gastrointestinal
HIV: human immundeficiency virus
L-AMB: liposomal amphotericin B
PJP: *Pneumocystis jirovecii* pneumonia
UPRTase: uracil phosphoribosyl transferase

ABCD. *Amphotericin B* colloidal dispersion contains roughly equimolar amounts of *amphotericin B* and cholesteryl sulfate formulated for injection. Like C-AMB, ABCD forms a colloidal solution when reconstituted in aqueous solution. ABCD provides much lower blood levels than C-AMB in humans, requiring administration of larger volumes to achieve equal blood levels. In a study of patients with neutropenic fever that compared daily ABCD (4 mg/kg) with C-AMB (0.8 mg/kg), chills and hypoxia were significantly more common in patients who received ABCD as compared with C-AMB (White et al., 1998). Hypoxia was associated with severe febrile reactions. In a study that compared ABCD (6 mg/kg) to C-AMB (1–1.5 mg/kg) in patients with invasive aspergillosis, ABCD was less nephrotoxic than C-AMB (15% vs. 49%) but caused more fever (27% vs. 16%) and chills (53% vs. 30%) (Bowden et al., 2002). ABCD is currently not commercially available in the U.S.

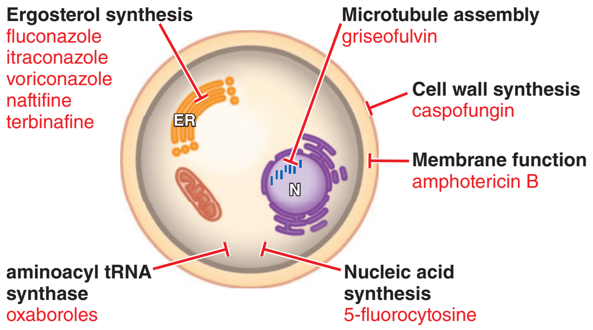


Figure 61–1 *Sites of action of antifungal agents.* Many antifungal agents act at sites involving cell wall and cell membrane function. *Amphotericin B* and other polyenes (e.g., *nystatin*) bind to ergosterol in fungal cell membranes and increase membrane permeability. The imidazoles and triazoles (*itraconazole*, etc.) inhibit 14-α-sterol demethylase, prevent ergosterol synthesis, and lead to the accumulation of toxic 14-α-methylsterols. The allylamines (e.g., *naftifine* and *terbinafine*) inhibit squalene epoxidase and prevent ergosterol synthesis. The echinocandins (e.g., *caspofungin*) inhibit the formation of glucans in the fungal cell wall. Metabolites of 5-fluorocytosine can disrupt fungal RNA and DNA synthesis. *Griseofulvin* inhibits microtubule assembly, thereby blocking fungal mitosis. Oxaboroles inhibit fungal aminoacyl tRNA synthase, thereby inhibiting fungal protein synthesis. ER, endoplasmic reticulum; N, nucleus, with microtubules.

L-AMB. Liposomal *amphotericin B* is a formulation in which *amphotericin B* is incorporated within a small, unilamellar liposomal vesicle formulation. The drug is supplied as a lyophilized powder and is reconstituted with sterile water for injection (Boswell et al., 1998). Blood levels following intravenous infusion are almost equivalent to those obtained with C-AMB, and because L-AMB can be given at higher doses, blood levels have been achieved that exceed those obtained with C-AMB (Boswell et al., 1998) (see Table 61–2).

ABLC. *Amphotericin B* lipid complex is a complex of *amphotericin B* with two phospholipids (dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol) (Slain, 1999). ABLC is given in a dose of 5 mg/kg in 5% dextrose in water, infused intravenously once daily over 2 h. Blood levels of *amphotericin B* are much lower with ABLC than with the same dose of C-AMB. ABLC is effective in a variety of mycoses, with the possible exception of cryptococcal meningitis.

Comparisons

Compared to C-AMB, all three of the *amphotericin B* lipid formulations appear to reduce the risk of acute kidney injury (defined as a doubling of patient serum creatinine) during therapy by 58% (Barrett et al., 2003). In patients at high risk for nephrotoxicity, ABLC has been observed to be more nephrotoxic than L-AMB (Wingard et al., 2000). Infusion-related reactions are not consistently reduced with the use of lipid preparations. ABCD causes more infusion-related reactions than C-AMB. Although L-AMB reportedly causes fewer infusion-related reactions than ABLC during the first dose (Wingard et al., 2000), the difference depends on whether premedication is given and varies considerably among patients. Infusion-related reactions typically decrease with subsequent infusions. While less toxic, the lipid formulations are much more costly than C-AMB, making them unavailable in many countries and dictating prudent use in the U.S. and other resource-rich areas. Interestingly, C-AMB is tolerated by premature neonates much better than by older children and adults; as a result, it remains an important part of the antifungal formulary in the critical care nursery (Downes et al., 2020).

ADME

Gastrointestinal absorption of all *amphotericin B* formulations is negligible, and intravenous delivery is indicated for systemic use. In plasma, *amphotericin B* is more than 90% bound to proteins. Pharmacokinetic properties differ among the preparations (see Table 61–2). Azotemia, liver failure, and hemodialysis do not have a measurable impact on plasma concentrations. The concentration of *amphotericin B* (via C-AMB) in fluids from inflamed pleura, peritoneum, synovium, and aqueous humor is approximately two-thirds that of trough concentrations in plasma. Regardless of formulation, very little *amphotericin B* penetrates into cerebrospinal fluid (CSF), vitreous humor, or normal amniotic fluid. Despite poor penetration into CSF, *amphotericin B* ± *flucytosine* is the treatment of choice for certain CNS fungal infections, such as cryptococcal meningitis and *Coccidioides* meningoencephalitis.

Antifungal Activity

Amphotericin B has useful clinical activity against a broad spectrum of pathogenic fungi, including *Candida* spp., *Cryptococcus neoformans*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Sporothrix schenckii*, *Coccidioides* spp., *Paracoccidioides braziliensis*, *Aspergillus* spp., *Penicillium marneffei (Talaromyces marneffei)*, *Fusarium* spp., and Mucorales. *Amphotericin B* has limited activity against the protozoa *Leishmania* spp. and *Naegleria fowleri*. The drug has no antibacterial activity.

Fungal Resistance

Isolates of *Candida lusitaniae* are frequently resistant to *amphotericin B*. *Aspergillus terreus* and *Aspergillus nidulans* likewise appear to be less susceptible to *amphotericin B* than other *Aspergillus* spp. (Steinbach et al., 2004). Mutants selected *in vitro* for resistance to nystatin (a related polyene antifungal used topically) or *amphotericin B* replace ergosterol with certain precursor sterols. Mutations in ergosterol biosynthesis genes *ERG2*, *ERG3*, *ERG5*, *ERG6*, and *ERG11* reduce susceptibility to *amphotericin B*, likely the result of reduced ergosterol in the cell membrane of these

TABLE 61–1 ■ PHARMACOTHERAPY OF MYCOSES[a]

DEEP MYCOSES	DRUGS
Invasive aspergillosis	
Immunosuppressed	Voriconazole, isavuconazole, amphotericin B
Nonimmunosuppressed	Voriconazole, isavuconazole, amphotericin B, itraconazole
Blastomycosis	
Rapidly progressive or CNS	Amphotericin B
Indolent and non-CNS	Itraconazole
Candidiasis	
Deeply invasive	Amphotericin B, fluconazole, voriconazole, caspofungin, micafungin, anidulafungin
Coccidioidomycosis	
Rapidly progressing	Amphotericin B
Indolent	Itraconazole, fluconazole
Meningeal	Fluconazole, intrathecal amphotericin B
Cryptococcosis	
Non-AIDS and initial AIDS	Amphotericin B, flucytosine
Maintenance AIDS	Fluconazole
Histoplasmosis	
Chronic pulmonary	Itraconazole
Disseminated	
Rapidly progressing or CNS	Amphotericin B
Indolent non-CNS	Itraconazole
Maintenance AIDS	Itraconazole
Mucormycosis	Amphotericin B, isavuconazole
Pseudallescheriasis	Voriconazole, itraconazole
Sporotrichosis	
Cutaneous	Itraconazole
Extracutaneous	Amphotericin B, itraconazole
Prophylaxis in the immunocompromised host	Fluconazole Posaconazole Micafungin
Empirical therapy in the immunocompromised host (*category not recognized by FDA*)	Amphotericin B Caspofungin Fluconazole
Microsporidia Infection	Albendazole Fumagillin
Pneumocystis jirovecii pneumonia	Trimethoprim-sulfamethoxazole Pentamidine

SUPERFICIAL MYCOSES	DRUGS *(Administration mode)*
Candidiasis	
Vulvovaginal	*Topical*
	Butoconazole, clotrimazole, miconazole, nystatin, terconazole, tioconazole
	Oral
	Fluconazole
Oropharyngeal	*Topical*
	Clotrimazole, nystatin
	Oral (systemic)
	Fluconazole, itraconazole
	Posaconazole
Cutaneous	*Topical*
	Amphotericin B, clotrimazole, ciclopirox, econazole, ketoconazole, miconazole, nystatin
Ringworm	*Topical*
	Butenafine, ciclopirox, clotrimazole, econazole, haloprogin, luliconazole, ketoconazole, miconazole, naftifine, oxiconazole, sertaconazole, sulconazole, terbinafine, tolnaftate, undecylenate
	Systemic
	Griseofulvin, itraconazole, terbinafine
Onychomycosis	*Systemic*
	Griseofulvin, itraconazole, terbinafine
	Topical
	Efinaconazole

[a] Not all formulations are available in all countries.

isolates (Geber et al., 1995; Hull et al., 2012; Martel et al., 2010). Resistance among clinical isolates of any fungal species is exceedingly rare, presumably because *amphotericin B* is fungicidal, and mutations that affect this critical membrane sterol are associated with significant fitness costs. *Candida auris* is an exception; nearly one-third of clinical isolates are considered resistant to *amphotericin B* based on tentative breakpoints proposed by the CDC (U.S. Centers for Diseases Control and Prevention) (CDC, 2020).

Therapeutic Uses

Intravenous administration of *amphotericin B* is the treatment of choice for invasive mucormycosis and in combination with *5-flucytosine* is the gold standard for induction treatment of cryptococcal meningitis. *Amphotericin B* is also indicated for the treatment of severe or rapidly progressive histoplasmosis, blastomycosis, coccidioidomycosis, and penicilliosis (talaromycosis). *Amphotericin B* is a salvage therapy for patients not responding to azole therapy for invasive aspergillosis, extracutaneous sporotrichosis, fusariosis, alternariosis, or trichosporonosis. *Amphotericin B* (C-AMB or L-AMB) can also be given to selected patients with profound neutropenia with fever who do not respond to broad-spectrum antibacterial agents over 5 to 7 days. However, the more recently developed azoles and echinocandins are generally the drugs of choice for such patients because of their reduced toxicity.

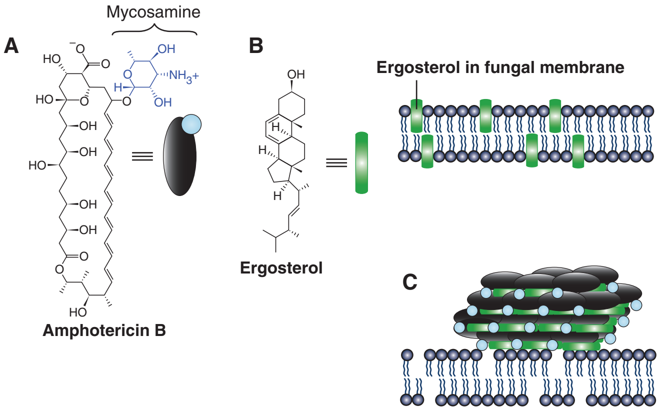


Figure 61–2 *Mechanism of action of amphotericin B.* The antifungal activity of *amphotericin B* depends on its capacity to bind ergosterol in the fungal cell membrane. **A.** *Amphotericin* is an amphipathic molecule with a mycosamine moiety (shown in blue) at one end of a 14-carbon hydrophobic chain. X-ray crystallography shows the molecule to be rigid and rod shaped, with the hydrophilic hydroxyl groups of the macrolide ring forming an opposing face to the lipophilic polyenic portion. **B.** Ergosterol, here depicted as a green rod, decorates both bilayers of the fungal membrane. **C.** *Amphotericin B* appears to form aggregates that sequester and effectively extract ergosterol from lipid bilayers, much like a selective sponge, disrupting membrane structure and resulting in fungal cell death.

Typical adult doses for each *amphotericin B* formulation are summarized in Table 61–2. *Candida* esophagitis responds to much lower doses than deeply invasive mycoses. Intrathecal infusion of C-AMB appears to be useful in patients with meningitis caused by *Coccidioides*. Small doses of C-AMB (from 0.01 to 1.5 mg, one to three times weekly) can be injected into the CSF of the lumbar spine, cisterna magna, or lateral cerebral ventricle. Fever and headache are common reactions that may be decreased by intrathecal administration of 10 to 15 mg of *hydrocortisone*. However, the general use of intrathecal C-AMB administration cannot be recommended due to a lack of clinical data. Local injections of *amphotericin B* into a joint or peritoneal dialysate fluid commonly produce irritation and pain. Intraocular injection following pars plana vitrectomy has been used to treat fungal endophthalmitis.

Adverse Effects

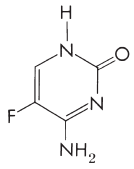
The major acute reactions to intravenous *amphotericin B* formulations are infusion-related fever and chills. These are due to the induction of a proinflammatory response in cells of the innate immune system signaling through the toll-like receptor 2 (TLR2) and CD14 (Rogers et al., 1998; Sau et al., 2003). Infusion-related reactions are most prominent with ABCD, whereas L-AMB administration appears to be less commonly associated with this adverse event. Tachypnea, respiratory stridor, or modest hypotension can also occur, but frank bronchospasm and anaphylaxis are rare. Patients with preexisting cardiac or pulmonary disease may tolerate the metabolic demands of the reaction poorly and develop hypoxia or hypotension. The reaction ends spontaneously in 30 to 45 min; treatment with *meperidine* may shorten it. Pretreatment with oral *acetaminophen* or *ibuprofen* or use of intravenous *hydrocortisone sodium succinate* (hemisuccinate), 0.7 mg/kg, at the start of the infusion decreases reactions. Febrile reactions tend to abate with subsequent infusions.

Azotemia occurs in 80% of patients who receive C-AMB for deep mycoses (Carlson and Condon, 1994). The lipid formulations are significantly less nephrotoxic than C-AMB. Toxicity is dose dependent, usually transient, and increased by concurrent therapy with other nephrotoxic agents, such as aminoglycosides or *cyclosporine*. Although permanent histological changes in renal tubules occur even during short courses of C-AMB, permanent functional impairment is uncommon in adults with normal renal function prior to treatment unless the cumulative dose exceeds 3 to 4 g. Renal tubular acidosis and renal wasting of K^+ and Mg^{2+} also may be seen during and for several weeks after therapy. Supplemental K^+ is required in one-third of patients on prolonged therapy. Saline loading has decreased nephrotoxicity, even in the absence of water or salt deprivation. Administration of 1 L of normal saline intravenously on the day that C-AMB is to be given has been recommended for adults who are able to tolerate the Na^+ load.

Hypochromic, normocytic anemia commonly occurs during treatment with C-AMB. Anemia is less with lipid formulations and usually not seen over the first 2 weeks. The anemia is most likely due to decreased production of erythropoietin and often responds to administration of recombinant erythropoietin. Headache, nausea, vomiting, malaise, weight loss, and phlebitis at peripheral infusion sites are common. Arachnoiditis has been observed as a complication of intrathecal administration of C-AMB.

Flucytosine

Flucytosine (5-fluorocytosine) is a fluorinated pyrimidine related to *fluorouracil* that has a limited role in the treatment of invasive fungal infections.



Flucytosine

Mechanism of Action

All susceptible fungi are capable of deaminating *flucytosine* to 5-*fluorouracil* (5FU) (Figure 61–3), a potent antimetabolite that is used in cancer chemotherapy. *Fluorouracil* is metabolized first to 5-fluorouracil-ribose monophosphate (5FUMP) by the enzyme uracil phosphoribosyl transferase (UPRTase). 5FUMP is then either incorporated into RNA (via synthesis of 5-fluorouridine triphosphate) or metabolized to 5-fluoro-2′-deoxyuridine-5′-monophosphate (5FdUMP), a potent inhibitor of *thymidylate synthase*, ultimately inhibiting DNA synthesis.

TABLE 61–2 ■ PHARMACOKINETIC DATA FOR AMPHOTERICIN B FORMULATIONS AFTER MULTIPLE ADMINISTRATIONS IN HUMANS

PRODUCT	DOSE (mg/kg)	C_{max} (μg/mL)	$AUC_{(1-24h)}$ (μg.hr/mL)	*V* (L/kg)	CL (mL/h/kg)
L-AMB	5	83 ± 35.2	555 ± 311	0.11 ± 0.08	11 ± 6
ABCD[a]	5	3.1	43	4.3	117
ABLC	5	1.7 ± 0.8	14 ± 7	131 ± 7.7	426 ± 188.5
C-AMB	0.6	1.1 ± 0.2	17.1 ± 5	5 ± 2.8	38 ± 15

[a] No longer marketed in the U.S.

For details, see the work of Boswell et al. (1998). From Boswell GW, et al. AmBisome (liposomal amphotericin B): a comparative review. *J Clin Pharmacol*, **1998**, *38*:583–592. © 1998 The American College of Clinical Pharmacology. Reprinted by permission of John Wiley and Sons.

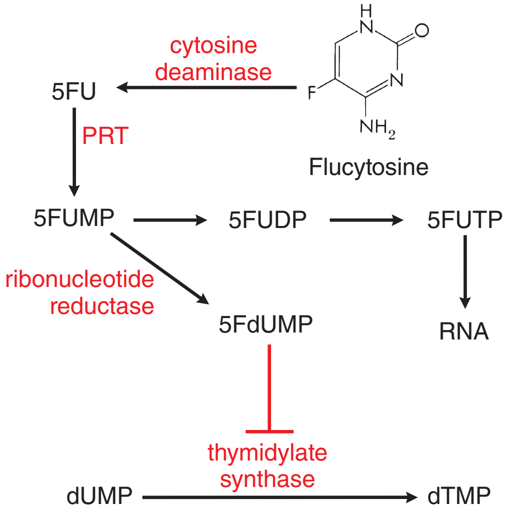


Figure 61–3 *Action of flucytosine in fungi. Flucytosine* is transported by cytosine permease into the fungal cell, where it is deaminated to 5FU. The 5FU is then converted to 5FUMP and then is either converted to 5FUTP (5-fluorouridine triphosphate) and incorporated into RNA or converted by ribonucleotide reductase to 5FdUMP, which is a potent inhibitor of thymidylate synthase. dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; PRT, phosphoribosyl pyrophosphate.

The selective action of *flucytosine* is due to the lack of *cytosine deaminase* in mammalian cells, which prevents metabolism to *fluorouracil*.

ADME

Flucytosine shows excellent bioavailability on oral administration and is absorbed rapidly from the gastrointestinal (GI) tract. It is widely distributed in the body, with a volume of distribution that approximates total body water, and is minimally bound to plasma proteins. The peak plasma concentration in patients with normal renal function is about 70 to 80 μg/mL, achieved 1 to 2 h after a dose of 37.5 mg/kg. The *flucytosine* concentration in CSF is about 65% to 90% of that found simultaneously in the plasma. The drug also appears to penetrate into the aqueous humor.

Approximately 80% of a given dose is excreted unchanged in the urine; concentrations in the urine range from 200 to 500 μg/mL. The $t_{1/2}$ of the drug is 3 to 6 h in normal individuals and may be as long as 200 h in patients with renal failure. The clearance of *flucytosine* is approximately equivalent to that of creatinine. In patients with decreased renal function, reduction of dosage is necessary; the plasma concentration (C_p) should be measured periodically. Peak concentrations should range between 50 and 100 μg/mL. *Flucytosine* is cleared by hemodialysis, and patients undergoing such treatment should receive a single dose of 37.5 mg/kg after dialysis. The drug also is removed by peritoneal dialysis.

Antifungal Activity and Fungal Resistance

Flucytosine is currently used primarily as an adjunctive agent with *amphotericin B* in the induction phase of cryptococcal meningoencephalitis therapy. It has *in vitro* activity against a number of pathogens, but the emergence of resistance limits its usefulness as single-agent therapy.

Drug resistance arising during therapy (secondary resistance) is an important cause of therapeutic failure when *flucytosine* is used alone for cryptococcosis and candidiasis. The mechanism for this resistance can be loss of the permease necessary for cytosine transport or decreased activity of either UPRTase or cytosine deaminase (see Figure 61–3).

In *Candida albicans,* substitution of thymidine for cytosine at nucleotide 301 in the gene encoding UPRTase (*FURl*) causes a cysteine to become an arginine, modestly increasing *flucytosine* resistance (Dodgson et al., 2004). *Flucytosine* resistance is further increased if both *FURl* alleles in the diploid fungus are mutated.

Therapeutic Uses

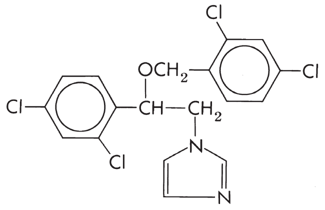
Flucytosine is given orally, 50 to 150 mg/kg per day, in four divided doses at 6-h intervals. Dosage must be adjusted for decreased renal function. *Flucytosine* is used almost exclusively in combination with *amphotericin B* for the treatment of cryptococcal meningitis, and this combination, as compared with *amphotericin B* alone, is associated with improved survival among patients with cryptococcal meningitis (Day et al., 2013). Based on this trial, the addition of *flucytosine* to *amphotericin B* is the current gold standard for the treatment of cryptococcal meningitis. Additional studies have also shown that a 2-week course of *fluconazole* plus *flucytosine* is as effective for cryptococcal meningitis induction therapy as 1 week of *amphotericin B* plus *flucytosine*. The *fluconazole/flucytosine* combination is an all-oral regimen and, therefore, much easier to administer in resource-limited regions with high burden of disease. The main limitation to broad implementation of this therapy in these regions is the expense and poor availability of *flucytosine* (Molloy et al., 2018).

Adverse Effects

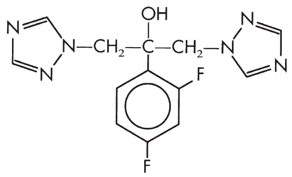
Flucytosine may depress the bone marrow and lead to leukopenia and thrombocytopenia. Patients are more prone to this complication if they have an underlying hematological disorder, are being treated with radiation or drugs that injure the bone marrow, or have a history of treatment with such agents. Other untoward effects, including rash, nausea, vomiting, diarrhea, and severe enterocolitis, have been noted. In about 5% of patients, plasma levels of hepatic enzymes are elevated, but this effect reverses when therapy is stopped. Toxicity is more frequent in patients with AIDS or azotemia (including those who are concurrently receiving *amphotericin B*) and when plasma drug concentrations exceed 100 μg/mL. Toxicity may result from conversion of *flucytosine* to 5FU by the microbial flora in the intestinal tract of the host.

Imidazoles and Triazoles

The azole antifungals include two broad classes, imidazoles and triazoles. Of the drugs now on the market in the U.S., *clotrimazole*, *miconazole*, *ketoconazole*, *econazole*, *butoconazole*, *oxiconazole*, *sertaconazole*, *sulconazole*, *tioconazole*, and *luliconazole* are *imidazoles*, and *efinaconazole*, *terconazole*, *itraconazole*, *fluconazole*, *voriconazole*, *posaconazole*, and *isavuconazole* are *triazoles*. The topical use of azole antifungals is described in the second section of this chapter.



MICONAZOLE



FLUCONAZOLE

Mechanism of Action

The major effect of imidazoles and triazoles on fungi is inhibition of 14-α-sterol demethylase, a cytochrome P450 (CYP) and the product of the gene *ERG11* (Figure 61–4). Imidazoles and triazoles thus impair the biosynthesis of ergosterol, resulting in depletion of membrane ergosterol and accumulation of the toxic product 14α-methyl-3,6-diol, leading to growth arrest (Kanafani and Perfect, 2008), possibly by disrupting the close packing of acyl chains of phospholipids and impairing the functions of membrane-bound enzyme systems. Some azoles directly increase

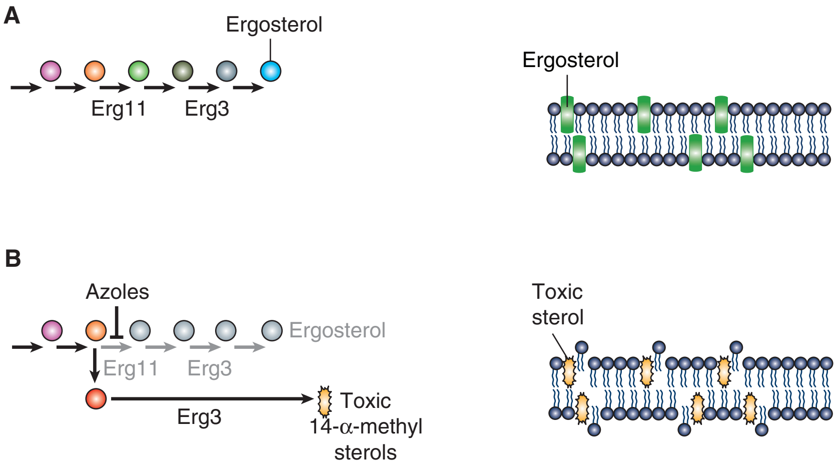


Figure 61–4 *Ergosterol biosynthesis and the mechanism of action of the azole antifungals.* **A.** Fungal ergosterol synthesis proceeds via a series of enzymic steps that include Erg11, a 14-α-sterol demethylase. The completed ergosterol is then inserted into both leaflets of the membrane bilayer. **B.** *Imidazole* and *triazole* antifungals inhibit the activity of 14-α-sterol demethylase, thereby reducing the biosynthesis of ergosterol and leading to the accumulation of 14-α-methylsterols. These methylsterols are toxic, disrupting the close packing of acyl chains of phospholipids, impairing the functions of certain membrane-bound enzyme systems, and thus inhibiting growth of the fungi.

permeability of the fungal cytoplasmic membrane, but the concentrations required are likely only obtained with topical use.

Antifungal Activity

Azoles as a group have clinically useful activity against *C. albicans, Candida tropicalis, Candida parapsilosis, C. neoformans, Blastomyces dermatitidis, H. capsulatum, Coccidioides* spp., *Paracoccidioides brasiliensis,* and ringworm fungi (dermatophytes). *Aspergillus* spp., *Scedosporium apiospermum* (*Pseudallescheria boydii*), *Fusarium*, and *Sporothrix schenckii* are intermediate in susceptibility. *Candida glabrata* exhibits reduced susceptibility to the azoles, whereas *Candida krusei* and the agents of mucormycosis are more resistant. *Posaconazole* and *isavuconazole* have modestly improved spectrum of activity *in vitro* against the agents of mucormycosis.

Resistance

C. glabrata and *C. krusei* are considered intrinsically resistant to *fluconazole*, whereas 90% of *C. auris* isolates are resistant based on tentative breakpoints proposed by the CDC (CDC, 2020). In *C. albicans*, azole resistance can be due in part to accumulation of mutations in *ERG11*, the gene encoding the azole target, 14-α-sterol demethylase. Increased azole efflux by overexpression of ABC (ATP-binding cassette) and/or major facilitator superfamily transporters impart azole resistance in *C. albicans* and *C. glabrata.* Overexpression of these genes is due to activating mutations in genes encoding their transcriptional regulators. Mutation of the C5,6 sterol desaturase gene *ERG3* also can increase azole resistance in some species (Nishimoto et al., 2020). Such mutations prevent formation of the toxic product 14α-methyl-3,6-diol from 14α-methylfecosterol; the resulting accumulation of 14α-methylfecosterol produces functional membranes and overcomes the effect of azoles. Increased production of 14-α-sterol demethylase due to overexpression of *ERG11* occurs, owing to activating mutations in the gene encoding its transcriptional regulator Upc2.

Azole resistance has been increasingly described in isolates of *Aspergillus fumigatus* with mutations in the genes encoding enzymes of the sterol biosynthesis pathway including the azole target, increased azole export, and decreased ergosterol content. The most commonly characterized mechanism of resistance is due to the TR(34)/L98H mutation in the promoter region of *CYP51A,* which encodes the target of azoles in *A. fumigatus* (Berkow et al., 2018). Patients with cryptococcal meningitis who are treated with *fluconazole* monotherapy frequently relapse, and their isolates show decreased *fluconazole* susceptibility, which is linked to aneuploidy (Stone et al., 2019).

Interaction of Azole Antifungals With Other Drugs

The azoles interact with hepatic CYPs as substrates and inhibitors (Table 61–3), providing myriad possibilities for the interaction of azoles with many other medications. Thus, azoles can elevate plasma levels of some coadministered drugs (Table 61–4). Other coadministered drugs can decrease plasma concentrations of azole antifungal agents (Table 61–5). As a consequence of these and other interactions, combinations of certain drugs with azole antifungal medications may be contraindicated (Table 61–6).

Available Agents

Ketoconazole. *Ketoconazole*, administered orally, has been replaced by *itraconazole* except when the lower cost of *ketoconazole* outweighs the advantage of *itraconazole*. *Ketoconazole* is available for topical use, as described further in this chapter.

Itraconazole. *Itraconazole* is a triazole that lacks the corticosteroid suppression associated with *ketoconazole* while retaining most of

TABLE 61–3 ■ INTERACTIONS OF AZOLE ANTIFUNGAL AGENTS WITH HEPATIC CYPs

FLUCONAZOLE	VORICONAZOLE	ITRACONAZOLE	POSACONAZOLE	ISAVUCONAZOLE
CYP3A4, 5, 7 inhibitor (moderate)	CYP2C9 inhibitor and substrate	CYP3A4, 5, 7 inhibitor and substrate	CYP3A4 inhibitor (potent)	CYP3A4 inhibitor and substrate
CYP2C9 inhibitor (strong)	CYP3A4, 5,7 inhibitor			CYP2B6 inhibitor
CYP2C19 inhibitor	CYP2C19 inhibitor and substrate			

TABLE 61–4 ■ DRUGS EXHIBITING ELEVATED C_p WHEN COADMINISTERED WITH AZOLE ANTIFUNGAL AGENTS

Alfentanil	Eplerenone	Losartan	Saquinavir
Alprazolam	Ergot alkaloids	Lovastatin	Sildenafil
Astemizole	Erlotinib	Methadone	Sirolimus
Buspirone	Eszopiclone	Methylprednisolone	Solifenacin
Busulfan	Felodipine	Midazolam	Sunitinib
Carbamazepine	Fexofenadine	Nevirapine	Tacrolimus
Cisapride	Gefitinib	Omeprazole	Triazolam
Cyclosporine	Glimepiride	Phenytoin	Vardenafil
Digoxin	Glipizide	Pimozide	Vinca alkaloids
Docetaxel	Halofantrine	Quinidine	Warfarin
Dofetilide	Haloperidol	Ramelteon	Zidovudine
Efavirenz	Imatinib	Ranolazine	Zolpidem
Eletriptan	Irinotecan	Risperidone	

Mechanism of interaction presumably occurs largely at the level of hepatic CYPs, especially CYPs 3A4, 2C9, and 2D6, but can also involve P-glycoprotein and other mechanisms. Not all drugs listed interact equally with all azoles.

ketoconazole's pharmacological properties and extending the antifungal spectrum. Importantly, *itraconazole* has activity against *Aspergillus* spp., whereas imidazoles do not. *Itraconazole* has been supplanted by other triazoles in the treatment of invasive mold infections but remains an important prophylactic agent in the prevention of mold infections in some patients (e.g., patients with chronic granulomatous disease [CGD]).

ADME. *Itraconazole* is available as a tablet, capsule, and a solution in hydroxypropyl-β-cyclodextrin for oral use. The capsule form of the drug is best absorbed in the fed state, but the oral solution is better absorbed in the fasting state, providing peak plasma concentrations more than 150% of those obtained with the capsule. The tablet formulation is approved only for onychomycosis. Super-bioavailable (SUBA)-*itraconazole*, a reformulation with enhanced GI absorption, has recently been approved by the FDA (U.S. Food and Drug Administration).

Itraconazole is metabolized in the liver. It is both a substrate for and a potent inhibitor of CYP3A4. *Itraconazole* is present in plasma with an approximately equal concentration of a biologically active metabolite, hydroxy-itraconazole. The native drug and metabolite are more than 99% bound to plasma proteins. Neither appears in urine or in CSF. The $t_{1/2}$ of *itraconazole* at steady state is about 30 to 40 h. Steady-state levels of *itraconazole* are not reached for 4 days and those of hydroxy-itraconazole for 7 days; thus, loading doses are recommended when treating deep mycoses. Severe liver disease will increase *itraconazole* plasma concentrations, but azotemia and hemodialysis have no effect.

Therapeutic Uses. *Itraconazole* is the drug of choice for patients with indolent, nonmeningeal infections due to *B. dermatitidis, H. capsulatum, P. brasiliensis,* and *Coccidioides immitis.* The drug also is useful in the therapy of indolent invasive aspergillosis outside the CNS, particularly after the infection has been stabilized with *amphotericin B*. Approximately half of the patients with distal subungual onychomycosis respond to *itraconazole* (Evans and Sigurgeirsson, 1999). Although not approved for these uses, *itraconazole* is a reasonable choice for the treatment of pseudallescheriasis, an infection that does not respond to *amphotericin B* therapy, as well as cutaneous and extracutaneous sporotrichosis, tinea corporis,

TABLE 61–5 ■ SOME DRUGS THAT DECREASE AZOLE CONCENTRATION WHEN COADMINISTERED[a]

DRUG	FLUCONAZOLE	VORICONAZOLE	ITRACONAZOLE	POSACONAZOLE	ISAVUCONAZOLE
Antacids (simultaneous)	–		+	–	–
Barbiturates		+	+[b]		+
Carbamazepine	+	+	+	+	+
H_2 antagonists			+	+	–
Didanosine			+		
Efavirenz		+	+		
Nevirapine		+	+		
Proton pump inhibitors	–	–[c]	+	+	–
Phenytoin	–	+	+	+	
Rifampin	+	+	+	+	+
Rifabutin		+	+	+	
Ritonavir		+			–[d]

[a] +, drug decreases (azole) when coadministered; –, drug does *not* decrease (azole) when coadministered.

[b] Phenobarbital only.

[c] Omeprazole (proton pump inhibitor) and voriconazole increase each other's concentrations in plasma; reduce omeprazole dose by 50% when initiating voriconazole therapy.

[d] With standard doses of ritonavir.

TABLE 61–6 ■ SOME CONTRAINDICATED AZOLE DRUG COMBINATIONS

DRUG	FLUCONAZOLE	VORICONAZOLE	ITRACONAZOLE	POSACONAZOLE	ISAVUCONAZOLE
Alfuzosin		x	x	x	
Artemether	x	x			
Bepridil	x				
Clopidogrel	x				
Conivaptan	x	x	x	x	
Dabigatran			x		
Darunavir		x			
Dronedarone	x	x	x	x	
Everolimus	x	x	x	x	
Lopinavir		x			
Lumefantrine	x	x			
Mesoridazine	x				
Nilotinib	x	x	x	x	
Nisoldipine	Use with caution	x	x	x	
Quinine	x	x			
Rifapentine		x	Use with caution	Use with caution	
Ritonavir		x	Use with caution	Use with caution	Use with caution
Rivaroxaban		x	x		
Salmeterol		x	x	x	
Silodosin		x	x	x	
Simvastatin	Use with caution		x	x	
St. John's wort		x			x
Tetrabenazine	x	x			
Thioridazine	x	x			
Tolvaptan	Avoid	x	x	x	Avoid
Topotecan			x		
Ziprasidone	x	x			

and extensive tinea versicolor. HIV-infected patients with disseminated histoplasmosis or penicilliosis have a decreased incidence of relapse if given prolonged *itraconazole* "maintenance" therapy. *Itraconazole* is not recommended for maintenance therapy of cryptococcal meningitis in HIV-infected patients because of a high incidence of relapse. Long-term *itraconazole* therapy has been used in non–HIV-infected patients with allergic bronchopulmonary aspergillosis to decrease the dose of glucocorticoids and reduce attacks of acute bronchospasm (Salez et al., 1999). *Itraconazole* solution is effective and approved for use in oropharyngeal and esophageal candidiasis. Because the solution has more GI side effects than *fluconazole* tablets, *itraconazole* solution usually is reserved for patients not responding to *fluconazole*. Finally, *itraconazole* is also used as *Aspergillus* prophylaxis in patients with CGD.

Dosage. In treating deep mycoses, a loading dose of 200 mg of *itraconazole* is administered three times daily for the first 3 days. After the loading doses, two 100-mg capsules are given twice daily with food. Divided doses may increase the AUC. For maintenance therapy of HIV-infected patients with disseminated histoplasmosis, 200 mg once daily is used. Onychomycosis can be treated with either 200 mg once daily for 12 weeks or, for infections isolated to fingernails, two monthly cycles consisting of 200 mg twice daily for 1 week followed by a 3-week period of no therapy—so-called pulse therapy (Evans and Sigurgeirsson, 1999). Once-daily *terbinafine* (250 mg), however, is superior to pulse therapy with *itraconazole*. For oropharyngeal candidiasis, *itraconazole* oral solution should be taken during fasting in a dose of 100 mg (10 mL) once daily and swished vigorously in the mouth before swallowing to optimize any topical effect. Patients with esophageal thrush unresponsive or refractory to treatment with *fluconazole* tablets are given 100 mg of the solution twice a day for 2 to 4 weeks. The typical dose for fungal prophylaxis in patients with CGD is 5 mg/kg per day. In pediatric patients, plasma levels are very erratic and therapeutic monitoring should be considered, particularly for patients being treated for systemic fungal infections (e.g., histoplasmosis and blastomycosis) (Downes et al., 2020).

Adverse Effects. *Itraconazole* carries an FDA boxed warning about possible serious adverse effects, including QT prolongation, heart failure, negative inotropic effects, and drug interactions. Serious hepatotoxicity has led, in rare cases, to hepatic failure and death. If symptoms of hepatotoxicity occur, the drug should be discontinued and liver function assessed. In the absence of interacting drugs, *itraconazole* capsules and suspension are well tolerated at 200 mg daily. Diarrhea, abdominal cramps, anorexia, and nausea are more common than with the capsules. Of patients receiving 50 to 400 mg of the capsules per day, nausea and vomiting, hypertriglyceridemia, hypokalemia, increased serum aminotransferase, and rash occurred in 2% to 10%. Occasionally, rash necessitates drug discontinuation, but most adverse effects can be handled with dose reduction. Profound hypokalemia has been seen in patients receiving 600 mg or more daily and in those who recently have received prolonged *amphotericin B* therapy. Doses of 300 mg twice daily have led to other side effects, including adrenal insufficiency, lower limb edema, hypertension, and in at least one case, rhabdomyolysis. Doses greater than 400 mg/day are not recommended for long-term use. Anaphylaxis has been observed rarely, as well as severe rash, including Stevens-Johnson syndrome. *Itraconazole* is contraindicated for the treatment of onychomycosis during pregnancy or for women contemplating pregnancy.

Drug Interactions. Tables 61–4, 61–5, and 61–6 list select interactions of azoles with other drugs. Many of the interactions can result in serious toxicity from the companion drug, such as inducing potentially fatal cardiac arrhythmias when used with *quinidine*, *halofantrine* (an orphan drug used for malaria), *levomethadyl* (an orphan drug used for heroin addiction), *pimozide*, or *cisapride* (available only under an investigational limited access program in the U.S.). Other drugs may decrease *itraconazole* serum levels below therapeutic concentrations (Table 61–5).

Fluconazole.

Fluconazole is a fluorinated bis-triazole.

ADME. *Fluconazole* is almost completely absorbed from the GI tract. Plasma concentrations are essentially the same whether the drug is given orally or intravenously, and its bioavailability is unaltered by food or gastric acidity. Peak plasma concentrations are 4 to 8 μg/mL after repetitive doses of 100 mg. Renal excretion accounts for more than 90% of elimination, and the elimination $t_{1/2}$ is 25 to 30 h. *Fluconazole* diffuses readily into body fluids, including breast milk, sputum, and saliva; concentrations in CSF can reach 50% to 90% of the simultaneous values in plasma. The dosage interval should be increased from 24 to 48 h with a creatinine clearance of 21 to 40 mL/min and to 72 h at 10 to 20 mL/min. A dose of 100 to 200 mg should be given after hemodialysis. About 11% to 12% of drug in the plasma is protein bound.

Therapeutic Uses

- **Candidiasis.** *Fluconazole*, 100 to 200 mg daily for 7 to 14 days, is effective in oropharyngeal candidiasis. A single dose of 150 mg is effective in uncomplicated vaginal candidiasis. A loading dose of 800 mg followed by 400 mg daily is useful in treating candidemia of nonimmunosuppressed patients (Pappas et al., 2007; Rex et al., 1994). Current treatment guidelines for candidemia indicate that *fluconazole* is an acceptable alternative to the first-line therapy of an echinocandin in select patients. *Fluconazole* is recommended as a step-down therapy provided the patient's isolate is susceptible to the azole and follow-up blood cultures are negative (Pappas et al., 2016).
- **Cryptococcosis.** *Fluconazole*, 400 mg daily, is used for the initial 8 weeks of the consolidation phase of the treatment of cryptococcal meningitis in patients with AIDS, after an induction course of at least 2 weeks of intravenous *amphotericin B*. If, after 8 weeks at 400 mg/day, the patient is no longer symptomatic, then the dose is decreased to 200 mg daily and continued indefinitely. If the patient has completed 12 months of treatment of cryptococcosis, responds to combination antiretroviral therapy, has a CD4 count maintained above 200/mm^3 for at least 6 months, and is asymptomatic from cryptococcal meningitis, it is reasonable to discontinue maintenance *fluconazole* as long as the CD4 response is maintained. *Fluconazole*, 400 mg daily, has been recommended as continuation therapy in patients without AIDS with cryptococcal meningitis who have responded to an initial course of C-AMB or L-AMB and for patients with pulmonary cryptococcosis (Perfect et al., 2010). Recent clinical trials indicate that *fluconazole* can be combined with *flucytosine* for induction therapy with efficacy similar to *amphotericin B* combined with *flucytosine* (Molloy et al., 2018).
- **Other Mycoses.** *Fluconazole* is the drug of choice for treatment of coccidioidal meningitis because of good penetration into the CSF and much lower morbidity compared to intrathecal *amphotericin B* (Galgiani et al., 2016). In other forms of coccidioidomycosis, *fluconazole* is comparable to *itraconazole*. Although *itraconazole* is the first-line therapy for blastomycosis, *fluconazole* is an alternative. *Fluconazole* has no useful activity against histoplasmosis or sporotrichosis, and is not effective in the prevention or treatment of aspergillosis. *Fluconazole* has no activity in mucormycosis.

Dosage. *Fluconazole* is marketed in the U.S. as tablets of 50, 100, 150, and 200 mg for oral administration, powder for oral suspension providing 10 and 40 mg/mL, and intravenous solutions containing 2 mg/mL in saline and in dextrose solution. The daily dose of *fluconazole* should be based on the infecting organism and the patient's response to therapy. Generally, recommended dosages are 50 to 400 mg once daily for either oral or intravenous administration. A loading dose of twice the daily maintenance dose is generally administered on the first day of therapy. Prolonged maintenance therapy may be required to prevent relapse. Children are treated with 12 mg/kg once daily (maximum 600 mg/day) without a loading dose. In adult patients, doses of up to 1200 mg have been safely administered in clinical trials for the treatment of cryptococcal meningitis.

Adverse Effects. Side effects in patients receiving more than 7 days of drug, regardless of dose, include nausea, headache, skin rash, vomiting, abdominal pain, and diarrhea (all at 2%–4%). Reversible alopecia may occur with prolonged therapy at 400 mg daily. Rare cases of deaths due to hepatic failure or Stevens-Johnson syndrome have been reported. *Fluconazole* has been associated with skeletal and cardiac deformities in at least three infants born to two women taking high doses during pregnancy. Although a recent clinical study found no association between *fluconazole* receipt by mothers and most birth defects in their children, this study did find a statistically significant increase in tetralogy of Fallot in babies born to mothers who received *fluconazole* (Mølgaard-Nielsen et al., 2013). *Fluconazole* should be avoided during pregnancy.

Drug Interactions. *Fluconazole* is an inhibitor of CYP3A4 and CYP2C9. *Fluconazole*'s drug-drug interactions are shown in Tables 61–4, 61–5, and 61–6. Patients who receive more than 400 mg daily or azotemic patients who have elevated *fluconazole* blood levels may experience drug interactions not otherwise seen.

Voriconazole.

Voriconazole is a triazole with a structure similar to *fluconazole* but with increased activity *in vitro*, an expanded spectrum, and poor aqueous solubility.

ADME. *Voriconazole* is available as 50- or 200-mg tablets or a suspension of 40 mg/mL when hydrated. The tablets, but not the suspension, contain lactose. Because high-fat meals reduce *voriconazole* bioavailability, oral drug should be given either 1 h before or 1 h after meals. Oral bioavailability is 96%; volume of distribution is high (4.6 L/kg), with extensive drug distribution in tissues. Metabolism occurs through CYPs 2C19 and 2C9; CYP3A4 plays a limited role. Plasma elimination $t_{1/2}$ is 6 h. *Voriconazole* exhibits nonlinear metabolism so that higher doses may cause greater-than-linear increases in systemic drug exposure. Genetic polymorphisms in CYP2C19 can cause up to 4-fold differences in drug exposure: About 20% of Asians are homozygous poor metabolizers, compared with 2% of whites and African Americans. Less than 2% of parent drug is recovered from urine; 80% of the inactive metabolites are excreted in the urine. The oral dose does not have to be adjusted for azotemia or hemodialysis. Patients with mild-to-moderate cirrhosis should receive the same loading dose of *voriconazole* but half the maintenance dose. The intravenous formulation of *voriconazole* contains sulfobutyl ether β-cyclodextrin (SBECD), which is excreted by the kidney. Significant accumulation of SBECD occurs with a creatinine clearance less than 50 mL/min; in that setting, oral *voriconazole* is preferred. Therapeutic drug monitoring is frequently used, with *target* serum concentrations between 1 and 5 mg/L thought to maximize efficacy and minimize adverse events. Monitoring is particularly important for children, who have very unpredictable plasma levels of *voriconazole* (Downes et al., 2020).

Therapeutic Uses. *Voriconazole* shows superior efficacy to C-AMB in the therapy of invasive aspergillosis using rate of response as the primary end point (Herbrecht et al., 2002); survival also is superior with *voriconazole*. *Voriconazole* was compared to L-AMB for empirical therapy of neutropenic patients whose fever did not respond to more than 96 h of antibacterial therapy. Because the 95% confidence interval in this noninferiority trial permitted the possibility that *voriconazole* might be more than 10% worse than L-AMB, the FDA did not approve *voriconazole* for this use

(Walsh et al., 2002); however, in a secondary analysis, there were fewer breakthrough infections with *voriconazole* (1.9%) than with L-AMB (5%).

Voriconazole is approved for use in esophageal candidiasis. In nonneutropenic patients with candidemia, *voriconazole* is comparable in efficacy and less toxic than initial C-AMB followed by *fluconazole* (Kullberg et al., 2005). *Voriconazole* is approved for initial treatment of candidemia and invasive aspergillosis, as well as for salvage therapy in patients with *P. boydii* (*S. apiospermum*) and *Fusarium* infections. Positive responses in patients with cerebral fungal infections suggest that the drug penetrates infected brain.

Dosage. Treatment is usually initiated with an intravenous infusion of 6 mg/kg every 12 h for two doses, followed by 3 to 4 mg/kg every 12 h, administered no faster than 3 mg/kg/h. As the patient improves, oral administration is continued as 200 mg every 12 h. Patients failing to respond may be given 300 mg every 12 h.

Adverse Effects. *Voriconazole* is teratogenic in animals and generally contraindicated in pregnancy. Although *voriconazole* is generally well tolerated, occasional cases of hepatotoxicity have been reported, and liver function should be monitored. *Voriconazole* can prolong the QTc interval, a significant issue in patients with other risk factors for torsades de pointes. Transient visual or auditory hallucinations are frequent after the first dose, usually at night and particularly with intravenous administration. Symptoms diminish with time. Patients receiving their first intravenous infusion have had anaphylactoid reactions. Rash occurs in 6% of patients. The cyclodextrin component of intravenous formulations may be toxic to the kidney; thus, intravenous *voriconazole* should be used with caution in patients with renal failure (Neofytos et al., 2012).

Drug Interactions. *Voriconazole* is metabolized by, and inhibits, CYPs 2C19, 2C9, and 3A4 (in that order of decreasing potency). The major metabolite of *voriconazole*, the *voriconazole* N-oxide, also inhibits these CYPs. Inhibitors or inducers of these CYPs may increase or decrease *voriconazole* plasma concentrations, respectively. *Voriconazole* and its major metabolite can increase the plasma concentrations of other drugs metabolized by these enzymes (see Tables 61–4, 61–5, and 61–6). Because the AUC of *sirolimus* increases 11-fold in the presence of *voriconazole*, coadministration is contraindicated. When starting *voriconazole* in a patient receiving 40 mg/day or more of *omeprazole*, the dose of *omeprazole* should be reduced by half.

Posaconazole. *Posaconazole* is a synthetic structural analogue of *itraconazole* with the same broad antifungal spectrum but with up to 4-fold greater activity *in vitro* against yeasts and filamentous fungi, including some, but not all, of the agents that cause mucormycosis (Frampton and Scott, 2008). Activity against yeasts *in vitro* is similar to *voriconazole*. The mechanism of action is the same as other imidazoles, inhibition of sterol 14-α-demethylase.

ADME. *Posaconazole* is available as a delayed-release tablet, intravenous formulations, and a flavored suspension. The delayed-release tablet and intravenous formulations provide a more consistent bioavailability in the presence of concomitant disease states, medications, and dietary considerations that alter concentrations achievable with the oral suspension (Guarascio and Slain, 2015). The bioavailability of the oral suspension (*posaconazole*, 40 mg/mL) is significantly enhanced by the concomitant presence of food (Courtney et al., 2003; Krieter et al., 2004). The drug has a long $t_{1/2}$ (25–31 h), a large volume of distribution (331–1341 L), and extensive protein binding (>98%). Systemic exposure is four times higher in homozygous CYP2C19 slow metabolizers than in homozygous wild-type metabolizers. Steady-state concentrations are reached in 7 to 10 days when dosed four times daily. Renal impairment does not alter plasma concentrations; hepatic impairment causes a modest increase. Almost 80% of the drug is excreted in the stool, with 66% as unchanged drug. The major metabolic pathway is hepatic UDP glucuronidation (Krieter et al., 2004). Hemodialysis does not remove drug from the circulation. Gastric acid improves absorption (Krishna et al., 2009); drugs that reduce gastric acid (e.g., *cimetidine* and *esomeprazole*) decrease *posaconazole* exposure by 32% to 50% (Frampton and Scott, 2008). Diarrhea reduces the average C_p by 37% (Smith et al., 2009).

Therapeutic Uses. *Posaconazole* is approved for treatment of oropharyngeal candidiasis, although *fluconazole* is the preferred drug because of safety and cost. *Posaconazole* is also approved for prophylaxis against candidiasis and aspergillosis in patients more than 13 years of age who have prolonged neutropenia or severe graft-versus-host disease (Ullmann et al., 2007). It is approved in the E.U. as salvage therapy for aspergillosis and several other infections, as are *itraconazole* and *voriconazole*. A recent comparison trial of *posaconazole* and *voriconazole* supports the use of *posaconazole* as a first-line treatment for invasive *Aspergillus* (Maertens et al., 2021). *Posaconazole* has increased activity against the molds that cause mucormycosis and is frequently used as an alternative to *amphotericin B* for intolerant patients (Cornely et al., 2019).

Dosage. For prophylaxis of invasive *Aspergillus* and *Candida* infections, the adult intravenous dose is 300 mg twice on day 1 and 300 mg daily thereafter. Duration of therapy is based on recovery from neutropenia or immunosuppression. The same dose is used for the delayed-release tablets. The dose for the oral suspension is 200 mg (5 mL) three times daily.

Adverse Effects. Common adverse effects include nausea, vomiting, diarrhea, abdominal pain, and headache (Smith et al., 2009). Although adverse effects occur in at least a third of patients, the rate of discontinuation due to adverse effects in long-term studies has been only 8%.

Drug Interactions. *Posaconazole* inhibits CYP3A4. Coadministration with *rifabutin* or *phenytoin* increases the plasma concentration of these drugs and decreases *posaconazole* exposure by 2-fold. *Posaconazole* increases the AUC of *cyclosporine*, *tacrolimus* (121%), *sirolimus* (790%), midazolam (83%), and other CYP3A4 substrates (Table 61–4) (Frampton and Scott, 2008; Krishna et al., 2009; Moton et al., 2009). *Posaconazole* can prolong the QTc interval and should not be coadministered with drugs that are CYP3A4 substrates that likewise prolong the QTc interval, such as *methadone*, *haloperidol*, *pimozide*, *quinidine*, *risperidone*, *sunitinib*, *tacrolimus*, and *halofantrine* (see Table 61–4).

Isavuconazole. *Isavuconazole* is a triazole that is administered as the isavuconazonium prodrug.

ADME. *Isavuconazole* is available in both oral and cyclodextrin-free intravenous formulations. It is highly bioavailable (98%) and is more than 99% protein bound in serum. Administration of oral isavuconazonium with food reduces AUC by about 20%. The parent form, isavuconazonium sulfate, is rapidly hydrolyzed to the active form, *isavuconazole*, which has a long plasma half-life (~130 h). Overall bioavailability of the active form is 98%. *Isavuconazole* is eliminated by hepatic metabolism, predominantly by CYP3A4 and CYP3A5. Less than 1% of *isavuconazole* is excreted unchanged in urine. No renal dose adjustments are needed (Rybak et al., 2015).

Therapeutic Uses. *Isavuconazole* exhibits a broad spectrum of activity against most yeast species, including *Candida* spp., *Cryptococcus gattii* and *C. neoformans*, and molds such as *Aspergillus* spp. and most Mucorales species complex. The drug is approved for the treatment of invasive aspergillosis and invasive mucormycosis.

Dosage. *Isavuconazole* is dosed as 372 mg isavuconazonium sulfate (equivalent to 200 mg of *isavuconazole*) every 8 h for six doses followed by 372 mg isavuconazonium sulfate by mouth or intravenously once daily starting 12 to 24 h after the last loading dose.

Adverse Effects. *Isavuconazole* is generally well tolerated. GI disorders, pyrexia, hypokalemia, headache, constipation, and cough are the most frequently reported adverse effects.

Drug Interactions. *Isavuconazole* is both a substrate and an inhibitor of CYP3A4. Consequently, a 5-fold increase in *isavuconazole* AUC results when it is administered with strong CYP inhibitors such as *ketoconazole*. Substantial reductions in *isavuconazole* AUC also result from coadministration of *isavuconazole* with *rifampin*. *Midazolam* and *sirolimus* AUCs are increased by coadministration with *isavuconazole*. *Isavuconazole* causes a dose-related shortening of QTc and is contraindicated in patients with familial short QT syndrome.

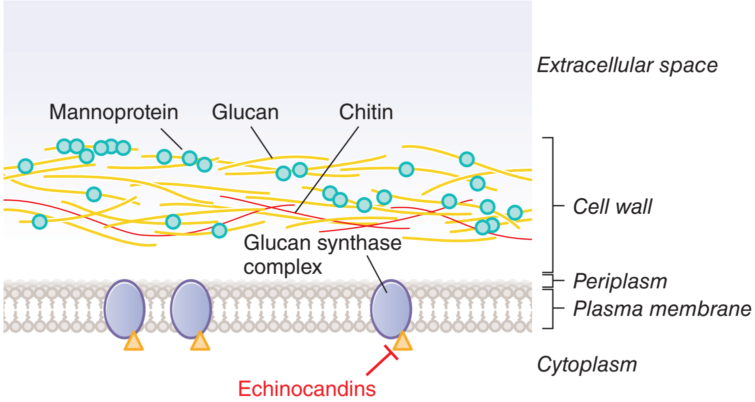


Figure 61–5 *The fungal cell wall and membrane and the action of echinocandins.* The strength of the fungal cell wall is maintained by fibrillar polysaccharides, largely β-1,3-glucan and chitin, which bind covalently to each other and to proteins. A glucan synthase complex in the plasma membrane catalyzes the synthesis of β-1,3-glucan; the glucan is extruded into the periplasm and incorporated into the cell wall. Echinocandins inhibit the activity of the glucan synthase complex, resulting in loss of the structural integrity of the cell wall. The Fks1p subunit of glucan synthase appears to be the target of echinocandins, and mutations in Fks1p cause resistance to echinocandins.

Echinocandins

Echinocandins are cyclic lipopeptides with a nucleus. Three echinocandins are approved for clinical use: *caspofungin*, *anidulafungin*, and *micafungin*. All act through the same mechanism but differ in pharmacological properties. Fungi that are susceptible to echinocandins include *Candida* and *Aspergillus* spp. (Bennett, 2006).

General Pharmacological Characteristics

Mechanism of Action. The echinocandins inhibit 1,3-β-D-glucan synthesis, which is an essential component of the fungal cell wall and is required for in cellular integrity (Figure 61–5).

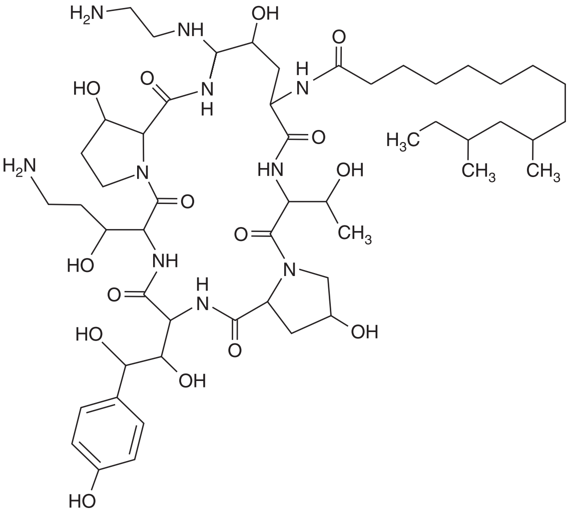
Antifungal Activity. Echinocandins exhibit fungicidal activity against *Candida* spp. In contrast, they are fungistatic against *Aspergillus* spp. and cause morphological changes to the filaments. Echinocandins do not appear to have clinically useful activity against dimorphic fungi such as *H. capsulatum* and do not have clinically useful activity against *C. neoformans*, *Trichosporon* spp., *Fusarium* spp., or agents of mucormycosis.

Resistance. Echinocandin resistance has emerged as a clinical problem and results from mutations leading to amino acid substitutions in the Fks subunits of glucan synthase (Perlin, 2015). Multidrug transporters do not appear to play a role in echinocandin resistance. Mutations conferring resistance occur in two conserved "hot spot" regions of *FKS1* in *C. albicans* and *C. auris*, and in *FKS1* and *FKS2* in *C. glabrata*. *Candida parapsilosis* complex and *Candida guilliermondii* display reduced *in vitro* echinocandin susceptibility as compared to other *Candida* spp. owing to inherently occurring polymorphisms in Fks hot spot regions. Species-specific clinical breakpoints for echinocandins have been recently described.

Echinocandins differ somewhat pharmacokinetically (Table 61–7), but all share extensive protein binding (>97%), inability to penetrate into CSF, lack of renal clearance, and only a slight-to-modest effect of hepatic insufficiency on plasma drug concentrations (Kim et al., 2007; Wagner et al., 2006). Currently available echinocandins also lack oral bioavailability and are available only for intravenous administration. Generally speaking, adverse effects are minimal and rarely lead to drug discontinuation (Kim et al., 2007). All three agents are well tolerated, with the exception of phlebitis at the infusion site. Histamine-like effects have been reported with rapid infusions. All three echinocandins are contraindicated in pregnancy.

Available Agents

Caspofungin. *Caspofungin acetate* is a water-soluble, semisynthetic lipopeptide synthesized from the fermentation product of *Glarea lozoyensis* (Johnson and Perfect, 2003; Keating and Figgitt, 2003).



CASPOFUNGIN

TABLE 61–7 ■ PHARMACOKINETIC DATA FOR ECHINOCANDINS IN HUMANS

DRUG	DOSE (mg)	C_{max} (μg/mL)	AUC_{0-24h} (mg • h/L)	$t_{1/2}$ (h)	CL (mL/min/kg)	V_d (L)
Caspofungin	70	12	93.5	10	0.15	9.5
Micafungin	75	7.1	59.9	13	0.16	14
Anidulafungin	200	7.5	104.5	25.6	0.16	33.4

For details, see Wagner C, et al. (2006). From Wagner C, et al. The echinocandins: comparison of their pharmacokinetics, pharmacodynamics and clinical applications. *Pharmacology*, **2006**, 78:161–177. Copyright © 2006. Reproduced by permission of Karger Publishers, Basel, Switzerland.

ADME. Catabolism is largely by hydrolysis and *N*-acetylation, with excretion of the metabolites in the urine and feces. Mild and moderate hepatic insufficiency increase the AUC by 55% and 76%, respectively.

Therapeutic Use. *Caspofungin* is approved for the treatment of invasive candidiasis. It is a first-line agent, along with other echinocandins, for the initial treatment of candidemia (Pappas et al., 2016). It is also approved as salvage therapy for patients with invasive aspergillosis who fail or are intolerant of approved drugs, such as *amphotericin B* formulations or *voriconazole*. *Caspofungin* is also approved for both esophageal and invasive candidiasis (Mora-Duarte et al., 2002; Villanueva et al., 2001) and for treatment of persistently febrile neutropenic patients with suspected fungal infections (Walsh et al., 2004).

Dosage. *Caspofungin* is administered intravenously once daily over 1 h. For candidemia and salvage therapy of aspergillosis, the initial dose is 70 mg, followed by 50 mg daily. The dose should be increased to 70 mg daily in patients receiving *rifampin* as well as in those failing to respond to 50 mg. Esophageal candidiasis is treated with 50 mg daily. In moderate hepatic failure, the dose should be reduced to 35 mg daily.

Drug Interactions. *Caspofungin* increases *tacrolimus* levels by 16%, which should be managed by standard monitoring. *Cyclosporine* slightly increases *caspofungin* levels. *Rifampin* and other drugs activating CYP3A4 can cause a slight reduction in *caspofungin* levels.

Micafungin. *Micafungin* is a water-soluble semisynthetic echinocandin derived from the fungus *Coleophoma empedri*.

ADME; Drug Interactions. *Micafungin* has linear pharmacokinetics over a large range of doses (1–3 mg/kg) and ages (premature infants to elderly). Small amounts of drug are metabolized in the liver by arylsulfatase and catechol *O*-methyltransferase. Hydroxylation by CYP3A4 is barely detectable. Unlike *caspofungin*, reduction of the *micafungin* dose in moderate hepatic failure is not required. *Micafungin* shows age-dependent clearance in children, with rapid clearance in premature infants and intermediate clearance in children 2 to 8 years of age, compared to older children and adults (Downes et al., 2020).

In normal volunteers, *micafungin* appears to be a mild inhibitor of CYP3A4, increasing the AUC of *nifedipine* by 18% and *sirolimus* by 21%. *Micafungin* has no effect on *tacrolimus* clearance.

Therapeutic Uses. *Micafungin* is approved for the treatment of invasive candidiasis (Fritz et al., 2008) and esophageal candidiasis and for prophylaxis in hematopoietic stem cell transplant recipients.

Dosage. *Micafungin* is administered intravenously as a 100-mg daily dose over 1 h for adults, with 50 mg recommended for prophylaxis and 150 mg for esophageal candidiasis. No loading dose is required.

Anidulafungin. *Anidulafungin* is a water-insoluble semisynthetic compound extracted from the fungus *A. nidulans*, from which the drug's name derives.

ADME; Drug Interactions. *Anidulafungin* is cleared from the body by slow chemical degradation (Vazquez and Sobel, 2006). No hepatic metabolism or renal excretion of active drug occurs; thus, no dose adjustment for hepatic or renal failure is needed. No clinically relevant drug-drug interactions have been observed with drugs likely to be coadministered with *anidulafungin*.

Therapeutic Use and Dosing. *Anidulafungin* is approved for the treatment of candidemia and other forms of *Candida* infections (Reboli et al., 2007), including intra-abdominal abscess, peritonitis, and esophageal candidiasis. For invasive candidiasis, *anidulafungin* is given daily as a loading dose of 200 mg followed by 100 mg daily. For esophageal candidiasis, a loading dose of 100 mg is followed by 50 mg daily. *Anidulafungin* is not approved for use in children; the other two currently available echinocandins are approved and frequently used in premature infants.

Other Systemic Antifungal Agents

Griseofulvin

Griseofulvin is an orally administered, fungistatic antifungal agent originally isolated from the mold *Penicillium griseofulvum*. It is practically insoluble in water.

Mechanism of Action. *Griseofulvin* inhibits microtubule function and thereby disrupts assembly of the mitotic spindle, which disrupts fungal cell division.

ADME. Blood levels after oral administration of *griseofulvin* are quite variable. Some studies have shown improved absorption when the drug is taken with a fatty meal. Because the rates of dissolution and disaggregation limit the bioavailability of *griseofulvin*, microsize and ultramicrosize powders are now used. *Griseofulvin* has a plasma $t_{1/2}$ of about 1 day; about 50% of the oral dose can be detected in the urine within 5 days, mostly in the form of metabolites. The primary metabolite is methylgriseofulvin. Barbiturates decrease *griseofulvin* absorption from the GI tract.

Griseofulvin is deposited in keratin precursor cells; when these cells differentiate, the drug is tightly bound to, and persists in, keratin, providing prolonged resistance to fungal invasion. For this reason, the new growth of hair or nails is the first to become free of disease. As the fungus-containing keratin is shed, it is replaced by normal tissue. *Griseofulvin* is detectable in the stratum corneum of the skin within 4 to 8 h of oral administration. Sweat and transepidermal fluid loss play an important role in the transfer of the drug in the stratum corneum. Only a very small fraction of a dose of the drug is present in body fluids and tissues.

Antifungal Activity. *Griseofulvin* is fungistatic *in vitro* for various species of the dermatophytes *Microsporum, Epidermophyton,* and *Trichophyton.* The drug has no effect on other fungi or on bacteria. Although failure of ringworm lesions to improve is not rare, isolates from these patients usually are still susceptible to *griseofulvin in vitro*.

Therapeutic Uses. Mycotic disease of the skin, hair, and nails due to *Microsporum, Trichophyton,* or *Epidermophyton* responds to *griseofulvin* therapy. For tinea capitis in children, *griseofulvin* remains the drug of choice for efficacy, safety, and availability as an oral suspension. Efficacy is best for tinea capitis caused by *Microsporum canis, Microsporum audouinii, Trichophyton schoenleinii,* and *Trichophyton verrucosum. Griseofulvin* is also effective for ringworm of the glabrous skin; tinea cruris and tinea corporis caused by *M. canis, Trichophyton rubrum, T. verrucosum,* and *Epidermophyton floccosum*; and tinea of the hands (*T. rubrum* and *Trichophyton mentagrophytes*) and beard (*Trichophyton* spp.). *Griseofulvin* also is highly effective in the treatment of tinea pedis, the vesicular form of which is most commonly due to *T. mentagrophytes* and the hyperkeratotic type to *T. rubrum.* Topical therapy is sufficient for most cases of tinea pedis. *T. rubrum* and *T. mentagrophytes* infections may require higher-than-conventional doses of *griseofulvin*. Treatment must be continued until infected tissue is replaced by normal hair, skin, or nails, which requires 1 month for scalp and hair ringworm, 6 to 9 months for fingernails, and at least a year for toenails. *Itraconazole* or *terbinafine* is much more effective for onychomycosis.

Adverse Effects. The incidence of serious reactions due to *griseofulvin* is very low: headache (15% of patients), GI and nervous system manifestations, and augmentation of the effects of alcohol. Hepatotoxicity has been observed. Hematological effects include leukopenia, neutropenia, punctate basophilia, and monocytosis; these often disappear despite continued therapy. Blood studies should be carried out at least once a week during the first month of treatment or longer. Common renal effects include albuminuria and cylindruria without evidence of renal insufficiency. Reactions involving the skin are cold and warm urticaria, photosensitivity, lichen planus, erythema, erythema multiforme–like rashes, and vesicular and morbilliform eruptions. Serum sickness syndromes and severe angioedema develop rarely. Estrogen-like effects have been observed in children. A moderate but inconsistent increase of fecal protoporphyrins has been noted with chronic use.

Drug Interactions. *Griseofulvin* induces hepatic CYPs and thereby increases the rate of metabolism of *warfarin*. Consequently, the dose of *warfarin* should be adjusted in some patients. The drug may also reduce the efficacy of low-estrogen oral contraceptive agents, probably by a similar mechanism.

Terbinafine

Terbinafine is a synthetic allylamine, structurally similar to the topical agent *naftifine* (see discussion that follows). It inhibits fungal squalene epoxidase and thereby reduces ergosterol biosynthesis.

ADME. *Terbinafine* is well absorbed, but bioavailability is about 40% due to first-pass metabolism in the liver. The drug accumulates in skin, nails, and fat. The initial $t_{1/2}$ is about 12 h but extends to 200 to 400 h at steady state. *Terbinafine* is not recommended in patients with marked azotemia or hepatic failure. *Rifampin* decreases and *cimetidine* increases plasma *terbinafine* concentrations.

Therapeutic Uses. *Terbinafine*, given as one 250-mg tablet daily for adults, is somewhat more effective than *itraconazole* for nail onychomycosis. Duration of treatment varies with the site of infection but typically ranges between 6 and 12 weeks. The efficacy for the treatment of onychomycosis can be improved by the simultaneous use of *amorolfine* 5% nail lacquer (*amorolfine* is not approved for use in the U.S.). *Terbinafine* is also effective for the treatment of tinea capitis and has been used for the off-label treatment of ringworm elsewhere on the body.

Adverse Effects. The drug is well tolerated, with a low incidence of GI distress, headache, or rash. Very rarely, fatal hepatotoxicity, severe neutropenia, Stevens-Johnson syndrome, or toxic epidermal necrolysis may occur. Systemic *terbinafine* therapy for onychomycosis should be postponed until after pregnancy is complete.

Agents Active Against Microsporidia and *Pneumocystis*

Microsporidia are spore-forming unicellular eukaryotic organisms that were once thought to be parasites but are now classified as fungi (Field and Milner, 2015). They can cause several disease syndromes, including diarrhea in immunocompromised individuals.

Albendazole

Intestinal infections with most microsporidia are treated with *albendazole*, an inhibitor of α-tubulin polymerization (see Chemotherapy of Helminth Infections, Chapter 68) (Anane and Attouchi, 2010).

Fumagillin

Fumagillin is an acyclic polyene macrolide produced by the fungus *A. fumigatus*. *Fumagillin* and its synthetic analogue TNP-470 are toxic to microsporidia.

Immunocompromised individuals with intestinal microsporidiosis due to *Enterocytozoon bieneusi* (which does not respond as well to *albendazole*) can be treated successfully with *fumagillin* (Didier et al., 2005; Rex and Stevens, 2014; Szumowski and Troemel, 2015). For the treatment of intestinal microsporidiosis caused by *E. bieneusi*, *fumagillin* is used at a dose of 20 mg orally three times daily for 2 weeks (Medical-Letter, 2013; Molina et al., 2002; Rex and Stevens, 2014). *Fumagillin* is used topically to treat keratoconjunctivitis caused by *Encephalitozoon hellem* at a dose of 3 to 10 mg/mL in a balanced salt suspension. Adverse effects of *fumagillin* may include abdominal cramps, nausea, vomiting, and diarrhea. Reversible thrombocytopenia and neutropenia also have been reported (Anane and Attouchi, 2010). *Fumagillin* is not approved for use in humans in the U.S.

Pentamidine

Pneumocystis jirovecii is another fungus that, until recently, was classified as a protozoan parasite. It is the causative agent of PJP (*Pneumocystis jirovecii* pneumonia), formerly known as PCP (*Pneumocystis carinii* pneumonia). *Pentamidine* is one of several drugs or drug combinations used to treat or prevent PJP, which is a major cause of mortality in immunocompromised individuals, including patients with AIDS. Note, however, that *trimethoprim-sulfamethoxazole* is the drug of choice for the treatment and prevention of PJP (see Chapter 57).

Therapy for PJP. *Pentamidine* as therapy for PJP is reserved for two indications:

- As a 4 mg/kg single daily intravenous dose for 21 days to treat severe PJP in individuals who cannot tolerate *trimethoprim-sulfamethoxazole* and are not candidates for alternative agent
- As a "salvage" agent for individuals with PJP who fail to respond to *trimethoprim-sulfamethoxazole* (*pentamidine* may be less effective than the combination of *clindamycin* and *primaquine* or *atovaquone* for this indication) (Gilroy and Bennett, 2011; Rex and Stevens, 2014)

Prophylaxis. *Pentamidine* administered as an aerosol preparation is used to prevent PJP in at-risk individuals who cannot tolerate *trimethoprim-sulfamethoxazole*, such as patients with severe bone marrow suppression. For prophylaxis, *pentamidine isethionate* is given monthly as a 300-mg dose in a 5% to 10% nebulized solution over 30 to 45 min (Gilroy and Bennett, 2011). Aerosolized *pentamidine* has several disadvantages, including its failure to treat any extrapulmonary sites of *Pneumocystis*, the lack of efficacy against any other potential opportunistic pathogens, and a risk for pneumothorax (Rex and Stevens, 2014). Recently, an intravenous dose of *pentamidine* every 28 days has been successfully used as PJP prophylaxis (Diri et al., 2016).

Adverse Effects. Reported adverse effects of inhaled *pentamidine* include bronchospasm (15%). For parenteral *pentamidine*, adverse effects (more likely with rapid injection) can include injection site irritation and pain, cardiac arrhythmias, severe hypotension, hypoglycemia, and acute pancreatitis. The FDA warns that administration of *pentamidine* should be limited to patients in whom *Pneumocystis* is identified and should proceed with careful monitoring for the development of adverse effects.

Topical Antifungal Agents

Topical agents are useful for the treatment of many superficial fungal infections, such as those confined to the stratum corneum, squamous mucosa, or cornea. Examples of infections that respond to topical therapy include dermatophytosis (ringworm), candidiasis, tinea versicolor, piedra, tinea nigra, and fungal keratitis. Preferred formulations for cutaneous application usually are creams or solutions. Ointments are inconvenient and can be too occlusive to the skin, particularly if the affected area is a macerated, fissured, or intertriginous lesion. Antifungal powders, whether applied by shake containers or aerosols, are useful only for lesions of the feet, groin, and similar intertriginous areas. With few exceptions, topical administration of antifungal agents usually is not successful for mycoses of the nails (onychomycosis) and hair (tinea capitis) and should not be used for the treatment of subcutaneous mycoses, such as sporotrichosis and chromoblastomycosis. Regardless of formulation, penetration of topical drugs into hyperkeratotic lesions often is poor. Removal of thick, infected keratin is sometimes a useful adjunct to therapy.

Topical Imidazoles and Triazoles

The imidazoles and triazoles are closely related classes of drugs that are synthetic antifungal agents used both topically and systemically. Indications for their topical use include ringworm, tinea versicolor, and mucocutaneous candidiasis. Resistance to imidazoles or triazoles is rare among the fungi that cause ringworm. Selection of one of these agents for topical use should be based on cost and availability because *in vitro* fungal susceptibility testing does not correlate with clinical responses. The mechanism of action of the azole antifungals was discussed previously in this chapter.

Modes of Administration

Cutaneous Application. The preparations for cutaneous use are effective for tinea corporis, tinea pedis, tinea cruris, tinea versicolor, and cutaneous candidiasis. They should be applied twice a day for 3 to 6 weeks. Cutaneous formulations are not suitable for oral, vaginal, or ocular use.

Vaginal Application. Vaginal creams, suppositories, and tablets for vaginal candidiasis are all used once a day for 1 to 7 days, preferably at bedtime to facilitate retention. None is useful in trichomoniasis. Most vaginal creams are administered in 5-g amounts. Three vaginal formulations—*clotrimazole* tablets (no longer marketed in the U.S.), *miconazole* suppositories, and *terconazole* cream—come in both low- and high-dose preparations. A shorter duration of therapy is recommended for the higher dose of each. These preparations are administered for 3 to 7 days. Approximately 3% to 10% of the vaginal dose is absorbed. Although some imidazoles are teratogenic in rodents, no adverse effects on the human fetus have been attributed to the vaginal use of imidazoles or triazoles. The most common side effect is vaginal burning or itching. A male sexual partner may experience mild penile irritation.

Oral Use. Use of the oral troche of *clotrimazole* is properly considered as topical therapy. The only indication for this 10-mg troche is oropharyngeal candidiasis. Antifungal activity is due entirely to the local concentration of the drug; there is no systemic effect.

Individual Agents

Clotrimazole

Absorption of *clotrimazole* is less than 0.5% after application to the intact skin; from the vagina, it is 3% to 10%. Fungicidal concentrations remain in the vagina for as long as 3 days after application of the drug. In adults, an oral dose of 200 mg/day will give rise initially to plasma concentrations of 0.2 to 0.35 μg/mL, followed by a progressive decline.

In a small fraction of patients, *clotrimazole* on the skin may cause skin irritation, stinging sensations, erythema, edema, vesication, desquamation, pruritus, or urticaria. When applied to the vagina, about 1.6% of patients experience a mild burning sensation. In rare instances, lower abdominal cramps, a slight increase in urinary frequency, or skin rash may occur. Occasionally, a patient's sexual partner may experience penile or urethral irritation. Oral *clotrimazole* troches cause GI irritation in about 5% of patients.

Therapeutic Uses. *Clotrimazole* is available as a 1% cream, lotion, powder (no longer marketed in the U.S.), aerosol solution, and solution; 1% or 2% vaginal cream; vaginal tablets (no longer marketed in the U.S.) of 100, 200, or 500 mg; and 10-mg troches. On the skin, applications are made twice a day. For the vagina, the standard regimens are one 100-mg tablet once a day at bedtime for 7 days, one 200-mg tablet daily for 3 days, one 500-mg tablet inserted only once, or 5 g of cream once a day for 3 days (2% cream) or 7 days (1% cream). For oropharyngeal candidiasis, troches are to be dissolved slowly in the mouth five times a day for 14 days.

Topical *clotrimazole* cures dermatophyte infections in 60% to 100% of cases. The cure rates in cutaneous candidiasis are 80% to 100%. In vulvovaginal candidiasis, the cure rate is usually greater than 80% when the 7-day regimen is used. A 3-day regimen of 200 mg once a day appears to be similarly effective, as does single-dose treatment (500 mg). Recurrences are common after all regimens. The cure rate with oral troches for oral and pharyngeal candidiasis may be as high as 100% in the immunocompetent host.

Econazole

Econazole is the deschloro-derivative of *miconazole*. *Econazole* readily penetrates the stratum corneum and achieves effective concentrations at the level of the middermis. Approximately 3% of recipients experience local erythema, burning, stinging, or itching. *Econazole nitrate* is available as a water-miscible cream (1%) to be applied twice a day.

Efinaconazole

Efinaconazole is an azoleamine derivative with excellent *in vitro* activity against *T. rubrum* and *T. mentagrophytes*. It is available as a 10% topical solution for the treatment of onychomycosis.

Miconazole

Miconazole readily penetrates the stratum corneum of the skin and persists for more than 4 days after application. Adverse effects from topical application to the vagina include burning, itching, or irritation in about 7% of recipients, as well as infrequent pelvic cramps (0.2%), headache, hives, or skin rash. Irritation, burning, and maceration are rare after cutaneous application. *Miconazole* is considered safe for use during pregnancy, although some experts advocate avoiding vaginal use during the first trimester.

Therapeutic Uses. *Miconazole nitrate* is available as a 2% cream, ointment, lotion, powder, gel, aerosol powder, and aerosol solution. To avoid maceration, only the lotion should be applied to intertriginous areas. *Miconazole* is available as a 2% and 4% vaginal cream and as 100-, 200-, or 1200-mg vaginal suppositories to be applied high in the vagina at bedtime for 7 or 3 days or 1 day, respectively.

In the treatment of tinea pedis, tinea cruris, and tinea versicolor, the cure rate exceeds 90%. In the treatment of vulvovaginal candidiasis, the mycological cure rate at the end of 1 month is about 80% to 95%. Pruritus sometimes is relieved after a single application. Some vaginal infections caused by *C. glabrata* also respond to this drug.

Luliconazole

Luliconazole is available as a 1% cream and is effective for the topical treatment of interdigital tinea pedis, tinea cruris, and tinea corporis caused by susceptible organisms. It should be applied to the affected area once daily for 2 weeks.

Terconazole and Butoconazole

Terconazole is a ketal triazole. The 80-mg vaginal suppository is inserted at bedtime for 3 days; the 0.4% vaginal cream is used for 7 days and the 0.8% cream for 3 days. Clinical efficacy and patient acceptance of both preparations are at least as good as for *clotrimazole* in patients with vaginal candidiasis.

Butoconazole is an imidazole that is pharmacologically comparable to *clotrimazole*. *Butoconazole nitrate* is available as a 2% vaginal cream; it is used at bedtime in nonpregnant females. Because of the slower response during pregnancy, a 6-day course is recommended (during the second and third trimesters).

Tioconazole

Tioconazole is an imidazole marketed for treatment of *Candida* vulvovaginitis. A single 4.6-g dose of ointment (300 mg of drug) is given at bedtime.

Oxiconazole, Sulconazole, and Sertaconazole

The imidazole derivatives *oxiconazole*, *sulconazole*, and *sertaconazole* are used for the topical treatment of infections caused by the common pathogenic dermatophytes. *Oxiconazole nitrate* is available as a 1% cream and lotion; *sulconazole nitrate* is supplied as a 1% solution or cream. *Sertaconazole* is a 2% cream marketed for tinea pedis.

Ketoconazole

The imidazole *ketoconazole* is available as a 0.5% cream, foam, gel, and shampoo for common skin dermatophyte infections, for tinea versicolor, and for seborrheic dermatitis.

Structurally Diverse Antifungal Agents

Ciclopirox Olamine

Ciclopirox olamine has broad-spectrum antifungal activity. It is fungicidal to *C. albicans, E. floccosum, M. canis, T. mentagrophytes,* and *T. rubrum.* It also inhibits the growth of *Malassezia furfur. Ciclopirox* appears to chelate trivalent metal cations and thereby inhibits metal-dependent enzymes required for degradation of peroxides within the fungal cell (Subissi et al., 2010). After application to the skin, it penetrates the epidermis to reach the dermis, but even under occlusion, less than 1.5% is absorbed into the systemic circulation. Furthermore, because the $t_{1/2}$ is 1.7 h, no systemic accumulation occurs. The drug penetrates hair follicles and sebaceous glands. It can sometimes cause hypersensitivity. It is available as a 0.77% cream, gel, suspension, and lotion for the treatment of cutaneous candidiasis and for tinea corporis, cruris, pedis, and versicolor. An 8% nail lacquer is available for onychomycosis. Cure rates in the dermatomycoses and candidal infections are 81% to 94%. No topical toxicity has been noted.

Ciclopirox 0.77% gel and 1% shampoo are also used for the treatment of seborrheic dermatitis of the scalp. An 8% topical solution is an effective treatment of mild-to-moderate superficial white onychomycosis.

Haloprogin

Haloprogin is a halogenated phenolic ether. It is fungicidal to various species of *Epidermophyton, Pityrosporum, Microsporum, Trichophyton,* and *Candida*. During treatment with this drug, irritation, pruritus, burning sensations, vesiculation, increased maceration, and "sensitization" (or exacerbation of the lesion) occasionally occur, especially on the foot if occlusive footgear is worn. *Haloprogin* is poorly absorbed through the skin; it is metabolized to trichlorophenol in the patient. However, systemic toxicity from topical application appears to be low. *Haloprogin* cream or solution is applied twice a day for 2 to 4 weeks. Its principal use is against tinea pedis, for which the cure rate is about 80%; it is thus approximately equal in efficacy to *tolnaftate*. It also is used against tinea cruris, tinea corporis, tinea manuum, and tinea versicolor. *Haloprogin* is no longer available in the U.S.

Tolnaftate

Tolnaftate is a thiocarbamate that is effective in the treatment of most cutaneous mycoses caused by *T. rubrum, T. mentagrophytes, Trichophyton tonsurans, E. floccosum, M. canis, M. audouinii, Microsporum gypseum,* and *M. furfur,* but it is ineffective against *Candida.* In tinea pedis, the cure rate is about 80%, compared with about 95% for *miconazole*. *Tolnaftate* is available in a 1% concentration as a cream, gel, powder, aerosol powder, topical solution, or topical aerosol liquid. The preparations are applied locally twice a day. Pruritus is usually relieved in 24 to 72 h. Involution of interdigital lesions caused by susceptible fungi is very often complete in 7 to 21 days. Toxic or allergic reactions to *tolnaftate* have not been reported.

Naftifine

Naftifine is a synthetic allylamine that inhibits squalene-2,3-epoxidase, a key enzyme in the fungal biosynthesis of ergosterol. The drug has broad-spectrum fungicidal activity *in vitro*. *Naftifine hydrochloride* is available as a 1% cream or gel. It is effective for the topical treatment of tinea cruris and tinea corporis; twice-daily application is recommended. The drug is well tolerated, although local irritation in 3% of treated patients and allergic contact dermatitis have been reported. *Naftifine* also may be efficacious for cutaneous candidiasis and tinea versicolor, although the drug is not approved for these uses.

Terbinafine

Like *naftifine*, *terbinafine* is an allylamine that targets ergosterol biosynthesis. *Terbinafine* 1% cream or spray, applied twice daily, is effective in tinea corporis, tinea cruris, and tinea pedis. *Terbinafine* is less active against *Candida* species and *Malassezia furfur,* but the cream also can be used in cutaneous candidiasis and tinea versicolor.

Butenafine

Butenafine hydrochloride is a benzylamine derivative with a mechanism of action similar to that of *terbinafine* and *naftifine*. Its spectrum of antifungal activity and use also are similar to those of the allylamines.

Tavaborole

Tavaborole is an oxaborole antifungal indicated for the topical treatment of onchomycosis of the toenails. The drug inhibits fungal leucyl-tRNA synthetase, thereby inhibiting protein synthesis and ultimately causing fungal cell death.

Nystatin

Nystatin, a tetraene macrolide produced by *Streptomyces noursei*, is structurally similar to *amphotericin B* and acts through the same mechanism. The drug is not absorbed from the GI tract, skin, or vagina. *Nystatin* is useful only for candidiasis and is supplied in preparations intended for cutaneous, vaginal, or oral administration for this purpose. The vaginal preparations are no longer marketed in the U.S. Infections of the nails and hyperkeratinized or crusted skin lesions do not respond. Powders are preferred for moist lesions such as diaper rash and are applied two to three times daily. Creams or ointments are used twice daily. Combinations of *nystatin* with corticosteroids also are available.

Allergic reactions to *nystatin* are uncommon. Although vaginal tablets of *nystatin* are well tolerated, imidazoles or triazoles are more effective agents than *nystatin* for vaginal candidiasis. *Nystatin* suspension is usually effective for oral candidiasis of the immunocompetent host and is widely used in neonates and infants for oral thrush. Patients should be instructed to swish the drug around in the mouth and then swallow; otherwise, the patient may expectorate the bitter liquid and fail to treat the infected mucosa in the posterior pharynx or esophagus. Other than the bitter taste and occasional complaints of nausea, adverse effects are uncommon.

Undecylenic Acid

Undecylenic acid is 10-undecenoic acid, an 11-carbon unsaturated compound. It is primarily fungistatic, although fungicidal activity may be observed with long exposure to high concentrations of the agent. The drug is active against a variety of fungi, including those that cause ringworm. *Undecylenic acid* is available in a cream, powder, spray powder, soap, and liquid. *Zinc undecylenate* is marketed in combination with other ingredients. The zinc provides an astringent action that aids in the suppression of inflammation. Compounded *undecylenic acid* ointment contains both *undecylenic acid* (~5%) and *zinc undecylenate* (~20%). *Calcium undecylenate* is available as a powder.

Undecylenic acid preparations are used in the treatment of various dermatomycoses, especially tinea pedis. Concentrations of the acid as high as 10%, as well as those of the acid and salt in the compounded ointment, may be applied to the skin. These preparations are usually not irritating to tissue, and sensitization to them is uncommon. This agent retards fungal growth in tinea pedis, but the infection frequently persists despite intensive treatment with preparations of the acid and the zinc salt. At best, the clinical "cure" rate is about 50%, which is much lower than that obtained with the imidazoles, *haloprogin*, or *tolnaftate*. Efficacy in the treatment of tinea capitis is marginal, and the drug is no longer used for that purpose. *Undecylenic acid* preparations also are approved for use in the treatment of diaper rash, tinea cruris, and other minor dermatologic conditions.

Benzoic and Salicylic Acids

An ointment containing *benzoic* and *salicylic acids* in a ratio of 2:1 (usually 6% and 3%) is known as Whitfield ointment. It combines the fungistatic action of benzoate with the keratolytic action of salicylate and is used mainly in the treatment of tinea pedis. Because *benzoic acid* is only fungistatic, eradication of the infection occurs only after the infected stratum corneum is shed; thus, continuous medication is required for several weeks to months. The *salicylic acid* accelerates desquamation. The ointment also is sometimes used to treat tinea capitis. Mild irritation may occur at the site of application.

Drug Facts for Your Personal Formulary: *Antifungal Agents*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Polyenes: Interact with ergosterol in the fungal cell membrane		
Amphotericin B deoxycholate (C-AMB)	• Invasive candidiasis and aspergillosis • Blastomycosis • Histoplasmosis • Coccidioidomycosis • Cryptococcosis • Mucormycosis • Sporotrichosis • Empirical therapy in the immunocompromised host	• Associated with significant nephrotoxicity, including azotemia, renal tubular acidosis, and hypochromic, normocytic anemia • Associated with acute reactions, including infusion-related fever and chills • C-AMB is better tolerated by premature neonates than by older children and adults
Amphotericin B colloidal dispersion (ABCD) (not available in the U.S.) Liposomal amphotericin B (L-AMB) Amphotericin B lipid complex (ABLC)		• All three amphotericin B lipid formulations are less nephrotoxic than C-AMB • Infusion-related reactions are highest with ABCD and lowest with L-AMB
Pyrimidines: Disrupt fungal RNA and DNA synthesis		
Flucytosine	• Cryptococcosis (with amphotericin B or fluconazole)	• Has broad activity but emergence of resistance limits usefulness as single-agent therapy • ↓ Dosage in patients with ↓ renal function • Toxicity more frequent in patients with AIDS or azotemia • May depress marrow → leukopenia & thrombocytopenia
Imidazoles and Triazoles: Inhibit ergosterol biosynthesis		
Ketoconazole	• Replaced by itraconazole for oral administration • Used topically for tinea versicolor, seborrheic dermatitis, common dermatophytes	• A reference substrate for and potent inhibitor of CYP3A4 • May cause redness in area of topical application • Topical preparations are available over the counter • Oral use can cause hepatotoxicity and adrenal insufficiency • In U.S., oral preparations available only with restrictions
Itraconazole	• Invasive aspergillosis • Blastomycosis • Coccidioidomycosis • Histoplasmosis • Pseudallescheriasis; Sporotrichosis • Ringworm; Onychomycosis	• Substrate for and potent inhibitor of CYP3A4 • Hepatotoxic • Contraindicated in pregnancy and in women considering becoming pregnant • Therapeutic monitoring useful, particularly in pediatric patients
Fluconazole	• Invasive candidiasis • Cryptococcosis • Oropharyngeal candidiasis • Coccidioidomycosis • Prophylaxis and empirical therapy in immunocompromised host	• Plasma concentrations are essentially the same whether the drug is given orally or intravenously • Concentrations in CSF = 50%–90% of C_P • Inhibitor of CYP3A4 and CYP2C9 • Contraindicated during pregnancy
Voriconazole	• Invasive aspergillosis • Invasive candidiasis • Pseudallescheriasis • Fusariosis	• Oral bioavailability is 96% • Monitor C_P; serum levels of 1–5 mg/L maximize efficacy and minimize toxicity • Metabolized by and inhibits CYPs (2C19 > 2C9 > 3A4) • Can prolong the QTc interval • Transient visual or auditory hallucinations are frequent after the first dose • Contraindicated in pregnancy
Posaconazole	• Oropharyngeal candidiasis • Mucormycosis • Prophylaxis in the immunocompromised host against aspergillosis and candidiasis	• Oral bioavailability enhanced by food • Drugs that ↓ gastric acid ↓ posaconazole exposure • Inhibits CYP3A4 • Can prolong the QTc interval • Adverse effects: headache and GI disorders
Isavuconazole (isavuconazonium prodrug)	• Invasive aspergillosis • Mucormycosis	• Oral bioavailability is 98% • Substrate of and inhibitor of CYP3A4 • Does not appear to prolong QTc

Drug Facts for Your Personal Formulary: *Antifungal Agents* (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Echinocandins: Inhibit 1,3-β-D-glucan synthesis in the fungal cell wall		
Caspofungin	• Invasive candidiasis • Salvage for aspergillosis • Empirical therapy in the immunocompromised host	• ↓ Dose in moderate hepatic impairment
Micafungin	• Invasive candidiasis • Prophylaxis in the immunocompromised host	• Reduction of micafungin dose in moderate hepatic failure is not required
Anidulafungin	• Invasive candidiasis	• No dose adjustment is needed for hepatic or renal failure
Griseofulvin: Inhibits microtubule function, disrupts assembly of the mitotic spindle		
Griseofulvin	• Ringworm • Onychomycosis	• Absorption is reduced by barbiturates • Induces hepatic CYPs
Allylamines: Inhibit fungal squalene epoxidase and reduce ergosterol biosynthesis		
Terbinafine	• Ringworm • Onychomycosis	• Bioavailability is ~40% due to first-pass metabolism in the liver. • The drug accumulates in skin, nails, and fat • The initial $t_{1/2}$ is ~12 h but extends to 200–400 h at steady state
Agents Active Against Microsporidia and *Pneumocystis*		
Albendazole	• Microsporidia infection	• Anthelmintic • Inhibitor of α-tubulin polymerization
Fumagillin	• Microsporidia infection	• Used in immunocompromised individuals with intestinal microsporidiosis due to *E. bieneusi* unresponsive to albendazole • Not approved for human use in the U.S.
Trimethoprim-sulfamethoxazole	• *Pneumocystis jiroveci* pneumonia	• See Chapter 57
Pentamidine	• *Pneumocystis jiroveci* pneumonia	• Prophylaxis use to prevent PJP in at-risk individuals who cannot tolerate trimethoprim-sulfamethoxazole
Topical Antifungal Agents		
Imidazoles and triazoles Clotrimazole, miconazole, ketoconazole, etc.	• Dermatophytosis (ringworm), candidiasis, tinea versicolor, piedra, tinea nigra, and fungal keratitis	• Available for cutaneous application as creams or solutions • Some are available as vaginal creams or suppositories or as oral troches
Tavaborole	• Toenail onychomycosis due to *T. rubrum* or *T. mentagrophytes*	• Apply daily for 48 weeks

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Chapter 62

Antiviral Agents (Nonretroviral)

Edward P. Acosta

VIRAL REPLICATION AND DRUG TARGETS

ANTIHERPESVIRUS AGENTS

- Acyclovir and Valacyclovir
- Cidofovir
- Famciclovir and Penciclovir
- Ganciclovir and Valganciclovir
- Letermovir
- Foscarnet
- Fomivirsen
- Docosanol
- Idoxuridine
- Trifluridine

ANTI-INFLUENZA AGENTS

- Amantadine and Rimantadine
- Oseltamivir
- Zanamivir
- Peramivir
- Baloxavir Marboxil
- Interferon

ANTI–ZAIRE EBOLAVIRUS AGENTS

- Inmazeb (Atoltivimab, Maftivimab, and Odesivimab) and Ebanga (Ansuvimab)

NOVEL 2019 CORONAVIRUS

- Remdesivir

Most antivirals currently available in the U.S. have been developed and approved in the last 35 years. This flurry of activity was driven by successes in rational drug design and approval that began with the antiherpesvirus nucleoside analogue *acyclovir* (Elion, 1986), whose discovery and development resulted in the awarding of the 1988 Nobel Prize in Physiology/Medicine to Gertrude Elion and George Hitchings, an award they shared with James Black (see Chapter 43) "for their discoveries of important principles for drug treatment." Because viruses are obligatory intracellular microorganisms and rely on host biosynthetic machinery to reproduce, there were doubts about the possibility of developing antiviral drugs with selective toxicity, but those doubts have long been erased. Viruses are now obvious targets for effective antimicrobial chemotherapy, and it is certain that the number of available agents in this category will continue to increase. Indeed, the recent development of agents that target the viral protein NS5A has revolutionized treatment of infections of hepatitis B virus (HBV) and hepatitis C virus (HCV), and these agents are now allotted a chapter of their own, Chapter 63. Chapter 64 describes chemotherapy for retroviruses. This chapter covers antiviral agents for nonretroviral infections other than HBV and HCV.

Viral Replication and Drug Targets

Viruses are simple microorganisms that consist of either double- or single-stranded DNA or RNA enclosed in a protein coat called a *capsid*. Some viruses also possess a lipid envelope derived from the infected host cell, which, like the capsid, may contain antigenic glycoproteins. Effective antiviral agents inhibit virus-specific replicative events or preferentially inhibit *virus-directed rather than host cell–directed* nucleic acid or protein synthesis (Table 62–1). Host cell molecules that are essential to viral replication also offer targets for intervention. Figure 62–1 gives a schematic diagram of the replicative cycle of typical DNA and RNA viruses with the sites of antiviral drugs indicated.

DNA viruses include poxviruses (smallpox), herpesviruses (chickenpox, shingles, oral and genital herpes); adenoviruses (conjunctivitis, sore throat); hepadnaviruses (HBV); and papillomaviruses (warts). Most DNA viruses enter the host cell nucleus, where the viral DNA is transcribed into mRNA (messenger RNA) by host cell polymerase; mRNA is translated in the usual host cell fashion into virus-specific proteins. Poxviruses are an exception; they carry their own RNA polymerase and replicate in the host cell cytoplasm.

For RNA viruses, the replication strategy either relies on enzymes in the virion to synthesize mRNA or has the viral RNA serving as its own mRNA. The mRNA is translated into various viral proteins, including RNA polymerase, which directs the synthesis of more viral mRNA and genomic RNA. Most RNA viruses complete their replication in the host cell cytoplasm, but some, such as influenza, are transcribed in the host cell nucleus. Examples of RNA viruses include rubella virus (German measles); rhabdoviruses (rabies); picornaviruses (poliomyelitis, meningitis, colds, hepatitis A); arenaviruses (meningitis, Lassa fever); flaviviruses (West Nile meningoencephalitis, yellow fever, hepatitis C, Zika virus); orthomyxoviruses (influenza); paramyxoviruses (measles, mumps); and coronaviruses (colds, severe acute respiratory syndrome [SARS]). Retroviruses are RNA viruses that include human immunodeficiency virus (HIV); chemotherapy for retroviruses is described in Chapter 64. Pharmacotherapy of viral hepatitis is covered separately in Chapter 63.

Table 62–2 summarizes currently approved drugs for nonretroviral infections, excluding those for viral hepatitis. Their pharmacological properties are presented in the material that follows, class by class, as listed in the table.

Antiherpesvirus Agents

Herpes simplex virus (HSV) type 1 typically causes diseases of the mouth, face, skin, esophagus, or brain. HSV-2 usually causes infections of the genitals, rectum, skin, hands, or meninges. Both cause serious infections in neonates. Agents used in treating HSV work by several mechanisms to inhibit viral DNA replication in the host cell (see Figure 62–1 and Table 62–1).

Acyclovir and Valacyclovir

Acyclovir is an acyclic guanine nucleoside analogue that lacks the 2′ and 3′ positions normally supplied by ribose. *Valacyclovir* is the L-valyl ester prodrug of *acyclovir*. *Acyclovir* is the prototype of a group of antiviral agents that are nucleoside congeners (Figure 62–2) that are phosphorylated intracellularly by a viral kinase and subsequently by host cell

Abbreviations

AIDS: acquired immune deficiency syndrome
AUC: area under curve of plasma drug concentration versus time
CDC: U.S. Centers for Disease Control and Prevention
Cl_{cr}: creatinine clearance
CMV: cytomegalovirus
CoV: coronavirus 2
CSF: cerebrospinal fluid
CYP: cytochrome P450 isozyme
EBV: Epstein-Barr virus
EIND: emergency investigational new drug
EVD: Ebola virus disease
FDA: U.S. Food and Drug Administration
G-CSF: granulocyte colony-stimulating factor
GI: gastrointestinal
HBV: hepatitis B virus
HCV: hepatitis C virus
HHV-6: human herpesvirus 6
HIV: human immunodeficiency virus
HSCT: hematopoietic stem cell transplantation
HSV: herpes simplex virus
IFN: interferon
mAb: monoclonal antibody
MERS: Middle East respiratory syndrome
mRNA: messenger RNA
NSAID: nonsteroidal anti-inflammatory drug
RdRp: RNA-dependent RNA polymerase
SARS: severe acute respiratory syndrome
TK: thymidine kinase
VZV: varicella zoster virus
WHO: World Health Organization

TABLE 62–1 ■ STAGES OF VIRUS REPLICATION AND POSSIBLE TARGETS OF ACTION OF ANTIVIRAL AGENTS

STAGE OF REPLICATION	CLASSES OF SELECTIVE INHIBITORS
Cell entry	
Attachment Penetration	Soluble receptor decoys, antireceptor antibodies, fusion protein inhibitors
Uncoating	
Release of viral genome	Ion channel blockers, capsid stabilizers
Transcription[a]	
Transcription of viral mRNA Replication of viral genome	Inhibitors of viral DNA polymerase, RNA polymerase, reverse transcriptase, helicase, primase, or integrase
Translation of viral proteins	
Regulatory proteins (early) Structural proteins (late)	Interferons, antisense oligonucleotides, ribozymes, inhibitors of regulatory proteins
Posttranslational modifications	
Proteolytic cleavage Myristoylation, glycosylation	Protease inhibitors
Assembly of virion components	Interferons, inhibitors of the assembly of viral proteins
Release	
Budding, cell lysis	Neuraminidase inhibitors, antiviral antibodies, cytotoxic lymphocytes

[a] Depends on specific replication strategy of virus, but virus-specified enzyme required for part of process.

enzymes to become inhibitors of viral DNA synthesis. Related agents include *penciclovir* and *ganciclovir*.

Mechanisms of Action and Resistance

Acyclovir inhibits viral DNA synthesis via a mechanism outlined in Figure 62–3. Its selectivity of action depends on interaction with HSV TK (thymidine kinase) and DNA polymerase. The initial phosphorylation of *acyclovir* is facilitated by HSV TK and thus occurs only in cells infected with the virus. The affinity of *acyclovir* for HSV TK is about 200 times greater than for the mammalian enzyme. Cellular enzymes convert the monophosphate to *acyclovir* triphosphate, which competes for endogenous dGTP. The immunosuppressive agent *mycophenolate mofetil* (see Chapter 39) potentiates the antiherpes activity of *acyclovir* and related agents by depleting intracellular dGTP pools. *Acyclovir triphosphate* competitively inhibits viral DNA polymerases and, to a much lesser extent, cellular DNA polymerases. *Acyclovir triphosphate* also is incorporated into viral DNA, where it acts as a chain terminator because of the lack of a 3′-hydroxyl group. By a mechanism termed *suicide inactivation*, the terminated DNA template containing *acyclovir* binds the viral DNA polymerase and leads to its irreversible inactivation.

Acyclovir resistance in HSV can result from impaired production of viral TK, altered TK substrate specificity (e.g., phosphorylation of thymidine but not *acyclovir*), or altered viral DNA polymerase. Alterations in viral enzymes are caused by point mutations and base insertions or deletions in the corresponding genes. Resistant variants are present in native virus populations and in isolates from treated patients. The most common resistance mechanism in clinical HSV isolates is absent or deficient viral TK activity; viral DNA polymerase mutants are rare. Phenotypic resistance typically is defined by *in vitro* inhibitory concentrations of more than 2 to 3 μg/mL, which predict failure of therapy in immunocompromised patients. *Acyclovir* resistance in isolates of varicella zoster virus (VZV) is caused by mutations in VZV TK and less often by mutations in viral DNA polymerase.

ADME

The oral bioavailability of *acyclovir* is about 10% to 30% and decreases with increasing dose (Wagstaff et al., 1994). Delivery of an oral dose can be enhanced by administration of the prodrug form, *valacyclovir*. *Valacyclovir* is an esterified version with higher bioavailability (55%–70%) than *acyclovir* (Steingrimsdottir et al., 2000); deesterification occurs rapidly and nearly completely following oral administration. Unlike *acyclovir*, *valacyclovir* is a substrate for intestinal and renal peptide transporters. *Acyclovir* distributes widely in body fluids, including vesicular fluid, aqueous humor, and CSF (cerebrospinal fluid). Compared with plasma, salivary concentrations are low, and concentrations in vaginal secretion vary widely. *Acyclovir* is concentrated in breast milk, amniotic fluid, and placenta. Newborn plasma levels are similar to maternal ones. Percutaneous absorption of *acyclovir* after topical administration is low. Renal excretion of unmetabolized *acyclovir* by glomerular filtration and tubular secretion is the principal route of elimination. The elimination $t_{1/2}$ of *acyclovir* is about 2.5 h (range 1.5–6 h) in adults with normal renal function. In neonates, the elimination $t_{1/2}$ of *acyclovir* is about 4 h and increases to 20 h in anuric patients.

Therapeutic Uses

Acyclovir's clinical use is limited to herpesviruses. *Acyclovir* is most active against HSV-1 (effective plasma drug concentration [C_p] range: 0.02–0.9 μg/mL), approximately half as active against HSV-2 (0.03–2.2 μg/mL), a tenth as potent against VZV (0.8–4.0 μg/mL) and Epstein-Barr virus (EBV), and least active against cytomegalovirus (CMV) (generally >20 μg/mL) and human herpesvirus 6 (HHV-6). Uninfected mammalian cell growth generally is unaffected by high *acyclovir* concentrations (>50 μg/mL).

In immunocompetent persons, the clinical benefits of *acyclovir* and *valacyclovir* are greater in initial HSV infections than in recurrent ones.

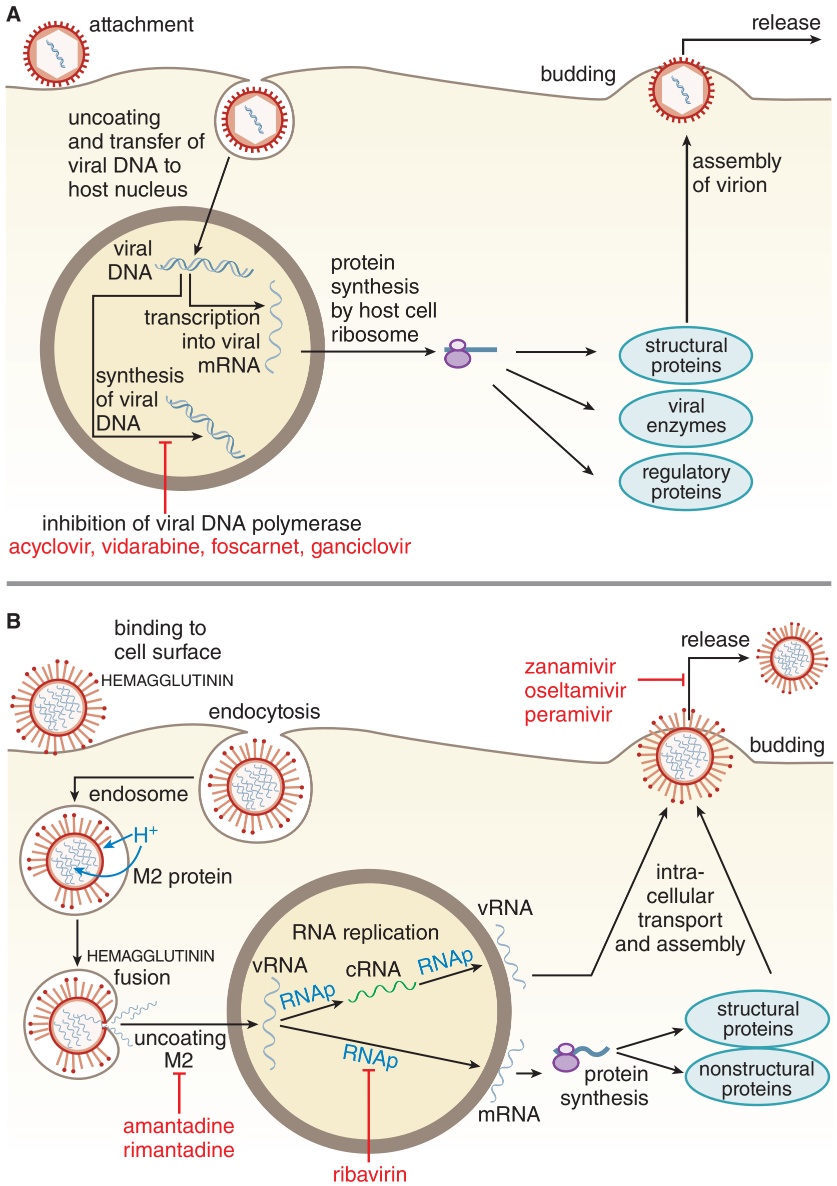

A
attachment
release
budding
uncoating and transfer of viral DNA to host nucleus
assembly of virion
viral DNA
protein synthesis by host cell ribosome
transcription into viral mRNA
synthesis of viral DNA
structural proteins
viral enzymes
regulatory proteins
inhibition of viral DNA polymerase
acyclovir, vidarabine, foscarnet, ganciclovir
B
binding to cell surface
HEMAGGLUTININ
release
zanamivir
oseltamivir
peramivir
endocytosis
budding
endosome
H+
M2 protein
intra-cellular transport and assembly
vRNA
RNA replication
HEMAGGLUTININ
fusion
vRNA
cRNA
RNAp
RNAp
RNAp
uncoating
M2
structural proteins
protein synthesis
nonstructural proteins
mRNA
amantadine
rimantadine
ribavirin

Figure 62–1 *Replicative cycles of DNA (**A**) and RNA (**B**) viruses.* The replicative cycles of herpesvirus (**A**) and influenza (**B**) are examples of DNA-encoded and RNA-encoded viruses, respectively. Sites of action of antiviral agents also are shown. The symbol ⊣ indicates a block to virus growth. **A.** *Replicative cycles of herpes simplex virus, a DNA virus, and the probable sites of action of antiviral agents.* Herpesvirus replication is a regulated multistep process. After infection, a small number of immediate-early genes are transcribed; these genes encode proteins that regulate their own synthesis and are responsible for synthesis of early genes involved in genome replication, such as TKs, DNA polymerases, and so on. After DNA replication, the bulk of the herpesvirus genes (called late genes) are expressed and encode proteins that either are incorporated into or aid in the assembly of progeny virions. **B.** *Replicative cycles of influenza, an RNA virus, and the loci for effects of antiviral agents.* The mammalian cell shown is an airway epithelial cell. The M2 protein of influenza virus allows an influx of hydrogen ions into the virion interior, which in turn promotes dissociation of the RNP (ribonuclear protein) segments and release into the cytoplasm (uncoating). Influenza virus mRNA synthesis requires a primer cleared from cellular mRNA and used by the viral RNA polymerase (RNAp) complex. The neuraminidase inhibitors *zanamivir* and *oseltamivir* specifically inhibit release of progeny virus.

These drugs are particularly useful in immunocompromised patients because these individuals experience both more frequent and more severe HSV and VZV infections. Because VZV is less susceptible than HSV to *acyclovir*, higher doses must be used for treating VZV infections. Oral *valacyclovir* is as effective as oral *acyclovir* in HSV infections and more effective for treating herpes zoster. *Acyclovir* is ineffective therapeutically in established CMV infections, but *ganciclovir* is effective for CMV prophylaxis in immunocompromised patients. EBV-related oral hairy leukoplakia may improve with *acyclovir*. Oral *acyclovir* in conjunction with systemic corticosteroids appears beneficial in treating Bell's palsy; *valacyclovir* is ineffective in acute vestibular neuritis.

Herpes Simplex Virus Infections

In initial genital HSV infections, oral *acyclovir* (200 mg five times daily or 400 mg three times daily for 7–10 days) and *valacyclovir* (1000 mg twice daily for 7–10 days) are associated with significant reductions in virus shedding, symptoms, and time to healing (Kimberlin and Rouse, 2004). Intravenous *acyclovir* (5 mg/kg every 8 h) has similar effects in patients hospitalized with severe primary genital HSV infections. Topical *acyclovir* is much less effective than systemic administration. None of these regimens reproducibly reduces the risk of recurrent genital lesions. *Acyclovir* (200 mg five times daily or 400 mg three times daily for 5 days or 800 mg three times daily for 2 days) or *valacyclovir*

TABLE 62–2 ■ NOMENCLATURE OF ANTIVIRAL AGENTS

GENERIC NAME	OTHER NAMES	DOSAGE FORMS AVAILABLE
Antiherpesvirus agents		
Acyclovir	ACV, acycloguanosine	IV, O, T, ophth[a]
Cidofovir	HPMPC, CDV	IV
Famciclovir	FCV	O
Foscarnet	PFA, phosphonoformate	IV, O[b]
Fomivirsena[b]	ISIS 2922	Intravitreal
Ganciclovir	GCV, DHPG	IV, O, intravitreal, ophthalmic gel
Idoxuridine[b]	IDUR	Ophth
Penciclovir	PCV	T, IV[b]
Trifluridine	TFT, trifluorothymidine	Ophth
Valacyclovir		O
Valganciclovir		O
Anti-influenza agents		
Amantadine		O
Oseltamivir	GS4104	O
Peramivir	BCX 1812	IV
Rimantadine		O
Zanamivir	GC167	Inhalation
Other antiviral agents		
Ribavirin	(see Chapter 63)	O, inhalation, IV[b]
Telbivudine[b]	(see Chapter 63)	O
Tenofovir disoproxil fumarate	TDF (see Chapter 63)	O
Imiquimod		Topical

O, oral; ophth, ophthalmic; IV, intravenous; T, topical.

[a] Availability pending in the U.S.

[b] Not available in the U.S.

(500 mg twice daily for 3 or 5 days) shortens the manifestations of recurrent genital HSV episodes by 1 to 2 days. Frequently recurring genital herpes can be suppressed effectively with chronic oral *acyclovir* (400 mg twice daily or 200 mg three times daily) or with *valacyclovir* (500 mg or, for very frequent recurrences, 1000 mg once daily). During use, the rate of clinical recurrences decreases by about 90%, and subclinical shedding is markedly reduced, although not eliminated. *Valacyclovir* suppression of genital herpes reduces the risk of transmitting infection to a susceptible partner by about 50% over an 8-month period (Corey et al., 2004). Chronic suppression may be useful in those with disabling recurrences of herpetic whitlow or HSV-related erythema multiforme.

Oral *acyclovir* is effective in primary herpetic gingivostomatitis (600 mg/m^2 four times daily for 10 days in children) but provides only modest clinical benefit in recurrent orolabial herpes. Short-term, high-dose *valacyclovir* (2 g twice over 1 day) shortens the duration of recurrent orolabial herpes by about 1 day (Elish et al., 2004). The U.S. Food and Drug Administration (FDA) has approved an *acyclovir/hydrocortisone* combination (Lipsovir) for early treatment of recurrent herpes cold sores. Topical *acyclovir* cream is modestly effective in recurrent labial (Spruance et al., 2002) and genital HSV infections. Preexposure *acyclovir* prophylaxis (400 mg twice daily for 1 week) reduces the overall risk of recurrence by 73% in those with sun-induced recurrences of HSV infections. *Acyclovir* during the last month of pregnancy reduces the likelihood of viral shedding and the frequency of cesarean delivery in women with primary or recurrent genital herpes (Corey and Wald, 2009).

In immunocompromised patients with mucocutaneous HSV infection, intravenous *acyclovir* (250 mg/m^2 every 8 h for 7 days) shortens healing time, duration of pain, and the period of virus shedding. Oral *acyclovir* (800 mg five times per day) and *valacyclovir* (1000 mg twice daily) for 5 to 10 days are also effective. Recurrences are common after cessation of therapy and may require long-term suppression. In those with very localized labial or facial HSV infections, topical *acyclovir* may provide some benefit. Intravenous *acyclovir* may be beneficial in viscerally disseminating HSV in immunocompromised patients and in patients with HSV-infected burn wounds.

Systemic *acyclovir* prophylaxis is highly effective in preventing mucocutaneous HSV infections in seropositive patients undergoing immunosuppression. Intravenous *acyclovir* (250 mg/m^2 every 8–12 h) begun prior to transplantation and continuing for several weeks prevents HSV disease in bone marrow transplant recipients. For patients who can tolerate oral medications, oral *acyclovir* (400 mg five times daily) is effective, and long-term oral *acyclovir* (200–400 mg three times daily for 6 months) also reduces the risk of VZV infection (Steer et al., 2000). In HSV encephalitis, *acyclovir* (10 mg/kg every 8 h for a minimum of 10 days) reduces mortality by more than 50% and improves overall neurological outcome compared with *vidarabine*. Higher doses (15–20 mg/kg every 8 h) and prolonged treatment (up to 21 days) are recommended by many experts. Intravenous *acyclovir* (20 mg/kg every 8 h for 21 days) is more effective than lower doses in viscerally invasive neonatal HSV infections (Kimberlin et al., 2001). In neonates and immunosuppressed patients and, rarely, in previously healthy persons, relapses of encephalitis following *acyclovir* may occur. The value of continuing long-term suppression with *valacyclovir* after completing intravenous *acyclovir* is under study.

An ophthalmic formulation of *acyclovir* (availability pending in the U.S.) is at least as effective as topical *vidarabine* or *trifluridine* in herpetic keratoconjunctivitis.

Infection owing to resistant HSV is rare in immunocompetent persons; however, in immunocompromised hosts, *acyclovir*-resistant HSV isolates can cause extensive mucocutaneous disease and, rarely,

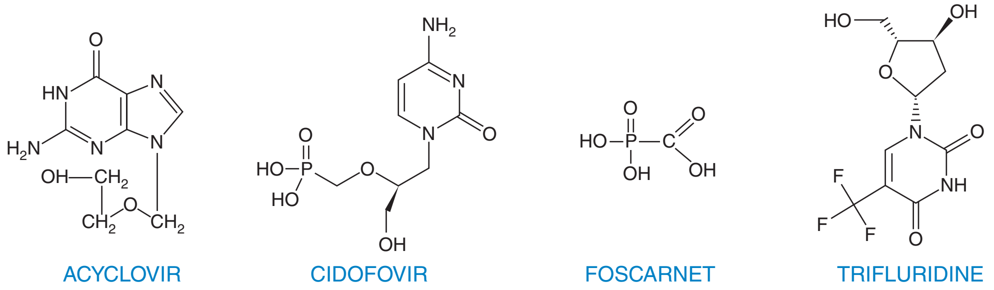
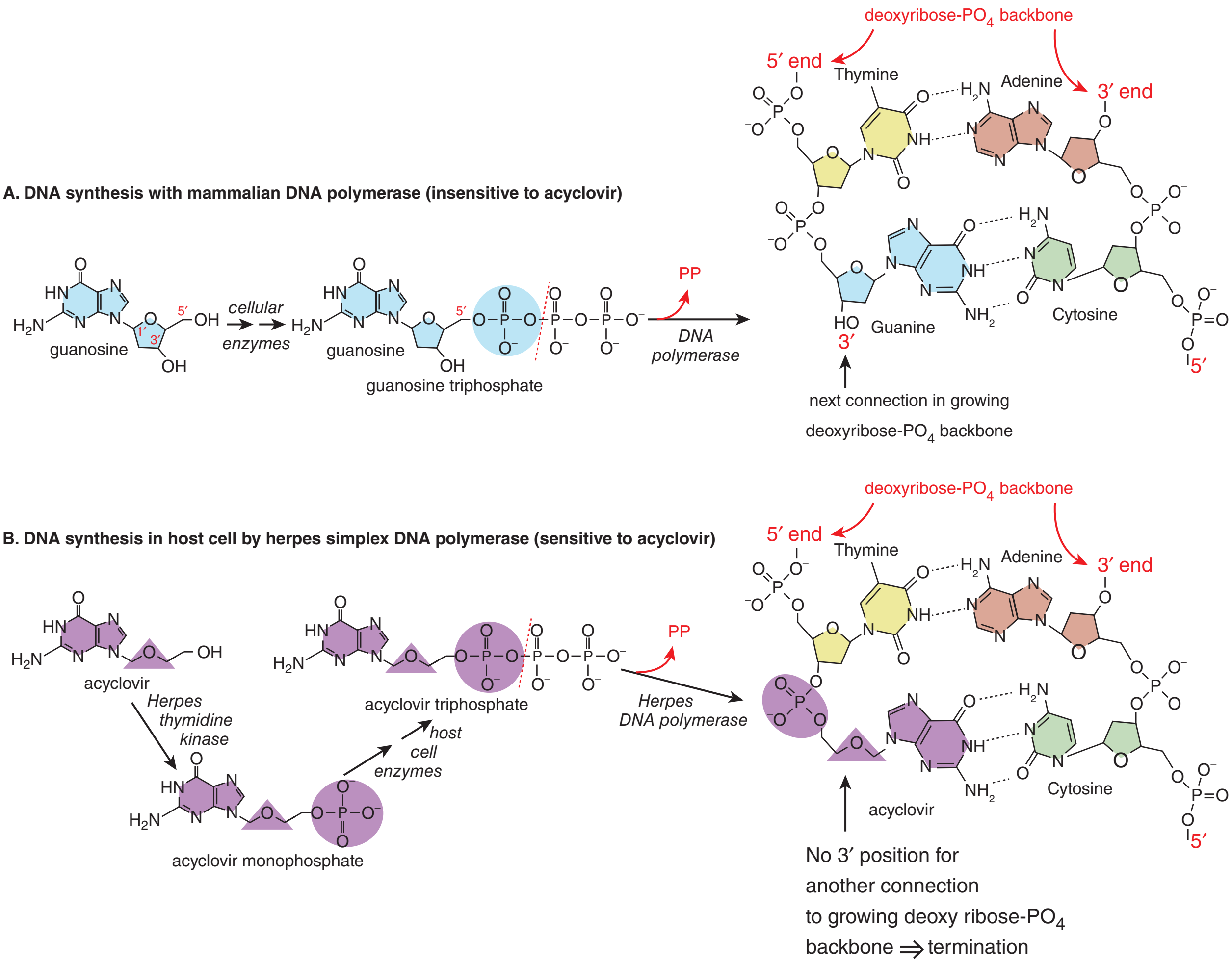


Figure 62–2 *Chemical structures of some antiherpes drugs.* Many antiherpes agents are nucleoside congeners that are phosphorylated sequentially by viral and host kinases to become triphosphate inhibitors of viral DNA synthesis (see Figure 62–3). *Foscarnet* is a pyrophosphate analogue that selectively blocks the pyrophosphate binding site on viral DNA polymerases, thereby inhibiting chain elongation.



A. DNA synthesis with mammalian DNA polymerase (insensitive to acyclovir)

B. DNA synthesis in host cell by herpes simplex DNA polymerase (sensitive to acyclovir)

Figure 62–3 *Acyclovir inhibits DNA synthesis by HSV DNA polymerase.* After penetrating the membrane of a susceptible mammalian host cell, an HSV virion releases its capsid, which delivers viral DNA into the host cell, initiating viral DNA synthesis. *Acyclovir*, a guanine analogue, inhibits viral but not mammalian DNA polymerase. **A.** *DNA synthesis with mammalian DNA polymerase (insensitive to acyclovir).* In the presence of *acyclovir*, human DNA synthesis proceeds normally. Here, mammalian DNA polymerase removes pyrophosphate (PP) (----), from dGTP and uses dGTP to add a dGMP to the 3′ end of a growing nucleic acid polymer, the guanine base pairing with a cytosine and dGMP's 5′PO_4 bonding to the 3′OH group on the ribose of the preceding base, thymine. A 3′OH on the sugar of the added dGMP is available to form a 3′-5′ bond with the next nucleotide added. **B.** *DNA synthesis in host cell by HSV DNA polymerase (sensitive to acyclovir).* The guanine analogue *acyclovir* inhibits viral DNA polymerase by acting as a terminal substrate, but to do so, *acyclovir* must be phosphorylated to *acyclovir* triphosphate. The first phosphate group is added by the HSV TK, which has an affinity for *acyclovir* that is about 200 times that of the mammalian enzyme for *acyclovir*. Host cell enzymes add the second and third phosphates, producing *acyclovir triphosphate*, which concentrates 40- to 100-fold in HSV-infected cells over the concentrations in uninfected cells. Thus, *acyclovir triphosphate* competes well for endogenous dGTP. HSV DNA polymerase cleaves PP_i (----) from *acyclovir triphosphate* and adds *acyclovir monophosphate* to the 3′ end of the growing DNA strand. *Acyclovir* lacks a hydroxyl group in the 3′ position (indeed, it lacks that 3′ position), and further addition to the polymer by HSV DNA polymerase is not possible. Furthermore, a viral exonuclease activity associated with viral DNA polymerase cannot remove the *acyclovir* moiety. Compare the actions of *acyclovir* to those of *ganciclovir* and *penciclovir*, which have 3′OH groups, and to *foscarnet*, which binds avidly at the PP_i cleavage site of HSV DNA polymerase, preventing cleavage of PP_i from nucleoside triphosphates.

meningoencephalitis, pneumonitis, or visceral disease. Resistant HSV can be recovered from 4% to 7% of immunocompromised patients receiving *acyclovir* treatment. Recurrences after cessation of *acyclovir* usually are due to sensitive virus but may be due to *acyclovir*-resistant virus in patients with acquired immune deficiency syndrome (AIDS). In patients with progressive disease, intravenous *foscarnet* therapy is effective, and *vidarabine* is considered only when all other therapies have failed (Chilukuri and Rosen, 2003).

Untoward Effects

Acyclovir generally is well tolerated. Chronic *acyclovir* suppression of genital herpes has been used safely for up to 10 years. No excess frequency of congenital abnormalities has been recognized in infants born to women exposed to *acyclovir* during pregnancy (Ratanajamit et al., 2003). Topical *acyclovir* in a polyethylene glycol base may cause mucosal irritation and transient burning when applied to genital lesions. Oral *acyclovir* has been associated infrequently with nausea, diarrhea, rash, or headache and very rarely with renal insufficiency or neurotoxicity. *Valacyclovir* also may be associated with headache, nausea, diarrhea, nephrotoxicity, and CNS (central nervous system) symptoms (confusion, hallucinations). Uncommon side effects include severe thrombocytopenic syndromes, sometimes fatal, in immunocompromised patients. *Acyclovir* has been associated with neutropenia in neonates. The principal dose-limiting toxicities of intravenous *acyclovir* are renal insufficiency and CNS side effects. Nephrotoxicity usually resolves with drug cessation and volume expansion. Hemodialysis may be useful in severe cases. Severe somnolence and lethargy may occur with combinations of *zidovudine* (see Chapter 64) and *acyclovir*. Concomitant *cyclosporine* and probably other nephrotoxic agents enhance the risk of nephrotoxicity. *Probenecid* decreases the *acyclovir* renal clearance and prolongs the elimination $t_{1/2}$.

Acyclovir may decrease the renal clearance of other drugs eliminated by active renal secretion, such as *methotrexate*.

Cidofovir

Cidofovir is a cytidine nucleotide analogue with inhibitory activity against human herpes, papilloma, polyoma, pox, and adenoviruses.

Because *cidofovir* is a phosphonate that is phosphorylated by cellular but not viral enzymes, it inhibits *acyclovir*-resistant TK-deficient or TK-altered HSV or VZV strains, *ganciclovir*-resistant CMV strains with UL97 mutations (but not those with DNA polymerase mutations), and some *foscarnet*-resistant CMV strains. *Cidofovir* synergistically inhibits CMV replication in combination with *ganciclovir* or *foscarnet*.

Mechanisms of Action and Resistance

Cidofovir inhibits viral DNA synthesis by slowing and eventually terminating chain elongation. *Cidofovir* is metabolized to its active diphosphate form by cellular enzymes; the levels of phosphorylated metabolites are similar in infected and uninfected cells. The diphosphate acts as both a competitive inhibitor with respect to dCTP and as an alternative substrate for viral DNA polymerase.

Cidofovir resistance in CMV is due to mutations in viral DNA polymerase. Low-level resistance to *cidofovir* develops in up to about 30% of patients with retinitis by 3 months of therapy. Highly *ganciclovir*-resistant CMV isolates that possess DNA polymerase and UL97 kinase mutations are resistant to *cidofovir*, and prior *ganciclovir* therapy may select for *cidofovir* resistance. Some *foscarnet*-resistant CMV isolates show cross-resistance to *cidofovir*, and triple-drug-resistant variants with DNA polymerase mutations occur.

ADME

Cidofovir has very low oral bioavailability. Penetration into the CSF is low. Topical *cidofovir* gel may result in low plasma concentrations (<0.5 μg/mL) in patients with large mucocutaneous lesions. Plasma levels after intravenous dosing decline in a biphasic pattern with a terminal $t_{1/2}$ that averages 2.6 h. The active form, *cidofovir diphosphate*, has a prolonged intracellular $t_{1/2}$ and competitively inhibits CMV and HSV DNA polymerases at concentrations one-eighth to one six-hundredth of those required to inhibit human DNA polymerases (Hitchcock et al., 1996), thereby providing a modest measure of selective toxicity. A phosphocholine metabolite of the drug also has a long intracellular $t_{1/2}$ (~87 h) and may serve as an intracellular reservoir of drug. The prolonged intracellular $t_{1/2}$ of *cidofovir diphosphate* allows infrequent (weekly or biweekly) dosing regimens. *Cidofovir* is cleared by the kidney via glomerular filtration and tubular secretion. Over 90% of the dose is recovered unchanged in the urine. *Probenecid* blocks tubular transport of *cidofovir* and reduces renal clearance and associated nephrotoxicity. Elimination relates linearly to creatinine clearance; the $t_{1/2}$ increases to 32.5 h in patients on chronic ambulatory peritoneal dialysis. Hemodialysis removes more than 50% of the administered dose.

Therapeutic Uses

Intravenous *cidofovir* is approved for the treatment of CMV retinitis in HIV-infected patients. Intravenous *cidofovir* has been used for treating *acyclovir*-resistant mucocutaneous HSV infection, adenovirus disease in transplant recipients, and extensive molluscum contagiosum in HIV patients. Reduced doses without *probenecid* may be beneficial in BK virus nephropathy in patients with a renal transplant. Topical *cidofovir* gel eliminates virus shedding and lesions in some HIV-infected patients with *acyclovir*-resistant mucocutaneous HSV infections and has been used in treating anogenital warts and molluscum contagiosum in immunocompromised patients and cervical intraepithelial neoplasia in women. Intralesional *cidofovir* induces remissions in adults and children with respiratory papillomatosis.

Untoward Effects

Nephrotoxicity is the principal dose-limiting side effect of intravenous *cidofovir*. Concomitant oral *probenecid* and saline prehydration reduce the risk of renal toxicity; however, *probenecid* alters renal clearance of many agents (see Figure 42–2), albeit not of *cidofovir*. For example, *probenecid* alters *zidovudine* pharmacokinetics such that *zidovudine* doses should be reduced when *probenecid* is present (as should the doses of other drugs whose renal secretion *probenecid* inhibits, e.g., β-lactam antibiotics, nonsteroidal anti-inflammatory drugs [NSAIDs], *acyclovir*, *lorazepam*, *furosemide*, *methotrexate*, *theophylline*, and *rifampin*).

On maintenance doses of 5 mg/kg every 2 weeks, up to 50% of patients develop proteinuria, 10% to 15% show an elevated serum creatinine concentration, and 15% to 20% develop neutropenia. Anterior uveitis that is responsive to topical corticosteroids and cycloplegia occurs commonly, and low intraocular pressure occurs infrequently with intravenous *cidofovir*. Administration with food and pretreatment with antiemetics, antihistamines, or *acetaminophen* may improve tolerance. Concurrent nephrotoxic agents are contraindicated, and at least 7 days should elapse before initiation of *cidofovir* treatment is recommended after prior exposure to aminoglycosides, intravenous *pentamidine*, *amphotericin B*, *foscarnet*, NSAID, or contrast dye. *Cidofovir* and oral *ganciclovir* are poorly tolerated in combination at full doses.

Topical application of *cidofovir* is associated with dose-related application site reactions (e.g., burning, pain, and pruritus) in up to one-third of patients and occasionally ulceration. *Cidofovir* is considered a potential human carcinogen. It may cause infertility and no adequate human data have established whether this drug poses a pregnancy risk (former pregnancy category C).

Famciclovir and Penciclovir

Famciclovir is the diacetyl ester prodrug of 6-deoxy penciclovir and lacks intrinsic antiviral activity. *Penciclovir* is an acyclic guanine nucleoside analogue. *Penciclovir* is similar to *acyclovir* in its spectrum of activity and potency against HSV and VZV. It also is inhibitory for HBV.

Mechanisms of Action and Resistance

Penciclovir is an inhibitor of viral DNA synthesis. In HSV- or VZV-infected cells, *penciclovir* is phosphorylated initially by viral TK. *Penciclovir triphosphate* is a competitive inhibitor of viral DNA polymerase (see Figure 62–3). Although *penciclovir triphosphate* is approximately one-hundredth as potent as *acyclovir triphosphate* in inhibiting viral DNA polymerase, it is present in infected cells at much higher concentrations and for more prolonged periods. The prolonged intracellular $t_{1/2}$ of *penciclovir triphosphate*, 7 to 20 h, is associated with prolonged antiviral effects. Because *penciclovir* has a 3′-hydroxyl group, it is not an obligate chain terminator but does inhibit DNA elongation. Resistance during clinical use is low. TK-deficient, *acyclovir*-resistant herpesviruses are cross-resistant to *penciclovir*.

ADME

Oral *penciclovir* has low (<5%) bioavailability. In contrast, *famciclovir* is well absorbed orally (bioavailability ~75%) and is converted rapidly to *penciclovir* by deacetylation of the side chain and oxidation of the purine ring during and following absorption. Food slows absorption but does not reduce overall bioavailability. The plasma elimination $t_{1/2}$ of *penciclovir* averages about 2 h, and more than 90% is excreted unchanged in the urine. Following oral *famciclovir* administration, nonrenal clearance accounts for about 10% of each dose, primarily through fecal excretion, but *penciclovir* (60% of dose) and its 6-deoxy precursor (<10% of dose) are eliminated primarily in the urine. The plasma $t_{1/2}$ averages 9.9 h in renal insufficiency (creatinine clearance [Cl_{cr}] <30 mL/min); hemodialysis efficiently removes *penciclovir*.

Therapeutic Uses

Oral *famciclovir*, topical *penciclovir*, and intravenous *penciclovir* are approved for managing HSV and VZV infections.

Oral *famciclovir* (250 mg three times a day for 7–10 days) is as effective as *acyclovir* in treating first-episode genital herpes (Kimberlin and Rouse, 2004). In patients with recurrent genital HSV, patient-initiated *famciclovir* treatment (125 or 250 mg twice daily for 5 days) reduces healing time and symptoms by about 1 day. *Famciclovir* (250 mg twice daily for up to 1 year) is effective for suppression of recurrent genital HSV, but single daily doses are less effective. Higher doses (500 mg twice daily) reduce HSV recurrences in HIV-infected persons. Intravenous *penciclovir* (5 mg/kg every 8 or 12 h for 7 days) (not available in the U.S.) is comparable to intravenous *acyclovir* for treating mucocutaneous HSV infections in immunocompromised hosts. In immunocompetent persons with recurrent orolabial HSV, topical 1% *penciclovir* cream (applied every 2 h while awake for 4 days) shortens healing time and symptoms by about 1 day (Raborn et al., 2002).

In immunocompetent adults with herpes zoster of 3 days' duration or less, *famciclovir* (500 mg three times a day for 10 days) is at least as effective as *acyclovir* (800 mg five times daily) in reducing healing time and zoster-associated pain, particularly in those 50 years or older. *Famciclovir* is comparable to *valacyclovir* in treating zoster and reducing associated pain in older adults (Tyring et al., 2000). *Famciclovir* (500 mg three times a day for 7–10 days) also is comparable to high-dose oral *acyclovir* in treating zoster in immunocompromised patients and in those with ophthalmic zoster (Tyring et al., 2001).

Famciclovir is associated with dose-related reductions in HBV DNA and transaminase levels in patients with chronic HBV hepatitis but is less effective than *lamivudine* (Lai et al., 2002). *Famciclovir* is also ineffective in treating *lamivudine*-resistant HBV infections owing to emergence of multiply resistant variants.

Untoward Effects

Oral *famciclovir* is associated with headache, diarrhea, and nausea. Urticaria, rash, and hallucinations or confusional states (predominantly in the elderly) have been reported. Topical *penciclovir* (~1%) rarely is associated with local reactions. The short-term tolerance of *famciclovir* is comparable with that of *acyclovir*. *Penciclovir* is mutagenic at high concentrations. Long-term administration (1 year) does not affect spermatogenesis in men. Safety during pregnancy has not been established.

Ganciclovir and Valganciclovir

Ganciclovir is an acyclic guanine nucleoside analogue that is similar in structure to *acyclovir*. *Valganciclovir* is the L-valyl ester prodrug of *ganciclovir*. *Ganciclovir* has inhibitory activity against all herpesviruses and is especially active against CMV.

Mechanisms of Action and Resistance

Ganciclovir inhibits viral DNA synthesis. It is monophosphorylated intracellularly by viral TK during HSV infection and by a viral phosphotransferase encoded by the *UL97* gene during CMV infection. *Ganciclovir diphosphate* and *ganciclovir triphosphate* are formed by host enzymes. At least 10-fold higher concentrations of *ganciclovir triphosphate* are present in CMV-infected than in uninfected cells. The triphosphate is a competitive inhibitor of dGTP incorporation into DNA and preferentially inhibits viral rather than host cellular DNA polymerases. Incorporation into viral DNA causes eventual cessation of DNA chain elongation (see Figures 62–1A and 62–3).

Cytomegalovirus can become resistant to *ganciclovir* by either of two mechanisms: reduced intracellular *ganciclovir* phosphorylation owing to mutations in the viral phosphotransferase or mutations in viral DNA polymerase. Highly resistant variants with both mutations are cross-resistant to *cidofovir* and variably to *foscarnet*. *Ganciclovir* also is much less active against *acyclovir*-resistant TK-deficient HSV strains.

ADME

The oral bioavailability of *ganciclovir* is low, only 6% to 9% following ingestion with food. On the other hand, oral doses of the prodrug *valganciclovir* are well absorbed and hydrolyzed rapidly to *ganciclovir*; thus, *valganciclovir* provides greater bioavailability of the *ganciclovir* moiety, about 60%. Food further increases the bioavailability of *valganciclovir* by about 25%. Following intravenous administration of *ganciclovir*, vitreous fluid levels are similar to or higher than those in plasma and decline with a $t_{1/2}$ of 23 to 26 h. Intraocular sustained-release *ganciclovir* implants provide vitreous levels of about 4.1 μg/mL. The plasma elimination $t_{1/2}$ is about 2 to 4 h. Intracellular *ganciclovir triphosphate* concentrations are 10-fold higher than those of *acyclovir* triphosphate and decline much more slowly, with an intracellular elimination $t_{1/2}$ longer than 24 h. These differences may account in part for *ganciclovir*'s greater anti-CMV activity and provide the rationale for single daily doses in suppressing human CMV infections. Over 90% of *ganciclovir* is eliminated unchanged by renal excretion. Plasma $t_{1/2}$ increases in patients with severe renal insufficiency.

Therapeutic Uses

In CMV retinitis, initial induction treatment (5 mg/kg IV every 12 h for 10–21 days) is associated with improvement or stabilization in about 85% of patients (Faulds and Heel, 1990). Reduced viral excretion is usually evident by 1 week, and funduscopic improvement is seen by 2 weeks. Because of the high risk of relapse, patients with AIDS with retinitis require suppressive therapy with high doses of *ganciclovir* (5 mg/kg per day). Oral *ganciclovir* (1000 mg three times daily) is effective for suppression of retinitis after initial intravenous treatment but has been replaced in practice by oral *valganciclovir*. Oral *valganciclovir* (900 mg twice daily for 21 days of initial treatment) is comparable to intravenous dosing for initial control and sustained suppression (900 mg daily) of CMV retinitis (Schreiber et al., 2009). Intravitreal *ganciclovir* injections have been used in some patients, and an intraocular sustained-release *ganciclovir* implant is more effective than systemic dosing in suppressing retinitis progression.

Ganciclovir therapy (5 mg/kg every 12 h for 14–21 days) may benefit other CMV syndromes in patients with AIDS or recipients of solid-organ transplants (Kotton et al., 2010). *Ganciclovir* has been used for both prophylaxis and preemptive therapy of CMV infections in transplant recipients (Schreiber et al., 2009).

A *ganciclovir* ophthalmic gel formulation is effective in treating HSV keratitis (Colin et al., 1997). Oral *ganciclovir* also reduces HBV DNA levels and aminotransferase levels in chronic HBV infection (Hadziyannis et al., 1999), but the drug is not approved for this indication.

Untoward Effects

Myelosuppression is the principal dose-limiting toxicity of *ganciclovir*. Neutropenia occurs in about 15% to 40% of patients and is observed most commonly during the second week of treatment and usually is reversible within 1 week of drug cessation. Persistent fatal neutropenia has occurred. Recombinant granulocyte colony-stimulating factor (G-CSF; *filgrastim*, *lenograstim*) may be useful in treating *ganciclovir*-induced neutropenia (see Chapter 45). Thrombocytopenia occurs in 5% to 20% of patients. *Zidovudine* and probably other cytotoxic agents increase the risk of myelosuppression, as do nephrotoxic agents that impair *ganciclovir* excretion. *Probenecid* and possibly *acyclovir* reduce renal clearance of *ganciclovir*. Oral *ganciclovir* increases the absorption and peak plasma concentrations of *didanosine* by approximately 2-fold and that of *zidovudine* by about 20%. CNS side effects (5%–15%) range in severity from headache to behavioral changes to convulsions and coma. About one-third of patients must interrupt or prematurely stop intravenous *ganciclovir* therapy because of bone marrow or CNS toxicity. Infusion-related phlebitis, azotemia, anemia, rash, fever, liver function test abnormalities, nausea or vomiting, and eosinophilia also have been described. Risk of *ganciclovir*'s use during pregnancy has not been ruled out.

Letermovir

Letermovir is the first newly licensed antiviral agent with CMV activity in over 20 years. It is indicated for prophylaxis of CMV infection and disease in adult CMV-seropositive recipients of an allogeneic hematopoietic stem cell transplantation (HSCT). The drug is also being evaluated in pediatric HSCT patients. Given *letermovir*'s efficacy and safety profile, the drug is being evaluated for treatment of neonatal symptomatic congenital CMV infection in combination with *ganciclovir*.

Mechanisms of Action and Resistance

Letermovir is a novel inhibitor of the CMV viral enzyme DNA terminase (UL56/UL89), which plays an important role in the cleavage of newly synthesized CMV DNA into individual viral genomes. These genomes are subsequently inserted into CMV procapsids to generate infectious CMV virions (Goldner et al., 2011). *Letermovir* has demonstrated potent, selective, and reversible inhibition of CMV activity in preclinical studies *in vitro* and efficacy against the virus *in vivo* (Goldner et al., 2011; Lischka et al., 2010). The median EC_{50} of *letermovir* against a collection of clinical

CMV isolates in cell culture was 2.1 nM (range, 0.7–6.1 nM; FDA, 2017). In a phase II trial, seven *letermovir*-treated participants experienced virological failure (Lischka et al., 2016). All but one of the resistance mutations were found to represent natural polymorphisms that did not affect susceptibility to *letermovir*. One subject from the 60-mg daily dose group exhibited the UL56 genotype, V236M, conferring *in vitro* resistance to *letermovir*. In a phase III trial, the protein subunit pUL56 substitutions V236M, E237G, C325W, and R369T were detected in three subjects, which significantly shifted the susceptibility (Douglas et al., 2020).

Cross-resistance is not likely with drugs outside of this class. *Letermovir* is fully active against viral populations with substitutions conferring resistance to CMV DNA polymerase inhibitors (*cidofovir*, *foscarnet*, and *ganciclovir*). These agents are expected to be fully active against *letermovir*-resistant viral populations.

ADME

Letermovir pharmacokinetics are nonlinear and time dependent and exhibit greater than dose-proportional changes in exposure. There is considerable variability in absorption after oral administration. The net effect of *letermovir* on CYP3A is moderate inhibition, and concentrations of CYP2C8 substrates are predicted to be increased by *letermovir* (Wang et al., 2019). *In vitro*, *letermovir* inhibits efflux transporters P-glycoprotein, multidrug resistance-associated protein 2 (MRP2), bile salt export pump (BSEP), breast cancer resistance protein (BCRP), hepatic uptake transporter OATP1B1/3, and organic anion transporter 3 (OAT3). Thus, one may expect drug-drug interactions involving those transporters.

Therapeutic Uses

Letermovir is dosed at 480 mg once daily orally or as an intravenous infusion over 1 h through 100 days after transplant. It is available as 240- and 480-mg oral tablets and a 20-mg/mL solution for infusion. The 240-mg dose is for patients receiving concomitant *cyclosporine*, which doubles *letermovir* exposure (assessed as AUC [area under curve of plasma drug concentration vs. time]) likely due to *cyclosporine*'s inhibition of liver uptake transporters OATP1B1/3. In a phase III trial in CMV-seropositive recipients of allogeneic HSCT, *letermovir* was superior to placebo in preventing clinically significant CMV infection through week 24 after transplantation (Marty et al., 2017). Among 495 patients with undetectable CMV DNA at baseline, 37.5% had clinically significant CMV infection or reached a primary endpoint by week 24 after transplantation compared to 60.6% of placebo recipients ($P < 0.001$).

Untoward Effects

Vomiting was reported in 18.5% of patients (vs. 13.5% for placebo). Edema was reported in 14.5% (vs. 9.4% for placebo), and atrial fibrillation or flutter was reported in 4.6% (vs. 1.0% for placebo). The rates of myelotoxic and nephrotoxic events were similar in the *letermovir* and placebo groups. All-cause mortality at 24 and 48 weeks after transplantation was 10.2% versus 15.9% ($P = 0.03$) and 20.9% versus 25.5% among *letermovir* versus placebo recipients, respectively. The frequency and severity of adverse events were similar in the two groups overall.

Foscarnet

Foscarnet (trisodium phosphonoformate) is an inorganic pyrophosphate analogue that is inhibitory for all herpesviruses and HIV.

Mechanisms of Action and Resistance

Foscarnet inhibits viral nucleic acid synthesis by interacting directly at HSV DNA polymerase or HIV reverse transcriptase (see Figures 62–1A and 62–3). *Foscarnet* reversibly blocks the pyrophosphate binding site of the viral DNA polymerase, inhibiting cleavage of pyrophosphate from deoxynucleotide triphosphates and thereby inhibiting chain elongation (deoxynucleotide triphosphate + $DNA_n \rightarrow$ diphosphate + DNA_{n+1}). *Foscarnet* has about 100-fold greater inhibitory effects against herpesvirus DNA polymerases than against cellular DNA polymerase α. Herpesviruses resistant to *foscarnet* have point mutations in the viral DNA polymerase.

ADME

Foscarnet is poorly soluble in aqueous solutions and requires large volumes for administration; in addition, the drug's oral bioavailability is low. Vitreous levels approximate those in plasma; CSF levels average 66% of those in plasma at steady state. Over 80% of *foscarnet* is excreted unchanged in the urine. Dose adjustments are necessary for small decreases in renal function. Plasma elimination has initial bimodal half-lives totaling 4 to 8 h and a prolonged terminal elimination $t_{1/2}$ of 3 to 4 days. Sequestration in bone with gradual release accounts for the fate of an estimated 10% to 20% of a given dose. *Foscarnet* is cleared efficiently by hemodialysis (~50% of a dose).

Therapeutic Uses

Intravenous *foscarnet* is effective for treatment of CMV retinitis, including *ganciclovir*-resistant infections, other types of CMV infection, and *acyclovir*-resistant HSV and VZV infections.

In CMV retinitis in patients with AIDS, *foscarnet* (60 mg/kg every 8 h or 90 mg/kg every 12 h for 14–21 days followed by chronic maintenance at 90–120 mg/kg every day in one dose) is associated with clinical stabilization in about 90% of patients. In CMV retinitis in patients with AIDS, *foscarnet* (60 mg/kg every 8 h or 90 mg/kg every 12 h for 14–21 days followed by chronic maintenance at 90–120 mg/kg every day in one dose) is associated with clinical stabilization in about 90% of patients. When used for preemptive therapy of CMV viremia in bone marrow transplant recipients, *foscarnet* (60 mg/kg every 12 h for 2 weeks followed by 90 mg/kg daily for 2 weeks) is as effective as intravenous *ganciclovir* and causes less neutropenia (Reusser et al., 2002). When used for CMV infections, *foscarnet* may reduce the risk of Kaposi sarcoma in HIV-infected patients. Intravitreal injections of *foscarnet* also have been used. In *acyclovir*-resistant mucocutaneous HSV infections, lower doses of *foscarnet* (40 mg/kg every 8 h for ≥7 days) are associated with cessation of viral shedding and with complete healing of lesions in about three-quarters of patients. *Foscarnet* also appears to be effective in *acyclovir*-resistant VZV infections. Topical *foscarnet* cream is ineffective in treating recurrent genital HSV in immunocompetent persons but appears to be useful in chronic *acyclovir*-resistant infections in immunocompromised patients.

Untoward Effects

Major dose-limiting toxicities are nephrotoxicity and symptomatic hypocalcemia. Increases in serum creatinine occur in up to one-half of patients but are generally reversible after cessation. High doses, rapid infusion, dehydration, prior renal insufficiency, and concurrent nephrotoxic drugs are risk factors. Saline loading may reduce the risk of nephrotoxicity. *Foscarnet* is highly ionized at physiological pH, and metabolic abnormalities are very common. These include increases or decreases in Ca^{2+} and phosphate, hypomagnesemia, and hypokalemia. Concomitant intravenous *pentamidine* administration increases the risk of symptomatic hypocalcemia. CNS side effects include headache (25%), tremor, irritability, seizures, and hallucinosis. Other reported side effects are generalized rash, fever, nausea or emesis, anemia, leukopenia, abnormal liver function tests, electrocardiographic changes, infusion-related thrombophlebitis, and painful genital ulcerations. Topical *foscarnet* may cause local irritation and ulceration, and oral *foscarnet* may cause gastrointestinal (GI) disturbance. Preclinical studies indicate that high *foscarnet* concentrations are mutagenic. Safety in pregnancy or childhood is uncertain.

Fomivirsen

Fomivirsen, a 21-base phosphorothioate oligonucleotide, provides antisense therapy. The drug is complementary to the mRNA sequence for the major immediate-early transcriptional region of CMV and inhibits CMV replication through sequence-specific and nonspecific mechanisms, including inhibition of virus binding to cells. *Fomivirsen* is active against CMV strains resistant to *ganciclovir*, *foscarnet*, and *cidofovir*. *Fomivirsen* is given by intravitreal injection in the treatment of CMV retinitis for patients intolerant of or unresponsive to other therapies. Following injection, it is cleared slowly from the vitreous ($t_{1/2}$ ~55 h) through distribution to the retina and probable exonuclease digestion. In HIV-infected patients with refractory, sight-threatening CMV retinitis, *fomivirsen* injections (330 μg weekly for 3 weeks and then every 2 weeks or on days 1 and 15 followed by monthly) significantly delay time to retinitis progression. Ocular side effects include iritis in up to one-quarter of patients, which can be managed with topical corticosteroids; vitritis; cataracts; and increases in intraocular pressure in 15% to 20% of patients. Recent *cidofovir* use may increase the risk of inflammatory reactions. *This drug is no longer available in the U.S.*

Docosanol

Docosanol is a long-chain saturated alcohol that is approved as an over-the-counter 10% cream for the treatment of recurrent orolabial herpes. *Docosanol* inhibits the *in vitro* replication of many lipid-enveloped viruses, including HSV. It does not inactivate HSV directly but appears to block fusion between the cellular and viral envelope membranes and inhibits viral entry into the cell. Topical treatment beginning within 12 h of prodromal symptoms or lesion onset reduces healing time by about 1 day and is well tolerated. Treatment initiation at papular or later stages provides no benefit.

Idoxuridine

Idoxuridine is an iodinated thymidine analogue that inhibits the *in vitro* replication of various DNA viruses, including herpesviruses and poxviruses. *Idoxuridine* lacks selectivity, in that low concentrations inhibit the growth of uninfected cells. The triphosphate inhibits viral DNA synthesis and is incorporated into both viral and cellular DNA. In the U.S., *idoxuridine* is approved only for topical (ophthalmic) treatment of HSV keratitis. *Idoxuridine* formulated in dimethylsulfoxide is available outside the U.S. for topical treatment of herpes labialis, genitalis, and zoster. Adverse reactions include pain, pruritus, inflammation, and edema of the eye or lids; allergic reactions are rare.

Trifluridine

Trifluridine is a fluorinated pyrimidine nucleoside that has *in vitro* inhibitory activity against HSV types 1 and 2, CMV, vaccinia, and to a lesser extent, certain adenoviruses. *Trifluridine* inhibits replication of herpesviruses, including *acyclovir*-resistant strains, and also inhibits cellular DNA synthesis at relatively low concentrations. *Trifluridine monophosphate* irreversibly inhibits thymidylate synthase, and *trifluridine triphosphate* is a competitive inhibitor of thymidine triphosphate incorporation into DNA; *trifluridine* is incorporated into viral and cellular DNA. *Trifluridine*-resistant HSV has been described.

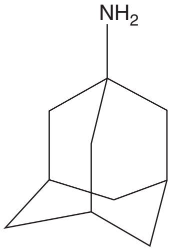
Trifluridine currently is used for treatment of primary keratoconjunctivitis and recurrent epithelial keratitis owing to HSV types 1 and 2. Topical *trifluridine* is more active than *idoxuridine* and comparable to *vidarabine* in HSV ocular infections. Adverse reactions include discomfort on instillation and palpebral edema. Hypersensitivity reactions and irritation are uncommon. Topical *trifluridine* also appears to be effective in some patients with *acyclovir*-resistant HSV cutaneous infections.

Anti-influenza Agents

Recently, there has been concern about the possibility of new influenza pandemics stemming from small but severe outbreaks of H5N1 avian influenza and the novel 2009 influenza A H1N1 that is thought to be of swine origin. Six drugs are currently approved for the treatment and prevention of influenza virus infection: the adamantane antivirals *amantadine* and *rimantadine*; *oseltamivir*; *zanamivir*; *peramivir*; and *baloxavir marboxil*. Resistance to these drugs has arisen as a consequence of their overuse, including in veterinary applications. Development of resistance and the spread of resistant viruses are major challenges in the chemotherapy and chemoprophylaxis of influenza and are likely to drive future recommendations for use of these drugs in global populations. The U.S. Centers for Disease Control and Prevention (CDC) annually issues recommendations for influenza vaccinations and comments on effective medications (CDC, 2021).

Amantadine and Rimantadine

Amantadine and its derivative *rimantadine* are uniquely configured tricyclic amines. *Rimantadine* has $H_3C-\underset{|}{CH}-NH_2$ in place of the $-NH_2$ group.



AMANTADINE

Mechanisms of Action and Resistance

Amantadine and *rimantadine* inhibit an early step in viral replication, probably viral uncoating; for some strains, they also have an effect on a late step in viral assembly, probably mediated through altering hemagglutinin processing. The primary locus of action is the influenza A virus M2 protein, an integral membrane protein that functions as an ion channel. By interfering with this function of the M2 protein, the drugs inhibit the acid-mediated dissociation of the ribonucleoprotein complex early in replication and potentiate acidic pH–induced conformational changes in hemagglutinin during its intracellular transport later in replication. Resistance to these drugs results from a mutation in the RNA sequence encoding for the M2 protein transmembrane domain; resistant isolates typically appear in the treated patient within 2 to 3 days of starting therapy.

ADME

Table 62–3 summarizes important pharmacokinetics properties of these antiviral agents. The two adamantanes differ in several respects. *Amantadine* is excreted largely unmetabolized in the urine ($t_{1/2}$ of elimination is ~12–18 h in young adults, increasing up to twice that in the elderly and even more in those with renal impairment). By contrast, elimination of *rimantadine* depends on hepatic function; the drug is subject to phase 1 and phase 2 reactions prior to renal excretion of metabolites (elimination $t_{1/2}$ ~24–36 h; 60%–90% is excreted in the urine as metabolites). Elderly patients require only one-half the weight-adjusted dose of *amantadine* needed for young adults. *Amantadine* is excreted in breast milk. *Rimantadine* concentrations in nasal mucus average 50% higher than those in plasma.

Therapeutic Uses

Although both drugs are useful for the prevention and treatment of infections caused by influenza A virus, vaccination against influenza is a more cost-effective means of reducing disease burden. *Amantadine* and *rimantadine* are active only against susceptible influenza A viruses (not influenza B); *rimantadine* is 4- to 10-fold more active than *amantadine*. Virtually all H3N2 strains of influenza circulating worldwide are resistant to these drugs.

Seasonal prophylaxis with either *amantadine* or *rimantadine* (a total of 200 mg/day in one or two divided doses in young adults) is about 70% to 90% protective against influenza A illness. These agents are efficacious in preventing nosocomial influenza and in curtailing nosocomial outbreaks during pandemic influenza. Doses of 100 mg/day are better tolerated and still appear to be protective against influenzal illness. Seasonal prophylaxis is an alternative in high-risk patients, if the influenza vaccine cannot be administered or may be ineffective (i.e., in immunocompromised patients). Prophylaxis should be started as soon as influenza is identified in a community or region and should be continued throughout the period of risk (usually 4–8 weeks) because any protective effects are lost several days after cessation of therapy. Alternatively, the drugs can be started in conjunction with immunization and continued for 2 weeks until protective immune responses develop.

TABLE 62–3 ■ PHARMACOLOGICAL CHARACTERISTICS OF ANTIVIRALS FOR INFLUENZA

	AMANTADINE	RIMANTADINE	ZANAMIVIR	OSELTAMIVIR	PERAMIVIR	BALOXAVIR
Spectrum[f]	A	A	A, B	A, B	A, B	A, B
Route/ formulations	Oral (tablet/ capsule/syrup)	Oral (tablet/syrup)	Inhaled (powder) Intravenous[a]	Oral (capsule/syrup) Intravenous[a]	Intravenous	Oral (tablet/ suspension)
Oral bioavailability	>90%	>90%	<5%[b]	80%[c]	Not applicable	>95%
Effect of meals on AUC	Negligible	Negligible	Not applicable	Negligible	Not applicable	↓ 36%
Elimination $t_{1/2}$, h	12–18	24–36	2.5–5	6–10[c]	20	79
Protein binding, %	67%	40%	<10%	3%[c]	<30%	93%
Metabolism, %	<10%	~75%	Negligible	Negligible	Negligible	80%
Renal excretion[e]	>90%	~25%	100%	95%[c]	90%	Negligible
Dose adjustments	$Cl_{cr} \le 50$ Age ≥ 65 years	$Cl_{cr} \le 10$ Age ≥ 65 years	None[d]	$Cl_{cr} \le 30$	$Cl_{cr} \le 50$	< 80 kg; 40 mg ≥80 kg; 80 mg

[a] Investigational.
[b] Systemic absorption 4%–17% after inhalation.
[c] For antivirally active oseltamivir carboxylate.
[d] Inhaled formulation only.
[e] % of parent drug.
[f] Types of influenza.

The adamantanes are effective against influenza A H1N1 if treatment is initiated within 2 days of the onset of symptoms (Schmidt, 2004). In uncomplicated influenza A illness of adults, early *amantadine* or *rimantadine* treatment (200 mg/day for 5 days) reduces the duration of fever and systemic complaints by 1 to 2 days, speeds functional recovery, and sometimes decreases the duration of virus shedding. The usual regimen in children (≥1 year of age) is 5 mg/kg per day, up to 150 mg, administered once or twice daily. Resistant variants have been recovered from about 30% of treated children or outpatient adults by the fifth day of therapy.

Untoward Effects

The most common side effects related to *amantadine* and *rimantadine* are minor dose-related CNS and GI effects: nervousness, light-headedness, difficulty concentrating, insomnia, loss of appetite, and nausea. CNS side effects (5%–33%) occur in patients treated with *amantadine* at doses of 200 mg/day but are significantly less frequent with *rimantadine*. The neurotoxic effects of *amantadine* appear to be increased by concomitant ingestion of antihistamines and psychotropic or anticholinergic drugs, especially in elderly patients. At comparable doses of 100 mg/day, *rimantadine* is significantly better tolerated in nursing home residents than *amantadine*. High *amantadine* plasma concentrations (1.0–5.0 μg/mL) have been associated with serious neurotoxic reactions, including delirium, hallucinosis, seizures, coma, and cardiac arrhythmias. Exacerbations of preexisting seizure disorders and psychiatric symptoms may occur with *amantadine* and possibly with *rimantadine*. No adequate human data have established whether the adamantanes pose a pregnancy risk (former pregnancy category C).

Oseltamivir

Oseltamivir carboxylate is a transition-state analogue of sialic acid that is a potent selective inhibitor of the neuraminidases of influenza A and B virus. *Oseltamivir phosphate* is an ethyl ester prodrug that lacks antiviral activity. *Oseltamivir carboxylate* has an antiviral spectrum and potency similar to that of *zanamivir*: It inhibits *amantadine*- and *rimantadine*-resistant influenza A viruses and some *zanamivir*-resistant variants.

Mechanisms of Action and Resistance

Influenza neuraminidase cleaves terminal sialic acid residues and destroys the receptors recognized by viral hemagglutinin, which are present on the cell surface, in progeny virions, and in respiratory secretions. This enzymatic action is essential for release of virus from infected cells. Interaction of *oseltamivir carboxylate* with the neuraminidase causes a conformational change within the enzyme's active site and inhibits its activity. Inhibition of neuraminidase activity leads to viral aggregation at the cell surface and reduced virus spread within the respiratory tract. Influenza variants selected *in vitro* for resistance to *oseltamivir carboxylate* contain hemagglutinin or neuraminidase mutations. Seasonal influenza A (H1N1) has become virtually 100% resistant to *oseltamivir* worldwide (Moscona, 2009; Schirmer and Holodniy, 2009). Importantly, novel H1N1 (nH1N1 or swine influenza) remains susceptible to *oseltamivir*.

ADME

Table 62–3 summarizes a number of pharmacokinetic properties of *oseltamivir carboxylate*. Oral *oseltamivir phosphate* is absorbed rapidly and cleaved by esterases in the GI tract and liver to the active carboxylate. Food does not decrease bioavailability but reduces the risk of GI intolerance. Bronchoalveolar lavage levels in animals and middle ear fluid and sinus concentrations in humans are comparable with plasma levels. *Probenecid* doubles the plasma $t_{1/2}$ of the carboxylate, which indicates tubular secretion by the anionic pathway. Children younger than 2 years exhibit age-related changes in *oseltamivir carboxylate* clearance and total drug exposure (Kimberlin et al., 2009).

Therapeutic Uses

Oral *oseltamivir* is effective in the treatment and prevention of influenza A and B virus infections. Treatment of previously healthy adults (75 mg twice daily for 5 days) or children 1 to 12 years of age (weight-adjusted dosing) with acute influenza reduces illness duration by about 1 to 2 days, speeds functional recovery, and reduces the risk of complications leading to antibiotic use by 40% to 50%. Treatment reduces by about 50% the risk of subsequent hospitalization in adults (Kaiser et al., 2003). When used for prophylaxis during the typical influenza season, *oseltamivir* (75 mg once daily) is effective (~70%–90%) in reducing the likelihood of influenza illness in both unimmunized working adults and in immunized nursing home residents; short-term use protects against influenza in household contacts (Schirmer and Holodniy, 2009).

Untoward Effects

Oral *oseltamivir* is associated with nausea, abdominal discomfort, and, less often, emesis. GI complaints typically resolve in 1 to 2 days despite continued dosing and are preventable by administration with food. An increased frequency of headache was reported in one prophylaxis study in elderly adults. Neither the phosphate nor the carboxylate form interacts with cytochrome P450 isozymes (CYPs) *in vitro*. *Oseltamivir* does not appear to impair fertility, but safety in pregnancy is uncertain.

Zanamivir

Zanamivir is a sialic acid analogue that potently and specifically inhibits the neuraminidases of influenza A and B viruses. *Zanamivir* inhibits *in vitro* replication of influenza A and B viruses, including *amantadine*- and *rimantadine*-resistant strains and several *oseltamivir*-resistant variants.

Mechanisms of Action and Resistance

Zanamivir inhibits viral neuraminidase and thus causes viral aggregation at the cell surface and reduced spread of virus within the respiratory tract. *In vitro* selection of viruses resistant to *zanamivir* is associated with mutations in the viral hemagglutinin or neuraminidase. Hemagglutinin variants are cross-resistant to other neuraminidase inhibitors. Neuraminidase variants contain mutations in the enzyme active site that diminish binding of *zanamivir*, but the altered enzymes show reduced activity or stability. *Zanamivir*-resistant variants usually have decreased infectivity in animals.

ADME

See Table 62–3 for pharmacokinetic properties of *zanamivir*. Oral bioavailability of *zanamivir* is less than 5%, and the commercial form is delivered by oral inhalation of dry powder in a lactose carrier. The proprietary inhaler device is breath actuated and requires a cooperative patient. Following inhalation of the dry powder, about 15% is deposited in the lower respiratory tract and about 80% in the oropharynx. Overall bioavailability is 4% to 17%.

Depending on the strain, *zanamivir* competitively inhibits influenza neuraminidase activity at concentrations of about 0.2 to 3 ng/mL but affects neuraminidases from other pathogens and mammalian sources only at 106-fold higher concentrations. *Zanamivir* inhibits *in vitro* replication of influenza A and B viruses, including *amantadine*- and *rimantadine*-resistant strains and several *oseltamivir*-resistant variants. It is active after topical administration in animal influenza models.

Therapeutic Uses

Inhaled *zanamivir* is effective for the prevention and treatment of influenza A and B virus infections. Early *zanamivir* treatment (10 mg [two inhalations] twice daily for 5 days) of febrile influenza in ambulatory adults and children 5 years or older shortens the time to illness resolution by 1 to 3 days and in adults reduces by 40% the risk of lower respiratory tract complications that require use of antibiotics. Once-daily inhaled *zanamivir* is highly protective against community-acquired influenza illness, and when given for 10 days, it protects against household transmission. Intravenous *zanamivir* ($t_{1/2}$ ~1.7 h) is available in the U.S. as an emergency investigational new drug (EIND) and in the E.U. on a compassionate use basis for life-threatening, resistant influenza.

Untoward Effects

Orally inhaled *zanamivir* generally is well tolerated in ambulatory adults and children with influenza. Wheezing and bronchospasm have been reported in some influenza-infected patients without known airway disease, and acute deteriorations in lung function, including fatal outcomes, have occurred in those with underlying asthma or chronic obstructive airway disease. *Zanamivir* is not generally recommended for treatment of patients with underlying airway disease because of the risk of serious adverse events. Preclinical studies of *zanamivir* revealed no evidence of mutagenic, teratogenic, or oncogenic effects (pregnancy risk not ruled out). No clinically significant drug interactions have been recognized to date. *Zanamivir* does not diminish the immune response to injected influenza vaccine.

Peramivir

Peramivir is an FDA-approved inhibitor of influenza virus neuraminidase indicated for the treatment of acute uncomplicated influenza in patients 18 years and older who have been symptomatic for no more than 2 days. While *peramivir* was in clinical development, the FDA authorized its emergency use for treatment of pandemic 2009 A/H1N1 in certain adult and pediatric patients.

Mechanisms of Action and Resistance

Peramivir has a mechanism of action similar to that of other neuraminidase inhibitors. Neuraminidase resistance can occur as the result of point mutations in either the neuraminidase or hemagglutinin genes or both. Structurally, *peramivir* differs somewhat from others in the class via a substitution resulting in multiple binding site interactions, which confers some activity against cross-resistant viruses. Antiviral resistance is currently low to the three available neuraminidase inhibitors among circulating influenza viruses. This will likely change with each influenza season. In general, cross-resistance across these agents exists, with the degree of cross-resistance depending on the viral strain and which point mutations occur.

ADME

Peramivir is not significantly metabolized in humans. It is not a substrate for CYPs and is neither a substrate nor an inhibitor of P-glycoprotein. The elimination half-life following intravenous administration of 600 mg as a single dose is approximately 20 h. Clearance is largely (90%) via renal excretion of the unchanged compound. Negligible accumulation is observed after repeated dosing. Following a 600-mg dose infused over 30 min, the end-of-infusion C_{max} was 46.8 μg/mL, and the $AUC_{0-\infty}$ was 102.7 μg*h/mL. Dosing should be adjusted in patients with altered Cl_{cr}. A single 200-mg dose should be administered for those with an estimated Cl_{cr} (Cockcroft-Gault) between 30 and 49 mL/min, and 100 mg should be administered for a Cl_{cr} of 10 to 29 mL/min. No clinically significant drug interactions have been recognized to date. Table 62–3 summarizes pharmacokinetic data for *peramivir*.

Therapeutic Uses

Peramivir is administered as a single 600-mg dose, administered via intravenous infusion over 15 to 30 min. A phase II clinical trial demonstrated that intravenous *peramivir* (300 or 600 mg as a single-dose infusion) reduced the time to alleviation of symptoms, from 82 h (placebo) to 59 h (treatment with 600 mg *peramivir*) (Kohno et al., 2010). A phase III trial demonstrated that *peramivir* (300 or 600 mg) was comparable in extent of symptom relief to oral *oseltamivir* (75 mg twice a day for 5 days) in patients with seasonal influenza A or B and with comparable rates of adverse events (Kohno et al., 2011). At present, *peramivir*'s primary use may be limited to patients with acute, uncomplicated influenza who cannot absorb or cannot take oral agents. Further studies are needed regarding the use of *peramivir* in severely ill hospitalized patients and in the pediatric population.

Untoward Effects

The most common adverse event (>2%) is diarrhea. Hypersensitivity reactions (e.g., Stevens-Johnson syndrome and erythema multiforme) have occurred, and treated patients with influenza may be at increased risk for neuropsychiatric events such as hallucinations, delirium, and abnormal behavior. Frequency and severity of adverse effects (*peramivir* 300 or 600 mg) are comparable to those with *oseltamivir* (75 mg twice daily for 5 days) (Kohno et al., 2011). Patients receiving 600 mg *peramivir* or *oseltamivir* had decreased neutrophil counts (10.4% vs. 9.3%), diarrhea (8.2% vs. 7.4%), and vomiting (1.6% vs. 4.1%), respectively.

Baloxavir Marboxil

Baloxavir marboxil is indicated for the treatment of acute uncomplicated influenza in patients 12 years of age and older who have been symptomatic for up to 2 days and for those at high risk of developing influenza-related complications. It is also approved for postexposure prophylaxis of influenza in patients 12 years of age or older following contact with an individual who has influenza. It is active against both type A and type B influenza.

Mechanisms of Action and Resistance

Baloxavir marboxil is an orally administered prodrug. Following absorption, the drug undergoes near complete hydrolysis to form active baloxavir acid. *Baloxavir* is a selective inhibitor of influenza cap-dependent endonuclease that blocks influenza proliferation by inhibiting the initiation of mRNA synthesis (Heo, 2018). *Baloxavir* has activity against *oseltamivir*-resistant viruses, and with its unique mechanism of action, it may act synergistically with other agents, particularly for uncomplicated infection. The emergence of polymerase acidic protein variants with I38T/M/F substitutions conferring reduced susceptibility to *baloxavir* occurred in 9.7% of *baloxavir* recipients in a phase III trial, typically at day 5 or later, but in none of 95 randomly selected placebo recipients (Hayden et al., 2018).

ADME

Baloxavir marboxil is rapidly and completely metabolized to baloxavir acid by hydrolysis in the intestine, blood, and liver mainly by arylacetamide deacetylase (AADAC). Baloxavir acid is primarily metabolized by UGT1A3 with a minor contribution from CYP3A4 and is primarily eliminated by biliary excretion (Ng, 2019). t_{max} occurs around 4 h after dose, and the drug undergoes a multiphasic decay with a terminal $t_{1/2}$ of approximately 80 h (Koshimichi et al., 2018). Coadministration of *baloxavir* with dairy products, calcium-fortified beverages, polyvalent cation-containing laxatives, antacids, or oral supplements (e.g., calcium, iron, magnesium, selenium, zinc) should be avoided since chelation with these polyvalent cations may decrease plasma exposure and curtail efficacy. The drug can be administered with or without food, but food decreases the C_{max} and AUC by 48% and 36%, respectively. Table 62–3 summarizes pharmacokinetic data for *baloxavir*.

Therapeutic Uses

Baloxavir marboxil is administered as a single oral dose: 40 mg for individuals weighing 40 to less than 80 kg and 80 mg for individuals weighing 80 kg or more (FDA, 2019a). A preservative-free (40 mg/20 mL) oral suspension formulation is also available; the *baloxavir* dose is the same for treatment and prophylaxis. The primary phase III trial was a double-blind, placebo- and *oseltamivir*-controlled, randomized trial that enrolled patients aged 12 to 64 years with influenza-like illness from the U.S. and Japan (Hayden et al., 2018). The median time to alleviation of symptoms was similar in the *baloxavir* (53.5 h) and *oseltamivir* (53.8 h) groups, relative to placebo (80.2 h). *Baloxavir* was associated with greater reductions in viral load at day 1 versus placebo or *oseltamivir*. Overall, the clinical benefits of *baloxavir* resemble those of *oseltamivir*.

Untoward Effects

Adverse events were reported in 20.7%, 24.6%, and 24.8% of *baloxavir*, placebo, and *oseltamivir* recipients, respectively. The primary *baloxavir* adverse event was diarrhea.

Interferon

Interferons (IFNs) are potent cytokines that possess antiviral, immunomodulatory, and antiproliferative activities (see Chapters 38 and 39). Three major classes of human IFNs with significant antiviral activity are α, β, and γ. Clinically used recombinant α-IFNs are nonglycosylated proteins of about 19,500 Da, with the pegylated forms predominating in the U.S. market. The mechanism of action, ADME, untoward effects, and therapeutic uses of IFNs are covered in Chapter 63. Recombinant, natural, and pegylated IFNs currently are approved in the U.S. for treatment of condyloma acuminatum, chronic HCV infection, chronic HBV infection, Kaposi sarcoma in HIV-infected patients, other malignancies, and multiple sclerosis. In addition, IFNs have been granted orphan drug status for a variety of rare disease states, including idiopathic pulmonary fibrosis, laryngeal papillomatosis, juvenile rheumatoid arthritis, and infections associated with chronic granulomatous disease.

Papillomavirus

In refractory condylomata acuminata (genital warts), intralesional injection of various natural and recombinant IFNs is associated with complete clearance of injected warts in 36% to 62% of patients, but other treatments are preferred. Relapse occurs in 20% to 30% of patients. Verruca vulgaris may respond to intralesional IFN-α. Intramuscular or subcutaneous administration is associated with some regression in wart size but greater toxicity. Systemic IFN may provide adjunctive benefit in recurrent juvenile laryngeal papillomatosis and in treating laryngeal disease in older patients. Chapter 40 presents information on the recommended schedule of immunizations against human papilloma virus (HPV).

Other Viruses

Interferons have been shown to have virological and clinical effects in various herpesvirus infections, including genital HSV infections, localized herpes zoster infection of cancer patients or of older adults, and CMV infections of renal transplant patients. However, IFN generally is associated with more side effects and inferior clinical benefits compared with conventional antiviral therapies. Topically applied IFN and *trifluridine* combinations appear active in *acyclovir*-resistant mucocutaneous HSV infections. In HIV-infected persons, IFNs have been associated with antiretroviral effects. In advanced infection, however, the combination of *zidovudine* and IFN is associated with only transient benefit and excessive hematological toxicity. IFN-α (3 million units thrice weekly) is effective for treatment of HIV-related thrombocytopenia that is resistant to *zidovudine* therapy.

Interferon has broad-spectrum antiviral activity against respiratory viruses other than adenovirus. However, prophylactic intranasal IFN-α is protective only against rhinovirus colds, and chronic use is limited by the occurrence of nasal side effects. Intranasal IFN is therapeutically ineffective in established rhinovirus colds.

Anti–Zaire Ebolavirus Agents

The family *Filoviridae* consists of three genera, *Ebolavirus*, *Marburgvirus*, and *Cuevavirus*. *Ebolavirus* is further subdivided into five species, each of which is represented by a unique virus: *Taï Forest ebolavirus*, *Reston ebolavirus*, *Sudan ebolavirus*, *Bundibugyo ebolavirus*, and *Zaire ebolavirus* (Baseler et al., 2017). Each species is named after the location in which it was first identified. Ebola virus was discovered in 1976, but it is believed to be an ancient virus that split from other viruses thousands of years ago. Current evidence suggests few differences in human pathology or pathogenesis among the ebolaviruses with the exception of members of the species *Reston ebolavirus*, which appear to be nonpathogenic for humans. Ebola (named after the Ebola River in Zaire) viruses cause a severe and often deadly illness known as Ebola virus disease (EVD), previously referred to as Ebola hemorrhagic fever. Fatality rates during EVD outbreaks average 50% but range from 25% to 90%. Viral particles contain one molecule of single-stranded negative-sense RNA enveloped in a lipid membrane. Ebola virus is spread through direct contact with blood or other bodily fluids, such as semen, feces, or vomit of infected persons (or animals), including close contact with deceased EVD victims, which are highly infectious. Infection can also be spread through needles, syringes, clothing, and bedding that have been contaminated with the virus.

Two monoclonal antibody (mAb) therapies are FDA-approved for *Zaire ebolavirus*. Both treatments were evaluated in a randomized controlled trial during the 2018–2020 Ebola outbreak in the Democratic Republic of the Congo (formerly Zaire). Overall survival was much higher for patients receiving either of the two treatments, but neither antibody has been evaluated for efficacy against species other than *Zaire ebolavirus*. A *Zaire ebolavirus* vaccine was FDA-approved in 2019 (FDA, 2019b).

Inmazeb (Atoltivimab, Maftivimab, and Odesivimab) and Ebanga (Ansuvimab)

Inmazeb and Ebanga (*ansuvimab*) are mAbs for the treatment of *Zaire ebolavirus*. Inmazeb is a cocktail of three recombinant human IgG1monoclonal antibodies, *atoltivimab, maftivimab,* and *odesivimab*. *Ansuvimab* is a single mAb. All four of the mAbs target the cell surface *Zaire ebolavirus* glycoprotein, which mediates virus attachment and membrane fusion with host cell membranes. The glycoprotein is expressed on the surface of a host cell infected with *Zaire ebolavirus*, making the cell a target for antibodies that mediate killing of these cells by antibody-dependent cellular cytotoxicity or other effector functions. The three mAbs in Inmazeb can bind the glycoprotein simultaneously (FDA, 2020a). *Ansuvimab* is a recombinant human IgG1κ monoclonal antibody that also targets the *Zaire ebolavirus* glycoprotein (FDA, 2020b).

Both agents were evaluated in the PALM trial, which was part of the emergency response to the ongoing EVD outbreak in the Democratic Republic of the Congo that started in August 2018 (Mulangu et al., 2019). Briefly, all patients received standard care and were randomly assigned in a 1:1:1:1 ratio to intravenous administration of the triple mAb ZMapp (triple mAb control), the antiviral agent *remdesivir*, the single mAb *ansuvimab*, or the triple IgG1 mAb cocktail (*atoltivimab, maftivimab,* and *odesivimab*). The primary endpoint was death at day 28. A total of 673 patients were included in the primary analyses. The case fatality rates at day 28 for ZMapp and *remdesivir* were 50% and 53%, respectively. The case fatality rate at day 28 was significantly reduced to 34% for patients receiving *ansuvimab* and to 35% for those receiving the three-mAb cocktail. However, in patients with a high viral load, these results were 70% and 64%, respectively, compared with 85% for the control and *remdesivir* groups, indicating essentially no activity. The dose for both agents is the same (50 mg/kg as a single dose by intravenous infusion), and their elimination half-lives are consistent with IgG antibodies (~24 days). A total of 29 serious adverse events were determined by investigators to be potentially related to study drugs, but these were difficult to extricate given the underlying EVD.

Novel 2019 Coronavirus

A SARS coronavirus 2 (SARS-CoV-2) viral outbreak, which rapidly became a worldwide pandemic, began in late 2019 in Wuhan, China. In February 2020, the World Health Organization (WHO) named the disease that SARS-CoV-2 causes COVID-19 (CO, corona; VI, virus; and D, disease). SARS-CoV-2 belongs to the β-coronavirus family, which also contains SARS-CoV and Middle East respiratory syndrome CoV (MERS-CoV). The first case in the U.S. was reported in January 2020. Older adults and people with severe underlying medical conditions (e.g., heart or lung disease, diabetes, obesity) were at higher risk for developing more serious complications from the infection. Early common symptoms included fever or chills, dry cough, shortness of breath, fatigue, body aches, loss of smell and/or taste, and headache, among many others. In May of 2020, the FDA granted emergency use authorization for *remdesivir* and full approval in October that same year. *Remdesivir* is currently the only FDA-approved small-molecule therapy for SARS-CoV-2. Vaccines (mRNA-type and traditional protein-based vaccines) became available during the 12 to 15 months following the start of the pandemic. Refer to Chapter 40 for information on these vaccines.

Remdesivir

Remdesivir was initially developed in response to the ongoing threat of RNA-based viruses that represented a potential for global pandemics, including viruses such as Ebola, MERS, and SARS. In early *in vitro* studies, *remdesivir* showed activity against Ebola and a broader range of viruses (Madelain et al., 2018; Warren et al., 2015). As such, *remdesivir* was included in a randomized controlled trial of Ebola virus therapeutics but was found to be inferior to the antibody-based therapeutics with respect to mortality. It was subsequently used in the first U.S. case of SARS-CoV-2, and no adverse events were reported (Holshue et al., 2020). Following multiple clinical trials, *remdesivir* was FDA-approved for COVID-19 treatment.

Mechanisms of Action and Resistance

Remdesivir, a nucleotide analogue, acts by inhibiting RNA-dependent RNA polymerase (RdRp). It is a phosphoramidate prodrug that is anabolized in cells to the active moiety, *remdesivir triphosphate*. Biochemical studies showed that the RdRp can use *remdesivir triphosphate* as a substrate, leading to the incorporation of *remdesivir monophosphate* into the growing RNA (Siegel et al., 2017). Following *remdesivir monophosphate* incorporation, the RdRp extends RNA by only three more nucleotides, after which chain elongation terminates, ending RNA synthesis (Gordon et al., 2020). Clinical resistance data are not available, but two substitutions in the viral RdRp at residues F476L and V553L together conferred a 5.6-fold reduction in susceptibility in cell culture resistance profiling (FDA, 2020c). *Remdesivir* has shown *in vitro* inhibitory activity against SARS-CoV-2 (Wang M et al., 2020).

ADME

Nonclinical metabolism studies indicate *remdesivir* is 80% metabolized in the liver by carboxylesterase 1 and 10% from cathepsin A and CYP3A each. Human mass balance data show that *remdesivir* is extensively metabolized and primarily eliminated in urine as the nucleoside metabolite GS-441524. *In vitro*, *remdesivir* is a substrate for CYP3A4 and for OATP1B1 and P-glycoprotein transporters. *In vitro*, it is an inhibitor of CYP3A4, OATP1B1, OATP1B3, and MATE1. Following multiple doses, the mean C_{max} and AUC_{tau} were 2229 ng/mL and 1585 ngxhr/mL, respectively; the C_{trough} was not quantifiable at 24 h post dose as the terminal $t_{1/2}$ is approximately 1 h (FDA, 2020c).

Therapeutic Uses

Remdesivir is indicated for adults and pediatric patients (12 years of age and older and weighing at least 40 kg) for the treatment of COVID-19 requiring hospitalization. The drug is infused over 30 to 120 min. Recommended dosage is a single loading dose of 200 mg on day 1 followed by 100-mg once-daily maintenance doses from day 2 onward. For patients not requiring mechanical ventilation, the recommended duration of treatment is 5 days; for patients not responding to initial treatment, 5 additional days are recommended.

Multiple phase III clinical trials have assessed the safety and efficacy of *remdesivir*. The primary study leading to its indication was from the ACTT-1 study group, which was a double-blind, randomized, placebo-controlled trial of intravenous *remdesivir* in adults hospitalized with COVID-19 and with evidence of lower respiratory tract infection (Beigel et al., 2020). The primary outcome was the time to recovery at 29 days. Patients who received *remdesivir* had a shorter time to recovery versus placebo recipients (10 days vs. 15 days, respectively; $P < 0.001$). Overall, day 15 mortality was 6.7% versus 11.9% and day 29 mortality was 11.4% versus 15.2% for *remdesivir* versus placebo, respectively. Prior to this study, a randomized, double-blind, placebo-controlled, multicenter trial at 10 hospitals in China evaluated *remdesivir* in adults with severe COVID-19 (Wang Y et al., 2020). The primary endpoint was time to clinical improvement up to day 28. Time to clinical improvement was similar in the *remdesivir* (median, 21 days) and placebo (median, 23 days) groups. A large trial conducted by the WHO compared *remdesivir*, *hydroxychloroquine*, *lopinavir* (without *interferon*), *interferon*, and *interferon* plus *lopinavir* against no trial therapy. The specified primary objective was in-hospital mortality at day 28. In the *remdesivir* arm, the mortality rate was 12.5%, versus 12.7% in the control arm ($P = 0.5$). The other drug regimens also did not demonstrate a mortality benefit. Another trial demonstrated that in patients with severe COVID-19 not requiring mechanical ventilation, there were no clinically significant differences between a 5-day versus a 10-day course of *remdesivir* therapy (Goldman et al., 2020). Collectively, these data suggest that *remdesivir* has marginal clinical benefit in patients with moderate to severe COVID-19 and that its definitive place in therapy has yet to be established.

Untoward Effects

Across all clinical trials to date, *remdesivir* has demonstrated a good safety profile, particularly given the patient populations within which it has been evaluated. From the ACTT-1 trial, serious adverse events were reported in 24.6% of patients receiving *remdesivir* versus 31.6% of patients receiving placebo. The most common nonserious adverse events occurring in at least 5% of all patients included decreased glomerular filtration rate, hemoglobin, and lymphocyte count; respiratory failure; pyrexia; hyperglycemia; and increased glucose. The incidence of these events was similar across both the placebo and *remdesivir*-treated groups.

Drug Facts for Your Personal Formulary: *Antiviral Agents for Herpes Virus and Influenza*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
ANTIHERPES AGENTS		
Guanine nucleoside analogues		
Acyclovir Valacyclovir (Val, an ester prodrug form of acyclovir)	• Clinical use limited to herpes viruses • Efficacy against: HSV-1 > HSV-2 > VZV > EBV > CMV = HHV-6	• Acyclovir has low bioavailability (~20%); Val has bioavailability of ~70% • Concentrates in breast milk • Clearance via renal excretion of acyclovir, requires good kidney function; $t_{1/2}$ prolonged in neonates and anuric patients • Safely used long term (10 years)
Cidofovir	• Active against human herpes, papilloma, polyoma, pox, adenoviruses	• Low oral bioavailability • Plasma $t_{1/2}$ ~2.6 h, but active diphosphate metabolite has long $t_{1/2}$ in cells, as does a phosphocholine metabolite ($t_{1/2}$ = 86 h) • Major risk: nephrotoxicity, reduced by oral probenecid and saline prehydration (beware interactions of probenecid and other medicines)
Famciclovir (Fam), a prodrug form, rapidly converted to penciclovir (Pen)	• Penciclovir similar to acyclovir against HSV and VZV; also inhibits HBV	• Oral bioavailabilities: Pen, <5%; Fam, ~75% • Food reduces rate but not extent of Pen absorption • Safety in pregnancy not established
Valganciclovir (Val), a prodrug valyl ester of ganciclovir (Gan)	• Gan has inhibitory activity against all herpesviruses, especially CMV	• Gan less active against acyclovir-resistant TK-deficient HSV strains • Active triphosphate form has long cellular $t_{1/2}$ • IV administration gives good levels in vitreous with long dwell time ($t_{1/2}$ ~25 h) • Major adverse effects: myelosuppression, neutropenia • Risk in pregnancy not ruled out
Pyrophosphate analogue		
Foscarnet	• Active against all herpesviruses and HIV	• Poorly soluble in water; requires large volumes of diluent • Adverse effects: nephrotoxicity, hypocalcemia • Safety in pregnancy and childhood uncertain
Other agents		
Fomivirsen (antisense oligonucleotide)	• Inhibits CMV replication	• No longer available in the U.S.
Docosanol (long-chain alcohol)	• 10% topical cream for labial herpes	• Treatment initiation at papular or later stages provides no benefit • Available over the counter
Idoxuridine (iodinated thymidine analogue)	• Ophthalmic HSV keratitis (in the U.S.)	• Adverse effects: pain, pruritus, inflammation, edema of eye/eyelid • Not available in the U.S.
Trifluridine (trifluoropyrimidine nucleoside)	• Ocular herpes; first-degree keratoconjunctivitis, recurrent epithelial keratitis from HSV1/2; for ophthalmic use	• More active than idoxuridine in HSV ocular infections • Triphosphate form incorporated into host and viral DNA, so not used systemically
ANTI-INFLUENZA AGENTS		
Inhibitors of viral M2 protein function		
Amantadine (Ama) Rimantadine (Rima)	• Active only against susceptible influenza A viruses (not B) • Seasonal prophylaxis against influenza A (70%–90% protective)	• Rima 4- to 10-fold more active than Ama • Resistant isolates appear after 2–3 days of therapy • Virtually all H3N2 strains of influenza are resistant to these drugs • Vaccination is more cost-effective
Inhibitors of viral neuraminidase (see pharmacokinetic data in Table 62–3)		
Oseltamivir	• Treatment and prevention of influenza A and B	• Probenecid doubles plasma $t_{1/2}$
Zanamivir	• Treatment and prevention of influenza A and B	• Inhalable formulation • IV formulation available as EIND • No clinically significant drug interactions
Peramivir	• Treatment of acute uncomplicated flu in patients ≥18 years and symptomatic ≤2 days	• Supplied as IV infusion; for patients who cannot use oral agents • Comparable in efficacy and adverse effects to oseltamivir • No clinically significant drug interactions reported

Drug Facts for Your Personal Formulary: *Antiviral Agents for Herpes Virus and Influenza* (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Inhibitor of Viral Cap-Dependent Endonuclease (blocks initiation of mRNA synthesis)		
Baloxavir marboxil	• Treatment of acute uncomplicated flu in patients ≥12 years and at risk of complications • As a postexposure preventative against developing influenza	• Do not administer with products or foods containing high amounts of calcium, iron, magnesium, aluminium, zinc, or selenium (dairy products, antacids, laxatives, etc.). These products will reduce absorption.
CYTOKINES		
Interferon (recombinant α-IFNs; natural and pegylated IFNs)	Treatment of condyloma acuminatum, chronic HCV and HBV infection, Kaposi sarcoma (in patients with HIV, other malignancies, multiple sclerosis)	See Chapter 63
AGENTS FOR CORONA VIRUS AND EBOLA VIRUS		
Remdesivir		• See text of this chapter
Vaccines against Ebola virus		• See text of this chapter
Vaccines against SARS-CoV-19		• See Chapter 40

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Chapter 63

Treatment of Viral Hepatitis (HBV/HCV)

Jennifer J. Kiser

HEPATITIS B VIRUS

- HBV Overview
- Genetic Heterogeneity of HBV
- HBV Genome and Life Cycle
- HBV Drug Targets and Treatment Approach
- Clinical Implications of HBV Resistance
- Hepatic Impairment: Implications for HBV Treatment
- Pharmacotherapy of HBV Infection
- Investigational Agents and the Future for Treatment of HBV

HEPATITIS C VIRUS

- HCV Overview
- Genetic Heterogeneity of HCV
- HCV Genome and Life Cycle
- HCV Drug Targets and Treatment Approach
- Clinical Implications of HCV Resistance
- Hepatic Impairment: Implications for HCV Treatment
- Pharmacotherapy of HCV Infection
- Investigational Agents and the Future for Treatment of HCV

Hepatitis viruses cause inflammation and necrosis of the liver. Hepatitis A and E, which are transmitted via the fecal-oral route, are typically self-limiting, although a small percentage (1%–2%) of those infected will develop fulminant hepatic failure. Hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis D viruses, however, are transmitted parenterally. Hepatitis B and C may or may not cause symptoms of acute infection, but both may progress to chronic infection. Individuals with chronic hepatitis B or C infection are at risk for cirrhosis, liver failure, and hepatocellular carcinoma. There are two notable differences between HBV and HCV. *First*, HBV is a vaccine-preventable illness, whereas there is no vaccine available to prevent HCV. *Second*, HCV can be cured with effective treatment, whereas the current treatments for HBV are not completely curative. The hepatitis D virus is defective and requires the presence of the HBV to propagate. Individuals coinfected with hepatitis B and D are at greater risk for cirrhosis and hepatocellular carcinoma compared with individuals with only hepatitis B infection, but fortunately, only about 5% of individuals with HBV are coinfected with hepatitis D.

There are no antiviral therapies available for the treatment of hepatitis A and E viruses. Limited options are available to treat hepatitis D, including *pegylated interferon α* (pegIFN-α), which is successful in only 20% to 35% of patients (Durantel and Zoulim, 2016) and *bulevirtide*, an agent recently approved in Europe (Kang and Syed, 2020). Multiple drugs are available for the treatment of hepatitis B and C. Available therapies for HBV include pegIFN-α and nucleoside and nucleotide analogues. Several investigational agents are in various stages of clinical development for the treatment of HBV (Soriano et al., 2020). Hepatitis C is treated with combinations of drugs that inhibit the RNA-dependent RNA polymerase (RdRp, NS5B), the NS5A replication complex, and the NS3 protease. Therapeutic strategies for these two chronic viral infections, hepatitis B and C, are very different and are described separately in this chapter.

Several agents employed against hepatitis viruses, including pegIFN-α, *ribavirin*, and the nucleoside/nucleotide analogues *lamivudine*, *emtricitabine*, and *tenofovir*, are also used to treat other conditions, described in Chapters 64 (Antiretroviral Agents and Treatment of HIV Infection), 74 (Ocular Pharmacology), and 75 (Dermatological Pharmacology).

Assessing the stage and severity of liver disease is an important aspect of treating individuals with chronic viral hepatitis. Individuals with cirrhosis require additional monitoring for potential complications (e.g., varices and hepatocellular carcinoma), and the approach to treatment differs in cirrhotics and decompensated cirrhotics compared to individuals without significant liver fibrosis. Cirrhosis can be diagnosed with histological, radiographic, or laboratory tests. However, decompensated cirrhosis (also known as end-stage liver disease) is a clinical diagnosis based on the presence of variceal hemorrhage, ascites, jaundice, or hepatic encephalopathy. Decompensated cirrhosis carries a high risk of mortality (median survival 2 years), and these individuals should be evaluated for liver transplantation.

The urgency for liver transplantation in individuals with decompensated cirrhosis is determined using the Model for End-Stage Liver Disease (MELD) score. A MELD score is calculated based on a patient's bilirubin, international normalized ratio (INR), and serum creatinine. Individuals with higher MELD scores have a higher risk for mortality and a more urgent need for transplantation. Cirrhosis may also be categorized based on the Child Pugh score. The Child-Pugh score accounts for bilirubin, albumin, and INR values and the presence/absence of ascites and encephalopathy. Higher scores are associated with more advanced disease: mild hepatic impairment (Child-Pugh category A; score of 5–6), moderate hepatic impairment (Child-Pugh category B; score of 7–9), and severe hepatic impairment (Child-Pugh category C; score of 10–15). Individuals with Child-Pugh B and C scores have decompensated cirrhosis. Familiarity with MELD and Child-Pugh scoring and the diagnoses of compensated versus decompensated cirrhosis is helpful for understanding the pharmacological basis of hepatitis B and C treatment.

Hepatitis B Virus

HBV Overview

Worldwide, an estimated 2 billion people have been infected with HBV and approximately 240 million have chronic disease (World Health Organization [WHO], 2015). Areas with the highest HBV endemicity are sub-Saharan Africa and countries in the WHO Western-Pacific region (e.g., China) (Schweitzer et al., 2015). In these regions, HBV is most likely acquired perinatally. Those infected at birth have a 90% risk of progressing to chronic disease; otherwise, the risk of chronic HBV disease in adults is 5% (Tong and Revill, 2016). The lifetime risk of developing cirrhosis, liver failure, or hepatocellular carcinoma in individuals with HBV is 15% to 40% (Lin et al., 2016). There are about 686,000 HBV-related deaths annually, which makes HBV the leading cause of liver-related deaths worldwide (WHO, 2020a).

Genetic Heterogeneity of HBV

There are 10 described HBV genotypes (A–J) and at least 35 subtypes with distinct geographical distributions (Tong and Revill, 2016). Genotype A is most frequent in North America and Africa, genotypes B and

Abbreviations

2–5(A): 2′-5′-oligoadenylate
ALT: alanine aminotransferase
AUC: area under the plasma concentration-time curve
BCRP: breast cancer resistance protein
cccDNA: covalently closed circular DNA
C_{max}, C_{Pmax}: maximal blood or plasma concentration
C_p: plasma concentration
Cl_{Cr}: creatinine clearance
CYP: cytochrome P450
DAA: direct-acting antiviral
dATP: deoxyadenosine triphosphate
DCV/SOF: daclatasvir/sofosbuvir
EBR: elbasvir
eGFR: estimated glomerular filtration rate
ER: endoplasmic reticulum
ESRD: end-stage renal disease
GLE: glecaprevir
GZR: grazoprevir
HBcAg: hepatitis B core antigen
HBeAg: hepatitis B e antigen
HBsAg: hepatitis B surface antigen
HBV: hepatitis B virus
HCV: hepatitis C virus
HSPGs: heparin sulfate proteoglycans
IFN: interferon
INR: international normalized ratio
LDV/SOF: ledipasvir/sofosbuvir
MELD: Model for End-Stage Liver Disease
MHC: major histocompatibility complex
MRP: multidrug resistance protein
MU: million units
NTCP: Na$^+$/taurocholate cotransporter
ORF: open reading frame
PEG: polyethylene glycol
pegIFN: pegylated interferon
PIB: pibrentasvir
P-gp: P-glycoprotein
PPI: proton pump inhibitor
RAS: resistance-associated substitution
RdRp: RNA-dependent RNA polymerase
SOF/VEL: sofosbuvir and velpatasvir
SR-B1: scavenger receptor B1
SVR: sustained virologic response
TAF: tenofovir alafenamide fumarate
TDF: tenofovir disoproxil fumarate
VEL: velpatasvir
VOX: voxilaprevir
WHO: World Health Organization

C are dominant throughout East Asia and are primarily transmitted via the perinatal route, and genotype D is most common in southern Europe and India. Genotype E is largely restricted to sub-Saharan Africa, while genotypes F and H cocirculate in indigenous peoples of South America. Genotypes I and J have been described in only three individuals and one individual, respectively.

Genotypes have relevance for the clinical manifestation of infection and the response to antiviral therapy. Clinically relevant features of HBV genotypes include the rate and durability of hepatitis B e antigen (HBeAg) (loss or seroconversion; A and D > B and C), the risk of developing aggressive HBeAg(−) chronic hepatitis B (C and D > A), spontaneous HBeAg loss (B > C), cirrhosis (C), hepatocellular carcinoma (C in Asians, F in Alaska Natives), and response to antivirals (A and B > C and D).

HBV Genome and Life Cycle

Hepatitis B virus is a small, enveloped, relaxed circular DNA virus with a complete minus strand and an incomplete plus strand, approximately 3200 kb in length (Tsukuda and Watashi, 2020). The viral nucleocapsid contains the hepatitis B core antigen (HBcAg), viral polymerase, and the viral DNA surrounded by an outer lipoprotein envelope containing the HBV surface antigen (HBsAg). HBV is compact and contains four overlapping open reading frames (ORFs). ORF C encodes the HBcAg and HBeAg, ORF P encodes the polymerase and terminal protein, ORF S/pre-S encodes HBsAg, and ORF X encodes the regulatory X protein.

Figure 63–1 depicts the HBV life cycle (Brahmania et al., 2016; Lin et al., 2016; Tong and Revill, 2016; Tsukuda and Watashi, 2020). The HBV life cycle is not fully defined, but current understanding is nicely reviewed by Tsukuda and Watashi (2020). Briefly, HBV initially attaches to heparin sulfate proteoglycans (HSPGs) on the hepatocyte surface and then to cell surface receptors like sodium taurocholate cotransporting peptide (NTCP/SLC10A1) with greater specificity and affinity. This triggers endocytosis of the nucleocapsid into the hepatocyte, where it is then transported to the nucleus and enters through the nuclear pore complex. The partial double strand is then repaired to form covalently closed circular DNA (cccDNA). The cccDNA is not eliminated by available treatments; thus, a complete cure is not currently possible. cccDNA complexes with host proteins to form a minichromosome that becomes a template for the transcription of the various virally derived RNAs. These RNAs are transported to the cytoplasm, and some of the smaller RNAs are then made into various viral proteins, including the envelope protein. These proteins can actually be released without any genetic material (known as subviral particles). Filamentous and spherical HBsAg particles are produced in 3 to 4 log excess over virions (complete virus particles). These subvirus particles are thought to contribute to immune tolerance and T-cell exhaustion by increasing the amount of antigen that is circulating. The larger pregenomic RNA is used to form the HBcAg protein, which is also encapsidated. In the capsid, HBV is reverse transcribed back to viral DNA. The nucleocapsids are then either returned to and recycled in the nucleus to make more cccDNA or they assemble with envelope protein for cellular release.

Some patients develop mutations in the HBV genome that prevent HBeAg formation despite active HBV replication; this condition is known as HBeAg(−) disease.

HBV Drug Targets and Treatment Approach

Hepatitis B virus is a dynamic condition. Individuals with chronic infection can pass through several phases of disease throughout their lifetimes. At present, treatment is typically reserved for individuals with high levels of circulating HBV DNA and alanine aminotransferase elevations, those with cirrhosis, or for prevention of reactivation of disease in those receiving immunosuppressive therapies (e.g., chemotherapy) (Terrault et al., 2018). When treatment is necessary, the approach to HBV therapy in children and adults is similar, but most children have normal alanine aminotransferase levels and do not require treatment.

Current HBV therapies cannot achieve a complete cure, and only a small proportion (~10%) of patients achieve a functional cure. A functional cure is defined as undetectable HBV DNA with seroconversion from HBsAg to the anti-HBs. This is analogous to a resolved acute infection, but cccDNA persists, which can lead to reactivation of the disease. Existing treatment options include pegIFN-α, which has nonspecific antiviral and immunomodulatory effects, or the nucleoside/-tide analogues (Figure 63–2) *adefovir*, *entecavir*, *lamivudine*, *telbivudine*, and *tenofovir*, which inhibit the HBV polymerase. IFN treatment is finite (48–52 weeks), whereas individuals may require lifelong treatment with nucleoside/-tide analogues. *Entecavir* and *tenofovir* (*tenofovir disoproxil fumarate* [TDF] or *tenofovir alafenamide fumarate* [TAF]) are the preferred nucleoside/-tide therapies due to their potency, good tolerability,

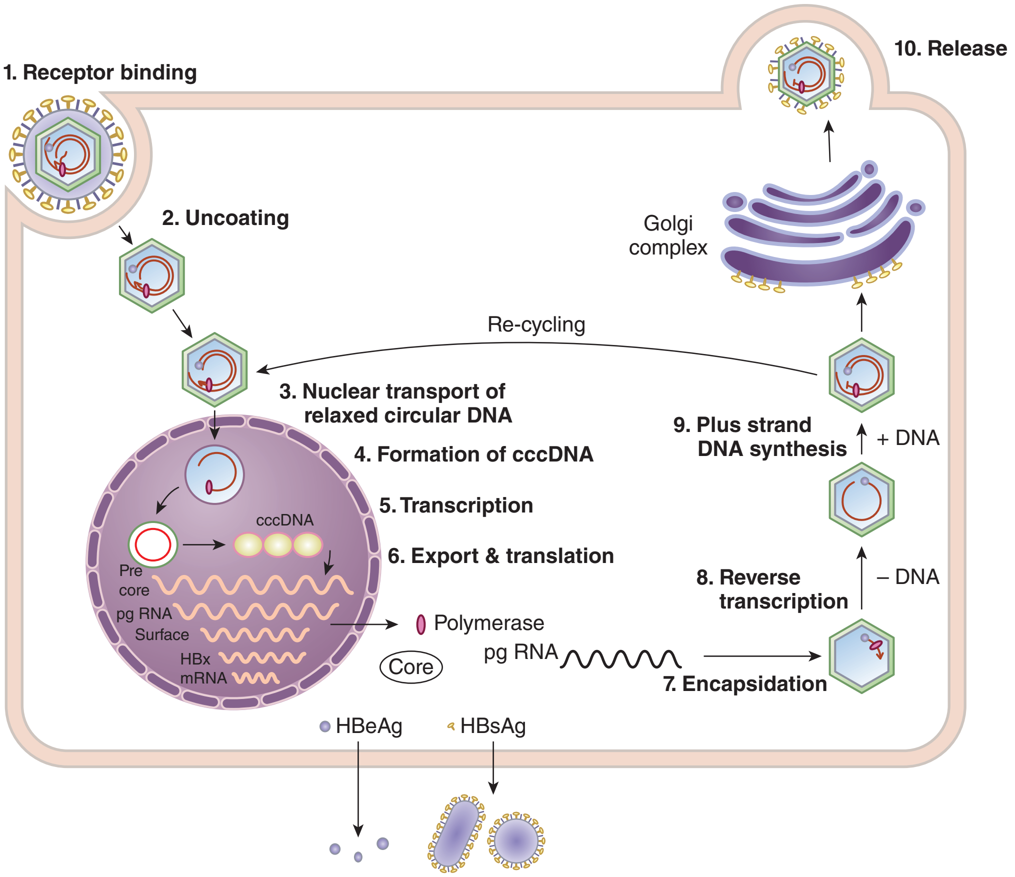
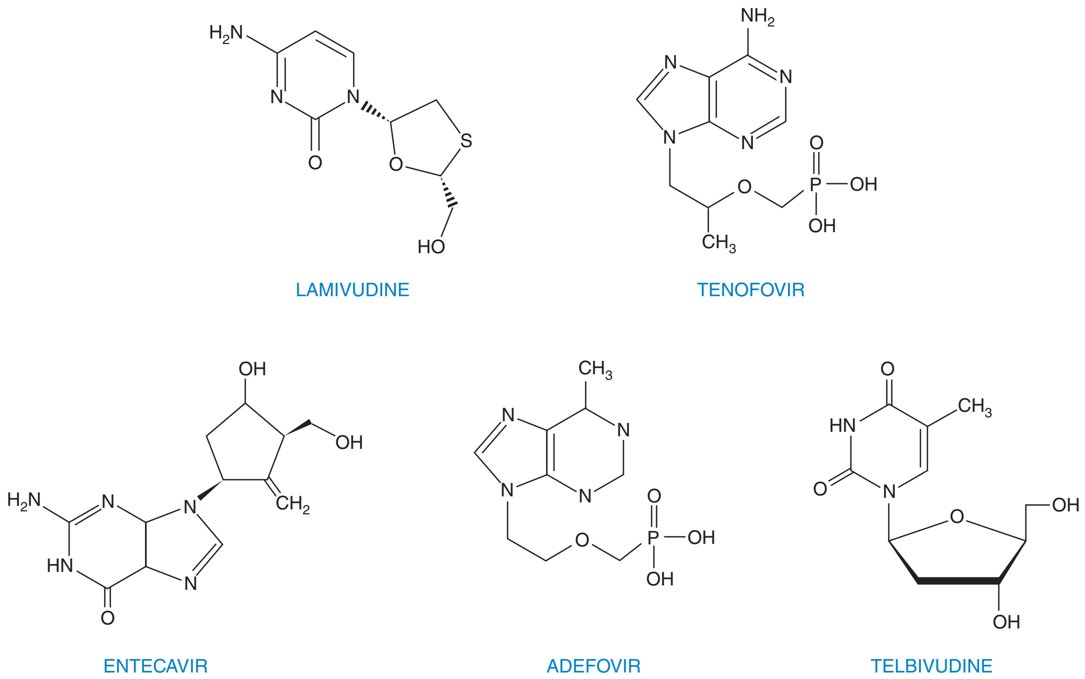

1. Receptor binding
10. Release
2. Uncoating
Golgi complex
Re-cycling
3. Nuclear transport of relaxed circular DNA
9. Plus strand DNA synthesis
+ DNA
4. Formation of cccDNA
5. Transcription
cccDNA
6. Export & translation
Pre core
pg RNA
Surface
HBx
mRNA
8. Reverse transcription
– DNA
Polymerase
Core
pg RNA
7. Encapsidation
HBeAg
HBsAg

Figure 63–1 *Hepatitis B life cycle.* Not all steps in the hepatitis B viral life cycle have been completely elucidated. The figure represents our current understanding. For further details, consult the following references: Brahmania et al., 2016; Liang et al., 2015; Tong and Revill, 2016; and Tsukuda and Watashi, 2020.


H_2N
N
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O
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HO
NH_2
N
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P
OH
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CH_3
OH
OH
H_2N
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CH_3
HN
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N
O
OH
OH

LAMIVUDINE

TENOFOVIR

ENTECAVIR

ADEFOVIR

TELBIVUDINE

Figure 63–2 *Chemical structures of nucleoside/-tide analogues used to treat hepatitis B.*

and low potential for the development of resistance (Martin et al., 2015). Considerations in selecting between the preferred therapies for HBV include the severity of liver disease, patient willingness, prior treatment, side effects, HBV genotype/serotype, comorbidities, and cost.

Because a functional cure occurs in only a small number of patients with current agents and a complete cure is not possible, treatment goals with either pegIFN-α or nucleoside/-tide analogues are to suppress HBV DNA replication and promote seroconversion from HBeAg to anti-HBe, which in turn will slow the progression of liver disease; reduce the risk for complications, including hepatocellular carcinoma; and prolong survival (Liang et al., 2015).

Clinical Implications of HBV Resistance

Hepatitis B virus replicates at a high rate. The viral polymerase also lacks proofreading capability. These conditions result in the generation of a large number of viral mutations. Mutations that confer a replication advantage are preferentially selected under pressure from antiviral therapy. Mutations in the reverse transcriptase/polymerase domain confer resistance to nucleoside analogues.

Lamivudine has the lowest genetic barrier to the development of resistance. After 4 years of treatment with *lamivudine*, 71% of patients have developed the M204V/I mutation, which renders this drug ineffective. Twenty-two percent of individuals treated with *telbivudine* develop this mutation after 2 years of therapy. With *adefovir*, about 29% of patients have developed an N236T mutation after 5 years of treatment. Resistance is uncommon with TDF and was not observed through 4 years with TAF. The rate of resistance with *entecavir* is 1% or less after 5 years in treatment-naïve individuals, whereas in those with preexisting M204V/I mutation, the prevalence of resistance is much higher, 51% after 5 years (Bhattacharya and Thio, 2010; Ghany and Doo, 2009).

The emergence of resistance has important clinical consequences. Resistance leads to therapeutic failure and a rapid resurgence of viral replication. This resurgence in HBV replication may predispose patients to hepatic decompensation. For this reason, monotherapy with *lamivudine*, *adefovir*, or *telbivudine* is not recommended for the treatment of hepatitis B (Terrault et al., 2018). Individuals taking these drugs require frequent monitoring of HBV DNA and counseling on the importance of excellent adherence to the daily therapy.

Hepatic Impairment: Implications for HBV Treatment

Prolonged suppression of HBV DNA production prevents disease progression in individuals with cirrhosis and aids reversal of liver damage. Although pegIFN-α can be used in individuals with compensated cirrhosis, it is contraindicated in those with decompensated cirrhosis. Nucleoside/-tide analogue therapy is the treatment of choice for decompensated disease (Martin et al., 2015). In general, nucleoside/-tide analogue treatment improves liver function and increases the 5-year survival rate in individuals with decompensated cirrhosis from 14% to 35% to 55% to 86% (Honda et al., 2015). It may also prevent the need for liver transplantation.

The nucleoside/-tide analogues of choice for the treatment of decompensated cirrhosis are *entecavir* and *tenofovir*. Both agents require adjustments for renal impairment. TDF has been associated with nephrotoxicity, and *entecavir* has been associated with lactic acidosis in this population, so careful monitoring is imperative. Also, a combination of nucleoside/-tide analogues may be needed to improve efficacy in those with preexisting viral resistance. All nucleoside/-tide analogues carry a black-box warning for the risk of lactic acidosis, a risk that may be greater in individuals with advanced liver disease.

Pharmacotherapy of HBV Infection

Practice guidelines are available from several authorities on the treatment of HBV (Martin et al., 2015; Terrault et al., 2018; WHO, 2015). A description of current HBV therapies follows, and a summary of the therapeutic uses and clinical pharmacology of these drugs is provided at the end of the chapter.

Interferons

Interferons (IFNs) are potent cytokines that possess antiviral, immunomodulatory, and antiproliferative effects (see Chapters 38 and 39). These proteins are synthesized by host cells in response to various inducers and, in turn, cause biochemical changes leading to an antiviral state in cells. Three major classes of human IFNs with significant antiviral activity currently are recognized: α (>18 individual species), β, and γ. Clinically used recombinant α-IFNs are nonglycosylated proteins of about 19,500 Da. Attachment of IFN proteins to large inert polyethylene glycol (PEG) molecules (pegylation) slows absorption, decreases clearance, and provides higher and more prolonged serum concentrations that enable once-weekly dosing. PegIFN-α is a first-line agent for the treatment of HBV.

Mechanisms of Action and Resistance. IFN-α may be produced by nearly all cells in response to viral infection and a variety of other stimuli, including double-stranded RNA and certain cytokines (e.g., interleukin-1, interleukin-2, and tumor necrosis factor). IFN-α exhibits antiviral and antiproliferative actions; stimulates the cytotoxic activity of lymphocytes, natural killer cells, and macrophages; and upregulates expression of class I major histocompatibility complex (MHC) antigens and other surface markers. In addition, IFNs downregulate production of a number of cellular proteins, which may be an equally important mediator of the pharmacological benefit of IFNs (Teijaro, 2016).

Following binding to specific cellular receptors, IFNs activate the Jak-STAT signal transduction pathway and lead to the nuclear translocation of a cellular protein complex that binds to genes containing an IFN-specific response element. This, in turn, leads to synthesis of over two dozen proteins that contribute to viral resistance mediated at different stages of viral penetration.

Inhibition of protein synthesis is the major inhibitory effect for many viruses. IFN-induced proteins include 2–5(A) synthetase and a protein kinase, either of which can inhibit protein synthesis in the presence of double-stranded RNA. The 2–5(A) synthetase produces adenylate oligomers that activate a latent cellular endoribonuclease (RNase L) to cleave both cellular and viral single-stranded RNAs. The protein kinase selectively phosphorylates and inactivates a protein involved in protein synthesis, eukaryotic initiation factor 2 (eIF-2). IFN-induced protein kinase also may be an important effector of apoptosis. In addition, IFN induces a phosphodiesterase that cleaves a portion of transfer RNA and thus prevents peptide elongation. A given virus may be inhibited at several steps, and the principal inhibitory effect differs among virus families.

Complex interactions exist between IFNs and other parts of the immune system, so IFNs may ameliorate viral infections by exerting direct antiviral effects or by modifying the immune response to infection. For example, IFN-induced expression of MHC antigens may contribute to the antiviral actions of IFN by enhancing the lytic effects of cytotoxic T lymphocytes. Conversely, IFNs may mediate some of the systemic symptoms associated with viral infections and contribute to immunologically mediated tissue damage in certain viral diseases.

Host genetics may predict IFN responsiveness. Single-nucleotide polymorphisms (rs12979860 and rs12980275) on or near the *IL-28B* gene on chromosome 19 have been associated with decreased responsiveness to IFN therapy with hepatitis C treatment (Sonneveld et al., 2012). Data are conflicting on whether *IL-28B* genetics are associated with response to IFN in individuals with HBV (Martin et al., 2015). This gene encodes IFN-λ-3. INF-λ enhances and sustains effects of INF-α on viral replication.

ADME. Oral administration of IFN-α does not result in detectable IFN levels in serum or increases in 2–5(A) synthetase activity in peripheral blood mononuclear cells (used as a marker of IFN's biological activity). After intramuscular or subcutaneous injection of IFN-α, absorption exceeds 80%. Pegylated IFN-α is dosed subcutaneously once per week. The dose of pegIFN-α2a is 180 μg and should be reduced for patients with Cl_{Cr} less than 30 mL/min. Plasma levels are dose related, peaking at 4 to 8 h and returning to baseline by 18 to 36 h. Levels of 2–5(A) synthetase in peripheral blood mononuclear cells show increases beginning at 6 h and lasting through 4 days after a single injection. An antiviral state in peripheral blood mononuclear cells peaks at 24 h and decreases slowly to baseline by 6 days after injection. After systemic administration, low levels of IFN are detected in respiratory secretions, cerebrospinal fluid, eye, and brain.

Two pegIFNs are available commercially: pegIFN-α2a and pegIFN-α2b. PegIFN-α2b has a straight-chain 12,000-Da type of PEG that increases the plasma $t_{1/2}$ from 2–3 to 30–54 h. PegIFN-α2a consists of an ester derivative of a branched-chain 40,000-Da PEG bonded to IFN-α2A and has a plasma $t_{1/2}$ of about 80 to 90 h. For pegIFN-α2a, peak serum concentrations occur up to 120 h after dosing and remain detectable throughout the weekly dosing interval; steady-state levels occur 5 to 8 weeks after initiation of weekly dosing. For pegIFN-α2A, dose-related maximum plasma concentrations occur at 15 to 44 h after dosing and decline by 96 to 168 h. Increasing PEG size is associated with longer $t_{1/2}$ and less renal clearance. About 30% of pegIFN-α2b is cleared by the kidneys; the remainder is cleared by both the liver and cellular degradation of IFN-receptor complexes. PegIFN-α2a is cleared by the liver and kidney. Patients with end-stage renal disease (ESRD) require dose reductions of both pegIFN products.

Therapeutic Uses. Nonpegylated forms of IFN may be used in certain clinical scenarios, but in the U.S., use of pegIFN-α predominates. PegIFN-α2a is FDA-approved for the treatment of hepatitis B and C. Its use in hepatitis C has been completely replaced by all oral direct-acting antiviral (DAA) agents (see further discussion). However, it remains a preferred agent for the treatment of HBV. IFNs are contraindicated in patients with advanced liver disease because they can precipitate clinical deterioration and increase the risk of bacterial infections.

Unlike nucleoside/-tide analogues, which may require lifelong administration for the treatment of hepatitis B, IFN (or pegIFN) treatment is finite. Plasma HBV DNA and polymerase activity decline promptly with α-IFN (pegIFN-α) in most patients, but complete disappearance is sustained in only about one-third of patients. The advantage of α-IFN (pegIFN-α) compared with nucleoside/-tide analogues, besides the finite treatment duration, is the higher rate of HBeAg loss (~32%–36%) and HBsAg loss (~4%–11%) (Terrault et al., 2018). However, not all patients respond to α-IFN, and there are significant toxicities associated with this therapy.

Low pretherapy serum HBV DNA levels and high aminotransferase levels are predictors of response. In addition, individuals with genotypes A and B are more likely to respond to α-IFN than genotypes C or D. Responses with seroconversion to anti-HBe usually are associated with aminotransferase elevations and often a hepatitis-like illness during the second or third month of therapy, likely related to immune clearance of infected hepatocytes.

Untoward Effects. Injection of recombinant IFN doses of 1 to 2 MU (million units) or more usually is associated with an acute influenza-like syndrome beginning several hours after injection. Symptoms include fever, chills, headache, myalgia, arthralgia, nausea, vomiting, and diarrhea. Fever usually resolves within 12 h. Tolerance develops gradually in most patients. Febrile responses can be moderated by pretreatment with antipyretics.

The principal dose-limiting toxicities of systemic IFN are depression, myelosuppression with granulocytopenia and thrombocytopenia; neurotoxicity manifested by somnolence, confusion, behavioral disturbance, and rarely, seizures; debilitating neurasthenia; autoimmune disorders, including thyroiditis and hypothyroidism; and uncommonly, cardiovascular effects with hypotension and tachycardia. Elevations in hepatic enzymes and triglycerides, alopecia, proteinuria and azotemia, interstitial nephritis, autoantibody formation, pneumonia, and hepatotoxicity may occur. Alopecia and personality change are common in IFN-treated children (Sokal et al., 1998). The development of serum-neutralizing antibodies to exogenous IFNs may be associated infrequently with loss of clinical responsiveness. IFN may impair fertility, and safety during pregnancy is not established. IFNs can increase the hematological toxicity of drugs such as *zidovudine* and *ribavirin* and may increase the neurotoxicity and cardiotoxic effects of other drugs. Thyroid function and hepatic enzymes should be monitored during IFN therapy.

Pegylated IFNs are generally better tolerated than standard IFNs, although the frequencies of fever, nausea, injection site inflammation, and neutropenia may be somewhat higher. Laboratory abnormalities, including severe neutropenia and the need for dose modifications, are higher in HIV-coinfected persons.

Pediatric and Geriatric Uses. Interferon-α2b is approved for children 1 year or older. The dose is 6 million IU/m^2 thrice weekly. PegIFN-α2a is not approved for children with HBV but is approved for treatment of children 5 years or older with HCV. Thus, the guidelines of the American Association for the Study of Liver Diseases state that providers may consider using this drug in children with HBV (Terrault et al., 2018). The dose of pegIFN-α2a in children with chronic HCV is 180 μg/1.73 m^2 × body surface area once weekly. PegIFN-α2a contains benzyl alcohol, which has been associated with neurological toxicities in infants and neonates.

Elderly patients may experience more toxicities from IFN treatment, and dose adjustments are necessary in renal impairment.

Entecavir

Entecavir is a guanosine analogue. *Entecavir* inhibited HBV DNA synthesis (50% reduction, EC$_{50}$) at a concentration of 0.004 μM in human HepG2 cells transfected with wild-type HBV. The median EC$_{50}$ value for *entecavir* against *lamivudine*-resistant HBV was 0.026 μM (range 0.010–0.059 μM).

Mechanisms of Action and Resistance. *Entecavir* requires intracellular phosphorylation. *Entecavir triphosphate* competes with endogenous deoxyguanosine triphosphate and inhibits all three activities of the HBV polymerase (reverse transcriptase):

- Base priming
- Reverse transcription of the negative strand from the pregenomic messenger RNA
- Synthesis of the positive strand of HBV DNA

Entecavir triphosphate is a weak inhibitor of cellular DNA polymerases α, β, and δ and mitochondrial DNA polymerase γ.

Susceptibility to *entecavir* is reduced with *lamivudine* and *telbivudine* resistance. In cell-based assays, 8- to 30-fold reductions in *entecavir* susceptibility were observed for *lamivudine*-resistant strains. In patients with preexisting *lamivudine* resistance, *entecavir* resistance emerged in 7% and 43% after 1 and 4 years, respectively.

Entecavir monotherapy in HIV/HBV-coinfected patients, including antiretroviral therapy–naïve patients, has significant anti-HIV activity and can result in the development of the M184V variant (Sasadeusz et al., 2008). Thus, *entecavir* should only be used in combination with fully suppressive antiretroviral therapy in individuals with HIV/HBV coinfection.

ADME. The recommended dose for nucleoside treatment–naïve adults without decompensated cirrhosis is 0.5 mg once daily. For adults with *lamivudine* or *telbivudine* resistance or for those with decompensated cirrhosis, the dose is 1 mg once daily. The dose must be reduced for individuals with Cl$_{Cr}$ less than 50 mL/min. Time to peak C_p occurs in 0.5 to 1.5 h. Steady state is reached after 6 to 10 days of once-daily dosing. The tablet and oral solution can be used interchangeably. Administration with food decreases C_{max} by 44% to 46% and AUC by 18% to 20%; thus, *entecavir* should be administered on an empty stomach.

Entecavir is extensively distributed in tissues and binds slightly (13%) to serum proteins. It is primarily eliminated unchanged in the kidney, probably by both glomerular filtration and net tubular secretion. *Entecavir* exhibits biphasic elimination, with a terminal $t_{1/2}$ of 128 to 149 h. Dose reductions are needed for patients with Cl$_{Cr}$ less than 50 mL/min, typically by extension of the dosing interval.

Therapeutic Uses. *Entecavir* is FDA-approved for the treatment of chronic HBV infection in adults and children 2 years or older with evidence of active viral replication and evidence of either persistent elevations in serum aminotransferases or histologically active disease. *Entecavir* is considered a first-line agent for HBV because of its potency, durability, and low genetic barrier to the development of resistance (Terrault et al., 2018). In HBeAg-positive individuals, 61% are virologically suppressed, 22% to 25% have HBeAg loss, 68% to 81% normalize alanine aminotransferase (ALT), and 4% to 5% have HBsAg loss after 2 to 3 years of continuous entecavir treatment. In HBeAg-negative individuals, 90% to 91% are virologically suppressed and 78% to 88% normalize ALT after 2 to 3 years of *entecavir* treatment, but fewer than 1% have HBsAg loss at 1 year. *Entecavir* use reduces the risk of hepatocellular carcinoma relative to untreated historical controls. Few studies have shown a benefit to combination treatment of HBV; however, one study found that in individuals with HBV DNA of 10^8 or greater, the combination of *entecavir* and TDF was superior to *entecavir* alone (Lok et al., 2012). Nucleoside/-tide combination therapy may also be used in the case of decompensated cirrhosis and in individuals with virological breakthrough on *entecavir* or TDF. The safety of *entecavir* in pregnancy is unknown.

Untoward Effects. Severe acute exacerbations of HBV have been reported in patients who have discontinued anti-HBV therapy, including *entecavir*. Hepatic function should be monitored closely with both clinical and laboratory follow-up for at least several months in patients who discontinue anti-HBV therapy. There is a potential for development of resistance to nucleoside reverse transcriptase inhibitors in HBV/HIV coinfection, especially if HIV is not being treated. Lactic acidosis and severe hepatomegaly with steatosis may occur in patients with decompensated cirrhosis receiving *entecavir*. More common adverse reactions include headache, fatigue, dizziness, and nausea (Sasadeusz et al., 2008).

Pediatric and Geriatric Uses. *Entecavir* oral solution is approved in children older than 2 years, and dosing is weight based up to 30 kg. *Entecavir* exposures following a single 1-mg dose were 29% higher in elderly versus young HBV-seronegative volunteers. This difference is likely explained by declines in renal function with age.

Tenofovir

Tenofovir, an acyclic nucleoside phosphonate, is available in two different prodrug formulations, TDF and TAF. TAF is described separately in a following discussion. With TDF, esterases cleave the diester, yielding *tenofovir* in plasma, which enters cells and is subsequently phosphorylated by cellular kinases to *tenofovir diphosphate* (see Figure 64–4). The drug has activity against both HIV-1 and HBV. In cell cultures *in vitro*, the EC_{50} for *tenofovir* against HBV ranges from 0.14 to 1.5 μM.

Mechanisms of Action and Resistance. Chapter 64 describes the mechanism of action of *tenofovir*. The genetic barrier to resistance for TDF is high, but a few reports have identified potential variants (Mokaya et al., 2020).

ADME. The TDF dose is 300 mg once daily; the dose is reduced in patients with renal impairment. The bioavailability of *tenofovir* is approximately 25%. *Tenofovir* exposures are increased 40% with a high-fat meal. Binding to plasma proteins is negligible (<8%). *Tenofovir* is not metabolized by CYP enzymes. The $t_{1/2}$ of *tenofovir* in plasma is 17 h, and the $t_{1/2}$ of the active form of the drug, *tenofovir diphosphate*, is 6 days and 17 days in peripheral blood and red blood cells, respectively. *Tenofovir* pharmacokinetics are not altered in hepatic impairment, but plasma exposures rise as Cl_{Cr} drops, becoming 2.8- and 7.3-fold higher in those with Cl_{Cr} of 30 to 49 mL/min and 12 to 29 mL/min compared to those with Cl_{Cr} greater than 80 mL/min. Doses should be reduced in those with Cl_{Cr} below 50 mL/min. Roughly 10% of the dose is removed by a 4-h hemodialysis session.

Therapeutic Uses. TDF is approved for the treatment of HBV infection in individuals 2 years or older. Due to its safety, efficacy, and resistance profile, TDF is a preferred agent for the treatment of HBV. In HBeAg-positive individuals, 76% are virologically suppressed, 21% have conversion from HBeAg to anti-HBe, 68% normalize ALT, and 8% have HBsAg loss after 3 years of continuous TDF treatment. In HBeAg-negative individuals, 93% are virologically suppressed and 76% normalize ALT after 2 to 3 years of treatment, but none have HBsAg loss at 1 year (Terrault et al., 2018).

Few studies have shown a benefit to combination treatment of HBV; however, one study found that in individuals with HBV DNA 10^8 or greater, the combination of *entecavir* and TDF was superior to *entecavir* alone (Lok et al., 2012). Nucleoside/-tide combination therapy may also be used in the case of decompensated cirrhosis and in individuals with virological breakthrough on *entecavir* or TDF. TDF has a pregnancy category of B, although a study found that infants born to HIV-infected women receiving TDF had 12% lower bone mineral density compared to infants born to women receiving other antiretroviral agents. HIV-infected individuals are generally treated with *tenofovir* and *emtricitabine*. *Emtricitabine* is a cytidine analogue with activity against HIV and HBV. Refer to Chapter 64 for additional information about *emtricitabine*.

Untoward Effects and Drug Interactions. *Tenofovir* has a low potential for interactions but is not devoid of interactions. *Tenofovir* is a substrate for several membrane transporters, including OAT1 and MRP4, and TDF is a substrate for P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), and thus, *tenofovir* may be affected by drugs that inhibit or induce these transporters. Some HCV therapies (e.g., *sofosbuvir*, *ledipasvir*, and *velpatasvir*) increase *tenofovir* exposures; patients receiving such combinations require more frequent renal monitoring.

Pediatric and Geriatric Uses. Primary considerations in treating the elderly with TDF are the bone and renal toxicities; a thorough study of TDF in the geriatric population has not been published. TDF has been studied in children and is approved for those 2 years or older. The dose of TDF in children is 8 mg/kg, with a maximum of 300 mg daily.

Adefovir

Adefovir dipivoxil is a diester prodrug of *adefovir*, an acyclic phosphonate nucleotide analogue of adenosine monophosphate. Inhibitory concentrations of *adefovir* against HBV range from 0.2 to 1.2 μM in cell culture.

Mechanisms of Action and Resistance. *Adefovir dipivoxil* is deesterified to *adefovir*. *Adefovir* is converted by cellular enzymes to the diphosphate, which acts as a competitive inhibitor of viral DNA polymerases and reverse transcriptases with respect to deoxyadenosine triphosphate (dATP) and also serves as a chain terminator of viral DNA synthesis.

Resistance to *adefovir* emerges in about 29% of patients after 5 years of treatment. The development of viral resistance may increase the risk of hepatic decompensation. Thus, *adefovir*, *lamivudine*, and *telbivudine* are not recommended for the treatment of HBV.

ADME. The *adefovir dipivoxil* dose is 10 mg once daily but must be reduced for those with renal impairment. The parent compound has low oral bioavailability (<12%), whereas the dipivoxil prodrug is absorbed rapidly and hydrolyzed by esterases in the intestine, liver, and blood to *adefovir*, providing a bioavailability of about 30% to 60%. Food does not affect bioavailability. *Adefovir* is scantily protein bound (<5%) and has a volume of distribution of about 0.4 L/kg.

The drug is eliminated unchanged by the kidney through a combination of glomerular filtration and tubular secretion. After oral administration of *adefovir* dipivoxil, about 30% to 45% of the dose is recovered within 24 h; the serum $t_{1/2}$ of elimination is 5 to 7.5 h. The intracellular $t_{1/2}$ of the active diphosphate form *in vitro* ranges from 5 to 18 h, although typically the half-lives are much longer *in vivo*. Dose reductions are recommended for Cl_{Cr} values below 50 mL/min. *Adefovir* is removed by hemodialysis, but the effects of peritoneal dialysis or severe hepatic insufficiency on pharmacokinetics are unknown.

Therapeutic Uses. *Adefovir dipivoxil* is approved for treatment of chronic HBV infections in individuals 12 years and older. However, *adefovir dipivoxil* is not a preferred agent for the treatment of HBV because it is associated with the development of viral resistance and nephrotoxicity.

Untoward Effects and Drug Interactions. *Adefovir dipivoxil* causes dose-related nephrotoxicity and tubular dysfunction, manifested by azotemia and hypophosphatemia, acidosis, glycosuria, and proteinuria, which usually are reversible months after discontinuation. Other adverse effects include headache, abdominal discomfort, diarrhea, and asthenia. Adverse events lead to premature discontinuation in about 2% of patients. After 2 years of dosing, the risk of serum creatinine levels rising above 0.5 mg/dL is approximately 2% but is higher in those with preexisting renal insufficiency. Acute, sometimes severe, exacerbations of hepatitis can occur in patients stopping *adefovir* or other anti-HBV therapies. Close monitoring is necessary, and resumption of antiviral therapy may be required in some patients.

No clinically important drug interactions have been recognized to date, although drugs that reduce renal function or compete for active tubular secretion could decrease *adefovir* clearance. *Ibuprofen* increases *adefovir* exposure modestly. An increased risk of lactic acidosis and steatosis may exist when *adefovir* is used in conjunction with other nucleoside analogues or antiretroviral agents. *Adefovir* is transported efficiently by renal tubular OAT1.

Adefovir is genotoxic, and high doses cause hepatotoxicity, lymphoid toxicity, and renal tubular nephropathy in animals. The diphosphate's inhibitory effects on renal adenylyl cyclase may contribute to nephrotoxicity (Shoshani et al., 1999). *Adefovir dipivoxil* is not associated with reproductive toxicity, although high intravenous doses of *adefovir* cause maternal and embryotoxicity with fetal malformations in rats (pregnancy category C).

Pediatric and Geriatric Uses. *Adefovir* should be avoided in elderly patients due to the risk for nephrotoxicity. This drug is approved in children 12 years or older at the same dose as that used in adults.

Lamivudine

Lamivudine, the (−)-enantiomer of 2′,3′-dideoxy-3′-thiacytidine, is a nucleoside analogue that inhibits HIV reverse transcriptase and HBV DNA polymerase. Details of its *mechanism of action, ADME, and drug interaction potential* are described in Chapter 64. *Lamivudine* inhibits HBV replication *in vitro* by 50% at concentrations of 4 to 7 ng/mL.

Mechanisms of Action and Resistance. Cellular enzymes convert *lamivudine* to the triphosphate, which competitively inhibits HBV DNA polymerase and causes chain termination.

After 5 years of treatment, approximately 71% of patients develop resistance to *lamivudine*. Point mutations in the *YMDD* motif of HBV DNA polymerase result in a 40- to 104-fold reduction in the *in vitro* susceptibility (Ono et al., 2001). *Lamivudine* resistance confers cross-resistance to related agents, such as *emtricitabine*. *Lamivudine*-resistant HBV retains susceptibility to *tenofovir* and partially to *adefovir* and *entecavir*. *Lamivudine* resistance is associated with elevated HBV DNA levels, decreased likelihood of HBeAg loss or seroconversion, hepatitis exacerbations, and progressive fibrosis and graft loss in transplant recipients.

Therapeutic Uses. *Lamivudine* is approved for the treatment of chronic HBV hepatitis in adults and children 2 years or older. However, *lamivudine* is not a preferred agent for the treatment of HBV because it is associated with a high rate of viral resistance.

The *lamivudine* dose is 100 mg once daily. In adults, doses of 100 mg/day for 1 year cause suppression of HBV DNA levels, normalization of aminotransferase levels in 41% or more of patients, and reductions in hepatic inflammation in more than 50% of patients. Seroconversion with antibody to HBeAg occurs in fewer than 20% of recipients at 1 year. In children 2 to 17 years of age, *lamivudine* (3 mg/kg per day to a maximum of 100 mg/day for 1 year) is associated with normalization of aminotransferase levels in about one-half and seroconversion to anti-HBe in about one-fifth of cases (Jonas et al., 2002). If resistant variants do not emerge, prolonged therapy is associated with sustained suppression of HBV DNA, continued histological improvement, and an increased proportion of patients experiencing a loss of HBeAg and undetectable HBV DNA. Prolonged therapy is associated with an approximate halving of the risk of clinical progression and development of hepatocellular carcinoma in those with advanced fibrosis or cirrhosis (Liaw et al., 2004). However, the frequency of *lamivudine*-resistant variants increases progressively with continued drug administration. Consider dose reduction for patients with renal impairment.

Untoward Effects. At the doses used for chronic HBV infection, *lamivudine* generally is well tolerated. Aminotransferase rises after therapy occur in *lamivudine* recipients, and flares in posttreatment aminotransferase elevations (>500 IU/mL) occur in about 15% of patients after cessation.

Pediatric and Geriatric Uses. The dose of *lamivudine* for use in children 2 to 17 years old is 3 mg/kg per day to a maximum of 100 mg/day. *Lamivudine* is not associated with increased toxicities in the elderly.

Telbivudine

Telbivudine is a synthetic thymidine nucleoside analogue with activity against HBV DNA polymerase. In a cell culture model, the EC_{50} for inhibition of viral DNA synthesis by *telbivudine* was 0.2 μM. The U.S. manufacturer has ceased producing *telbivudine*.

Mechanisms of Action and Resistance. *Telbivudine* is phosphorylated by cellular kinases to the active triphosphate form, telbivudine 5′-triphosphate, which inhibits HBV DNA polymerase (reverse transcriptase) by competing with the natural substrate, thymidine 5′-triphosphate. Incorporation of telbivudine 5′-triphosphate into viral DNA causes chain termination.

Resistance to *telbivudine* emerges in about 22% of patients after 2 years of treatment. The development of viral resistance may increase the risk for hepatic decompensation. Thus, this drug, along with *lamivudine* and *adefovir*, is not recommended for the treatment of HBV.

ADME. The standard *telbivudine* dose is 600 mg once daily. The bioavailability of *telbivudine* is 68% (Zhou et al., 2006), and the drug is widely distributed into tissues. Food does not affect the pharmacokinetics of *telbivudine*. The drug is eliminated unchanged in the urine. *Telbivudine* concentrations decline biexponentially with an elimination $t_{1/2}$ of 40 to 49 h. Patients with moderate-to-severe renal dysfunction and those undergoing hemodialysis require dose adjustments.

Therapeutic Uses. *Telbivudine* is indicated for the treatment of chronic HBV in adult patients (≥16 years) with evidence of viral replication and either evidence of persistent elevations in serum aminotransferases (ALT or aspartate aminotransferase [AST]) or histologically active disease. Although *telbivudine* has superior efficacy compared with *lamivudine* and *adefovir*, its use is associated with the development of resistance. Thus, *telbivudine* is not a preferred agent for the treatment of HBV.

Untoward Effects and Drug Interactions. *Telbivudine* is generally well tolerated and safe. The most common adverse events resulting in *telbivudine* discontinuation included increased creatine kinase, nausea, diarrhea, fatigue, myalgia, and myopathy. Elevations of creatine kinase activity, mostly asymptomatic grade 3 to 4, are more common in *telbivudine*-treated than in *lamivudine*-treated patients after 2 years of therapy. The risk of peripheral neuropathy is increased when *telbivudine* is used with IFN-α, so this combination should be avoided.

Pediatric and Geriatric Uses. *Telbivudine* is not approved for children younger than 16 years. The primary consideration in treating geriatric patients with *telbivudine* is renal function.

Tenofovir Alafenamide

A different prodrug of *tenofovir*, TAF, is a phosphonate ester of *tenofovir* (Ray et al., 2016).

Mechanisms of Action and Resistance. The active ingredient of TAF is *tenofovir*, an inhibitor of HBV reverse transcriptase and HIV-1 reverse transcriptase. TAF is relatively more stable in the plasma than TDF; it is taken up into cells (e.g., hepatocytes), where it is deesterified, concentrated, and phosphorylated to *tenofovir diphosphate*. *Tenofovir diphosphate* is a competitive inhibitor of reverse transcriptase, competing with the physiological substrate dATP; when incorporated into DNA, the drug results in chain termination. No resistance to TAF has been observed after 96 weeks of treatment (Cathcart et al., 2018).

ADME. The dose of TAF for hepatitis B is 25 mg once daily. Plasma concentrations of *tenofovir* when administered as TAF are 90% less than when administered as TDF. The renal adverse effects of *tenofovir* are mediated through uptake by OAT1 in the kidney. TAF is not a substrate for OAT1, so less *tenofovir* is delivered to the kidneys, and there is less renal toxicity with this agent. However, TAF is preferentially taken up by several cell types, including peripheral blood mononuclear cells, and cellular concentrations of the active form, *tenofovir diphosphate*, are actually higher than those achieved with TDF.

Therapeutic Uses. TAF is used at a daily dose of 25 mg to treat HBV. Two studies have found TAF noninferior to TDF in terms of suppressed HBV DNA after 48 weeks of treatment with smaller declines in bone mineral density and estimated glomerular filtration rate (eGFR).

Untoward Effects and Drug Interactions. TAF has similar efficacy to TDF with fewer adverse effects on bone mineral density and kidney function (Cl_{Cr}, eGFR, proteinuria). Common side effects of *tenofovir* include nausea, rash, diarrhea, depression, and weakness. HBV "flare up" can result from sudden discontinuation of the drug.

Investigational Agents and the Future for Treatment of HBV

Several studies are investigating whether the nucleoside/-tide analogues can be safely discontinued in persons who are noncirrhotic and have converted from HBeAg positive to negative in order to stimulate HBsAg loss (Hadziyannis and Hadziyannis, 2020). Preliminary data suggest this approach may lead to HBsAg loss in a small percentage (~5%) of patients within 48 weeks of discontinuing nucleoside reverse transcriptase inhibitors (Hall et al., 2020).

Current therapies provide a functional cure (i.e., a cure that mimics naturally acquired immunity off therapy) in only a minority of patients, and none provides a complete cure because cccDNA persists in cells. Thus, new agents are needed to treat HBV. Multiple antiviral and immune modulators are in various stages of clinical development for HBV. Antiviral targets include entry inhibitors, cccDNA disruptors, translation inhibitors, capsid assembly inhibitors, polymerase inhibitors, and secretion inhibitors. Antivirals in more advanced stages of clinical development include *bulevirtide*, JNJ-6379, ABI-H0731, ARO-HBV, and REP-2139 (Soriano et al., 2020). *Bulevirtide*, an entry inhibitor, irreversibly binds to NTCP. It is given as a daily subcutaneous injection and recently received regulatory approval in Europe for treatment of hepatitis D. It is moving into phase III development for treatment of HBV. Capsid inhibitors, such as JNJ-6379 and ABI-H0731, destabilize HBcAg assembly, leading to aberrant capsids or capsids devoid of genetic material. ARO-HBV contains two RNA interference compounds that silence mRNA produced by cccDNA or viral DNA integrated into host. REP-2139 reduces HBsAg subparticle release, preventing these particles from exhausting adaptive immune responses. Immunomodulators in development include checkpoint inhibitors and toll-like receptor or RIG-1 agonists, among others (Soriano et al., 2020). It is likely that combinations of these compounds with or without IFN and/or the nucleoside/-tide analogues will be required for optimal efficacy.

Hepatitis C Virus

HCV Overview

Approximately 71 million people have chronic HCV infection. Africa and Central and East Asia have the highest prevalence of HCV. The majority of individuals infected with HCV, about 70%, will develop chronic infection, which may progress to cirrhosis (WHO, 2020b). Approximately 6% of cirrhotic individuals will develop symptoms of decompensated liver disease (e.g., ascites, hepatic encephalopathy, or variceal bleeding) each year, and 4% will develop hepatocellular carcinoma. These long-term complications carry a high risk of mortality and generally occur more than 20 years after infection (Freeman et al., 2001). There are approximately 400,000 HCV-related deaths annually.

Genetic Heterogeneity of HCV

Hepatitis C virus exhibits remarkable within- and between-subject genetic heterogeneity, which is a major obstacle to the development of a universal preventive vaccine. At least six HCV genotypes have been identified. HCV strains belonging to different genotypes differ at 30% to 35% of nucleotide sites (Messina et al., 2015). Within each genotype, HCV is further classified into subtypes that differ at fewer than 15% of nucleotide sites. Among genotypes, transmissibility does not seem to differ, whereas the rate of disease progression and response to treatment with current therapies does differ. Globally, genotype 1 is most prevalent (Messina et al., 2015), followed by genotype 3. Genotypes 2, 4, and 6 together account for 25% of those living with HCV. Within the U.S., 75% of HCV isolates are genotype 1a or 1b, and the remainder are primarily genotype 2 or 3 (Zein et al., 1996).

HCV Genome and Life Cycle

The HCV genome consists of a positive-sense, single-stranded RNA, about 9600 nucleotides long, which encodes three structural proteins (core, E1, and E2); the ion channel protein p7; and six nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (Tang and Grise, 2009). HCV replicates entirely within the cytoplasm (Figure 63–3); it does not establish latency, and it is curable. Cure of HCV is synonymous with achieving a sustained virologic response (SVR). SVR is defined as having no measurable HCV RNA in the blood 12 weeks following treatment cessation. Achieving SVR decreases the progression of liver disease and reduces liver-related and all-cause mortality.

The HCV life cycle is shown in Figure 63–3. Not all steps in the hepatitis C viral life cycle have been completely elucidated. HCV binds two host receptors on the hepatocyte surface, low density lipoprotein (LDLr) and HSPGs. This initiates binding of E2 with CD81 and scavenger receptor B1 (SR-B1). The N-terminal hypervariable region of E2 interacts with SR-B1 (Kalemera et al., 2019). Proteins in the tertiary structure of E2 bind the large extracellular loop of CD81. These interactions create a tight junction between hepatocytes. Within the tight junction, HCV interacts with claudin-1 (CLDN1) and folds up with the hepatocyte membrane. This clathrin-covered endosome disperses inside the cell and fuses with host membranes, which permits uncoating of the capsid shell and release of the HCV RNA into the cytosol. HCV polyprotein translation begins when the ribosomal subunits bind the HCV RNA in the endoplasmic reticulum (ER). The polyprotein is co- and posttranslationally cleaved by both host and viral proteases to produce the 10 proteins described above. HCV proteins and host factors rearrange the host cell membranes to form the membranous web. In this web, the NS5B RdRp catalyzes the synthesis of a negative-sense RNA intermediate (template) that is used to create copies of positive-sense progeny HCV RNA. These newly made HCV RNAs are either used for RNA translation and replication or incorporated into nucleocapsid particles. Following RNA replication, HCV RNA is shuttled to the lipid droplet where core protein accumulates, oligomerizes, and engages the NS3 and NS5A proteins (Roder et al., 2019). Core oligomers bound to viral RNA bud into the ER lumen, acquiring an ER-derived lipid bilayer envelope that contains E1 and E2. After budding into the ER lumen, the virion moves through the very-low-density lipoprotein (VLDL) secretory pathway, acquiring apolipoproteins and other lipids en route. The lipoviroparticle fuses with the hepatocyte membrane and is subsequently released into extracellular compartment.

HCV Drug Targets and Treatment Approach

For many years, HCV was treated with a prolonged regimen of subcutaneously administered IFN-α (or pegIFN-α) with or without the purine nucleoside analogue *ribavirin*. SVR rates with this therapy were low, and tolerability was poor. Now, several antivirals that directly target various steps in the HCV life cycle (so-called DAAs) are the mainstay of HCV treatment. DAAs are administered orally, have few side effects, are typically taken for a period of 8 or 12 weeks, and achieve SVR rates of approximately 95% in most patient populations. Use of pegIFN-α for the treatment of HCV has been replaced by DAAs, although *ribavirin* is still used in combination with DAAs to improve SVR rates in certain clinical scenarios.

Detailed knowledge of the HCV life cycle and the structures of HCV proteins has permitted development of targeted inhibitors of HCV replication and revolutionized pharmacotherapy of HCV infection. Available DAAs target three major sites in the HCV life cycle: the NS3 protease, the NS5B polymerase, and NS5A (see Figure 63–3) (Ciesek and Manns,

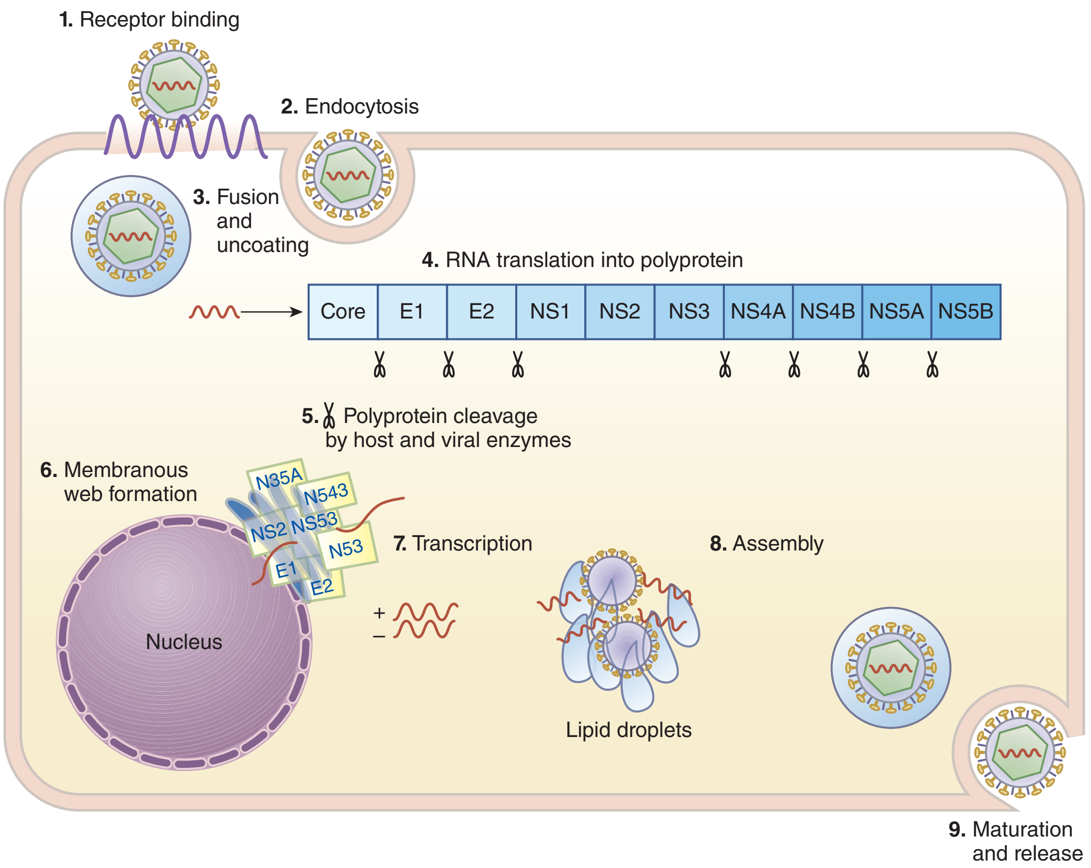
1. Receptor binding
2. Endocytosis
3. Fusion and uncoating
4. RNA translation into polyprotein
Core
E1
E2
NS1
NS2
NS3
NS4A
NS4B
NS5A
NS5B
5. Polyprotein cleavage by host and viral enzymes
6. Membranous web formation
N35A
N543
NS2
NS53
N53
E1
E2
Nucleus
7. Transcription
+
–
8. Assembly
Lipid droplets
9. Maturation and release

Figure 63–3 *Hepatitis C life cycle.* Not all steps in the hepatitis C viral life cycle have been completely elucidated. This figure represents our current understanding. For further details, consult the following references: Ciesek and Manns, 2011; Dubuisson and Cosset, 2014; and Holmes and Thompson, 2015.

2011; Dubuisson and Cosset, 2014; Holmes and Thompson, 2015). Inhibition of the NS3 protease prevents the cleavage of the viral polyprotein and formation of the replication complex. The NS5B enzyme is essential for HCV replication as it catalyzes the synthesis of the complementary minus-strand RNA and subsequent genomic plus-strand RNA. There are two types of NS5B RdRp inhibitors: nucleotide and nonnucleoside inhibitors. The nucleotide inhibitors are active site inhibitors, whereas the nonnucleoside inhibitors are allosteric inhibitors. Another target, NS5A, encodes a protein that appears essential to the replication machinery of HCV and critical in the assembly of new infectious viral particles (Gish and Meanwell, 2011). However, the specific functions of this protein have not been established.

Clinical Implications of HCV Resistance

On average, almost a trillion HCV particles are produced in an infected individual each day (Neumann et al., 1998). The HCV NS5B polymerase enzyme lacks a proofreading function and has relatively poor fidelity (Brown, 2009); therefore, there is a continuous generation of a large variety of spontaneous viral mutations. Thus, even untreated persons have HCV genomes that harbor preexisting resistance-associated substitutions (RASs). These naturally occurring RASs may affect the chances of achieving a cure with DAA treatment.

The likelihood that a DAA will select for and allow outgrowth of viral populations carrying RASs during treatment depends on several factors, including the DAA's genetic barrier to resistance (the number and type of base-pair mutations needed to result in amino acid substitutions that confer resistance), the level of drug exposure(s), and the viral fitness (replicative capacity) of the RAS. Nucleotide NS5B polymerase inhibitors have a high genetic barrier to resistance; three or more RASs are required for full resistance. In contrast, the nonnucleoside NS5B polymerase inhibitors. NS3 protease inhibitors, and NS5A inhibitors generally have a low genetic barrier to the development of resistance; that is, only one or two amino acid substitutions result in lack of drug efficacy. For drugs in these classes, there is swift emergence of RASs during monotherapy. Thus, as with HIV treatment, HCV treatment requires a combination of agents to optimize viral suppression and protect against the development of resistance. Data are emerging on the impact of treatment-emergent NS5A RASs on the likelihood of achieving SVR with current therapies. NS5A RASs have been shown to persist for several years after treatment cessation, and some NS5A inhibitors suffer from broad cross-resistance at key positions (Q30R, L31M/V, Y93H/N).

Hepatic Impairment: Implications for HCV Treatment

The vast majority of HCV-infected individuals have at least a 90% chance of achieving SVR with current therapies. There are some groups, however, such as those with decompensated cirrhosis, who have lower rates of SVR. Treating those with decompensated cirrhosis and achieving SVR have been shown to improve MELD scores and reverse fibrosis, but significant morbidity may persist (Bittermann and Reddy, 2021; Verna et al., 2020).

Studies indicated the optimal time to treat HCV is early in the course of illness. The risk of developing decompensated cirrhosis or hepatocellular carcinoma, requiring a liver transplant, or dying from HCV-related complications is significantly lower in those treated with minimal fibrosis compared to those treated once bridging fibrosis or cirrhosis has developed. Treating all patients early in the course of disease is challenging, however, because many patients with HCV are unaware of their disease.

Pharmacotherapy of HCV Infection

A description of current DAA therapies follows, and a summary of the therapeutic uses and clinical pharmacology of these drugs is provided in the Drug Facts table at the end of this chapter. Up-to-date information on testing, managing, and treating HCV can be found in guidelines from the American Association for the Study of Liver Diseases and Infectious Diseases Society of America (http://www.hcvguidelines.org). An important aspect of treating HCV is identification and management of drug-drug interactions with DAAs. The University of Liverpool offers a free, comprehensive, and reliable web-based drug interactions resource (available at http://www.hep-druginteractions.org).

Sofosbuvir

Sofosbuvir is a prodrug based on a uridine analogue. In cells, *sofosbuvir* is metabolized to an active form (known as GS-461203) that competes with uridine triphosphate for incorporation into HCV RNA by NS5B polymerase.

Mechanisms of Action and Resistance. *Sofosbuvir* is a prodrug, a 5′ monophosphorylated uridine analogue on which the charges of the phosphate group are masked by groups that are readily removed in the body. The active drug, generated in the host mammalian cell, inhibits HCV RNA polymerase. Figure 63–4 shows the cellular pharmacology of *sofosbuvir*.

Sofosbuvir provides a high barrier to the development of resistance, but the S282T RAV has been reported in patients who relapsed to *sofosbuvir*-based treatment. L159F, V321A, and C316N/H/F have also been observed (Lontok et al., 2015).

ADME. The dose of *sofosbuvir* is 400 mg taken once daily. The absolute bioavailability of *sofosbuvir* is estimated to be at least 80% based on recovery of *sofosbuvir* and its primary metabolite, GS-331007, following administration of a radiolabeled dose. A high-fat meal increases *sofosbuvir*'s AUC by 67% to 91%, but, based on the drug's high therapeutic index, the increased exposure is not thought to increase the likelihood of toxicities (Kirby et al., 2015). *Sofosbuvir* exhibits time-independent, near-linear pharmacokinetics across a range of doses. *Sofosbuvir* is 63% protein bound, whereas protein binding of the deesterified GS-331007 is minimal.

The primary metabolic route of *sofosbuvir* is hydrolysis to GS-331007. GS-331007 lacks antiviral activity and cannot be rephosphorylated. The majority of a *sofosbuvir* dose is converted to GS-331007 and eliminated in the urine via a combination of tubular secretion and glomerular filtration. *Sofosbuvir* AUC is increased 2.3-fold and 2.5-fold in patients with

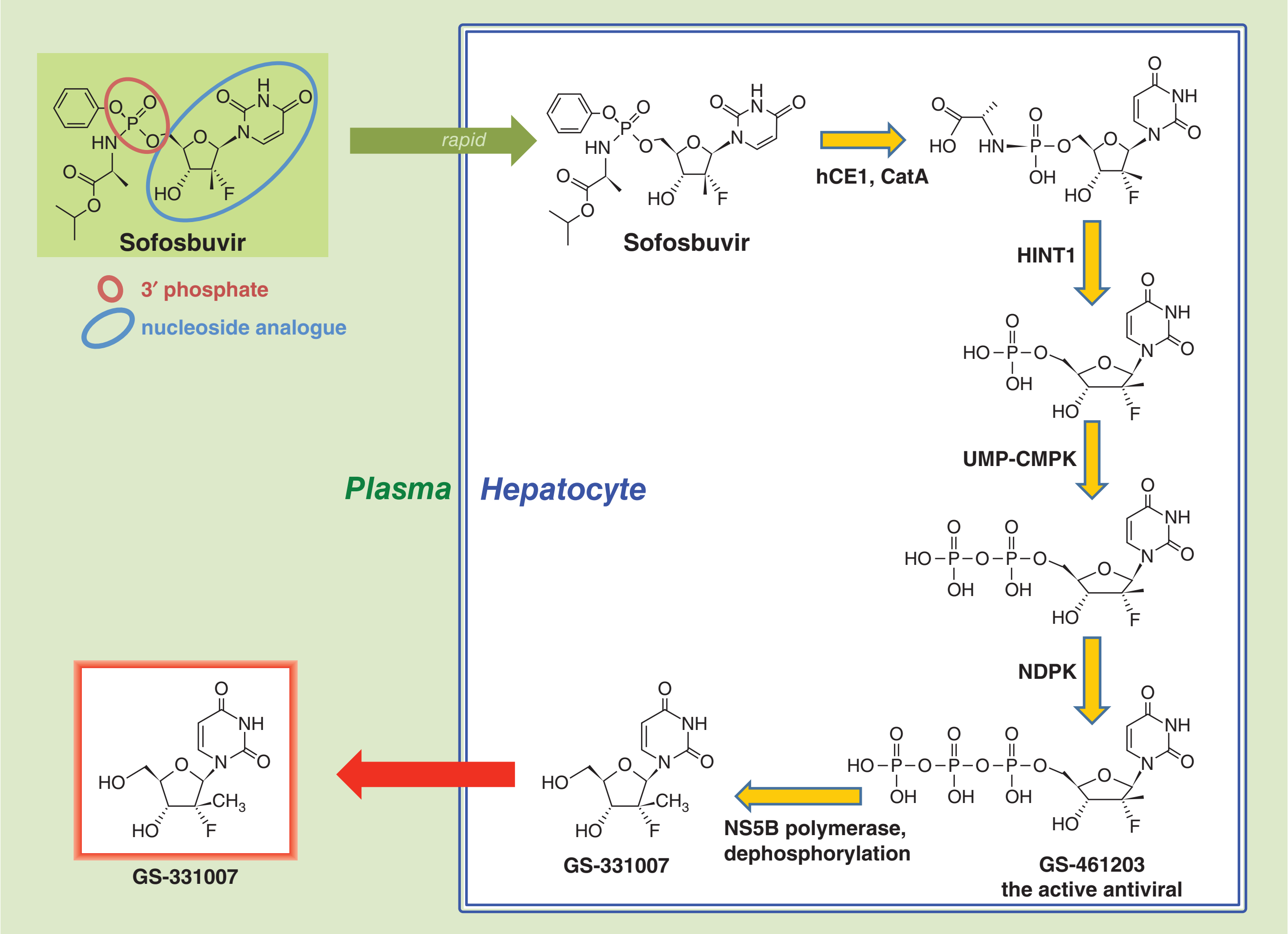

Figure 63–4 *Cellular pharmacology of sofosbuvir.* Some nucleoside analogues act as antiviral agents in infected cells, but first they must cross the plasma membrane and then be phosphorylated, starting at the 5′ position, a slow reaction catalyzed by nucleoside kinase. Use of a monophosphorylated nucleoside avoids the slow nucleoside kinase reaction, but the charge on the phosphate groups would greatly slow passage of the molecule across the plasma membrane. *Sofosbuvir* circumvents these issues by having a phosphate (red ellipse) at the 5′ position of the nucleoside analogue (blue ellipse) but masking the two negative charges of the phosphate group with adducts that esterases/amidases can readily remove. *Sofosbuvir* crosses the plasma membrane rapidly. The carboxyl ester moiety is hydrolyzed by CatA or hCE1. Histidine triad nucleotide-binding protein 1 (HINT1) catalyzes the phosphoramidate cleavage, yielding the monophosphate form, retained in the cell by ion trapping. Subsequent phosphorylation occurs via the pyrimidine nucleotide biosynthetic pathway. The triphosphate (GS-461203) is the active form responsible for the antiviral activity of *sofosbuvir*. GS-461203 acts as a defective deoxynucleotide triphosphate (dNTP) substrate and inhibits HBV RNA polymerase (NS5B); it may also act as a chain terminator. Dephosphorylation of GS-461203 yields a uridine analogue metabolite, GS-331007, that cannot be readily rephosphorylated, lacks antiviral activity, and exits the cell. Most (90%) of the drug-related material measured in plasma is GS-331007. CatA, cathepsin A; hCE1, human carboxylesterase 1; NDPK, nucleoside diphosphate kinase; UMP-CMPK, uridine/cytidine monophosphate kinase.

moderate and severe hepatic impairment (i.e., Child-Pugh B and C), respectively, but GS-331007 AUC is unchanged. No dose adjustment is needed for this population; available data suggest *sofosbuvir* is safe and effective in individuals with decompensated cirrhosis when combined with appropriate DAAs (Charlton et al., 2015; Manns et al., 2016).

In contrast, *sofosbuvir* and GS-331007 pharmacokinetics are affected by renal impairment. *Sofosbuvir* and GS-331007 AUC increased about 60% in those with eGFR of 50 to 80 mL/min/1.73 m^2, 107% in those with eGFR of 30 to 50 mL/min/1.73 m^2, and 171% in those with an eGFR less than 30 mL/min/1.73 m^2. Despite these increased exposures, there are no renal restrictions on the use of *sofosbuvir*-based therapies to treat HCV since emerging data indicate the drug's safety and efficacy in this population. *Sofosbuvir*'s AUC is 60% higher in HCV-infected persons, and the AUC of GS-331007 is 39% lower compared with HCV-seronegative volunteers. The mechanism for this is unclear. The median terminal half-lives of *sofosbuvir* and GS-331007 are 0.4 and 27 h, respectively.

Therapeutic Uses. The NS5B polymerase region is well conserved across genotypes. Thus, *sofosbuvir* is active against all HCV genotypes. In individuals with genotype 1 HCV, 7 days of *sofosbuvir* monotherapy reduces HCV RNA by 4.65 logs (Lawitz et al., 2013). *Sofosbuvir* is used in combination with the NS5A inhibitors *daclatasvir*, *ledipasvir*, or *velpatasvir*. It is also used with *velpatasvir* and the NS3 protease inhibitor, *voxilaprevir*, as a salvage regimen for those who previously failed NS5A-based HCV treatment. The efficacy and tolerability of *sofosbuvir* in these combination therapies are described in subsequent sections.

Untoward Effects and Drug Interactions. There are no hallmark toxicities associated with *sofosbuvir* use. Side effects observed when *sofosbuvir* is administered as part of combination DAA treatment are described in the material that follows with the concomitant antiviral.

Sofosbuvir is not a cytochrome P450 (CYP) substrate, inhibitor, or inducer and therefore has a low potential for CYP-mediated drug interactions. However, *sofosbuvir* is a substrate for the efflux transporters P-gp and BCRP and should not be used with potent inducers of these transporters (e.g., *rifampin*, St. John's wort, *phenytoin*, *carbamazepine*). *Sofosbuvir* is also an inhibitor of carboxylesterase-2 (CES-2), an enzyme involved in the hydrolysis of many drugs, including TDF (Shen and Yan, 2017). *Sofosbuvir*-containing DAA treatments have been associated with bradycardia, the requirement for pacemaker insertion, and fatal cardiac arrest in patients taking *amiodarone*. The mechanism(s) for this is unclear, but the interaction appears to be more pharmacodynamic rather than pharmacokinetic in nature (Regan et al., 2016). If the combination cannot be avoided, cardiac monitoring in an inpatient setting is advised for the first 48 h of *sofosbuvir*-based treatment, with daily heart rate monitoring for an additional 2 weeks.

Pediatric and Geriatric Uses. *Sofosbuvir/ledipasvir* and *sofosbuvir/velpatasvir* are approved in children as described in the *ledipasvir* and *velpatasvir* sections that follow. In licensing trials, the response rates of adults under 65 years and over 65 years of age were similar.

Ribavirin

Ribavirin, a purine nucleoside analogue with a modified base and D-ribose sugar, inhibits the replication of a wide range of RNA and DNA viruses, including orthomyxo-, paramyxo-, arena-, bunya-, and flaviviruses *in vitro*.

Mechanisms of Action and Resistance. Host cell enzymes phosphorylate *ribavirin* to mono-, di-, and triphosphate derivatives. The exact mechanism(s) of action of *ribavirin* or its phosphorylated derivatives *in vivo* is unknown, but several immunomodulatory and antiviral effects have been observed *in vitro*, including:

1. Inhibiting the HCV RdRp
2. Depleting guanosine triphosphate (and thus nucleic acid synthesis in general) through inhibition of inosine 5′-monophosphate dehydrogenase
3. Enhancing viral mutagenesis
4. Converting the T-helper cell phenotype from 2 to 1
5. Inducing IFN-stimulated genes
6. Modulating natural killer cell response

Ribavirin may select for HCV resistance mutations *in vitro*, but this has not been observed *in vivo*.

ADME. *Ribavirin* dosing is typically weight based. Individuals weighing less than 75 kg receive 1000 mg daily in two divided doses. Individuals weighing at least 75 kg receive 1200 mg daily in two divided doses. Dose reductions are necessary for those with renal impairment, and individuals with renal impairment or decompensated cirrhosis may have difficulty tolerating 1000 or 1200 mg of *ribavirin*. Decompensated cirrhotics are often initiated on a lower dose of 600 mg daily and increased as tolerated.

Ribavirin oral bioavailability averages 50%. Food increases plasma levels substantially. *Ribavirin* is a substrate for equilibrative and concentrative nucleoside uptake transporters (ENT1 [SLC29A1], CNT2 [SLC28A2], and CNT3 [SLC28A3]) and thus is widely distributed throughout the body. The half-lives of the mono-, di-, and triphosphate derivatives in red blood cells and peripheral blood mononuclear cells mirror the plasma $t_{1/2}$ of parent *ribavirin* (7–10 days) (Wu et al., 2015). Hepatic metabolism (deribosylation and hydrolysis to a triazole carboxamide) and renal excretion of *ribavirin* and its metabolites are the principal routes of elimination. The dose of *ribavirin* should be reduced in patients with renal impairment. Even with a reduced dose, patients with renal impairment have difficulty tolerating *ribavirin*.

Therapeutic Uses. *Ribavirin* can be administered orally, intravenously, and by inhalation. The aerosolized form of *ribavirin* is used in the treatment of a variety of respiratory viruses, including respiratory syncytial virus. Intravenous *ribavirin* has been used to treat hemorrhagic fever and severe influenza. Oral *ribavirin* is used in combination with DAAs for the treatment of chronic HCV infection in certain scenarios (e.g., with *sofosbuvir* and *ledipasvir* or *velpatasvir* in the setting of decompensated cirrhosis, with *grazoprevir/elbasvir* in individuals with genotype 1a HCV and preexisting NS5A RASs). The efficacy of *ribavirin* in combinations with these DAAs is described in subsequent sections.

Untoward Effects and Drug Interactions. Aerosolized *ribavirin* may cause conjunctival irritation, rash, transient wheezing, and occasional reversible deterioration in pulmonary function. When used in conjunction with mechanical ventilation, equipment modifications and frequent monitoring are required to prevent plugging of valves and tubing with *ribavirin*. Healthcare workers should use techniques to reduce environmental exposure; pregnant women should not directly care for patients receiving *ribavirin* aerosol due to the possibility of fetal harm from exposure to the aerosol. Bolus intravenous infusion may cause rigors. Systemic *ribavirin* causes dose-related reversible hemolytic anemia, with associated increases in reticulocyte counts and in serum bilirubin, iron, and uric acid concentrations. The *ribavirin* dose is often reduced when hemoglobin decreases to less than 10 g/dL or declines by more than 3 g/dL and discontinued if hemoglobin becomes less than 8.5 g/dL.

Anemia occurs in 5% to 11% of patients receiving *ribavirin* in combination with DAAs (clinical trials of mostly noncirrhotic subjects); 18% to 40% of patients post–liver transplant or with decompensated cirrhosis receiving *ribavirin* with DAAs experienced anemia, with many receiving *ribavirin* doses lower than the typical 1000 to 1200 mg daily. Two polymorphisms in the gene encoding inosine triphosphatase protect against *ribavirin*-induced hemolytic anemia; the frequency of the protective alleles in the general population is about 10%.

Other *ribavirin* toxicities include fatigue, cough, rash, and pruritus. Preclinical studies indicated that *ribavirin* is teratogenic, embryotoxic, oncogenic, and possibly gonadotoxic. *Ribavirin* is present in higher amounts in sperm than plasma. Thus, *ribavirin* is contraindicated in men and women who are attempting conception and for up to 6 months following cessation of treatment. *Ribavirin* has minimal drug interactions but should not be used with the HIV nucleoside analogue *didanosine*.

Pediatric and Geriatric Uses. *Ribavirin* is indicated for the treatment of HCV in children 5 years and older. Doses range from 400 to 1200 mg daily depending on body weight, administered in two divided doses.

Specific pharmacokinetic evaluations for *ribavirin* in the elderly have not been performed. The primary consideration in treating older individuals with *ribavirin* is renal function. The dose of *ribavirin* should be reduced in those with an eGFR less than or equal to 50 mL/min/1.73 m^2.

Ledipasvir

Ledipasvir, an NS5A inhibitor, is available only as part of a fixed-dose combination tablet with the NS5B nucleotide polymerase inhibitor, *sofosbuvir* (LDV/SOF).

Mechanisms of Action and Resistance. *Ledipasvir* is an inhibitor of NS5A. Baseline NS5A RASs were detected in 16% of the 2144 participants in the phase II and III studies of LDV/SOF (Sarrazin et al., 2016). For treatment-naïve patients, slightly lower rates of SVR are observed among persons with HCV genotype 1a *with* versus *without* baseline NS5A RASs (92% vs. 98%, respectively). Treatment-experienced patients with baseline NS5As RASs achieve lower SVR rates compared with treatment-experienced patients with no baseline RASs. NS5A RASs are detected in the majority of patients who fail to respond to LDV/SOF treatment. These NS5A RASs persist for at least 2 years and may affect the success of future HCV treatments.

ADME. *Ledipasvir*, 90 mg, is available as part of a fixed-dose combination tablet with *sofosbuvir* (German et al., 2016). This tablet is administered once daily without regard to meals. LDV/SOF should not be used in individuals with an eGFR less than 30 mL/min/1.73 m^2.

Ledipasvir absorption is pH dependent. Concomitant use of antacids, H_2 receptor antagonists, and proton pump inhibitors (PPIs) is problematic. The bioavailability of *ledipasvir* in humans is unknown (30%–50% in rats, monkeys, and dogs). *Ledipasvir* concentrations are similar when given fasted versus a high-fat (1000 kcal) meal. *Ledipasvir* is greater than 99.8% bound to human plasma proteins. *Ledipasvir* is primarily eliminated unchanged in the feces. Approximately 30% is metabolized via uncertain pathways. *Ledipasvir* pharmacokinetics are not significantly altered by hepatic or renal impairment. The $t_{1/2}$ of *ledipasvir* is 47 h. *Ledipasvir* AUC and C_{Pmax} are 24% and 32% lower in HCV-infected subjects, respectively, compared with HCV-seronegative volunteers.

Therapeutic Uses. LDV/SOF is FDA-approved for individuals with HCV (genotype 1, 4, 5, and 6 disease) and those with decompensated cirrhosis. *In vitro*, *ledipasvir* has limited activity against genotype 3; its activity against genotype 2 is reduced by the highly present L31M RAV; thus, LDV/SOF is not recommended for this genotype.

The SVR rates with 12 weeks of LDV/SOF therapy were 96% and 99% in clinical trials of HCV treatment-naïve patients and 95% in treatment-experienced patients without cirrhosis. Treatment-experienced individuals with cirrhosis should be treated for either 24 weeks with LDV/SOF or 12 weeks with LDV/SOF plus *ribavirin* to improve the likelihood of achieving a cure. LDV/SOF for 12 weeks achieved an SVR rate of 95% in two studies with a small number of individuals with genotype 4 disease. SVR was 93% in 44 patients with genotype 5 and 96% in 25 patients with genotype 6 receiving 12 weeks of LDV/SOF.

Treatment of individuals with HIV/HCV coinfection for 12 weeks yielded similarly high SVRs. However, SVR rates with 8 weeks of LDV/SOF in persons with HIV were slightly lower; thus, this group should be treated for 12 weeks. In treating patients with decompensated cirrhosis, *ribavirin* is added to LDV/SOF treatment to increase SVR rates, usually at a *ribavirin* dose of 600 mg daily due to poor tolerability of *ribavirin* in this patient population.

Untoward Effects and Drug Interactions. Among patients receiving 12 weeks of LDV/SOF in phase III clinical trials, 13% to 14% reported fatigue and headache. The addition of *ribavirin* to LDV/SOF in cirrhotic patients increased the number and frequency of adverse effects.

Ledipasvir relies on an acidic environment for optimal absorption; thus, gastric acid modifiers should be used with caution. In one large cohort, use of PPIs was found to be an independent predictor of relapse to LDV/SOF treatment (Terrault et al., 2016). If gastric acid modifiers must be used, temporal separation is necessary with antacids (by 4 h); H_2 blocker and PPI doses should not exceed the equivalent of 40 mg *famotidine* twice daily and 20 mg *omeprazole* once daily. The *omeprazole* must be administered simultaneously with LDV/SOF in the fasted state. Like *sofosbuvir*, *ledipasvir* is a substrate for P-gp and BCRP and thus cannot be used with potent inducers of these transporters. The CYP3A inducer *efavirenz* reduces ledipasvir concentrations by 30%, and the pharmacokinetic enhancer *cobicistat* increases *ledipasvir* concentrations by 2-fold; given the rather high therapeutic index of *ledipasvir*, these changes are not expected to have clinical relevance. *Ledipasvir* inhibits P-gp and BCRP and may increase the concentrations of *rosuvastatin* via inhibition of BCRP; thus, this combination is not recommended. LDV/SOF increases the exposure to *tenofovir*, which may increase the risk of renal toxicity in HIV-infected individuals taking TDF with a boosting agent such as *ritonavir* or *cobicistat*. Use of TAF instead of TDF is an option for patients taking an antiretroviral regimen, which includes *ritonavir* and *cobicistat*.

Pediatric and Geriatric Uses. LDV/SOF is approved in children 3 years and older with HCV genotypes 1, 4, 5, or 6. LDV/SOF dosing in children is weight based and administered as tablets or oral pellets. The SVR rate was 98% in 100 adolescents 12 to 17 years receiving the FDA-approved adult dose of LDV/SOF with good tolerability. Two trials of children 3 to 11 years showed similar SVR rates to adults. Age is not associated with *ledipasvir* exposures in population pharmacokinetic analyses over the range of 18 to 80 years. No differences in safety or efficacy have been observed between individuals 65 years and older and younger patients.

Daclatasvir

Daclatasvir is an NS5A inhibitor.

Mechanism of Action and Resistance. *Daclatasvir* binds to the N-terminus of NS5A and inhibits both viral RNA replication and virion assembly. The Y93H RAV is detected in most patients who fail *daclatasvir/sofosbuvir* (DCV/SOF) treatment. This variant has been shown to persist for several years after treatment cessation.

ADME. *Daclatasvir* is available as 30-mg and 60-mg tablets. The standard dose of *daclatasvir* is 60 mg, but the dose should be reduced to 30 mg with strong CYP3A inhibitors and increased to 90 mg with moderate CYP3A inducers (Table 63–1). The absolute bioavailability of *daclatasvir* is 67%. A high-fat, high-calorie meal reduces *daclatasvir* exposure by 23%, but a low-fat meal has no effect. The drug is approved for administration without regard to meals. *Daclatasvir* is highly protein bound (99%).

Daclatasvir is metabolized by CYP3A and is therefore susceptible to the effects of potent inhibitors and inducers of this enzyme, but the drug itself does not appear to inhibit or induce any CYPs. *Daclatasvir* is a substrate for P-gp. *Daclatasvir* inhibits P-gp, BCRP, and OATP1B1/3 and thus may increase exposures of drugs that are substrates for these transporters. Total exposure to *daclatasvir* is about 37% lower in patients with Child-Pugh B and C decompensated cirrhosis, but unbound concentrations are unchanged, and no dose adjustment is needed in such patients. *Daclatasvir* AUC increases in those with ESRD; with an eGFR of 15 to 29 mL/min/1.73 m^2, AUC is roughly doubled. Given the high therapeutic index of *daclatasvir*, this change is unlikely to have clinical relevance. The half-life of *daclatasvir* is 12 to 15 h. *Daclatasvir* pharmacokinetics are similar in HCV-seropositive and -seronegative individuals.

Therapeutic Uses. *Daclatasvir* is FDA-approved for use in combination with *sofosbuvir* in those with genotype 1 and 3 disease, in individuals with HIV coinfection regardless of HCV genotype, and in persons with advanced liver disease (Keating, 2016). It is rarely used in the U.S., however, because there are coformulated and cheaper alternatives. In Japan, *daclatasvir* is also approved in combination with the HCV NS3/4A protease inhibitor asunaprevir; this combination is not available in the U.S.

The SVR rates are 86% to 90% in treatment-naïve and treatment-experienced individuals receiving 12 weeks of DCV/SOF. Lower SVR rates occur in those with cirrhosis (63%). Thus, cirrhotic patients with genotype 3 disease may benefit from the addition of *ribavirin*. In HIV-coinfected individuals with HCV genotypes 1 to 4, 96% achieved SVR with 12 weeks of *daclatasvir* and *sofosbuvir* administration. In a group

TABLE 63–1 ■ DACLATASVIR (DCV) DOSING WITH INDUCERS AND INHIBITORS OF CYP3A4

STRONG CYP3A INHIBITORS	MODERATE CYP3A INHIBITORS	STRONG CYP3A INDUCERS	MODERATE CYP3A INDUCERS
Decrease DCV Dose to 30 mg	**Standard DCV Dose, 60 mg**	**DCV Contraindicated**	**Increase DCV Dose to 90 mg**
Ritonavir-boosted atazanavir	Ritonavir-boosted darunavir	Rifamycins	Bosentan
Clarithromycin	Ritonavir-boosted lopinavir	St. John's wort	Dexamethasone
Itraconazole	Ciprofloxacin	Antiepileptics	Efavirenz
Ketoconazole	Diltiazem		Etravirine
Nefazodone	Erythromycin		Modafinil
Nelfinavir	Fluconazole		Nafcillin
Posaconazole	Fosamprenavir		Rifapentine
Telithromycin	Verapamil		
Voriconazole			

of individuals primarily (75%) infected with HCV genotype 1 with advanced liver disease and with Child-Pugh A (n = 12), B (n = 32), and C (n = 16) cirrhosis, 92%, 94%, and 56%, respectively, achieved SVR with 12 weeks of DCV/SOF plus *ribavirin* treatment.

Untoward Effects and Drug Interactions. *Daclatasvir* is well tolerated. The most commonly reported adverse events in genotype 3 patients receiving DCV/SOF were headache and fatigue (14% each). HIV-coinfected patients reported fatigue (17%), nausea (13%), and headache (11%). When combined with *ribavirin* in patients with advanced cirrhosis, the most common adverse effects were anemia (20%), fatigue (18%), nausea (17%), and headache (15%).

Daclatasvir is primarily a victim rather than a perpetrator in drug-drug interactions (Garimella et al., 2016). *Daclatasvir* cannot be used with potent CYP3A inducers but may be used with moderate inducers, if the *daclatasvir* dose is increased from 60 to 90 mg. The *daclatasvir* dose must be reduced from 60 to 30 mg with potent CYP3A inhibitors. Table 63–1 lists some comedications necessitating a *daclatasvir* dose modification.

Pediatric and Geriatric Uses. *Daclatasvir* exposures in adolescents receiving the adult dose (60 mg daily) are similar to historical data in adults. *Daclatasvir* has not been studied in children younger than 12 years and has not been evaluated in the elderly. Age was not significantly associated with *daclatasvir* pharmacokinetics in population modeling over the range of 18 to 79 years; no unique safety concerns were evident in those over 65 years of age, and SVR rates were comparable in older and younger subjects in trials.

Velpatasvir

Velpatasvir (VEL) is an NS5A inhibitor available as part of a fixed-dose combination product with *sofosbuvir* (SOF/VEL).

Mechanism of Action and Resistance. Baseline RASs do not appear to influence the likelihood of achieving SVR with SOF/VEL except in cirrhotic patients with genotype 3 disease where the SVR rate was 73% compared with 93% in those without baseline RASs (Foster et al., 2015).

ADME. SOF/VEL is a fixed-dose combination tablet containing 400 mg of *sofosbuvir* and 100 mg of VEL taken once daily. As with *ledipasvir*, VEL absorption is pH dependent, and gastric acid modifiers require special dosing considerations with this agent; food has little effect on VEL's absorption. VEL is greater than 99.5% protein bound. VEL is predominantly excreted in feces as parent and metabolite; less than 1% of a dose appears in urine. VEL AUC changes only modestly (−17% to +14%) with moderate and severe hepatic impairment. In patients with severe renal impairment (eGFR <30 mL/min/1.73 m^2), the AUC of VEL increases by 50%. The VEL $t_{1/2}$ is 15 h. VEL AUC and C_{max} are reduced by approximately 40% in HCV-infected individuals compared with healthy volunteers.

Therapeutic Uses. SOF/VEL is active against all HCV genotypes (1–6). SVR rates were 99% in patients with genotypes 1, 2, 4, 5, and 6 and 95% in genotype 3 patients with 12 weeks of SOF/VEL in trials. Patients with genotype 3 and cirrhosis should undergo resistance testing prior to initiating SOF/VEL. Cirrhotic genotype 3 patients with the Y93H RAS have diminished SVR with 12 weeks of SOF/VEL and should receive an alternative treatment. In decompensated patients, 83% achieved SVR with SOF/VEL for 12 weeks, 94% achieved SVR with SOF/VEL plus *ribavirin*, and 86% achieved SVR with 24 weeks of SOF/VEL.

Untoward Effects and Drug Interactions. The most common adverse reactions with SOF/VEL are headache (22%), fatigue (15%), nausea (9%), asthenia (5%), and insomnia (5%). When *ribavirin* is given with SOF/VEL to patients with decompensated cirrhosis, adverse events are more frequent: fatigue (32%), anemia (26%), nausea (15%), headache (11%), insomnia (11%), and diarrhea (10%).

Velpatasvir has a pharmacological profile similar to that of *ledipasvir* but is subject to more CYP3A-mediated interactions. As with *ledipasvir*, antacids should be separated by 4 h, and H_2 blocker doses should not exceed the equivalent of 40 mg of *famotidine* twice daily, and PPI doses should not exceed the equivalent of 20 mg of *omeprazole* daily. The timing of the PPI in relation to SOF/VEL differs from LDV/SOF and is important to adhere to. SOF/VEL should be taken with food 4 h prior to a PPI dose. VEL is a substrate for CYPs 3A4, 2C8, and 2B6 but has not been shown to inhibit any CYPs. VEL is a substrate for and weak inhibitor of P-gp and a weak inhibitor of BCRP and OATP1B1/1B3. In terms of its potential to act as a perpetrator of drug interactions, VEL causes the following changes to transporter probe substrates (Mogalian et al., 2016): The AUC of *pravastatin* (an OATP1B1 substrate) increases by 35% and the AUC of *rosuvastatin* (an OATP1B1 and BCRP substrate) increases by approximately 170% when coadministered with VEL in healthy volunteers. Similarly, the AUC of *digoxin* (a P-gp substrate) increases by 34%. Single-dose *rifampin*, an OATP1B1 inhibitor, increases the AUC of VEL by 47%; however, multiple doses of rifampin, which induces CYP metabolism and transporter expression, reduces the AUC of VEL by about 82%. Single-dose *cyclosporine* (a mixed OATP/P-gp/MRP2 inhibitor) doubles the VEL AUC. *Ketoconazole* (CYP3A inhibitor) increases the VEL AUC by 70%. *Efavirenz* reduces the VEL AUC by 50%. *Efavirenz*, *rifampin*, and other potent inducers should be avoided.

Pediatric and Geriatric Uses. SOF/VEL is approved in children with any HCV genotype aged 6 years or older and weighing 17 kg or more. Children weighing 30 kg or more receive the adult dose, and those weighing 17 kg to less than 30 kg receive SOF/VEL 200 mg/50 mg once daily. Twelve percent of participants in phase III trials (156 subjects) were age 65 or older; no differences in efficacy or safety were observed in this group compared with those younger than 65 years.

Voxilaprevir

Voxilaprevir (VOX) is an NS3 protease inhibitor available as part of a fixed dose combination product with SOF/VEL (SOF/VEL/VOX).

Mechanism of Action and Resistance. SOF/VEL/VOX is used for second-line treatment of patients who have previously failed a DAA regimen. The presence of NS3, NS5A, or NS5B RASs prior to treatment does not influence the likelihood of achieving SVR.

ADME. The fixed-dose tablet contains 400 mg of *sofosbuvir*, 100 mg of VEL, and 100 mg of VOX. The absolute bioavailability of VOX in humans is unknown, but it is 83% in rats and 27% in dogs. VOX exposures are increased 112% with a light-fat meal, 185% with a moderate-fat meal, and 435% with a high-fat meal relative to fasting. The $t_{1/2}$ of VOX is 33 h. VOX is primarily eliminated through biliary excretion; 94% is excreted in the feces (40% as parent drug). VOX is more than 99% protein bound. VOX exposures are 71% higher in persons with eGFR less than 30 mL/min/1.73 m^2, but this is unlikely to have clinical relevance unless combined with other risk factors for increased VOX exposures. However, VOX exposures are increased 299% with moderate and 500% with severe hepatic impairment. Higher VOX exposures may increase the potential for hepatotoxicity or liver failure, and this treatment should therefore not be used in patients with decompensated cirrhosis. This is a class effect for HCV NS3 protease inhibitors. VOX exposures are 260% higher in persons with HCV versus those without HCV.

Therapeutic Uses. SOF/VEL/VOX for 12 weeks is used in patients with genotypes 1 to 6 who have previously failed DAA treatment. Among those who previously failed an NS5A-containing regimen, SVR was 96% after 12 weeks of SOF/VEL/VOX (Bourliere et al., 2017). Patients with genotype 3 and cirrhosis have lower SVR rates and require the addition of *ribavirin* to SOF/VEL/VOX to maximize SVR.

Untoward Effects and Drug Interactions. The most common adverse reactions with SOF/VEL/VOX are headache (21%), fatigue (17%), diarrhea (13%), and nausea (13%).

The same drug interactions with *sofosbuvir* and VEL apply to SOF/VEL/VOX. Since it is administered with VEL, careful adherence to dosing limitations for the gastric acid modifiers is necessary, but there are no temporal separation requirements with PPIs as there are with LDV/SOF or SOF/VEL. VOX is a substrate for P-gp, BCRP, OATP1B1/3, CYP1A2, CYP2C8, and CYP3A4. Potent OATP1B1 inhibitors (e.g., *cyclosporine*) should not be used with VOX. VOX is an inhibitor of P-gp, BCRP, and OATP1B1/3. VOX increases exposures of BCRP substrates and should be avoided with *methotrexate*, *mitoxantrone*, *imatinib*, *irinotecan*, *lapatinib*, *rosuvastatin*, *sulfasalazine*, and *topotecan*. VOX does not cause significant increases in ethinyl estradiol exposures, but a few cases of liver enzyme elevations have been noted during drug interaction trials with ethinyl estradiol–containing hormonal contraception and SOF/VEL/VOX. This effect has also been observed with other HCV protease inhibitors, necessitating caution with the use of this combination.

Pediatric and Geriatric Uses. SOF/VEL/VOX has not been evaluated in children. In SOF/VEL/VOX registrational trials, there was no signal for differences in efficacy or safety in those 65 and older versus younger subjects.

Glecaprevir/Pibrentasvir

Glecaprevir (GLE) is an NS3 protease inhibitor; *pibrentasvir* (PIB) is an NS5A inhibitor.

Mechanism of Action and Resistance. Baseline RASs do not appear to influence the likelihood of achieving SVR with GLE/PIB in patients with HCV genotypes 1, 2, 4, 5, and 6. Among noncirrhotic treatment-naïve genotype 3 patients, 78% of those with the A30K RAS achieved SVR12 with 8 weeks of GLE/PIB. There are insufficient data to characterize the impact of A30K in genotype 3 patients with cirrhosis or prior treatment experience.

ADME. Each fixed-dose combination tablet contains 100 mg of GLE and 40 mg of PIB; patients take three tablets each day. Moderate- and high-fat meals increase GLE 83% to 163% and PIB 40% to 53%. Absolute bioavailabilities of GLE/PIB in humans are unknown. The half-lives for GLE and PIB are 6 and 13 h, respectively. GLE/PIB is eliminated largely via the biliary-fecal route (>92%); renal excretion accounts for less than 1%. Both GLE and PIB are highly protein-bound. In persons with HCV, GLE and PIB exposures are elevated (86% and 54%, respectively) in ESRD, with or without dialysis, compared to subjects with normal renal function. GLE exposures are doubled in Child-Pugh B subjects and 11-fold higher in Child-Pugh C subjects relative to persons with no hepatic impairment. PIB exposures are 26% higher in Child-Pugh B subjects and 114% higher in Child-Pugh C subjects. NS3 protease–containing DAA therapies, such as GLE/PIB, are contraindicated in decompensated cirrhosis. Based on a population pharmacokinetic analysis, PIB exposures are 51% higher in persons without HCV relative to those with HCV, and GLE exposures do not differ in HCV-seropositive versus -seronegative individuals.

Therapeutic Uses. GLE/PIB is pangenotypic. For treatment-naïve individuals, SVR rates of 95% or greater were observed in multiple trials with 8 weeks of dosing, including in compensated cirrhotics. In treatment-experienced patients, SVR rates are 92% and 94% in patients with prior NS3 protease and NS5A failures with 12 and 16 weeks of dosing, respectively. GLE/PIB is a treatment of choice in persons with renal impairment. SVR rates of 97% or greater have been observed in trials of GLE/PIB in patients with chronic kidney disease.

Untoward Effects and Drug Interactions. The most common adverse reactions with GLE/PIB are headache (13%), fatigue (11%), and nausea (8%).

The GLE/PIB combination can act as a perpetrator in drug interactions. GLE/PIB inhibits P-gp, BCRP, and OATP1B1/3, and weakly inhibits CYP3A, CYP1A2, and UGT1A1. As victims of interactions, GLE/PIB are substrates of P-gp and/or BCRP. GLE is a substrate of CYP3A and OATP1B1/3. Thus, drugs that inhibit P-gp, BCRP, or OATP1B1/3 may increase the plasma concentrations of GLE and/or PIB. Potent inducers, such as antiepileptics, St. John's wort (hypericum), *rifampin*, and *efavirenz*, are not recommended with GLE/PIB. Although GLE has pH-dependent absorption and exposures are reduced by 29% to 51% with *omeprazole* 20 to 40 mg daily, clinical data support efficacy even in presence of gastric acid modifiers as long as the *omeprazole* dose equivalent does not exceed 40 mg daily (Flamm et al., 2019). Ethinyl estradiol–containing contraception should not be used during and for 2 weeks after GLE/PIB. Alanine aminotransferase was increased in 5 of 26 women in drug interaction studies of ethinyl estradiol and GLE/PIB. HIV protease inhibitors and *cyclosporine* should not be used with GLE/PIB as the increase in GLE exposures may predispose to hepatotoxicity.

Pediatric and Geriatric Uses. GLE/PIB, at the adult dose, is approved for use in children weighing at least 45 kg. A pediatric formulation of GLE/PIB has proven efficacious and well tolerated in chronic HCV-infected children 3 to < 12 years in a phase 2/3 study (Jonas et al., 2021). Among children ages 3 to 11 years, weight-based doses of small film-coated pellets of GLE/PIB are as follows: 250 mg GLE plus 100 mg PIB (in children weighing ≥30 kg to <45 kg), 200 mg GLE plus 80 mg PIB (≥20 kg to <30 kg), and 150 mg GLE plus 60 mg PIB (12 kg to <20 kg). Pellets are mixed with 1 to 2 teaspoons of a soft food vehicle such as hazelnut spread, yogurt, or peanut butter. Ninety six percent of children ages 3 to 11 years achieved SVR with these weight-based doses (8 weeks of GLE/PIB).

In GLE/PIB licensing trials, no overall differences in safety or effectiveness were observed between subjects over 65 years of age and younger subjects.

Grazoprevir/Elbasvir

Grazoprevir (GZR) is an NS3 protease inhibitor; *elbasvir* (EBR) is an NS5A inhibitor. These drugs are available in a single fixed-dose combination tablet.

Mechanism of Action and Resistance. *Grazoprevir* and EBR are inhibitors of the NS3 and NS5A viral enzymes, respectively. In trials, preexisting NS3 RASs were detected in 57% and 19% of individuals with genotype 1a and 1b, respectively, but the presence of preexisting NS3 RASs does not reduce the likelihood of high SVR rates. The presence of preexisting NS5A RASs did reduce SVR rates in genotype 1a patients. SVR rates were 58% and 68% in genotype 1a treatment-naïve and treatment-experienced patients, respectively, with preexisting NS5A RASs. Adding *ribavirin* and extending the treatment duration greatly increase SVR rates in patients with preexisting NS5A RASs.

ADME. GZR/EBR is dosed as a single 100-mg/50-mg fixed-dose combination tablet taken once daily without regard to meals, although a high-fat meal will increase the AUC and C_{max} of GZR by 1.5- and 2.8-fold. The bioavailability of EBR is 30%; that of GZR ranges from 10% to 40%. The $t_{1/2}$ of GZR is about 30 h. EBR has a $t_{1/2}$ of 23 h. Less than 1% of GZR and EBR are renally eliminated. Both drugs are extensively (~99%) bound. GZR exposures are increased 62% in those with mild (Child-Pugh A) and 388% in those with moderate (Child-Pugh B) hepatic impairment relative to those with no hepatic impairment. Total concentrations of EBR are 24% and 14% lower in patients with mild and moderate hepatic insufficiency, respectively, likely a reflection of reduced serum protein levels. GZR and EBR pharmacokinetics in individuals with ESRD on hemodialysis are comparable to individuals without hepatic impairment. The AUCs of GZR and EBR are increased, however, by 65% and 86%, respectively, in those with eGFR less than 30 mL/min/1.73 m^2 not receiving dialysis. As with other hepatically metabolized DAAs, this increase may be due to accumulation of uremic toxins, parathyroid hormone, or cytokines that can impair hepatic metabolism. The GZR AUCs are 1.2- to 2.1-fold higher in HCV-infected individuals compared with HCV-uninfected individuals. There are no differences in EBR pharmacokinetics in HCV-seropositive versus -seronegative individuals.

Therapeutic Uses. GZR/EBR is FDA-approved for HCV genotypes 1 and 4 and in those with renal impairment.

Based on clinical trials, 95% of treatment-naïve individuals with primarily genotype 1 disease will achieve SVR after 12 weeks of GZR/EBR (SVR rates: 92% with genotype 1a, 99% with genotype 1b, 100% with genotype 4, and 80% with genotype 6). There are no differences in response between cirrhotics and noncirrhotics, but lower SVR rates (58% vs. 99%) are observed in patients with preexisting NS5A RASs. Thus, NS5A resistance testing needs to be performed for individuals with genotype 1a before starting GZR/EBR. In genotype 1a individuals with preexisting NS5A RASs, adding *ribavirin* and treating for an additional 4 weeks for a total of 16 weeks improves SVR rates. SVR rates are similar in patients with HIV coinfection, but drug interactions with antiretroviral therapy are an important consideration in this population.

Grazoprevir/elbasvir has demonstrated safety and efficacy in the treatment of HCV-infected patients with impaired renal function. In a trial of individuals (14% cirrhotic) with eGFR less than 30 mL/min/1.73 m^2 (76% of whom were hemodialysis dependent), the SVR after 12 weeks of treatment was 94%. The percentage of patients reporting any adverse event (~75%) was similar to studies of GZR/EBR in patients without renal impairment. More serious events occurred in these patients (15% vs. 3%), but many were not considered related to GZR/EBR treatment (Roth et al., 2015).

Untoward Effects and Drug Interactions. The most common side effects with GZR/EBR are headache (17%), fatigue (16%), and nausea (9%–15%). Higher exposures of GZR are associated with liver function test elevations. GZR may increase bilirubin concentrations through inhibition of OATP1B1.

Grazoprevir is a substrate for CYP3A4, P-gp, and OATP1B1. OATP1B1 inhibitors (e.g., HIV protease inhibitors) and moderate/strong CYP3A and P-gp inducers (including *efavirenz*) are not recommended for coadministration with GZR/EBR. EBR is a substrate for CYP3A4 and P-gp and an inhibitor of BCRP and P-gp.

Pediatric and Geriatric Uses. GZR/EBR has not been studied in children, and no dedicated studies have been performed in older patients. No age effect was observed in EBR pharmacokinetics in young (22–45 years) versus elderly (65–78 years) males; elderly females had a 33% higher EBR AUC compared with elderly men even after adjustment for body weight.

Investigational Agents and the Future for Treatment of HCV

Available agents achieve cure in the vast majority of patients; thus, HCV drug development has slowed considerably. The remaining challenges for this disease are testing and diagnosis, linkage to care, increasing access to therapy, and management of special patient populations, including patients with acute HCV, pregnant women, and after transplant for persons who received HCV-positive organs.

Drug Facts for Your Personal Formulary: *Viral Hepatitis (HBV/HCV)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Hepatitis B Therapy		
Pegylated interferon alfa	• Preferred agent • Approved for adult patients with compensated liver disease and evidence of viral replication and liver inflammation • Administered SC weekly for 48–52 weeks	• Adverse reactions (>40%): fatigue/asthenia, pyrexia, myalgia, and headache • May cause fatal neuropsychiatric, autoimmune, ischemic, and infectious disorders • Frequent hematological monitoring required • Contraindicated in advanced liver disease and in pregnancy
Entecavir	• Preferred agent • Approved for individuals ≥2 years old • Indefinite treatment for patients with cirrhosis	• Use higher dose for decompensated cirrhosis and patients with lamivudine or telbivudine resistance • Take on an empty stomach • Monitor for lactic acidosis in decompensated cirrhosis • Adverse reactions (≥3%): headache, fatigue, dizziness, nausea
Tenofovir disoproxil fumarate	• Preferred agent • Approved for individuals ≥2 years old • Indefinite treatment for patients with cirrhosis	• Dose reduction in renal impairment • Monitor renal function • May decrease bone mineral density • Adverse reactions (≥10%) in decompensated cirrhosis: abdominal pain, nausea, insomnia, pruritus, vomiting, dizziness, and pyrexia
Tenofovir alafenamide fumarate	• Preferred agent • Approved for ages 18 and older • Indefinite treatment for patients with cirrhosis	• Avoid drugs that strongly affect P-gp and BCRP • Most common adverse reaction (≥10%) is headache • Monitor renal function • Not recommended for eGFR <15 mL/min/1.73 m^2 • Take with food

Drug Facts for Your Personal Formulary: *Viral Hepatitis (HBV/HCV)* (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Adefovir Lamivudine Telbivudine	• Alternative agents due to high incidence of HBV resistance with monotherapy • Indefinite treatment for patients with cirrhosis	• Dose adjust for renal impairment • Abrupt discontinuation causes hepatitis flares • Common adverse reactions: • *Adefovir*: asthenia and impaired renal function • *Lamivudine*: ear, nose, and throat infections; sore throat; and diarrhea • *Telbivudine*: increased creatinine kinase, nausea, diarrhea, fatigue, myalgia, and myopathy
Hepatitis C Therapy		
Sofosbuvir/ledipasvir	• HCV genotypes 1, 4, 5, and 6 • Administered as fixed-dose combination tablet for 8 or 12 weeks • Use with ribavirin for 12 weeks in treatment-experienced patients with cirrhosis • Weight-based dosing approved in children aged ≥3 years with genotype 1, 4, 5, or 6	• Ledipasvir should not be used with potent P-gp inducers • Ledipasvir absorption requires acid gastric pH • Coadministration of sofosbuvir and amiodarone may cause severe bradycardia and fatal cardiac arrest • Adverse reactions (≥10%): fatigue, headache
Sofosbuvir/ daclatasvir	• Activity against all genotypes; seldom used in the U.S. due to availability of cheaper fixed-dose combinations • 12-week treatment in patients without cirrhosis • Coadministered with ribavirin in patients with cirrhosis for 12 weeks	• Daclatasvir should not be used with potent CYP3A inducers • Daclatasvir dose reduction needed with strong CYP3A inhibitors • Coadministration of sofosbuvir and amiodarone may cause severe bradycardia and fatal cardiac arrest • Adverse reactions (≥10%): fatigue, headache
Sofosbuvir/ velpatasvir	• Approved for use in all HCV genotypes • Administered as fixed-dose combination tablet for 12 weeks • Cirrhotic patients with genotype 3 and Y93H RAS have ↓ SVR and require alternative therapy • Used with ribavirin for patients with decompensated cirrhosis • Approved for use in children ≥6 years or weighing ≥17 kg	• Do not use with potent P-gp or CYP3A inducers • Velpatasvir requires acidic gastric pH • Coadministration of sofosbuvir and amiodarone may cause severe bradycardia and fatal cardiac arrest • Adverse reactions (≥10%): fatigue and headache
Sofosbuvir/ velpatasvir/ voxilaprevir	• Used for second-line treatment of patients who have previously failed a DAA regimen • All genotypes receive one fixed-dose combination tablet once daily for 12 weeks • Patients with genotype 3 and cirrhosis require the addition of ribavirin	• Do not use with potent P-gp or CYP3A inducers or potent OATP1B1 inhibitors • Do not use with BCRP substrates with narrow therapeutic indices • Velpatasvir requires acidic gastric pH • Coadministration of sofosbuvir and amiodarone may cause severe bradycardia and fatal cardiac arrest • Avoid ethinyl estradiol–containing hormonal contraception during and for 2 weeks after treatment due to potential hepatotoxicity • Adverse reactions (≥10%): headache, fatigue, diarrhea, nausea • Contraindicated in advanced liver disease
Glecaprevir/ pibrentasvir	• Approved for use in all HCV genotypes aged >12 years or ≥45 kg • Administered as three fixed-dose combination tablets once a day for 8 weeks • Preferred treatment in renal impairment • Weight-based dosing in children ≥3 years, FDA-approved, June 2021	• Do not use with potent P-gp or CYP3A inducers or potent OATP1B1 inhibitors • Avoid ethinyl estradiol–containing hormonal contraception during and for 2 weeks after treatment due to potential hepatotoxicity • Adverse reactions (≥10%): headache and fatigue • Contraindicated in advanced liver disease
Grazoprevir/elbasvir	• 12-week therapy for patients without baseline NS5A RASs • 16-week combined therapy with ribavirin for patients with baseline NS5A RASs • Preferred treatment in renal impairment	• Should not be used with moderate and strong CYP3A and P-gp inducers • Should not be used with OATP1B1 inhibitors • Adverse reactions (≥10%): headache, fatigue, nausea • Contraindicated in advanced liver disease
Ribavirin	• Used in combination with other HCV regimens to boost therapeutic efficacy	• May cause hemolytic anemia • Teratogenic • Wide tissue distribution • Long half-life (7–10 days) • Dose adjustment needed for renal impairment

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Chapter 64

Antiretroviral Agents and Treatment of HIV Infection

Charles W. Flexner

PATHOGENESIS OF HIV-RELATED DISEASE
- Virus Structure
- Virus Life Cycle
- How the Virus Causes Disease

PRINCIPLES OF HIV CHEMOTHERAPY

DRUGS USED TO TREAT HIV INFECTION
- Nucleoside and Nucleotide Reverse Transcriptase Inhibitors
- Nonnucleoside Reverse Transcriptase Inhibitors
- HIV Protease Inhibitors
- Entry Inhibitors
- Integrase Inhibitors
- Long-Acting and Extended-Release Antiretroviral Formulations
- Future Treatment Guidelines

Safe and effective pharmacotherapy to treat and prevent human immunodeficiency virus (HIV) is one of the greatest achievements of science and public health in the past century. Infected individuals who maintain a daily oral regimen now have a normal or near-normal life expectancy. More than 30 approved drugs and dozens of formulations have produced thousands of possible drug combinations, but most patients receive one of a handful of well-tolerated and rigorously tested regimens. Unique features of antiretroviral therapy include the need for lifelong treatment to control virus replication and the possibility of rapid emergence of permanent drug resistance if these agents are not used properly. Although three-drug and two-drug combination oral regimens have radically altered the course of this epidemic, future options will include long-acting injectable and implantable formulations.

Pathogenesis of HIV-Related Disease

Human immunodeficiency viruses are lentiviruses, a family of retroviruses evolved to establish chronic persistent infection with gradual onset of clinical symptoms. Replication is constant following infection, and although some infected cells may harbor nonreplicating virus for years, in the absence of treatment, there is generally no true period of latency following infection (Deeks et al., 2015). Humans and nonhuman primates are the only natural hosts for these viruses.

There are two major families of HIV. Most of the epidemic involves HIV-1; HIV-2 is more closely related to simian immunodeficiency virus (SIV) and is concentrated in western Africa. HIV-1 is genetically diverse, with at least five distinct subfamilies or clades. HIV-1 and HIV-2 have similar sensitivity to most antiretroviral drugs, although the nonnucleoside reverse transcriptase inhibitors (NNRTIs) are HIV-1 specific and have no activity against HIV-2.

Virus Structure

HIV is a typical retrovirus with a small RNA genome of 9300 base pairs. Two copies of the genome are contained in a nucleocapsid core surrounded by a lipid bilayer, or envelope, that is derived from the host cell plasma membrane (Figure 64–1). The viral genome encodes three major open reading frames: *gag* encodes a polyprotein that is processed to release the major structural proteins of the virus; *pol* overlaps *gag* and encodes three important enzyme activities (an RNA-dependent DNA polymerase or reverse transcriptase with RNAase activity, protease, and the viral integrase); and *env* encodes the large transmembrane envelope protein responsible for cell binding and entry. Several small genes encode regulatory proteins that enhance virion production or combat host defenses. These include *tat*, *rev*, *nef*, and *vp* .

Virus Life Cycle

Understanding the HIV life cycle (Figure 64–1) is crucial to understanding rational therapy of the infection. HIV tropism is controlled by the envelope protein gp160 (*env*). The major target for *env* binding is the CD4 receptor present on lymphocytes and macrophages, although cell entry also requires binding to a coreceptor, generally the chemokine receptors CCR5 or CXCR4. CCR5 is present on macrophage lineage cells. Most infected individuals harbor predominantly the CCR5-tropic virus; HIV with this tropism is responsible for nearly all naturally acquired infections. A shift from CCR5 to CXCR4 utilization is associated with advancing disease, and the increased affinity of HIV-1 for CXCR4 allows infection of T-lymphocyte lines. A phenotypic switch from CCR5 to CXCR4 heralds accelerated loss of $CD4^+$ helper T cells and increased risk of immunosuppression. Whether coreceptor switch is a cause or a consequence of advancing disease is still controversial, but it is possible to develop clinical AIDS without this switch (Deeks et al., 2015).

The gp41 domain of *env* controls the fusion of the virus lipid bilayer with that of the host cell. Following fusion, full-length viral RNA enters the cytoplasm, where it undergoes replication to a short-lived RNA-DNA duplex; the original RNA is degraded by the RNase H activity of reverse transcriptase to allow creation of a full-length double-stranded DNA copy of the virus. Because the HIV reverse transcriptase is error prone and lacks a proofreading function, mutation is frequent and occurs at about three bases for every full-length (9300-base-pair) replication (Coffin, 1995). Virus-derived DNA is transported into the nucleus, where it is integrated into a host chromosome by the viral integrase in a random or quasi-random location (Greene and Peterlin, 2002).

Following integration, the virus may remain quiescent, not producing RNA or protein but replicating as the cell divides. When a cell that harbors the viral DNA is activated, viral RNA and proteins are produced. Structural proteins assemble around full-length genomic RNA to form a nucleocapsid. The envelope and structural proteins assemble at the cell surface, concentrating in cholesterol-rich lipid rafts. The nucleocapsid cores are directed to these sites and bud through the cell membrane, creating new enveloped HIV particles containing two complete single-stranded RNA genomes. Reverse transcriptase is incorporated into virus particles, so replication can begin immediately after the virus enters a new cell.

How the Virus Causes Disease

Sexual acquisition of HIV infection is likely mediated by one or, at most, a handful of infectious virus particles. Soon after infection, there is a rapid burst of replication peaking at 2 to 4 weeks, with $\geq 10^9$ $CD4^+$ cells

Abbreviations

ABC: abacavir
ADME: absorption, distribution, metabolism, excretion
AIDS: acquired immunodeficiency syndrome
5′-AMP: adenosine 5′-monophosphate
AUC: area under plasma concentration-time curve
CBT: cabotegravir
cDNA: complementary DNA
CL_{cr}: creatinine clearance
CMP: cytidine monophosphate
CNS: central nervous system
CSF: cerebrospinal fluid
CYP: cytochrome P450
dCMP: deoxycytidine monophosphate
ddC: dideoxycytidine
ddI: didanosine
DF: disoproxil fumarate
DRESS: drug reaction with eosinophilia and systemic symptoms
d4T: stavudine
eCL_{cr}: estimated creatinine clearance
***env*:** envelope protein gp160
FDA: Food and Drug Administration
FTC: emtricitabine
GI: gastrointestinal
HBV: hepatitis B virus
HCV: hepatitis C virus
HIV: human immunodeficiency virus
HTLV: human T-cell lymphotrophic virus
IMP: inosine 5′-monophosphate
InSTI: integrase strand transfer inhibitor
IRIS: immune reconstitution inflammatory syndrome
LA: long-acting
LTR: long terminal repeat
NDP: nucleoside diphosphate
NNRTI: nonnucleoside reverse transcriptase inhibitor
NRTI: nucleos(t)ide reverse transcriptase inhibitor
OATP: organic anion-transporting polypeptide
PI: protease inhibitor
PK: pharmacokinetic
PRPP: phosphoribosyl pyrophosphate
QTc: corrected cardiac QT interval
RNase H: ribonuclease H
RPV: rilpivirine
RT: reverse transcriptase
SIV: simian immunodeficiency virus
$t_{1/2}$: half-life of elimination
TAF: tenofovir alafenamide
TAM: thymidine analogue mutation
3TC: lamivudine
TDF: tenofovir disoproxil fumarate
vRNA: viral RNA
ZDV: zidovudine

becoming infected. This peak is associated with a transient dip in the number of peripheral CD4$^+$ (helper) T lymphocytes. With a balance of new host immune responses and target cell depletion, the number of infectious virions as reflected by the plasma HIV RNA concentration (also known as viral load) declines to a quasi-steady state or set point. This set point reflects the interplay between host immunity and the pathogenicity of the infecting virus (Coffin, 1995). In the average infected individual, several billion infectious virus particles are produced every few days.

Eventually, the host CD4$^+$ T-lymphocyte count begins a steady decline, accompanied by a rise in the plasma HIV RNA concentration. Once the peripheral CD4 cell count falls below 200 cells/mm^3, there is an increasing risk of opportunistic diseases and, ultimately, death. Sexual acquisition of CCR5-tropic HIV-1 is associated with a median time to clinical AIDS of 8 to 10 years if the patient is not treated. Some patients, termed long-term nonprogressors, can harbor HIV for more than two decades without significant decline in peripheral CD4 cell count or clinical immunosuppression; this may reflect a combination of favorable host immunogenetics and immune responses.

An important question relevant to treatment is whether AIDS is a consequence of CD4$^+$ lymphocyte depletion alone. Most natural history data suggest that this is true. Regardless, successful therapy is based on inhibition of HIV replication; interventions designed specifically to boost the host immune response without exerting a direct antiviral effect have had no reliable clinical benefit (Deeks et al., 2015).

Principles of HIV Chemotherapy

Current treatment assumes that all aspects of disease derive from the direct toxic effects of HIV on host cells, mainly CD4$^+$ T lymphocytes. *The goal of therapy is to suppress virus replication as much as possible for as long as possible.* The current standard of care is to use three different antiretroviral drugs simultaneously for initial treatment and two to three drugs for the remaining duration of treatment (Flexner, 2019).

Two large randomized clinical trials found substantial clinical benefit from initiating antiretroviral therapy regardless of baseline CD4 count (INSIGHT START Study Group, 2015; TEMPRANO ANRS 12136 Study Group, 2015); thus, the current global standard of care is to offer treatment to all infected individuals whenever possible (World Health Organization, 2021). Substantial evidence confirms the value of antiretroviral therapy in preventing transmission of the virus from person to person (Cohen et al., 2011) and suggests that infected individuals with an undetectable viral load are incapable of transmitting the virus to others.

Drug resistance remains a key problem. There is a high likelihood that all untreated infected individuals harbor viruses with single-amino-acid mutations that confer some degree of resistance to every known antiretroviral drug because of the high mutation rate of HIV and the tremendous number of infectious virions (Coffin, 1995). Thus, a combination of active agents is required to prevent drug resistance, analogous to strategies employed in the treatment of tuberculosis (see Chapter 65). Prolonged drug holidays may allow the virus to replicate anew, increasing the risk of drug resistance and disease progression; such breaks in treatment are associated with increased morbidity and mortality and are not generally recommended (Lawrence et al., 2003).

The expected outcome of initial therapy in a previously untreated patient is an undetectable viral load (plasma HIV RNA <50 copies/mL) within 24 weeks of starting treatment. In treatment-naïve patients, a regimen containing a nonnucleoside plus two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) is as effective as a regimen containing an additional nucleoside (Shafer et al., 2003), indicating the equivalence of these three-drug and four-drug regimens. Four or more drugs may be used simultaneously in patients harboring drug-resistant virus, but the number of agents a patient can tolerate is limited by toxicity and inconvenience. Most patients today can be maintained on a two- or three-drug regimen, even if they are highly treatment experienced.

Failure of an antiretroviral regimen is defined as a persistent increase in plasma HIV RNA of greater than 200 copies/mL in a patient with previously undetectable virus, despite continued treatment with that regimen. This is associated with a high probability of resistance to one or more of the drugs in the regimen and necessitates a change in treatment. The selection of new agents is informed by the patient's treatment history and viral resistance testing. Treatment failure indicates the need for a completely new combination of drugs if possible (Department of Health and Human Services, 2019). Adding a single active agent to a failing regimen is functional monotherapy if the patient is resistant to all other drugs

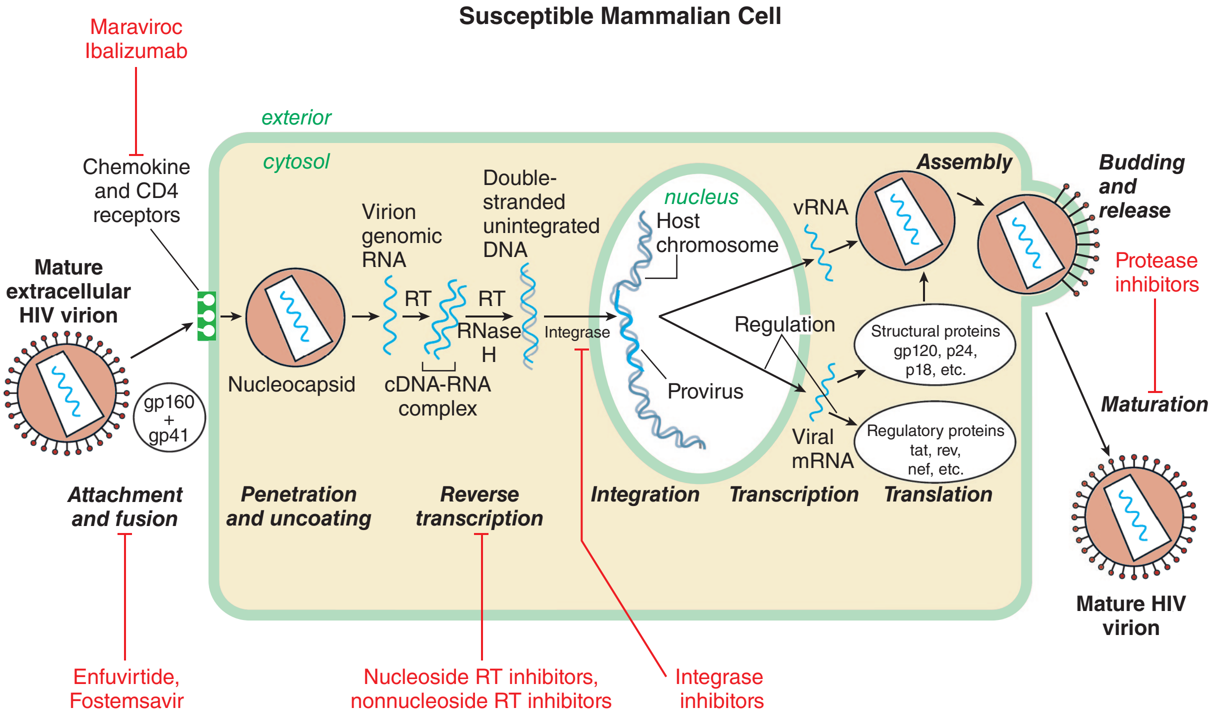

Susceptible Mammalian Cell
Maraviroc
Ibalizumab
exterior
cytosol
Chemokine and CD4 receptors
Mature extracellular HIV virion
gp160 + gp41
Nucleocapsid
Virion genomic RNA
RT
RT
RNase H
cDNA-RNA complex
Double-stranded unintegrated DNA
Integrase
nucleus
Host chromosome
Provirus
Regulation
vRNA
Viral mRNA
Assembly
Structural proteins gp120, p24, p18, etc.
Regulatory proteins tat, rev, nef, etc.
Budding and release
Protease inhibitors
Maturation
Mature HIV virion
Attachment and fusion
Penetration and uncoating
Reverse transcription
Integration
Transcription
Translation
Enfuvirtide, Fostemsavir
Nucleoside RT inhibitors, nonnucleoside RT inhibitors
Integrase inhibitors

Figure 64–1 *Replicative cycle of HIV-1 showing the sites of action of available antiretroviral agents.* Available antiretroviral agents are shown in red. In this figure, gp120 + gp41 indicates extracellular and intracellular domains, respectively, of envelope glycoprotein.

in the regimen. The risk of failing a regimen also depends on the percentage of prescribed doses taken during a given period of treatment.

As antiretroviral therapy becomes more effective and easier to take, long-term toxicity of these drugs is of great importance. Several approved antiretroviral agents (*lamivudine*, *emtricitabine*, and the integrase strand transfer inhibitors [InSTIs]) have few or no clinically significant toxicities in most patients. Protease inhibitors (PIs) and NNRTIs can produce clinically significant pharmacokinetic drug interactions, since all agents in these two drug classes can act as inhibitors and/or inducers of hepatic CYPs, other drug-metabolizing enzymes, and drug transport proteins (University of Liverpool, 2021; see also Chapters 4 and 5). An uncommon complication of initiating antiretroviral therapy is accelerated inflammatory reaction to overt or subclinical opportunistic infections. This immune reconstitution inflammatory syndrome (IRIS) is most commonly seen when initiating therapy in individuals with very low CD4 counts or advanced HIV disease. Infections commonly associated with IRIS include tuberculosis and other mycobacterial diseases, cryptococcosis, hepatitis virus infections, and *Pneumocystis* pneumonia (Manabe et al., 2007).

Drugs Used to Treat HIV Infection

Nucleoside and Nucleotide Reverse Transcriptase Inhibitors

Overview

HIV-encoded, RNA-dependent DNA polymerase, also called reverse transcriptase, converts viral RNA into proviral DNA, which is then incorporated into a host cell chromosome. Available inhibitors of this enzyme are either nucleoside/nucleotide analogues or nonnucleoside inhibitors (Table 64–1). NRTIs prevent infection of susceptible cells but do not eradicate the virus from cells that already harbor integrated proviral DNA. Nearly all patients starting antiretroviral treatment do so with at least one agent from this class. Figure 64–2 shows the mechanism of action of NRTIs, which involves phosphorylation by host cells to the active inhibitory form. All but one of the drugs in this class (see structures in Figure 64–3) are nucleosides that must be triphosphorylated at the 5′-hydroxyl to exert activity. The sole exception, *tenofovir*, is a nucleotide monophosphate analogue that requires two additional phosphates to acquire full activity. These compounds inhibit both HIV-1 and HIV-2, and several have broad-spectrum activity against other human and animal retroviruses; *emtricitabine*, *lamivudine*, and *tenofovir* are active against hepatitis B virus (HBV; see Chapter 63), whereas *tenofovir* also has activity against herpesviruses (see Chapter 62).

The selective toxicity of these drugs depends on their ability to inhibit the HIV reverse transcriptase without inhibiting host cell DNA polymerases. Although the intracellular triphosphates for all these drugs have low affinity for human DNA polymerase α and polymerase β, some can inhibit human DNA polymerase γ, which is the mitochondrial enzyme. As a result, the important toxicities common to older representatives of this class of drugs result largely from the inhibition of mitochondrial DNA synthesis (Lee et al., 2003). These toxicities include anemia, granulocytopenia, myopathy, peripheral neuropathy, and pancreatitis. Lactic acidosis with or without hepatomegaly and hepatic steatosis is a rare but potentially fatal complication seen with *stavudine*, *zidovudine*, and *didanosine*. Phosphorylated *emtricitabine*, *lamivudine*, and *tenofovir* have low affinity for DNA polymerase γ and are largely devoid of mitochondrial toxicity.

Table 64–2 summarizes the pharmacokinetic properties of NRTIs approved for treating HIV infection. Figure 64–4 shows the cellular routes that catalyze the activation of NRTIs. In general, the phosphorylated anabolites are eliminated from cells much more gradually than the parent drug is eliminated from the plasma. As a result, available NRTIs are dosed once or twice daily. These drugs are not major substrates for hepatic CYPs. Pharmacokinetic drug interactions involving *tenofovir* and PIs are likely explained by inhibition of OATP and other drug transporters (Chapman et al., 2003; see also Chapter 4). High-level resistance to NRTIs, especially thymidine analogues, occurs slowly by comparison to NNRTIs and first-generation PIs. High-level resistance can occur rapidly with *lamivudine* and *emtricitabine*. Cross-resistance is common but often confined to drugs having similar chemical structures. Several nucleoside analogues have favorable safety and tolerability profiles and are useful in suppressing the emergence of HIV isolates resistant to the more potent drugs in combination regimens (Kuritzkes, 2011).

Zidovudine

Zidovudine is a synthetic thymidine analogue (Figure 64–3) with potent activity against a broad spectrum of retroviruses, including HIV-1,

TABLE 64–1 ■ ANTIRETROVIRAL AGENTS APPROVED FOR USE IN THE U.S.

GENERIC NAME	ABBREVIATION; CHEMICAL NAME
Nucleoside/Nucleotide Reverse Transcriptase Inhibitors	
Zidovudine[a]	ZDV; azidothymidine (AZT)
Didanosine[b]	ddI; dideoxyinosine
Stavudine	d4T; didehydrodeoxythymidine
Zalcitabine[c]	ddC; dideoxycytidine
Lamivudine[a]	3TC; dideoxythiacytidine
Abacavir[a]	ABC; amino-cyclopropylamino-purinylcyclopentenylmethanol
Tenofovir disoproxil[a,d] Tenofovir alafenamide[a,d]	TDF; TAF; phosphono-methoxypropyladenine (PMPA)
Emtricitabine[a]	FTC; fluorooxathiolanyl cytosine
Nonnucleoside Reverse Transcriptase Inhibitors	
Nevirapine	NVP
Efavirenz[a]	EFV
Delavirdine[b]	DLV
Etravirine	ETV
Rilpivirine[a]	RPV
Doravirine[a]	DOR
Protease Inhibitors	
Saquinavir	SQV
Indinavir[b]	IDV
Ritonavir[a]	RTV
Nelfinavir	NFV
Amprenavir[c]	APV
Lopinavir[e]	LPV/r
Atazanavir	ATV
Fosamprenavir	FPV
Tipranavir	TPV
Darunavir[a]	DRV
Entry Inhibitors	
Enfuvirtide	T-20
Maraviroc	MVC
Fostemsavir	FTR
Ibalizumab	
Integrase Inhibitors	
Raltegravir[a]	RAL
Elvitegravir[a,f]	EVG
Dolutegravir[a]	DTG
Bictegravir[f]	BTG
Cabotegravir	CBT

[a] A number of fixed-dose coformulations are available; these are described in more detail in the text.
[b] No longer marketed in the U.S.
[c] No longer marketed worldwide.
[d] Tenofovir disoproxil fumarate is a nucleotide prodrug of the nucleoside tenofovir.
[e] Lopinavir is available only as part of a fixed-dose coformulation with ritonavir.
[f] Only available as a coformulation with other antiretroviral agents.

HIV-2, and HTLVs I and II. It was the first FDA-approved antiretroviral drug. *Zidovudine* is active in lymphoblastic and monocytic cell lines but has no impact on cells already infected with HIV. *Zidovudine* appears to be more active in activated than in resting lymphocytes because the phosphorylating enzyme, thymidine kinase, is S-phase specific (Cihlar and Ray, 2010). *Zidovudine* is FDA-approved for the treatment of adults and children with HIV infection and for preventing mother-to-child transmission; it was previously recommended for postexposure prophylaxis in HIV-exposed healthcare workers but is rarely used for this indication because of its potential toxicity as compared to alternatives. *Zidovudine* is marketed in oral tablets, capsules, and solution as well as a solution for intravenous injection. *Zidovudine* is available in coformulated tablets with *lamivudine* or with *lamivudine* and *abacavir*. *Zidovudine* is mainly used in pediatric applications and is less commonly used in adults because of its potential toxicity.

Mechanisms of Action and Resistance. Intracellular *zidovudine* is phosphorylated to zidovudine 5′-triphosphate. Zidovudine triphosphate terminates the elongation of proviral DNA because it is incorporated by reverse transcriptase into nascent DNA but lacks a 3′-hydroxyl group. The monophosphate competitively inhibits cellular thymidylate kinase, and this may reduce the amount of intracellular thymidine triphosphate formed. Zidovudine triphosphate only weakly inhibits cellular DNA polymerase α but is a more potent inhibitor of mitochondrial polymerase γ. Because the conversion of zidovudine monophosphate to diphosphate is inefficient, high concentrations of the monophosphate accumulate inside cells and may serve as a precursor depot for formation of triphosphate. Consequently, there is little correlation between extracellular concentrations of parent drug and intracellular concentrations of triphosphate, and higher plasma concentrations of *zidovudine* do not increase intracellular triphosphate concentrations proportionately.

Resistance to *zidovudine* is associated with mutations at reverse transcriptase codons 41, 44, 67, 70, 210, 215, and 219. These mutations are referred to as thymidine analogue mutations (TAMs) because of their ability to confer cross-resistance to other thymidine analogues, such as *stavudine*. The M184V substitution in the reverse transcriptase gene associated with the use of *lamivudine* or *emtricitabine* greatly restores sensitivity to *zidovudine* (Kuritzkes, 2011). The combination of *zidovudine* and *lamivudine* produces greater long-term suppression of plasma HIV RNA than does *zidovudine* alone.

ADME. *Zidovudine* is absorbed rapidly and reaches peak plasma concentrations within 1 h. Table 64–2 summarizes the drug's pharmacokinetic profile, which is not altered significantly during pregnancy; drug concentrations in the newborn approach those of the mother. Parent drug crosses the blood-brain barrier relatively well and is also detectable in breast milk, semen, and fetal tissue.

Untoward Effects and Drug Interactions. Patients initiating *zidovudine* often complain of fatigue, malaise, myalgia, nausea, anorexia, headache, and insomnia; these symptoms usually resolve within a few weeks. Erythrocytic macrocytosis is seen in about 90% of patients but usually is not associated with anemia. Chronic *zidovudine* administration has been associated with nail hyperpigmentation. Skeletal myopathy can occur and is associated with depletion of mitochondrial DNA, most likely as a consequence of inhibition of DNA polymerase γ. Serious hepatic toxicity, with or without steatosis and lactic acidosis, is rare but can be fatal.

Probenecid, fluconazole, atovaquone, and *valproic acid* may increase plasma concentrations of *zidovudine*, probably through inhibition of glucuronosyl transferases. *Zidovudine* is neither a prominent substrate nor potent inhibitor of CYPs. *Zidovudine* can cause bone marrow suppression and should be used cautiously in patients with preexisting anemia or granulocytopenia and in those taking other marrow-suppressive drugs. *Stavudine* and *zidovudine* compete for intracellular phosphorylation and should not be used concomitantly.

Lamivudine

Lamivudine is a cytidine analogue reverse transcriptase inhibitor that is active against HIV-1, HIV-2, and hepatitis B virus (HBV). *Lamivudine* is

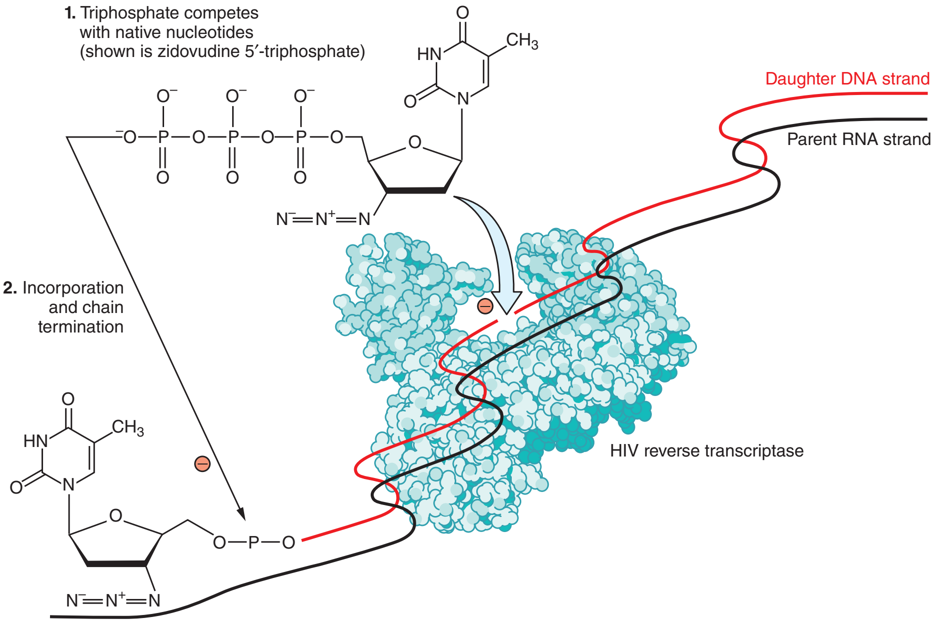

1. Triphosphate competes with native nucleotides (shown is zidovudine 5′-triphosphate)
Daughter DNA strand
Parent RNA strand
2. Incorporation and chain termination
HIV reverse transcriptase

Figure 64–2 *Mechanism of NRTIs. Zidovudine* is depicted; Table 64–1 lists other agents in the NRTI class. Nucleoside and nucleotide analogues must enter cells and be phosphorylated to generate synthetic substrates for reverse transcriptase. The fully phosphorylated analogues block replication of the viral genome both by competitively inhibiting incorporation of native nucleotides and by terminating elongation of nascent proviral DNA because they lack a 3′-hydroxyl group.

approved for HIV in adults and children aged 3 months or older. *Lamivudine* has been effective in combination with other antiretroviral drugs in both treatment-naïve and -experienced patients and is a common component of therapy, given its safety, convenience, and efficacy (Cihlar and Ray, 2010). *Lamivudine* is also approved for treatment of chronic HBV (see Chapter 63).

Mechanisms of Action and Resistance. *Lamivudine* enters cells by passive diffusion and is sequentially phosphorylated to lamivudine 5′-triphosphate, which is the active anabolite. *Lamivudine* has low affinity for human DNA polymerases, explaining its low toxicity to the host. High-level resistance to *lamivudine* occurs with single-amino-acid substitutions, M184V or M184I. These mutations can reduce *in vitro* sensitivity to *lamivudine* as much as 1000-fold. The M184V mutation restores *zidovudine* susceptibility in *zidovudine*-resistant HIV and partially restores *tenofovir* susceptibility in *tenofovir*-resistant HIV harboring the K65R mutation (Kuritzkes, 2011). This effect may contribute to the sustained virologic benefits seen with combinations of *zidovudine* and *lamivudine*.

ADME. Table 64–2 summarizes the pharmacokinetic parameters for this drug. *Lamivudine* is excreted primarily unchanged in the urine; dose adjustment is recommended for patients with a creatinine clearance less than 50 mL/min. *Lamivudine* freely crosses the placenta into the fetal circulation.

Untoward Effects and Precautions. *Lamivudine* is one of the least toxic antiretroviral drugs. Neutropenia, headache, and nausea have

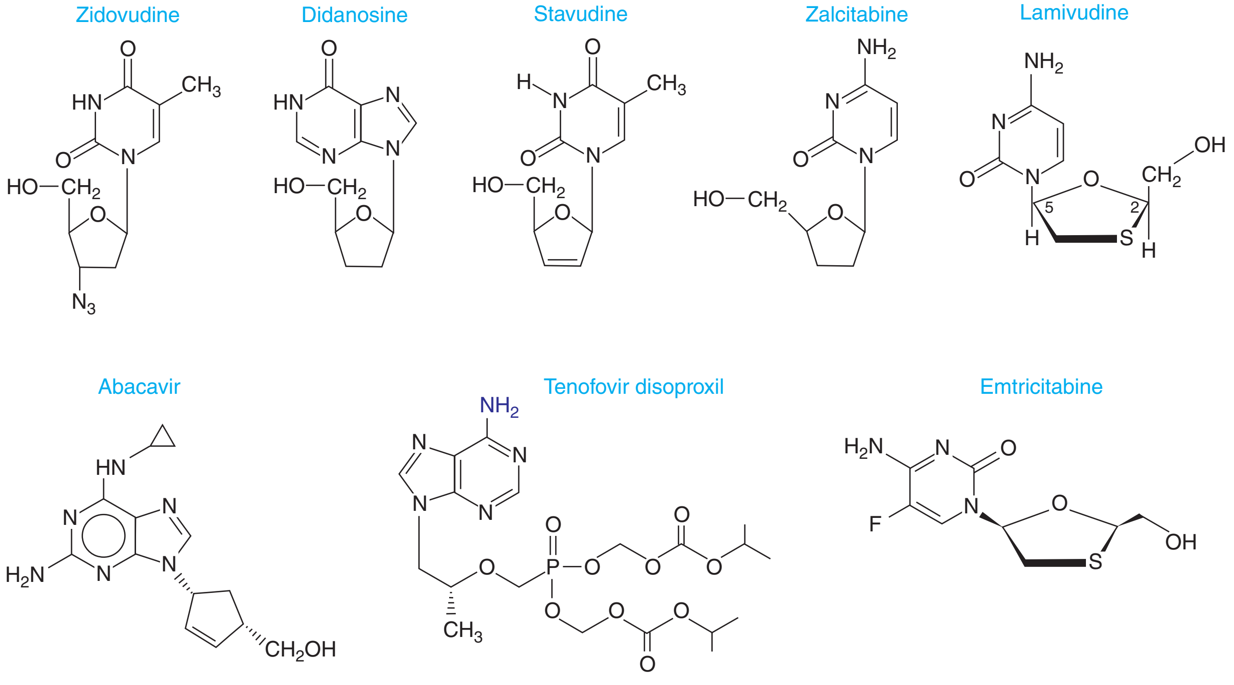

Zidovudine
Didanosine
Stavudine
Zalcitabine
Lamivudine
Abacavir
Tenofovir disoproxil
Emtricitabine

Figure 64–3 *Structures of NRTIs.*

TABLE 64–2 ■ PHARMACOKINETIC PROPERTIES OF NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS[a]

PARAMETER	ZIDOVUDINE	LAMIVUDINE	STAVUDINE[b]	DIDANOSINE[c]	ABACAVIR	TENOFOVIR[d]	EMTRICITABINE
Oral bioavailability, %	64	86–87	86	42	83	25	93
Effect of meals on AUC	↓ 24% (high fat)	↔	↔	↓ 55% (acidity)	↔	↑ 40% (high fat)	↔
Plasma $t_{1/2}$, h	1.0	5–7	1.1–1.4	1.5	0.8–1.5	14–17	10
Intracellular $t_{1/2}$ of triphosphate, h	3–4	12–18	3.5	25–40	21	60–100	39
Plasma protein binding, %	20–38	<35	<5	<5	50	<8	<4
Metabolism, %	60–80 (glucuronidation)	<36	ND	50 (purine metabolism)	>80 (dehydrogenation; glucuronidation)	ND	13
Renal excretion of parent drug, %	14	71	39	18–36	<5	70–80	86

↑, increase; ↓, decrease; ↔, no effect; ND, not determined; AUC, area under plasma concentration-time curve.

[a] Reported mean values in adults with normal renal and hepatic function.

[b] Parameters reported for the stavudine capsule formulation.

[c] Parameters reported for the didanosine chewable tablet formulation.

[d] Reported values for oral tenofovir disoproxil fumarate.

been reported at higher-than-recommended doses. Pancreatitis has been reported in pediatric patients. Because *lamivudine* also has activity against HBV, caution is warranted in using this drug in patients coinfected with HBV or in HBV-endemic areas: Abrupt discontinuation of *lamivudine* may be associated with a rebound of HBV replication and exacerbation of hepatitis.

Abacavir

Abacavir, a synthetic purine analogue, is approved for the treatment of HIV-1 infection in combination with other antiretroviral agents. *Abacavir* is available in a coformulation with *zidovudine* and *lamivudine* for twice-daily dosing, and in a coformulation with *lamivudine*, or *lamivudine* and *dolutegravir*, for once-daily dosing. *Abacavir* is approved for use in adult and pediatric patients aged 3 months or older, with dosing in the latter based on body weight.

Mechanisms of Action and Resistance. *Abacavir* is the only approved antiretroviral that is active as a guanosine analogue. It is sequentially phosphorylated in the host cell to carbovir 5′-triphosphate, which terminates the elongation of proviral DNA because it is incorporated by reverse transcriptase into nascent DNA but lacks a 3′-hydroxyl group (Cihlar and Ray, 2010). Clinical resistance to *abacavir* is associated with four specific substitutions: K65R, L74V, Y115F, and M184V. In combination, these substitutions can reduce susceptibility by up to 10-fold. K65R confers cross-resistance to all nucleosides except *zidovudine*. An alternate pathway for *abacavir* resistance involves mutations at RT codons 41, 210, and 215 (Kuritzkes, 2011).

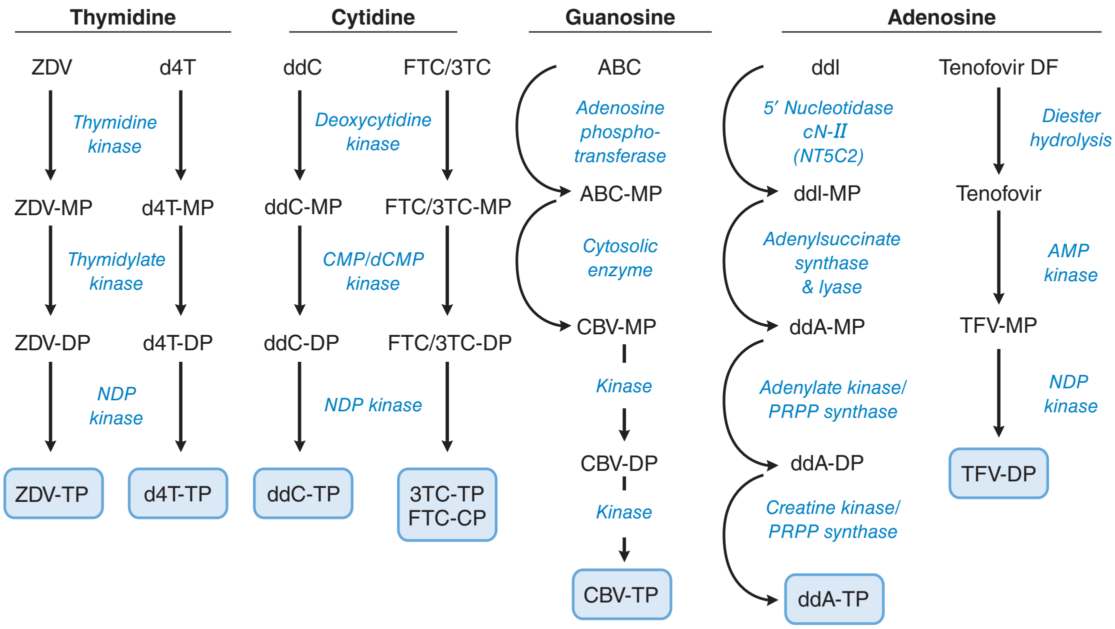

Thymidine
ZDV
d4T
Thymidine kinase
ZDV-MP
d4T-MP
Thymidylate kinase
ZDV-DP
d4T-DP
NDP kinase
ZDV-TP
d4T-TP
Cytidine
ddC
FTC/3TC
Deoxycytidine kinase
ddC-MP
FTC/3TC-MP
CMP/dCMP kinase
ddC-DP
FTC/3TC-DP
NDP kinase
ddC-TP
3TC-TP
FTC-CP
Guanosine
ABC
Adenosine phospho-transferase
ABC-MP
Cytosolic enzyme
CBV-MP
Kinase
CBV-DP
Kinase
CBV-TP
Adenosine
ddI
5′ Nucleotidase cN-II (NT5C2)
ddI-MP
Adenylsuccinate synthase & lyase
ddA-MP
Adenylate kinase/ PRPP synthase
ddA-DP
Creatine kinase/ PRPP synthase
ddA-TP
Tenofovir DF
Diester hydrolysis
Tenofovir
AMP kinase
TFV-MP
NDP kinase
TFV-DP

Figure 64–4 *Intracellular activation of nucleoside analogue reverse transcriptase inhibitors.* Drugs and phosphorylated anabolites are abbreviated (see Table 64-1); the enzymes responsible for each conversion are spelled out. The active antiretroviral anabolite for each drug is shown in the blue box. (Adapted with permission from Khoo SH, et al. Pharmacology. In: Boucher CAB, Galasso GAJ, eds. *Practical Guidelines in Antiviral Therapy*. Elsevier, New York, **2002**, 13–35. Copyright © Elsevier.) MP, monophosphate; DP, diphosphate; TP, triphosphate.

ADME. Table 64–2 summarizes the pharmacokinetic parameters for this agent. The presence of food does not affect the oral bioavailability of *abacavir*. *Abacavir* is neither a substrate nor an inhibitor of CYPs. Its CSF/plasma AUC ratio is about 0.3.

Untoward Effects and Drug Interactions. The most important adverse effect of *abacavir* is a unique and potentially fatal hypersensitivity syndrome characterized by fever, abdominal pain, and other GI complaints; a mild maculopapular rash; and malaise or fatigue. Respiratory complaints (cough, pharyngitis, dyspnea), musculoskeletal complaints, headache, and paresthesias are less common. The presence of concurrent fever, abdominal pain, and rash within 6 weeks of starting *abacavir* is diagnostic and necessitates immediate discontinuation of the drug. Once discontinued for hypersensitivity, *abacavir* must never be restarted. The hypersensitivity syndrome (2%–9% of patients) results from a genetically mediated immune response linked to both the HLA-B*5701 locus and the M493T allele in the heat-shock locus Hsp70-Hom. The *Hsp* gene is implicated in antigen presentation, and this haplotype is associated with aberrant tumor necrosis factor α release after exposure of human lymphocytes to *abacavir ex vivo* (White et al., 2015). *Abacavir* should not be used in anyone known to carry the HLA-B*5701 locus (~10% of Caucasians).

Abacavir is not associated with any clinically significant pharmacokinetic drug interactions. However, a large dose of ethanol (0.7 g/kg) increases the *abacavir* plasma AUC by 41% and prolongs the elimination $t_{1/2}$ by 26%, possibly owing to competition for alcohol dehydrogenase, which produces the dehydro-metabolite of the drug (see Table 64–2).

Tenofovir

Tenofovir is a derivative of 5′-AMP that lacks a complete ribose ring; it is the only nucleotide analogue currently marketed for the treatment of HIV infection. *Tenofovir* is available as the disoproxil or alafenamide prodrugs, which substantially improve oral absorption. *Tenofovir* is active against HIV-1, HIV-2, and HBV. *Tenofovir disoproxil fumarate* (TDF) is FDA-approved for treating HIV infection in adults and children older than 2 years in combination with other antiretroviral agents and for the treatment of chronic hepatitis B in adults and children older than 12. It is also approved for HIV preexposure prophylaxis (in combination with *emtricitabine*) in adults at high risk of acquiring the infection. TDF is available in a variety of oral coformulations and is the most widely used antiretroviral globally. *Tenofovir alafenamide* (TAF) is FDA-approved for the treatment of HIV and HBV and for HIV prophylaxis in combination with *emtricitabine*. The advantages of TAF over TDF include lower dose and less long-term renal and bone toxicity (Sax et al., 2015). TAF is currently available in coformulation with *elvitegravir, cobicistat*, and *emtricitabine*; with *bictegravir* and *emtricitabine*; with *rilpivirine* and *emtricitabine*; or with *emtricitabine*.

Mechanisms of Action and Resistance. *Tenofovir disoproxil* is hydrolyzed rapidly to *tenofovir*, which is phosphorylated by cellular kinases to its active metabolite, tenofovir diphosphate, which is actually a triphosphate: The parent drug is a monophosphate (Figure 64–3) (Cihlar and Ray, 2010). The disposition of oral *tenofovir alafenamide* is similar, but it circulates largely as the uncleaved prodrug, which is taken up into cells and then converted to the parent nucleotide; consequently, circulating concentrations of *tenofovir*, which may contribute to renal toxicity, are much lower than produced by the disoproxil fumarate prodrug. Tenofovir diphosphate is a competitive inhibitor of viral reverse transcriptases and is incorporated into HIV DNA to cause chain termination because it has an incomplete ribose ring. Although tenofovir diphosphate has broad-spectrum activity against viral DNA polymerases, it has low affinity for human DNA polymerase α, polymerase β, and polymerase γ, which is the basis for its selective toxicity.

Specific resistance occurs with a K65R substitution in RT that has been associated with clinical failure of *tenofovir*-containing regimens. *Tenofovir* sensitivity and virologic efficacy also are reduced in patients harboring HIV isolates with high-level resistance to *zidovudine* or *stavudine*. The M184V mutation associated with *lamivudine* or *emtricitabine* resistance partially restores susceptibility in *tenofovir*-resistant HIV harboring the K65R mutation (Kuritzkes, 2011).

ADME. Table 64–2 shows pharmacokinetic data for *tenofovir*. Following an intravenous dose, 70% to 80% of the drug is recovered unchanged in the urine; thus, doses should be decreased in those with renal insufficiency. *Tenofovir* is not known to inhibit or induce CYPs.

Untoward Effects and Drug Interactions. *Tenofovir* generally is well tolerated, with few significant symptoms reported except for flatulence. Rare episodes of acute renal failure and Fanconi syndrome have been reported, and this drug should be used with caution in patients with preexisting renal disease. *Tenofovir* use is associated with small declines in eCL_{Cr} (estimated creatinine clearance) after months of treatment in some patients; because the dose needs to be reduced in renal insufficiency, renal function (creatinine and phosphorus) should be monitored regularly. Because *tenofovir* also has activity against HBV, caution is warranted in using this drug in patients coinfected with HBV: Abrupt discontinuation of *tenofovir* may be associated with a rebound of HBV replication and exacerbation of hepatitis. *Tenofovir* can increase the AUC of *didanosine*, and the two drugs should not be used together (Cihlar and Ray, 2010).

Tenofovir plasma concentrations are increased by 30% to 50% if combined with the pharmacokinetic enhancers *ritonavir* or cobicistat due to inhibition of renal drug transporters. Similar effects are seen with the HCV NS5A inhibitors *ledipasvir* and *velpatasvir* (see Chapter 63). Regardless, these combinations are generally well tolerated and without producing significant renal injury. However, acute renal insufficiency has been reported when TDF is combined with the oral nonsteroidal *diclofenac*.

Emtricitabine

Emtricitabine is a cytidine analogue that is chemically related to *lamivudine* and shares many of its properties. *Emtricitabine* is active against HIV-1, HIV-2, and HBV. The drug is FDA-approved for treating HIV infection in adults, children, and infants, in combination with other antiretroviral agents, and is available coformulated with TDF with or without *efavirenz* or with either *tenofovir* prodrug plus *elvitegravir* and *cobicistat*; *bictegravir* and *cobicistat*; or *rilpivirine*. *Emtricitabine* is approved for HIV preexposure prophylaxis (in combination with *tenofovir*) in adults at high risk of acquiring the infection.

Mechanisms of Action and Resistance. *Emtricitabine* enters cells by passive diffusion and is sequentially phosphorylated to its active metabolite, emtricitabine 5′-triphosphate. High-level resistance to *emtricitabine* occurs with the same mutations affecting *lamivudine* (mainly M184I/V), although these appear to occur less frequently with *emtricitabine*. The M184V mutation restores sensitivity to *zidovudine* in *zidovudine*-resistant HIV and partially restores sensitivity to *tenofovir* in *tenofovir*-resistant HIV harboring the K65R mutation (Kuritzkes, 2011). The same K65R mutation confers resistance to *emtricitabine* and the other cytidine analogue *lamivudine*, as well as *didanosine*, *stavudine*, and *abacavir*.

ADME. Table 64–2 summarizes pharmacokinetic data for *emtricitabine*. Orally administered drug is rapidly absorbed; the drug can be taken without regard to meals. *Emtricitabine* is excreted primarily unchanged in the urine; thus, the dose should be reduced in patients with CL_{Cr} below 50 mL/min.

Untoward Effects and Drug Interactions. *Emtricitabine* is one of the least-toxic antiretroviral drugs and has few significant adverse effects (Cihlar and Ray, 2010). Prolonged exposure has been associated with hyperpigmentation of the skin, especially in sun-exposed areas. Because *emtricitabine* also has *in vitro* activity against HBV, caution is warranted in using this drug in patients coinfected with HBV and in regions with high HBV seroprevalence; abrupt discontinuation of *emtricitabine* may be associated with a rebound of HBV replication and exacerbation of hepatitis.

Emtricitabine is not metabolized to a significant extent by CYPs, and it is not susceptible to known metabolic drug interactions.

Older NRTIs

Stavudine (d4T) and *didanosine* (ddI) are no longer widely used because of their relative toxicity as compared to other NRTIs. *Dideoxycytidine* (ddC) is no longer marketed for the same reason. Detailed information about these drugs is available in previous editions of this book.

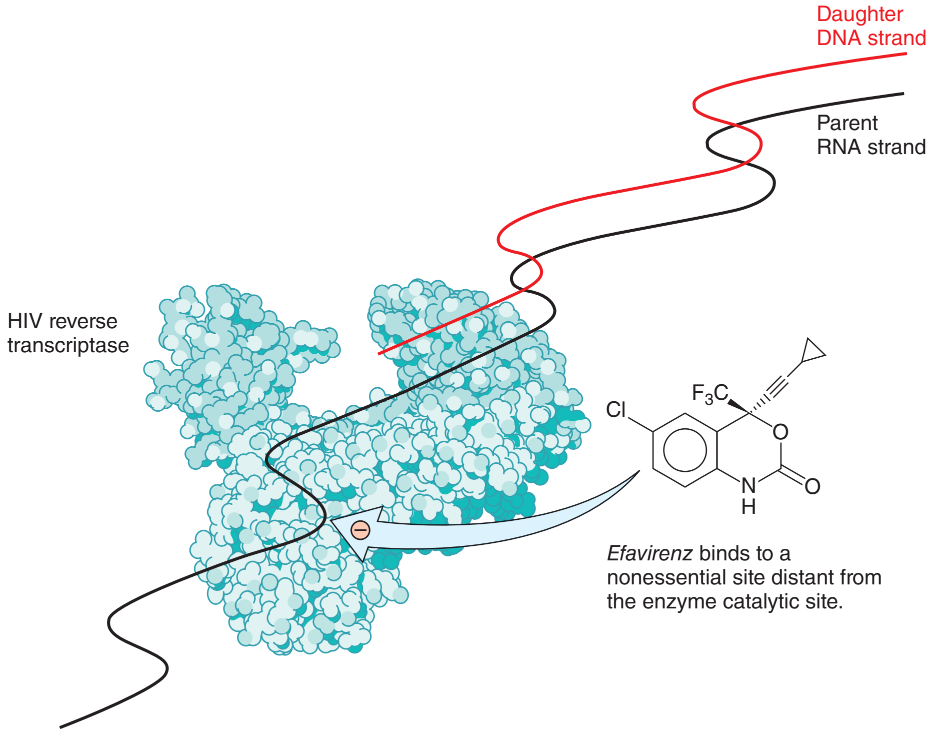

Daughter DNA strand
Parent RNA strand
HIV reverse transcriptase
Cl
F_3C
O
N
H
O
Efavirenz binds to a nonessential site distant from the enzyme catalytic site.

Figure 64–5 *Mechanism of NNRTIs.*

Nonnucleoside Reverse Transcriptase Inhibitors

Overview

The NNRTIs include a variety of chemical substrates that bind to a hydrophobic pocket in the p66 subunit of the HIV-1 reverse transcriptase, in a site distant from the active site (Figure 64–5). These compounds induce a conformational change in the three-dimensional structure of the enzyme that greatly reduces its activity, and thus, they act as noncompetitive inhibitors. Because the binding site for NNRTIs is virus-strain specific, the approved agents are active against HIV-1 but not HIV-2 or other retroviruses and should not be used to treat HIV-2 infection. These compounds also have no activity against host cell DNA polymerases (de Bethune, 2010). The six approved NNRTIs are *nevirapine, efavirenz, etravirine, rilpivirine*, *delavirdine*, and *doravirine*. Table 64–3 summarizes their pharmacokinetic properties.

Agents in this class share a number of properties. All approved NNRTIs are eliminated from the body by hepatic metabolism. *Efavirenz, etravirine*, and *nevirapine* are moderately potent inducers of hepatic drug-metabolizing enzymes, including CYP3A4; *delavirdine* is mainly a CYP3A4 inhibitor. Pharmacokinetic drug interactions are thus an important consideration with this class of compounds. All NNRTIs except *etravirine* and *rilpivirine* are susceptible to high-level drug resistance caused by single-amino-acid changes in the NNRTI-binding pocket (usually in codons 103 or 181; Kuritzkes, 2011). Exposure to even a single dose of *nevirapine* in the absence of other antiretroviral drugs is associated with resistance mutations in up to one-third of patients (Eshleman et al., 2004). These agents are potent and highly effective but should be combined with other active agents to avoid resistance. *NNRTIs should never be used as single agents in monotherapy or as the sole addition to a failing regimen.*

NNRTIs in combination with other antiretroviral drugs are associated with favorable long-term suppression of viremia and elevation of $CD4^+$ lymphocyte counts. Rashes occur frequently with all NNRTIs except doravirine, usually during the first 4 weeks of therapy. Rare cases of potentially fatal drug reaction with eosinophilia and systemic symptoms (DRESS) or Stevens-Johnson syndrome have been reported with *nevirapine, efavirenz, rilpivirine*, and *etravirine*. Fat accumulation can be seen after long-term use of some NNRTIs, and fatal hepatitis has been associated with *nevirapine* use (de Bethune, 2010).

TABLE 64–3 ■ PHARMACOKINETIC PROPERTIES OF NONNUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS[a]

PARAMETER	NEVIRAPINE[b]	EFAVIRENZ[b]	ETRAVIRINE	RILPIVIRINE	DORAVIRINE
Oral bioavailability, %	90–93	50	NR	NR	64
Effect of meals on AUC	↔	↑ 17%–28%	↑ 33%–102%	↑ 40%–50%	↔
Plasma $t_{1/2}$, h	25–30	40–55	41	50	15
Plasma protein binding, %	60	99	99.9	99.7	76
Metabolism by CYPs and UGT	3A4 > 2B6	2B6 > 3A4	3A4, 2C9, 2C19, UGT	3A4, 3A5	3A4, 3A5
Renal excretion of parent drug, %	<3	<3	1%	6%	6%
Autoinduction of metabolism	Yes	Yes	NR	No	No
Inhibition of CYP3A	No	Yes	No	No	No

↑, increase; ↓, decrease; ↔, no effect; NR, not reported.

[a] Reported mean values in adults with normal renal and hepatic function.

[b] Values at steady state after multiple oral doses.

Nevirapine

Nevirapine is a dipyridodiazepinone NNRTI with potent activity against HIV-1. The drug is FDA-approved for the treatment of HIV-1 infection in adults and children in combination with other antiretroviral agents. *Nevirapine* is approved for use in infants and children 15 days old or older, with dosing based on body surface area. Single-dose *nevirapine* was used widely in pregnant HIV-infected women to prevent mother-to-child transmission, but this practice is declining as more pregnant HIV-infected women are being placed on permanent multidrug antiretroviral regimens (de Bethune, 2010). *Nevirapine* for HIV treatment has largely been replaced by the less toxic *efavirenz* and is less widely used now as more patients begin therapy with or switch to InSTI-based regimens.

ADME. Table 64–3 summarizes the pharmacokinetic data for this agent. The drug readily crosses the placenta and has been found in breast milk. *Nevirapine* is a moderate inducer of CYPs and induces its own metabolism. To compensate for this, the drug should be initiated at a dose of 200 mg once daily for 14 days, with the dose then increased to 200 mg twice daily if no adverse reactions have occurred.

Untoward Effects and Drug Interactions. The most frequent adverse events associated with *nevirapine* are rash (in ~16% of patients) and pruritus. In most patients, the rash resolves with continued administration of drug; administration of glucocorticoids may cause a more severe rash. Life-threatening Stevens-Johnson syndrome is rare but occurs in up to 0.3% of recipients. Clinical hepatitis occurs in up to 1% of patients. Severe and fatal hepatitis has been associated with *nevirapine* use, and this may be more common in women with CD4 counts greater than 250 cells/mm^3, especially during pregnancy (de Bethune, 2010). Other reported side effects include fever, fatigue, headache, somnolence, and nausea.

Because *nevirapine* induces CYP3A4, this drug may lower plasma concentrations of coadministered CYP3A4 substrates. *Methadone* withdrawal has been reported in patients receiving *nevirapine*, presumably as a consequence of enhanced *methadone* clearance. Plasma *ethinyl estradiol* and *norethindrone* concentrations decrease by 20% with *nevirapine*; alternative methods of birth control are advised.

Efavirenz

Efavirenz (see structure in Figure 64–5) is an NNRTI with potent activity against HIV-1. The drug should be used only in combination with other effective agents and should not be added as the sole new agent to a failing regimen. *Efavirenz* is approved for adult and pediatric patients aged 3 months and older and weighing at least 3.5 kg. *Efavirenz* was widely used because of its convenience, effectiveness, and long-term tolerability. Especially popular was the once-daily, single-pill coformulation of *efavirenz*, TDF, and *emtricitabine* or *lamivudine*. A 400-mg daily dose of *efavirenz* may be equally efficacious and better tolerated than the standard 600-mg dose (ENCORE1 Study Group, 2014). *Efavirenz* can be safely combined with *rifampin* and is useful in patients also being treated for tuberculosis. *Efavirenz* use is likely to decline in the coming years as more patients begin therapy with or switch to InSTI-based regimens.

ADME. Table 64–3 summarizes the pharmacokinetic data for this agent. *Efavirenz* is well absorbed from the GI tract, but there is diminished absorption of the drug with increasing doses. Bioavailability (AUC) is increased by 22% with a high-fat meal. *Efavirenz* is more than 99% bound to plasma proteins and, consequently, has a low CSF-to plasma ratio of 0.01. The drug should be taken initially on an empty stomach at bedtime to reduce side effects. The long elimination $t_{1/2}$ permits once-daily dosing.

Untoward Effects and Drug Interactions. The most important adverse effects of *efavirenz* involve the CNS. Up to 53% of patients report some CNS or psychiatric side effects, but fewer than 5% discontinue the drug for this reason. Patients commonly report dizziness, impaired concentration, dysphoria, vivid or disturbing dreams, and insomnia. CNS side effects generally become more tolerable and resolve within the first 4 weeks of therapy. Rash occurs frequently with *efavirenz* (27%), usually in the first few weeks of treatment, resolving spontaneously and rarely requiring drug discontinuation. Life-threatening skin eruptions such as Stevens-Johnson syndrome are rare (de Bethune, 2010). Other side effects reported with *efavirenz* include headache, increased hepatic transaminases, and elevated serum cholesterol. *Efavirenz* is the only antiretroviral drug that is teratogenic in primates, but careful clinical studies suggested that it is no more teratogenic in humans than other antiretroviral drugs (Ford et al., 2014).

Efavirenz is a moderate inducer of hepatic enzymes, especially CYP3A4, but also a weak to moderate CYP inhibitor. *Efavirenz* decreases concentrations of *phenobarbital*, *phenytoin*, and *carbamazepine*; the *methadone* AUC is reduced by 33% to 66% at steady state. *Efavirenz* reduces the *rifabutin* AUC by about 38%. *Efavirenz* has a variable effect on HIV PIs: *Indinavir*, *saquinavir*, and *amprenavir* concentrations are reduced; *ritonavir* and *nelfinavir* concentrations are increased. Drugs that induce CYPs 2B6 or 3A4 (e.g., *phenobarbital*, *phenytoin*, and *carbamazepine*) would be expected to increase the clearance of *efavirenz* and should be avoided (de Bethune, 2010).

Rilpivirine

Rilpivirine is a diarylpyrimidine NNRTI with potent activity against HIV-1; Table 64–3 presents some of the drug's pharmacokinetic values. The drug should be used only in combination with other effective agents and should not be added as the sole new agent to a failing regimen. *Rilpivirine* has one of the best side effect profiles of available NNRTIs and has few or no CNS side effects compared to *efavirenz*. *Rilpivirine* retains efficacy against many HIV strains that are resistant to *efavirenz* and other earlier NNRTIs. However, higher concentrations of *rilpivirine* are associated with prolongation of the QTc interval, and at the approved dose of 25 mg/day, it is less effective than *efavirenz* in patients with baseline viral load greater than 100,000 copies/mL or CD4 count below 200 cells/mm^3 (Sharma and Saravolatz, 2013). Failure of a *rilpivirine*-containing regimen generally results in resistance that precludes the use of other NNRTIs. *Rilpivirine* is approved for adult and pediatric patients 12 years of age and older and weighing at least 35 kg. *Rilpivirine* is also available as once-daily, single-tablet coformulations with TDF and *emtricitabine*, TAF and *emtricitabine*, or *dolutegravir*. A nanocrystalline long-acting injectable formulation of *rilpivirine* was FDA-approved in early 2021 (see section on Long-Acting and Extended-Release Antiretroviral Formulations).

ADME. Table 64–3 summarizes the pharmacokinetic data for *rilpivirine*. Absorption of *rilpivirine* is both food and pH dependent. Bioavailability (AUC) is decreased by 40% in the fasted state or by 50% with a high-protein, low-fat meal; the drug should only be given with food and not with high-protein, low-fat supplements. *Rilpivirine* should not be given with proton pump inhibitors, and H_2 receptor antagonists should be administered 12 h before or 4 h after dosing (Sharma and Saravolatz, 2013). The long elimination $t_{1/2}$ permits once-daily dosing.

Untoward Effects and Drug Interactions. Rash (<5%) occurs less frequently with *rilpivirine* than with other NNRTIs. Although CNS adverse effects are much less common with *rilpivirine* than with *efavirenz*, depressive symptoms have been reported, including in pediatric and adolescent patients. Elevated hepatic transaminases may occur, especially in those with underlying HBV or HCV infection. *Rilpivirine* is considered generally safe in pregnancy (former FDA category B).

Rilpivirine concentrations can be reduced by coadministered CYP inducers, and this drug should not be given with *rifampin*; anticonvulsants such as *carbamazepine, phenobarbital*, or *phenytoin*; or any products containing St. John's wort. At the 25-mg daily dose, *rilpivirine* is not a clinically significant inhibitor or inducer of hepatic enzymes. Because *rilpivirine* can prolong the QTc in a concentration-dependent fashion, caution is advised when combining this drug with any known CYP3A4 inhibitors or other drugs that can increase *rilpivirine* concentrations or with other drugs known to prolong the QTc.

Etravirine

Etravirine is a diarylpyrimidine NNRTI that is active against HIV-1. The drug is unique in its ability to inhibit reverse transcriptase that is resistant to other NNRTIs. *Etravirine* appears to have conformational and positional flexibility in the NNRTI-binding pocket that allows it to inhibit the HIV-1 RT in the presence of common NNRTI resistance mutations (de Bethune, 2010). *Etravirine* is approved for use only in treatment-experienced adults and children aged 2 years or older and weighing at least 10 kg with viral strains resistant to another NNRTI. NNRTI-experienced patients should not receive *etravirine* plus NRTIs alone.

ADME. Table 64–3 summarizes the pharmacokinetic data for this agent. Food increases the *etravirine* AUC by about 50%; thus, the drug should be administered with food. Methyl- and dimethyl-hydroxylated metabolites are produced in the liver primarily by CYPs 3A4, 2C9, and 2C19, accounting for most of the elimination of this drug.

Untoward Effects and Drug Interactions. The only notable side effect of *etravirine* is rash (17% vs. placebo value of 9%), usually occurring within a few weeks of starting therapy and resolving within 1 to 3 weeks if therapy is continued. Severe rash, including Stevens-Johnson syndrome and toxic epidermal necrolysis, have been reported (de Bethune, 2010).

Etravirine is an inducer of CYP3A4 and glucuronosyl transferases and an inhibitor of CYPs 2C9 and 2C19; it can therefore be involved in a number of clinically significant pharmacokinetic drug interactions. *Etravirine* can be combined with *darunavir/ritonavir*, *lopinavir/ritonavir*, and *saquinavir/ritonavir* without the need for dose adjustments. The dose of *maraviroc* should be doubled when these two drugs are combined. *Etravirine* should not be administered with *tipranavir/ritonavir*, *fosamprenavir/ritonavir*, or *atazanavir/ritonavir* in the absence of better data to guide dosing. *Etravirine* should not be combined with other NNRTIs. Unlike other NNRTIs, *etravirine* does not appear to alter the clearance of *methadone*.

Doravirine

Doravirine is a benzonitrile NNRTI with potent activity against HIV-1; Table 64–3 presents some of the drug's pharmacokinetic properties. The drug should be used only in combination with other effective agents and should not be added as the sole new agent to a failing regimen. *Doravirine* has the best side effect profile of available NNRTIs and has few or no CNS side effects. *Doravirine* retains efficacy against many HIV strains that are resistant to *efavirenz* and other earlier NNRTIs. However, treatment failure has been associated with the acquisition of V106I, H221Y, and F227C mutations (Blevins et al., 2020). *Doravirine* is approved only for treatment-naïve adults. *Doravirine* is also available as a once-daily, single-tablet coformulation with TDF and *lamivudine*.

ADME. Table 64–3 summarizes the pharmacokinetic data for *doravirine*. *Doravirine* is mainly metabolized by CYP3A4 and is not subject to significant renal clearance. Unlike *rilpivirine*, *doravirine* absorption is not significantly altered by coadministration with food or proton pump inhibitors (Blevins et al., 2020). The long elimination $t_{1/2}$ of 12 to 21 h allows once-daily dosing.

Untoward Effects and Drug Interactions. In comparative clinical studies, *doravirine* was better tolerated than *efavirenz* and had a side effect profile equivalent to or better than that of *darunavir/ritonavir*-containing regimens (Blevins et al., 2020). *Doravirine* is not teratogenic in animals but has not been adequately studied in pregnant women.

Coadministration of CYP inducers will reduce *doravirine* concentrations; thus, this drug should not be given with *rifampin*; anticonvulsants such as *carbamazepine*, *phenobarbital*, or *phenytoin*; or any products containing St. John's wort. *Doravirine* may be administered in patients with tuberculosis taking *rifabutin* if the *doravirine* dose is doubled to 100 mg twice per day (Blevins et al., 2020). At the 100-mg daily dose, *doravirine* is not a clinically significant inhibitor or inducer of hepatic enzymes. Unlike *rilpivirine*, *doravirine* does not significantly prolong the QTc interval.

Delavirdine

Delavirdine, a bisheteroarylpiperazine NNRTI that selectively inhibits HIV-1, is rarely used because it requires for thrice-daily dosing and is more toxic and less convenient than other NNRTIs. A more detailed description of this drug is available in previous editions of this book.

HIV Protease Inhibitors

Overview

The HIV PIs are peptide-like chemicals that competitively inhibit the action of the HIV aspartyl protease (Figure 64–6). This protease is a homodimer consisting of two 99-amino-acid monomers; each monomer contributes an aspartic acid residue that is essential for catalysis. The preferred cleavage site for this enzyme is the N-terminal side of proline residues, especially between phenylalanine and proline. Human aspartyl proteases (i.e., renin, pepsin, gastricsin, and cathepsins D and E) contain only one polypeptide chain and are not significantly inhibited by HIV PIs (Wensing et al., 2010).

Table 64–4 summarizes pharmacokinetic data for these agents. Clearance is mainly through hepatic oxidative metabolism. All except *nelfinavir* are metabolized predominantly by CYP3A4 (and *nelfinavir*'s major metabolite is cleared by CYP3A4). All approved HIV PIs have the potential for metabolic drug interactions. Most of these drugs inhibit CYP3A4 at clinically achieved concentrations, although the magnitude of inhibition varies greatly, with *ritonavir* by far the most potent. It is now a common practice to combine HIV PIs with a low dose of *ritonavir* or *cobicistat* to take advantage of either drug's remarkable capacity to inhibit CYP3A4-mediated metabolism.

"Boosting" PIs With a CYP Inhibitor. The metabolic clearance of HIV PIs is inhibited by *cobicistat*, a ritonavir analogue that lacks antiretroviral activity and that was developed for exclusive use as a pharmacokinetic enhancer (Sherman et al., 2015). *Cobicistat* is a potent and selective inhibitor of CYP3A4 that is better tolerated than *ritonavir* and is not a CYP enzyme inducer. Doses of *ritonavir* of 100 or 200 mg once or twice daily, or *cobicistat* 150 mg daily, are sufficient to inhibit CYP3A4 and increase ("boost") the concentrations of most concurrently administered CYP3A4 substrates. The enhanced pharmacokinetic profile of HIV PIs administered with *ritonavir* or *cobicistat* reflects inhibition of both first-pass and systemic clearance, resulting in improved oral bioavailability and a longer elimination $t_{1/2}$ of the coadministered drug. This allows a reduction in both drug dose and dosing frequency while increasing systemic concentrations. Combinations of either *atazanavir*, *darunavir*, *fosamprenavir*, or *lopinavir* with *ritonavir*, or *cobicistat* with *atazanavir* or *darunavir*, are approved for once-daily administration.

A Note on Cobicistat. *Cobicistat* selectively inhibits CYP3A4 with potency similar to that of *ritonavir* but without anti-HIV activity (Sherman et al., 2015). Additional potential advantages compared to *ritonavir* include greater specificity for CYP3A4, better tolerability, reduced impact on plasma lipids, and lack of P450 enzyme induction. However, *cobicistat* can inhibit some drug transport proteins and blocks the tubular transport of creatinine, resulting in a small and reversible increase in serum creatinine. *Cobicistat* is available only in coformulation with *elvitegravir*, *atazanavir*, or *darunavir*.

Other Shared Properties of PIs

Most HIV PIs are substrates for the P-glycoprotein efflux transporter (the multidrug resistance transporter, also known as MDR1 and ABCB1; see Chapter 4). These agents generally penetrate less well into semen than do NRTIs and NNRTIs. HIV PIs have high interindividual serum concentration variability that may reflect differential interactions with intestinal and hepatic CYPs and drug transport proteins. The speed with which HIV develops resistance to unboosted PIs is intermediate between that of nucleoside analogues and NNRTIs. Initial (primary) resistance mutations in the enzymatic active site confer only a 3- to 5-fold drop in sensitivity to most drugs; these are followed by secondary mutations often distant from the active site that compensate for the reduction in proteolytic efficiency. Accumulation of secondary resistance mutations increases the likelihood of cross-resistance to other PIs. This relatively favorable resistance profile makes HIV PIs attractive agents for use as second-line therapy in patients who have failed previous antiretroviral regimens (Wensing et al., 2010). Patients failing PI-containing regimens in the clinic often do so without any evidence of resistance to the PI in the regimen. Gastrointestinal side effects, including nausea, vomiting, and

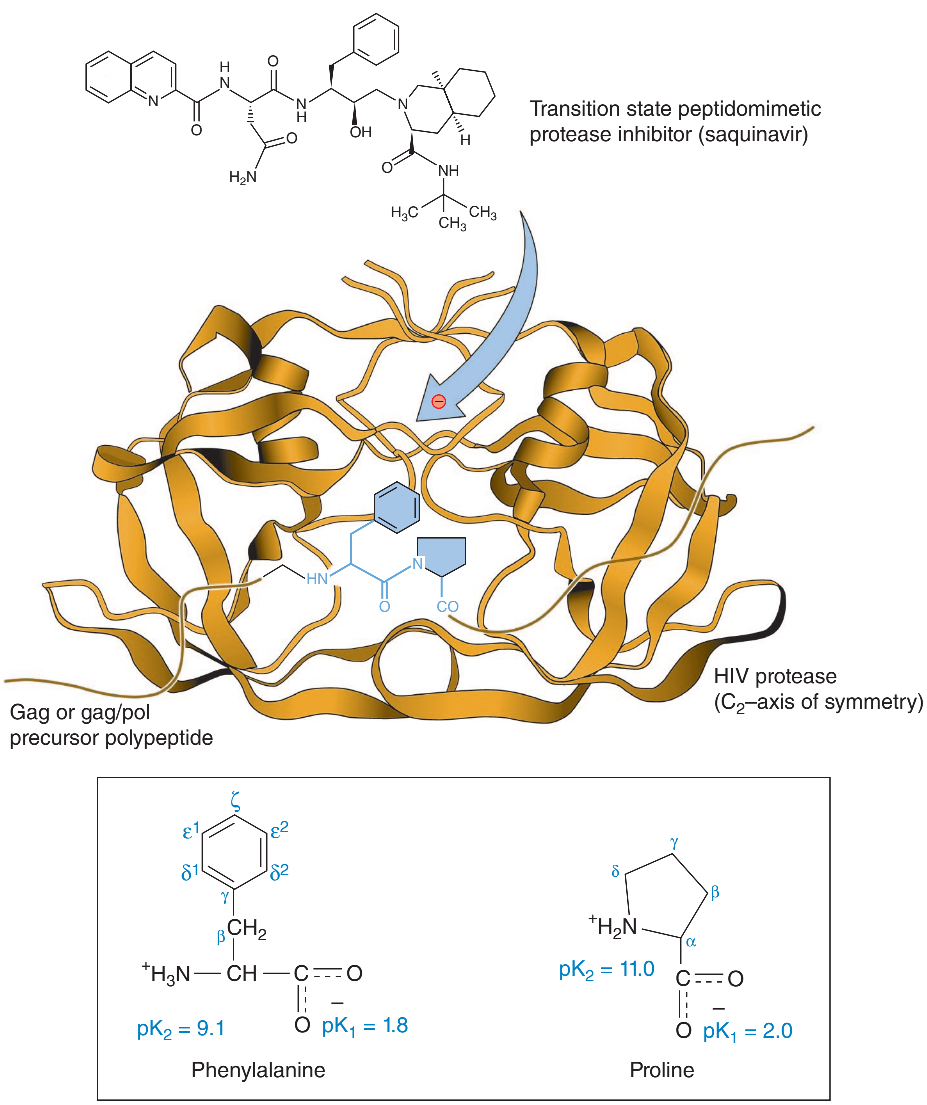


Figure 64–6 *Mechanism of action of an HIV PI.* Shown here is a phenylalanine-proline target peptide sequence (in blue) for the protease enzyme (in golden brown) with chemical structures of the native amino acids (in lower box) to emphasize homology of their structures to that of *saquinavir* (at top).

diarrhea, were common with older HIV PIs but are much less likely to occur with the newer drugs *atazanavir* and *darunavir*.

Ritonavir

Ritonavir is a peptidomimetic HIV PI designed to complement the C2 axis of symmetry of the enzyme active site. *Ritonavir* is active against both HIV-1 and HIV-2 (perhaps slightly less active against HIV-2). Today, *ritonavir* is mainly used as a pharmacokinetic enhancer (CYP3A4 inhibitor); the low doses used for this purpose are not known to induce *ritonavir* resistance mutations in HIV. The drug is used infrequently as the sole PI in combination regimens because of GI toxicity (Wensing et al., 2010).

ADME. Table 64–4 summarizes the pharmacokinetic profile of *ritonavir*. Interindividual variability in pharmacokinetics is high, with variability exceeding 6-fold in trough concentrations among patients given 600 mg of *ritonavir* every 12 h as capsules.

Untoward Effects and Drug Interactions. The major side effects of *ritonavir* are GI and include dose-dependent nausea, vomiting, diarrhea, anorexia, abdominal pain, and taste perversion. GI toxicity may be reduced if the drug is taken with meals. Peripheral and perioral paresthesias can occur at the therapeutic dose of 600 mg twice daily. These side effects generally abate within a few weeks of starting therapy. *Ritonavir* also causes dose-dependent elevations in serum total cholesterol and triglycerides, as well as other signs of lipodystrophy.

Ritonavir is one of the most potent known inhibitors of CYP3A4. Thus, *ritonavir* must be used with caution in combination with any CYP3A4 substrate and should not be combined with a drug that is a substrate for CYP3A and has a narrow therapeutic index, such as *midazolam, triazolam, fentanyl*, and ergot derivatives. *Ritonavir* is a mixed competitive and irreversible inhibitor of CYP3A4; its effects can persist for 2 to 3 days after the drug is discontinued (Washington et al., 2003). *Ritonavir* is also a weak inhibitor of CYP2D6. Potent inducers of CYP3A4 activity such as *rifampin* may lower *ritonavir* concentrations and should be avoided or the dosages adjusted. Capsule and solution formulations of *ritonavir* contain alcohol and should not be administered with *disulfiram* or *metronidazole*. *Ritonavir* is also a moderate inducer of CYP3A4, glucuronosyl-S-transferase, and possibly other hepatic enzymes and drug transport proteins. The concentrations of some drugs therefore will be decreased in the presence of *ritonavir*. *Ritonavir* reduces the AUC of *ethinyl estradiol* by 40%; thus, alternative forms of contraception should be used.

Use of Ritonavir as a CYP3A4 Inhibitor. *Ritonavir* inhibits the metabolism of all current HIV PIs and is frequently used in combination with most of these drugs (with the exception of *nelfinavir*) to enhance their

TABLE 64–4 ■ PHARMACOKINETIC PROPERTIES OF HIV-1 PROTEASE INHIBITORS[a]

PARAMETER	SAQUINAVIR[b]	INDINAVIR	RITONAVIR
Bioavailability (oral), %	13	60–65	>60
Effect of meals on AUC	↑ 570% (high fat)	↓ 77% (high fat)	↑ 13% (capsule)
Plasma $t_{1/2}$, h	1–2	1.8	3–5
Plasma protein binding, %	98	60	98–99
Metabolism by CYPs	3A4	3A4	3A4 > 2D6
Autoinduction of metabolism	No	No	Yes
Renal excretion of parent drug, %	<3	9–12	3.5
Inhibition of CYP3A4	+	++	+++

PARAMETER	NELFINAVIR	FOSAMPRENAVIR	LOPINAVIR[c]
Bioavailability (oral), %	20–80 (formulation and food dependent)	ND	ND
Effect of meals on AUC	↑ 100%–200%	↔	↑ 27% (moderate fat)
Plasma $t_{1/2}$, h	3.5–5	7.7	5–6
Plasma protein binding, %	>98	90	98–99
Metabolism by CYPs	2C19 > 3A4	3A4	3A4
Autoinduction of metabolism	Yes	No	Yes
Renal excretion of parent drug, %	1–2	1	<3
Inhibition of CYP3A4	++	++	+++

PARAMETER	ATAZANAVIR	TIPRANAVIR	DARUNAVIR
Bioavailability (oral), %	ND	ND	82
Effect of meals on AUC	↑ 70% (light meal)	↔	↑ 30%
Plasma $t_{1/2}$, h	6.5–7.9	4.8–6.0	15
Plasma protein binding, %	86	99.9	95
Metabolism by CYPs	3A4	3A4	3A4
Autoinduction of metabolism	No	Yes	ND
Renal excretion of parent drug, %	7	0.5	8
Inhibition of CYP3A4	++	+++	+++

↑, increase; ↓, decrease; ↔, no effect; ND, not determined; +, weak; ++, moderate; +++, substantial.

[a] Reported mean values in adults with normal renal and hepatic function.

[b] Parameters reported for the saquinavir soft-gel capsule formulation.

[c] Values for lopinavir, tipranavir, and darunavir reflect coadministration with ritonavir.

pharmacokinetic profiles and allow a reduction in their doses and dosing frequencies. *Ritonavir* also overcomes the deleterious effect of food on *indinavir* bioavailability. Low doses of *ritonavir* (100 or 200 mg once or twice daily) are just as effective at inhibiting CYP3A4 and are much better tolerated than the 600-mg twice-daily dose (Wensing et al., 2010).

Lopinavir

Lopinavir is structurally similar to *ritonavir* but is 3- to 10-fold more potent against HIV-1. This agent is active against both HIV-1 and HIV-2. *Lopinavir* is available only in coformulation with low doses of *ritonavir* (as a CYP3A4 inhibitor). *Lopinavir* has antiretroviral activity comparable with that of other potent HIV PIs and better than that of *nelfinavir*. *Lopinavir* also has considerable and sustained antiretroviral activity in patients who have failed previous HIV PI-containing regimens (Wensing et al., 2010).

Treatment-naïve patients who fail a first regimen containing *lopinavir* generally do not have detectable HIV protease mutations but may have genetic resistance to the other drugs in the regimen (Wensing et al., 2010). For treatment-experienced patients, accumulation of four or more HIV PI resistance mutations is associated with a reduced likelihood of virus suppression after starting *lopinavir*.

ADME. Table 64–4 summarizes the pharmacokinetic profile of this agent. The adult *lopinavir/ritonavir* dose is 400/100 mg (2 tablets) twice daily or 800/200 mg (4 tablets) once daily. *Lopinavir/ritonavir* should not be dosed once daily in treatment-experienced patients. *Lopinavir/ritonavir* is approved for use in pediatric patients aged 14 days or older, with dosing based on either weight or body surface area. A pediatric tablet formulation is available for use in children older than 6 months of age who reliably demonstrate the capacity to swallow the intact tablet; a pediatric-friendly *lopinavir/ritonavir* oral solution is also available. *Lopinavir* is absorbed rapidly after oral administration. Food has a minimal effect on bioavailability. Although the tablets contain *lopinavir/ritonavir* in a fixed 4:1 ratio, the observed plasma concentration ratio for these two drugs following oral administration is nearly 20:1, reflecting the sensitivity of *lopinavir* to the inhibitory effect of *ritonavir* on CYP3A. Both *lopinavir* and *ritonavir* are highly bound to plasma proteins, mainly to α_1-acid glycoprotein, and have a low fractional penetration into CSF and semen.

Untoward Effects and Drug Interactions. The most common adverse events reported with the *lopinavir/ritonavir* coformulation are GI: loose stools, diarrhea, nausea, and vomiting. Laboratory abnormalities include elevated total cholesterol and triglycerides. It is unclear whether these side effects are due to *ritonavir*, *lopinavir*, or both.

Concomitant administration of agents that induce CYP3A4, such as *rifampin*, may lower plasma *lopinavir* concentrations considerably. St. John's wort is a known inducer of CYP3A4, leading to lower concentrations of *lopinavir* and possible loss of antiviral effectiveness. Coadministration of other antiretrovirals that can induce CYP3A4 (e.g., *amprenavir*, *nevirapine*, or *efavirenz*) may require increasing the dose of *lopinavir*. The liquid formulation of *lopinavir* contains 42% ethanol and should not be administered with *disulfiram* or *metronidazole*. *Ritonavir* is also a moderate CYP inducer at the dose employed in the coformulation and can adversely decrease concentrations of some coadministered drugs (e.g., oral contraceptives). There is no direct proof that *lopinavir* is a CYP inducer *in vivo*; however, concentrations of some coadministered drugs (e.g., *amprenavir* and *phenytoin*) are lower with the *lopinavir/ritonavir* coformulation than would have been expected with low-dose *ritonavir* used alone or in combinations with other PIs.

Atazanavir

Atazanavir is an azapeptide PI that is active against both HIV-1 and HIV-2. A coformulation with *cobicistat* is also available.

Therapeutic Use. *Atazanavir*, with or without *ritonavir*, is approved for treatment of adults and pediatric patients older than 3 months of age and weighing at least 5 kg; in the pediatric population, dosing is based on weight. In treatment-experienced adult patients, *atazanavir* 400 mg once daily without *ritonavir* was inferior to the *lopinavir/ritonavir* coformulation given twice daily. The combination of *atazanavir* and low-dose *ritonavir* had a similar viral load effect as the *lopinavir/ritonavir* coformulation in one study, suggesting that this drug should be combined with *ritonavir* or *cobicistat* in treatment-experienced patients and perhaps in treatment-naïve patients with high baseline viral loads. The primary *atazanavir* resistance mutation occurs at HIV protease codon 50 and confers about 9-fold decreased susceptibility. High-level resistance is more likely if five or more additional mutations are present (Wensing et al., 2010).

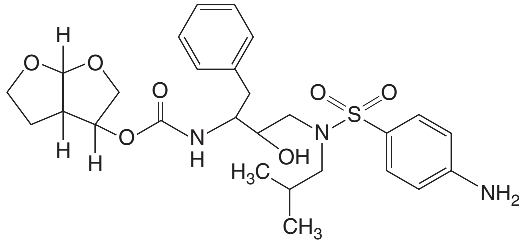
ADME. Table 64–4 summarizes the pharmacokinetic profile of this agent. *Atazanavir* is absorbed rapidly after oral administration. A light meal increases the AUC; thus, the drug should be administered with food. Absorption is pH dependent, and proton pump inhibitors or other acid-reducing agents substantially reduce *atazanavir* concentrations after oral dosing (Wensing et al., 2010). The mean elimination $t_{1/2}$ of *atazanavir* increases with dose, from 7 h at the standard 400-mg once-daily dose to nearly 10 h at a dose of 600 mg. The recommended *atazanavir* dose is 400 mg once daily in adults if given without a pharmacokinetic enhancer (*ritonavir* or *cobicistat*) and 300 mg if given with *ritonavir* 100 mg or *cobicistat* 150 mg. The drug is present in CSF at less than 3% of plasma concentrations but has excellent penetration into seminal fluid.

Untoward Effects and Drug Interactions. *Atazanavir* frequently causes unconjugated hyperbilirubinemia due to inhibition of UGT1A1, although this is not associated with hepatotoxicity. Postmarketing reports include hepatic adverse reactions of cholecystitis, cholelithiasis, cholestasis, and other hepatic function abnormalities. *Atazanavir* can precipitate in urine, causing increased risk of kidney stones. Other side effects reported with *atazanavir* include diarrhea and nausea, mainly during the first few weeks of therapy. Overall, 6% of patients discontinue *atazanavir* because of side effects during 48 weeks of treatment. Patients treated with *atazanavir* have significantly lower fasting triglyceride and cholesterol concentrations than patients treated with *nelfinavir, lopinavir*, or *efavirenz*.

Because *atazanavir* is metabolized by CYP3A4, concomitant administration of agents that induce CYP3A4 (e.g., *rifampin*) is contraindicated. *Atazanavir* is also a moderate inhibitor of CYP3A4 and may alter plasma concentrations of other CYP3A4 substrates. *Atazanavir* is a moderate UGT1A1 inhibitor and increases the *raltegravir* AUC by 41% to 72%. *Ritonavir* significantly increases the *atazanavir* AUC and reduces *atazanavir* systemic clearance. Concomitantly administered proton pump inhibitors reduce *atazanavir* concentrations substantially. Proton pump inhibitors and H_2 receptor antagonists should be avoided in patients receiving *atazanavir* without *ritonavir* (Wensing et al., 2010).

Darunavir

Darunavir is a nonpeptidic PI that is active against both HIV-1 and HIV-2. *Darunavir* binds tightly but reversibly to the active site of HIV protease but has also been shown to prevent protease dimerization. At least three *darunavir*-associated resistance mutations are required to confer resistance (Wensing et al., 2010). *Darunavir* in combination with *ritonavir* or *cobicistat* is approved for use in HIV-infected adults and children older than 3 years old. Coformulations with *cobicistat* or with *cobicistat*, TAF, and *emtricitabine* are also available.



DARUNAVIR

ADME. Table 64–4 presents some pharmacokinetic data on this agent. *Darunavir/ritonavir* can be used as a once-daily (800/100-mg) regimen with nucleosides in treatment-naïve adults or as a twice-daily (600/100-mg) regimen in treatment-experienced adults with at least one *darunavir*-associated resistance mutation; this drug should be taken with food. Pediatric dosing is based on body weight. *Darunavir* is absorbed rapidly after oral coadministration with *ritonavir*, with peak concentrations occurring after 2 to 4 h. *Ritonavir* increases *darunavir* bioavailability by up to 14-fold. When combined with *ritonavir*, the mean elimination $t_{1/2}$ of *darunavir* is about 15 h, and the AUC is increased by an order of magnitude; *cobicistat* produces similar results.

Untoward Effects and Drug Interactions. Because *darunavir* must be combined with *cobicistat* or a low dose of *ritonavir*, drug administration can be accompanied by all of the side effects caused by those agents, including drug interactions and GI complaints in up to 20% of patients taking *ritonavir*. *Darunavir*, like *fosamprenavir*, contains a sulfa moiety, and rash has been reported in up to 10% of recipients. *Darunavir/ritonavir* is associated with increases in plasma triglycerides and cholesterol, although the magnitude of increase is lower than that seen with *lopinavir/ritonavir*. *Darunavir* has been associated with episodes of hepatotoxicity.

Because *darunavir* is metabolized by CYP3A4, concomitant administration of agents that induce CYP3A4 (e.g., *rifampin*) is contraindicated. The drug interaction profiles of *darunavir/ritonavir* or *darunavir/cobicistat* are dominated by those expected with the pharmacokinetic enhancer. *Darunavir/ritonavir* 600/100 twice daily increases the *maraviroc* AUC by 340%; the *maraviroc* dose should be reduced to 150 mg twice daily when combined *with darunavir* (Wensing et al., 2010).

Older Protease Inhibitors

Saquinavir, indinavir, nelfinavir, fosamprenavir, and *tipranavir* are rarely used because of greater toxicity and intolerability as compared to other PIs. Detailed information about these drugs is available in previous editions of this book.

Entry Inhibitors

Overview

The entry of HIV into targets cells involves sequential binding of the virus to both a main receptor (CD4) and a chemokine co-receptor (CCR5 or CXCR4), followed by membrane fusion and release of virion contents into the cytoplasm (see Figure 64–1). This creates several targets for pharmacologic intervention (Tilton and Doms, 2010).

The four drugs available in this class have different mechanisms of action (see Figure 64–1). *Fostemsavir* binds to the virus envelope protein gp120 and prevents binding to CD4. *Ibalizumab* binds to host CD4 and thus prevents virus from entering target cells. *Maraviroc* is a chemokine receptor antagonist and binds to the host cell CCR5 receptor to block binding of viral gp120. *Enfuvirtide* inhibits fusion of the viral and cell membranes mediated by gp41 and CD4 interactions (Tilton and Doms, 2010).

Fostemsavir

Fostemsavir is a methylphosphate prodrug of *temsavir*. *Fostemsavir* is rapidly hydrolyzed to the parent compound, which binds to the viral envelope protein gp120, preventing attachment to CD4 and subsequent cell entry. *Fostemsavir* is approved only for use in heavily treatment-experienced adults with multidrug-resistant HIV-1 infection. It retains activity against viruses that have become resistant to antiretroviral agents of other classes because of its unique mechanism of action. Given its relatively short $t_{1/2}$ (7–14 h), *fostemsavir* must be given twice daily. Neither food nor increased gastric pH significantly alters the plasma pharmacokinetic (PK) profile of *fostemsavir* (Hiryak and Koren, 2021).

Fostemsavir resistance is complex and related to polymorphisms in the C1–C5 domains of gp120 (Hiryak and Koren, 2021). Amino acid substitutions that contribute to resistance include S375T, M426L, M434I, S375H/M, and L116P; M426L can be found in up to 15% of populations that have not been treated with *fostemsavir*. In clinical trials in heavily treatment-experienced patients, suppression rates at 48 weeks ranged from 38% to 54%, and 51% of subjects with virologic failure in randomized cohorts had treatment-emergent *temsavir* resistance mutations (Hiryak and Koren, 2021). This may reflect the difficulty of finding effective agents to use in combination with *fostemsavir* or nonadherence issues common in such patients.

ADME. *Temsavir* metabolism is complex and includes contribution from both esterases and CYP3A4. Fifty-one percent of *temsavir* is excreted unchanged in the urine. No clinically significant changes in pharmacokinetic parameters are seen in patients with renal or hepatic impairment, and no dose adjustments are needed in such patients (Hiryak and Koren, 2021).

Untoward Effects and Drug Interactions. *Fostemsavir* is generally well tolerated, but its use has been associated with elevations of hepatic transaminases, particularly in patients with concurrent HBV or HCV infection. In addition, high doses of *fostemsavir* (four times the approved daily dose of 600 mg twice per day) cause QTc prolongation. This drug should therefore be used with caution in patients at risk of QT-associated arrhythmias.

Temsavir is a CYP3A4 and P-glycoprotein substrate and is susceptible to PK drug interactions involving hepatic enzyme inhibitors or inducers. *Fostemsavir* should not be combined with *rifampin* or other potent CYP inducers, but it can be combined with *rifabutin* in patients with tuberculosis. *Temsavir* inhibits OATP1B1 and OATP1B3 and should not be combined with statins that are substrates for these transporters.

Ibalizumab

Ibalizumab is a humanized monoclonal antibody that binds to the CD4 extracellular domain 2, causing steric hindrance of a conformational change in the gp120-CD4 complex necessary for HIV fusion and entry (Chahine and Durham, 2021). *Ibalizumab* does not inhibit gp120 attachment to CD4. The *ibalizumab* binding site on CD4 is separate from the major histocompatibility complex class II binding site, and thus, *ibalizumab* does not interfere with host immune responses. *Ibalizumab* is approved for use in heavily treatment-experienced adults with multidrug-resistant HIV-1 infection who are failing their current antiretroviral regimen. The drug must be given as an intravenous infusion every 2 weeks, limiting its use to this small population of patients (Chahine and Durham, 2021). *Ibalizumab* retains activity against viruses that have become resistant to antiretroviral agents of other classes because of its unique mechanism of action.

Resistance to *ibalizumab* can precede exposure to the drug and can develop rapidly if the drug is not combined with other active antiretroviral agents. Resistance is mediated by decreased expression of N-linked glycosylation sites in the V5 loop of gp120 (Chahine and Durham, 2021). *Ibalizumab* has been studied only in small groups of heavily treatment-experienced patients. In one small study, when *ibalizumab* was combined with an optimized background regimen, the percentage of patients with an undetectable viral load (<50 copies/mL of HIV RNA) at week 48 ranged from 47% to 56%, and at week 96, it was 52% (Chahine and Durham, 2021).

Untoward Effects and Drug Interactions. *Ibalizumab* is generally well tolerated with few side effects. The most common adverse effects reported in clinical trials were diarrhea (8%), dizziness (8%), nausea (5%), and rash (5%). Antidrug antibody responses were rarely seen in clinical trial participants and appeared to have no clinical consequences (Chahine and Durham, 2021). Based on its pharmacologic properties, no drug-drug interactions are expected with *ibalizumab*.

Maraviroc

Maraviroc blocks the binding of the HIV outer envelope protein gp120 to the CCR5 chemokine co-receptor (Figure 64–7). *Maraviroc* is approved for use in HIV-infected adults who have baseline evidence of predominantly CCR5-tropic virus. The drug has no activity against viruses that are CXCR4-tropic or dual-tropic (Tilton and Doms, 2010). *Maraviroc* retains activity against viruses that have become resistant to antiretroviral agents of other classes because of its unique mechanism of action.

HIV can develop resistance to this drug through two distinct pathways. A patient starting *maraviroc* therapy with HIV that is predominantly CCR5-tropic may experience a shift in tropism to CXCR4 or dual/mixed tropism predominance. This is especially likely in patients harboring low-level but undetected CXCR4- or dual/mixed-tropic virus prior to initiation of *maraviroc*. Alternatively, HIV can retain its CCR5 tropism but gain resistance to the drug through specific mutations in the V3 loop of gp120 that allow virus binding to CCR5 in the presence of inhibitor (Kuritzkes, 2011).

ADME. *Maraviroc* is the only antiretroviral drug approved at three different starting doses, depending on concomitant medications. When combined with most CYP3A inhibitors, the starting dose is 150 mg twice daily; when combined with most CYP3A inducers, the starting dose is 600 mg twice daily; for other concomitant medications, the starting dose is 300 mg twice daily. Once-daily *maraviroc* has been studied but is not FDA-approved. The oral bioavailability of *maraviroc*, 23% to 33%, is dose dependent. Food decreases bioavailability, but there are no food requirements for drug administration.

Untoward Effects and Drug Interactions. *Maraviroc* is generally well tolerated but can cause dose- and concentration-dependent orthostatic hypotension. Although one case of serious hepatotoxicity with allergic features has been reported, significant (grade 3 or 4) hepatotoxicity was no more frequent with *maraviroc* than with placebo in controlled trials.

Maraviroc is a CYP3A4 substrate and susceptible to pharmacokinetic drug interactions involving CYP3A4 inhibitors or inducers. *Maraviroc* itself is not a CYP inhibitor or inducer *in vivo*, although high-dose *maraviroc* (600 mg daily) can increase concentrations of the CYP2D6 substrate *debrisoquine*.

Enfuvirtide

Enfuvirtide is a 36-amino-acid synthetic peptide that is not active against HIV-2 but is broadly effective against laboratory and clinical isolates of HIV-1. *Enfuvirtide* is FDA-approved for use only in treatment-experienced adults and children weighing 11 kg or more who have evidence of HIV replication despite ongoing antiretroviral therapy. The drug's cost and

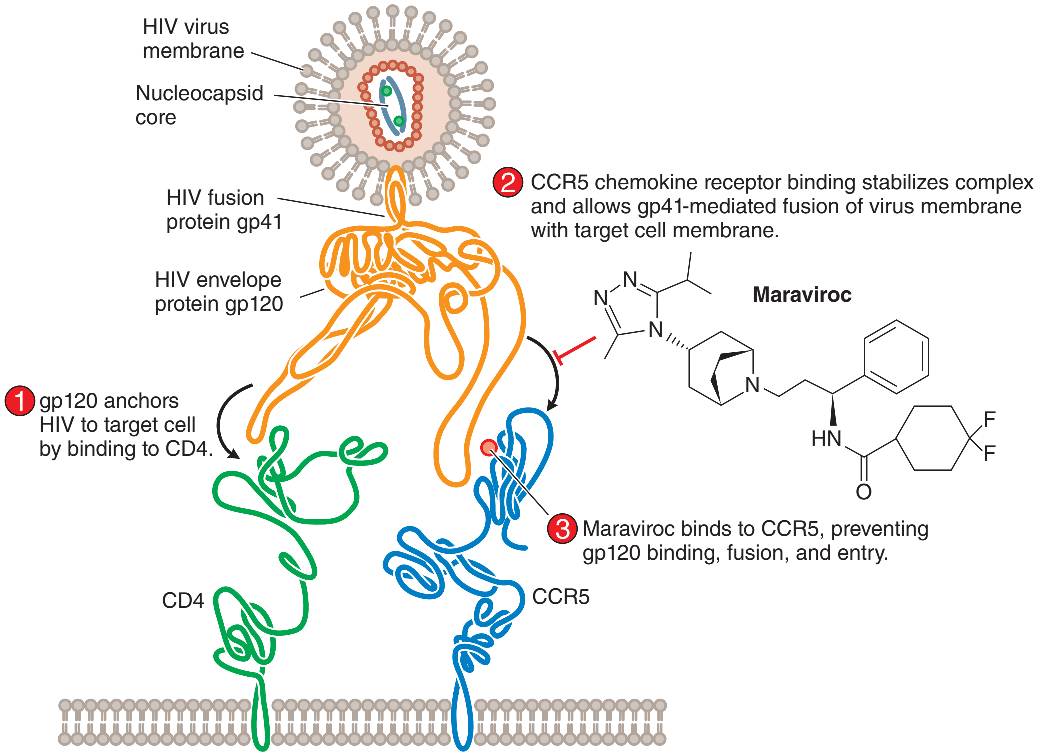

HIV virus membrane
Nucleocapsid core
HIV fusion protein gp41
HIV envelope protein gp120
1 gp120 anchors HIV to target cell by binding to CD4.
2 CCR5 chemokine receptor binding stabilizes complex and allows gp41-mediated fusion of virus membrane with target cell membrane.
Maraviroc
N
N
N
N
HN
O
F
F
3 Maraviroc binds to CCR5, preventing gp120 binding, fusion, and entry.
CD4
CCR5

Figure 64–7 *Mechanism of action of the HIV entry inhibitor maraviroc.*

route of administration (subcutaneous injection twice daily) limit its use to those with no other treatment options (Tilton and Doms, 2010).

Mechanisms of Action and Resistance. The amino acid sequence of *enfuvirtide* derives from the transmembrane gp41 region of HIV-1 that is involved in fusion of the viral membrane lipid bilayer with that of the host cell membrane. The peptide blocks the interaction between the N36 and C34 sequences of the gp41 glycoprotein by binding to a hydrophobic groove in the N36 coil. This prevents formation of a six-helix bundle critical for membrane fusion and viral entry into the host cell (Tilton and Doms, 2010). *Enfuvirtide* inhibits infection of CD4$^+$ cells by free virus particles. *Enfuvirtide* retains activity against viruses that have become resistant to antiretroviral agents of other classes. HIV can develop resistance to this drug through specific mutations in the *enfuvirtide*-binding domain of gp41.

ADME. The bioavailability of subcutaneous *enfuvirtide* is 84% compared with an intravenous dose. Pharmacokinetics of the subcutaneous drug are not affected by site of injection. The major route of elimination for *enfuvirtide* has not been determined. The mean elimination $t_{1/2}$ of parenteral drug is 3.8 h, necessitating twice-daily administration, which may create adherence issues for the patient.

Untoward Effects and Drug Interactions. The most prominent adverse effects of *enfuvirtide* are injection-site reactions. Most patients (98%) develop local side effects, including pain, erythema, and induration at the site of injection; 80% of patients develop nodules or cysts. Use of *enfuvirtide* has been associated with a higher incidence of lymphadenopathy and pneumonia. *Enfuvirtide* is not known to alter the concentrations of any coadministered drugs.

Integrase Inhibitors

Overview

Chromosomal integration is a defining characteristic of retrovirus life cycles and allows viral DNA to remain in the host cell nucleus for a prolonged period of inactivity or latency (Figure 64–1). Because human DNA is not known to undergo excision/reintegration, this process is an excellent target for selective antiviral intervention. The HIV integrase inhibitors prevent formation of covalent bonds between host and viral DNA—a process known as strand transfer—presumably by interfering with essential divalent cations in the enzyme's catalytic core (Figure 64–8). In clinical trials, HIV integrase inhibitors produce a more rapid decline in plasma viral RNA over the first 3 to 4 months of therapy than other antiretroviral agents and are generally better tolerated than comparator agents (Scarsi et al., 2020). Because of their unique mechanism of action, these agents retain activity against viruses that have become resistant to antiretroviral agents of other classes.

Integrase inhibitors have radically altered strategies for HIV treatment, given their high degree of efficacy and tolerability and very low propensity for selecting drug-resistant mutations. The coformulation of *dolutegravir* and *lamivudine* is the only two-drug regimen approved for first-line use in treatment-naïve patients.

Raltegravir

Raltegravir blocks the catalytic activity of the HIV-encoded integrase, preventing integration of viral DNA into the host chromosome (see Figure 64–8). *Raltegravir* has potent activity against both HIV-1 and HIV-2. *Raltegravir* is approved for use in HIV-infected adults and children weighing 2 kg or more. A 400-mg twice-daily oral formulation and a 1200-mg once-daily oral formulation are both available, but the once-daily formulation should be used only in treatment-naïve patients or those already virologically suppressed on the twice-daily formulation.

ADME. Peak concentrations of *raltegravir* occur about 1 h after oral dosing. Elimination is biphasic, with an α phase $t_{1/2}$ of about 1 h and a terminal β phase $t_{1/2}$ of about 12 h, with the α phase predominating. The pharmacokinetics of *raltegravir* are highly variable. Moderate- and high-fat meals increase *raltegravir*'s AUC by as much as 2-fold; a low-fat meal decreases AUC modestly (↓ 46%); however, there are no food requirements for *raltegravir* administration. The drug is 83% protein bound in human plasma. *Raltegravir* is eliminated mainly via glucuronidation by UGT1A1 (Scarsi et al., 2020).

Untoward Effects and Drug Interactions. *Raltegravir* generally is well tolerated, with little clinical toxicity. The most common complaints

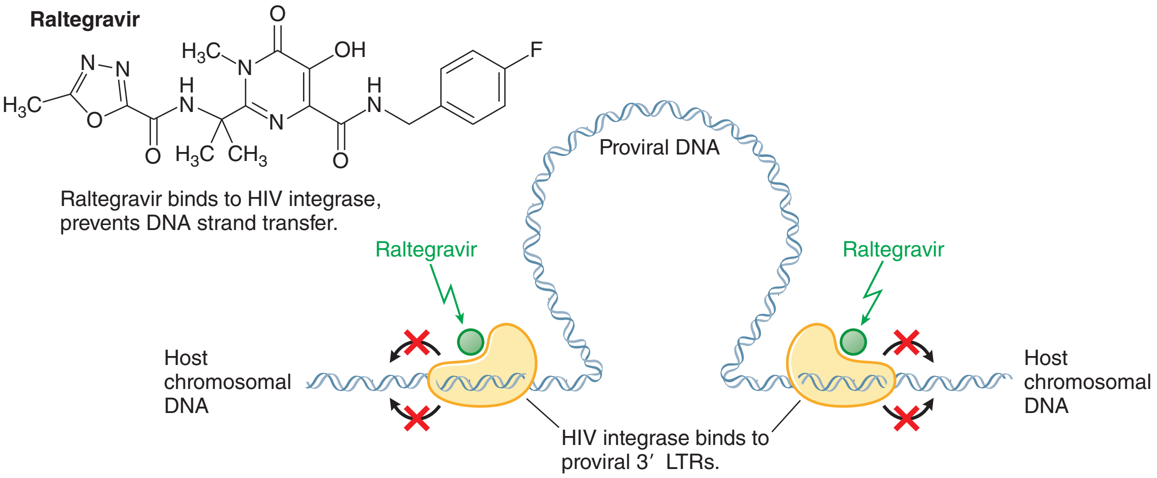

Raltegravir
Raltegravir binds to HIV integrase, prevents DNA strand transfer.
Raltegravir
Raltegravir
Proviral DNA
Host chromosomal DNA
Host chromosomal DNA
HIV integrase binds to proviral 3′ LTRs.

Figure 64–8 *Mechanism of action of the HIV integrase inhibitor raltegravir.*

are headache, nausea, asthenia, and fatigue, which are also associated with coadministered NRTIs. Creatine kinase elevations, myopathy, and rhabdomyolysis have been reported, as has exacerbation of depression, but their relationship to *raltegravir*'s use is not clear (Scarsi et al., 2020). *Raltegravir* is rarely associated with DRESS (Ripamonti et al., 2014).

As a UGT1A1 substrate, *raltegravir* is susceptible to pharmacokinetic drug interactions involving inhibitors or inducers of this enzyme. *Atazanavir*, a moderate UGT1A1 inhibitor, increases *raltegravir*'s AUC by 41% to 72%. *Tenofovir* increases *raltegravir*'s AUC by 49%, but the mechanism for this interaction is unknown. When *raltegravir* is combined with the CYP inducer *rifampin*, the *raltegravir* dose should be doubled to 800 mg twice daily. *Raltegravir* has little effect on the pharmacokinetics of coadministered drugs. Because the mechanism of action of HIV integrase strand transfer inhibitors involves chelation with a divalent cation (Mg^{2+}) in the enzyme active site, chelation by divalent cations may occur in the GI tract following oral administration. To minimize this interaction, any use of magnesium- or aluminum-containing antacids and *sucralfate* must occur 6 h before or 2 h after *raltegravir*. Administration of calcium- and iron-containing supplements should also be separated from *raltegravir* by at least 2 h, unless given with food (Scarsi et al., 2020).

Elvitegravir

Elvitegravir blocks the catalytic activity of the HIV-encoded integrase, preventing integration of viral DNA into the host chromosome for both HIV-1 and HIV-2. *Elvitegravir* retains activity against viruses that have become resistant to antiretroviral agents of other classes but shares cross-resistance with mutations affecting the HIV integrase inhibitor *raltegravir* (Scarsi et al., 2020). *Elvitegravir* is available only in single-tablet, once-daily coformulations with *cobicistat*, *emtricitabine*, and *tenofovir disoproxil fumarate* (STRIBILD) or *tenofovir alafenamide* (Genvoya). These coformulations are approved for use in HIV-infected adults and children older than 12 years of age.

ADME. Peak concentrations of *elvitegravir* occur about 4 h after oral dosing. The plasma $t_{1/2}$ of *elvitegravir* (administered as STRIBILD) is approximately 13 h. Because light or high-fat meals increase the AUC of *elvitegravir* by 34% or 87%, respectively, *elvitegravir* coformulations should be given with food. The drug is largely (98%–99%) protein bound in human plasma. Unlike *raltegravir* and *dolutegravir*, *elvitegravir* is eliminated mainly by CYP3A4, thus explaining the substantial pharmacokinetic benefit of combining it with *cobicistat*. *Elvitegravir* coformulations should not be given to patients with baseline estimated creatinine clearance below 70 mL/min (for STRIBILD) or 30 mL/min (for Genvoya) because of the potential for reduced clearance of the NRTI components of the tablet (Scarsi et al., 2020).

Untoward Effects and Drug Interactions. Like other HIV integrase inhibitors, *elvitegravir* is well tolerated. In clinical trials, the only side effects reported in more than 10% of *elvitegravir* recipients were nausea and diarrhea; fewer than 1% of patients discontinued treatment due to adverse drug effects.

Although *elvitegravir* by itself is not likely to cause clinically significant drug-drug interactions, the drug interaction profile of the coformulation is dominated by the effects of *cobicistat*, a potent inhibitor of CYP3A4 and a weak inhibitor of CYP2D6. Caution is warranted in administering *elvitegravir* coformulations with other CYP3A4 substrates with low therapeutic indices or with CYP3A4 inducers that could lower concentrations of *cobicistat* or *elvitegravir*. This includes rifamycins and St. John's wort. As with other integrase inhibitors, *elvitegravir* coformulations are subject to similar restrictions for coadministration of oral divalent cations (Mg^{2+}, Al^{2+}, Ca^{2+}, Fe^{2+}). Because *tenofovir* plus *emtricitabine* is effective suppressive therapy for chronic HBV infection (see Chapter 63), these coformulations should not be discontinued abruptly in coinfected patients because of the risk of rebound HBV replication and acute liver damage.

Dolutegravir

Dolutegravir blocks the catalytic activity of the HIV-encoded integrase of HIV-1 and HIV-2, preventing integration of viral DNA into the host chromosome. *Dolutegravir* retains activity against some viruses that are resistant to *raltegravir* or *elvitegravir*. *Dolutegravir* is approved for use in HIV-infected adults and children older than 4 weeks of age and weighing at least 3 kg. *Dolutegravir* is also available as a single-tablet, once-daily coformulation with *lamivudine*, with *abacavir* and *lamivudine*, with *rilpivirine*, or in a generic form with TDF and *lamivudine*. Given its high efficacy, tolerability, and convenience, *dolutegravir* is increasingly popular as a starting agent in many parts of the world, especially in its generic form (Scarsi et al., 2020). Resistance to *dolutegravir* is rare, and most patients who fail a *dolutegravir*-containing regimen do so without evidence of *dolutegravir* resistance.

ADME. Peak concentrations of *dolutegravir* occur 3 to 4 h after oral dosing without regard to concurrent food. The drug is 99% protein bound in human plasma; its plasma elimination $t_{1/2}$ is about 12 h. Like *raltegravir*, *dolutegravir* is eliminated mainly by UGT1A1. Subjects with UGT1A1 slow metabolism genotypes had a 32% lower clearance of *dolutegravir* and 46% higher AUC, although this is not thought to have clinical significance (Scarsi et al., 2020).

Untoward Effects and Drug Interactions. Like other HIV integrase inhibitors, *dolutegravir* is generally well tolerated. *Dolutegravir*-containing regimens may be associated with significant long-term weight gain; this effect may be more prominent in women than in men and in individuals of African descent (Scarsi et al., 2020). One large observational study

TABLE 64–5 ■ PHARMACOKINETIC PROPERTIES OF INTEGRASE STRAND TRANSFER INHIBITORS[a]

PARAMETER	RALTEGRAVIR	ELVITEGRAVIR[b]	DOLUTEGRAVIR	BICTEGRAVIR
Oral bioavailability, %	NR	NR	NR	NR
Effect of meals on AUC	↔	↑ 34%–87%	↑ 33%–66%	↑ 24%
Plasma $t_{1/2}$, h	9	12.9	14	17.3
Plasma protein binding, %	83	99	98.9	>99
Metabolism by CYPs and UGT	UGT1A1	CYP3A4 (major) UGT1A1/3 (minor)	UGT1A1 > CYP3A4	CYP3A4, UGT1A1
Renal excretion of parent drug, %	9	6.7	<1%	NR
Inhibition of CYP3A	No	Yes	No	No

↑, increase; ↓, decrease; ↔, no effect; NR, not reported.

[a] Reported mean values in adults with normal renal and hepatic function.

[b] Values when elvitegravir is combined with the CYP3A4 inhibitor cobicistat.

found an increased risk of neural tube defects in infants whose mothers were taking *dolutegravir* at the time of conception; more recent data suggests that this risk—if real—is likely to be quite small (Scarsi et al., 2020). Hypersensitivity reactions, including rash and organ dysfunction, have been reported rarely (<1% of recipients). Because *dolutegravir* inhibits the renal organic cation transporter OCT2, it causes small increases in serum creatinine (0.1–0.2 mg/dL) that persist during therapy but are immediately reversible once the drug is stopped.

Dolutegravir has low potential to cause clinically significant drug-drug interactions. Its capacity to inhibit the renal organic cation transporter OCT2 likely explains why it doubles concentrations of coadministered *metformin*. *Dolutegravir* is susceptible to decreases in plasma concentrations caused by inducers of drug-metabolizing enzymes, and it should not be coadministered with *etravirine*, *nevirapine*, or St. John's wort. For some enzyme inducers such as *rifampin*, *efavirenz*, and *ritonavir*-boosted HIV PIs, the *dolutegravir* dose can be increased from 50 mg once daily to 50 mg twice daily to counter this effect (Dooley et al., 2013). Similar to other HIV integrase inhibitors, *dolutegravir* should be taken 2 h before or 6 h after cation-containing antacids or laxatives, *sucralfate*, or oral supplements containing Fe^{2+} or Ca^{2+}, unless combined with food (Scarsi et al., 2020). Because *lamivudine* is no longer considered adequate suppressive therapy for chronic HBV infection, the coformulation of *dolutegravir* with *abacavir* and *lamivudine* should not be considered adequate treatment of HBV in coinfected patients (see Chapter 63). If a patient is coinfected with HBV or becomes infected during treatment, abrupt discontinuation of any *dolutegravir* coformulation with *lamivudine* or *tenofovir* may result in rebound HBV replication and acute liver damage. The *dolutegravir/abacavir/lamivudine* combination should not be given to patients who are known to carry the HLA-B*5701 allele because of the risk of *abacavir* hypersensitivity.

Bictegravir

Bictegravir is a close structural analogue of *dolutegravir* and shares many of its pharmacological properties. *Bictegravir* blocks the catalytic activity of the HIV-encoded integrase of HIV-1 and HIV-2, preventing integration of viral DNA into the host chromosome. The drug retains activity against some viruses that are resistant to *raltegravir* or *elvitegravir*. *Bictegravir* is approved for use in HIV-infected adults who are treatment naïve or suppressed on current antiretroviral therapy without known resistance to *bictegravir*, *tenofovir*, or *emtricitabine*. *Bictegravir* is available only as a single-tablet, once-daily coformulation with *tenofovir alafenamide* and *emtricitabine*. Given its high efficacy, tolerability, and convenience, the *bictegravir* coformulation is popular as a starting agent in high-income countries (Scarsi et al., 2020). Resistance to *bictegravir* is rare, and most patients who fail a *bictegravir*-containing regimen do so without evidence of *bictegravir* resistance.

ADME. Peak concentrations of *bictegravir* occur 2 to 4 h after oral dosing with or without food. The drug is 99% protein bound in human plasma; its plasma elimination $t_{1/2}$ is about 17 h. Like *dolutegravir*, *bictegravir* is eliminated mainly by CYP3A4 and UGT1A1.

Untoward Effects and Drug Interactions. Like other HIV integrase inhibitors, *bictegravir* is generally well tolerated. Because *bictegravir* inhibits the renal organic cation transporter OCT2, it can cause small increases in serum creatinine (0.1–0.2 mg/dL) that persist during therapy but are immediately reversible once the drug is stopped. Long-term *bictegravir* use has not been associated with significant weight gain, although studies of the drug in the international setting are limited. The safety of *bictegravir* in pregnancy is not yet established (Scarsi et al., 2020).

Bictegravir has low potential to cause clinically significant drug-drug interactions. It increases *metformin* concentrations (mean AUC) by about 39%, which is less than the effect seen with *dolutegravir* (79%). *Bictegravir* is more susceptible than *dolutegravir* to decreases in plasma concentrations caused by inducers of drug-metabolizing enzymes, and it should not be coadministered with rifamycins, *carbamazepine*, or St. John's wort (Scarsi et al., 2020). As with other HIV integrase inhibitors, *bictegravir* should be taken 2 h before or 6 h after cation-containing antacids or laxatives, *sucralfate*, or oral supplements containing Fe^{2+} or Ca^{2+}, unless given with food. If a patient is coinfected with HBV or becomes infected during treatment, abrupt discontinuation of *bictegravir/tenofovir alafenamide/emtricitabine* may result in rebound HBV replication and acute liver damage.

Long-Acting and Extended-Release Antiretroviral Formulations

Although oral antiretroviral therapy has been a huge success by any measure, 30% or more of HIV-infected persons do not maintain lifelong suppression of HIV with oral regimens, putting them at risk of morbidity and mortality and placing others at risk of drug-resistant infections. The major factor preventing more widespread therapeutic success is suboptimal adherence. Parenteral long-acting (LA) formulations could improve treatment success and help lower rates of HIV transmission. By analogy to other chronic conditions whose management has been transformed with LA formulations (e.g., hormonal contraception, schizophrenia, osteoporosis), LA formulations are often more convenient, are better tolerated, and can radically improve adherence as compared to daily oral therapy. Patient surveys consistently show a high level of interest in and preference for LA treatment regimens (Flexner, 2019).

Most now see InSTI-based oral coformulations as the pinnacle of safety, efficacy, convenience, and tolerability, and difficult to improve upon. Consequently, almost all antiretroviral agents in current development are LA/extended release, a fact likely to continue into the foreseeable future.

The two-drug combination of LA *cabotegravir* and LA *rilpivirine* was approved by the FDA in early 2021 for HIV treatment, and two other LA agents, islatravir and lenacapavir, are in phase III clinical trials (Thornhill and Orkin, 2021).

LA Cabotegravir

Cabotegravir is a close structural analogue of *dolutegravir* and shares many of its pharmacological properties. The chemical structure of *cabotegravir* makes it more suitable for nanoformulation and LA parenteral administration (Scarsi et al., 2020). LA *cabotegravir* is a nanocrystalline suspension of the integrase inhibitor *cabotegravir*, formulated for intramuscular injection. It is available only for combination administration with LA *rilpivirine* in adults with a chronically suppressed plasma HIV RNA (<50 copies/mL) who are stable on an antiretroviral regimen with no history of treatment failure and with no known or suspected resistance to either *cabotegravir* or *rilpivirine*. In two large, prospective, double-blind, randomized trials for HIV prevention, *cabotegravir* injected every 8 weeks was substantially more effective than daily oral coformulated TDF and *emtricitabine*, reducing infections by 68% in men and 89% in women as compared to standard of care.

Cabotegravir blocks the catalytic activity of the HIV-encoded integrase of HIV-1 and HIV-2, preventing integration of viral DNA into the host chromosome. *Cabotegravir* retains activity against viruses that have become resistant to antiretroviral agents of other classes and also remains active against some viruses that are resistant to *raltegravir* or *elvitegravir*. The initial approval of *cabotegravir* required administration of a 30-mg daily oral formulation combined with NRTIs or *rilpivirine* for 28 days to assure tolerability prior to starting injections. Recent data suggest that this oral lead-in may not be required in many settings (Thornhill and Orkin, 2021). Treatment failure with *cabotegravir* drug resistance was rare in clinical trials, but it did occur, and especially in those with subtype A virus.

ADME. Peak concentrations after beginning LA *cabotegravir* occur 7 days after injection. The drug is 99.8% protein bound in human plasma; its elimination $t_{1/2}$ as the injectable nanoformulation ranges from 5.6 to 11.5 weeks, although drug can be detected in many recipients for more than a year after a single injection. Like *dolutegravir*, *cabotegravir* is mainly metabolized by UGT1A1. Despite metabolism, 27% of an oral dose is eliminated unchanged in the urine. Because initial release of drug from the intramuscular depot is slow, a loading dose of 600 mg is required prior to beginning the monthly maintenance dose of 400 mg. Patients who expect to miss a scheduled injection by more than 7 days should resume oral therapy until the next injection.

Untoward Effects and Drug Interactions. The combination of injected *cabotegravir* and injected *rilpivirine* was generally well tolerated. More than 80% of study participants reported injection site reactions, but only 1% discontinued injections as a result. Other reported adverse effects included pyrexia, fatigue, headache, and musculoskeletal pain. Hepatotoxicity and depressive symptoms were also reported with the combination of LA *cabotegravir* and *rilpivirine*.

Cabotegravir is not known to cause clinically significant drug-drug interactions. However, *cabotegravir* is susceptible to decreases in plasma concentrations caused by inducers of drug-metabolizing enzymes, and it should not be coadministered with rifamycins, *carbamazepine*, St. John's wort, or other potent inducers. Neither *cabotegravir* nor *rilpivirine* has activity against hepatitis B virus; switching an HBV-coinfected patient from a *tenofovir*-containing regimen to the combination of injectable *cabotegravir* and *rilpivirine* may result in rebound HBV replication and acute liver damage.

LA Rilpivirine

Long-acting *rilpivirine* is a nanocrystalline suspension of the NNRTI *rilpivirine*, formulated for intramuscular injection. It is available only for combination administration with LA *cabotegravir* in adults with a chronically suppressed plasma HIV RNA (<50 copies/mL) who are stable on an antiretroviral regimen and with no history of treatment failure and known or suspected resistance to either *cabotegravir* or *rilpivirine*. Treatment failure with *rilpivirine* drug resistance was rare in clinical trials, but it did occur, especially in those with subtype A virus. For a more complete description of the pharmacology of *rilpivirine*, see the section on oral NNRTIs. *Rilpivirine* has no activity against HIV-2; thus, the combination of injectable *cabotegravir* and *rilpivirine* should not be used as treatment for those infected with HIV-2.

ADME. Peak concentrations after beginning LA *rilpivirine* occur 3 to 4 days after injection. The drug is 99.7% protein bound in human plasma; its elimination $t_{1/2}$ as the injectable nanoformulation ranges from 13 to 28 weeks, and drug can be detected in many recipients for more than a year after a single injection. *Rilpivirine* is mainly metabolized by CYP3A4. Because initial release of drug from the intramuscular depot is slow, a loading dose of 900 mg is required prior to beginning the monthly maintenance dose of 600 mg. Patients who expect to miss a scheduled injection by more than 7 days should resume oral therapy until the next injection.

Untoward Effects and Drug Interactions. The combination of injected *cabotegravir* and injected *rilpivirine* is generally well tolerated. More than 80% of patients report injection site reactions, but only 1% discontinue injections as a result. Adverse effects include pyrexia, fatigue, headache, and musculoskeletal pain. Hepatotoxicity and depressive symptoms are also reported. Hypersensitivity reactions including DRESS have been reported with *rilpivirine*-containing regimens. Rare but serious postinjection reactions have been reported within minutes after receiving LA *rilpivirine*; these have included dyspnea, agitation, abdominal cramping, flushing, sweating, oral numbness, and changes in blood pressure. These events occurred in less than 1% of subjects and began to resolve within a few minutes after the injection. Oral *rilpivirine* is known to produce dose- and concentration-dependent prolongation of the QTc interval, and caution is warranted in using this drug in those otherwise at risk of QT-associated arrhythmias.

Rilpivirine in its injectable form has low potential to cause clinically significant drug-drug interactions. However, *rilpivirine* is susceptible to decreases in plasma concentrations caused by inducers of drug-metabolizing enzymes and should not be coadministered with rifamycins, *carbamazepine*, St. John's wort, or other potent inducers. Neither *cabotegravir* nor *rilpivirine* has activity against hepatitis B virus; switching an HBV-coinfected patient from a *tenofovir*-containing regimen to the combination of injectable *cabotegravir* and *rilpivirine* may result in rebound HBV replication and acute liver damage.

LA Drugs and Formulations in Late-Stage Clinical Development

Two additional LA antiretroviral agents are in phase III clinical trials and likely to be approved as early as 2022. *Islatravir* is an NRTI with a unique mechanism of action. Because its ribose ring contains a 3′-hydroxyl group, it does not act directly as a chain terminator like other NRTIs; rather, it blocks HIV replication by inhibiting translocation of the RT/RNA duplex (Thornhill and Orkin, 2021). *Islatravir* is more potent than existing NRTIs, and its active metabolite, the intracellular triphosphate, has a $t_{1/2}$ of 78 to 128 h, making it suitable for LA strategies. *Islatravir* is under evaluation as a once-monthly oral preparation and a subcutaneous nonerodable implant formulation. The latter can deliver effective concentrations of drug for at least 12 months. Oral *islatravir* is well tolerated in daily combination therapy and has a high genetic barrier to drug resistance (Thornhill and Orkin, 2021).

Lenacapavir is a first-in-class inhibitor of assembly of the HIV capsid protein. Like *islatravir*, this drug is exceedingly potent and, when injected subcutaneously in seronegative volunteers, has a plasma half-life of 38 days (Thornhill and Orkin, 2021). *Lenacapavir* is under evaluation for HIV treatment and prevention at a subcutaneous dose of 900 mg every 6 months. Because of its unique mechanism of action, *lenacapavir* remains active against virus isolates that have developed resistance to any other antiretroviral drug class.

Several other LA drugs and formulations are in advanced clinical development. This includes a number of *broadly neutralizing antiretroviral monoclonal antibodies* that can produce a significant reduction in plasma HIV RNA and may be able to control HIV replication long term if used in combination. A leucine-serine (LS) mutation in the Fc binding domain of these antibodies can extend their plasma half-life to more than

2 months, allowing intravenous administration as infrequently as once every 6 months (Thornhill and Orkin, 2021).

Future Treatment Guidelines

Several expert panels issue periodic recommendations for use of antiretroviral drugs for treatment-naïve and treatment-experienced adults and children. In the U.S., the Department of Health and Human Services Panel on Antiretroviral Guidelines for Adults and Adolescents issues regularly updated guidelines that are accessible at https://clinicalinfo.hiv.gov/en/guidelines/adult-and-adolescent-arv/whats-new-guidelines (Department of Health and Human Services, 2022).

Treatment recommendations compare various regimens currently available for treatment-naïve patients and address when to change therapy in individuals who are failing their current regimen. The specific drugs recommended may change as new choices become available and clinical research data accumulate. Selection of drugs in treatment-experienced patients will be driven by genotypic and phenotypic resistance testing, when available. However, future treatment guidelines will likely continue to be driven by three principles:

- Use of combination therapy to prevent the emergence of resistant virus
- Emphasis on regimen convenience, tolerability, and adherence in order to chronically suppress HIV replication
- Realization of the need for lifelong treatment under most circumstances

Treatment guidelines are not sufficient to dictate all aspects of patient management. Prescribers of antiretroviral therapy must maintain a comprehensive and current fund of knowledge regarding this disease and its pharmacotherapy. Because the treatment of HIV infection is a long-lived and complex affair, and because mistakes can have dire and irreversible consequences for the patient, the prescribing of these drugs should be limited to those with specialized training.

Drug Facts for Your Personal Formulary: *Antiretroviral Agents and Treatment of HIV Infection*

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (phosphorylated to active form to prevent infection of susceptible cells; do not eradicate virus from cells with integrated proviral DNA): Active against HIV-1 and HIV-2 and in some cases HBV		
Zidovudine (AZT) (thymidine analogue)	• HIV in adults and children • Preventing mother-to-child transmission	• Adverse effects: bone marrow (anemia, neutropenia) and muscle toxicity (myopathy); inhibits mitochondrial DNA polymerase γ • Do not use with stavudine
Lamivudine (cytidine analogue)	• HIV in adults and children ≥3 months • Chronic hepatitis B (adults, children)	• Essentially nontoxic
Abacavir (only guanosine analogue antiretroviral)	• HIV in adults and children ≥3 months • Not active against HBV	• Bioavailability not affected by food • Adverse effects: hypersensitivity syndrome (fever, abdominal pain, rash), associated with HLA B*5701 genotype; discontinue drug immediately and never use again as this is potentially fatal
Tenofovir (5′-AMP derivative; supplied as prodrugs: TDF or TAF)	• HIV infection (adults, children >2 years, in combination with other antiretrovirals) • Chronic hepatitis B (adults, children >2 years) • HIV preexposure prophylaxis (with emtricitabine) in adults at high risk of infection	• Nephrotoxicity: small decreases in estimated creatinine clearance are common; Fanconi syndrome rare • Decreases in bone mineral density with chronic use
Emtricitabine (cytidine analogue)	• HIV infection (adults, children, in combination with other antiretrovirals) • Chronic hepatitis B (off-label; adults, children) • HIV preexposure prophylaxis (with tenofovir) in adults at high risk of infection	• Generally nontoxic
Nonnucleoside Reverse Transcriptase Inhibitors: Do not require metabolic activation; HIV-1 specific and not active against HIV-2		
Nevirapine	• HIV-1 infection in infants ≥15 days, children, and adults • Single-dose prevention of mother-to-child transmission	• Autoinducer of metabolism • Commonly produces rash that usually resolves with continued treatment • Can rarely produce life-threatening skin eruptions such as Stevens-Johnson syndrome • Rarely produces life-threatening hepatitis
Efavirenz	• HIV-1 infection in children ≥3 months and adults	• Commonly causes CNS toxicity that usually resolves with continued treatment but can be severe enough to warrant discontinuation • Moderate hepatic enzyme inducer
Rilpivirine	• HIV-1 infection in children >12 years and adults	• Must be given with food • Avoid proton pump inhibitors because of reduced absorption • May cause prolonged QTc interval if concentrations are too high
Etravirine	• Treatment-experienced adults and children ≥2 years	• Commonly produces rash that usually resolves with continued treatment • Can rarely produce life-threatening skin eruptions such as Stevens-Johnson syndrome • Moderate inducer of hepatic enzymes
Doravirine	• Treatment-naïve adults	• Avoid coadministration of CYP3A inducers

Drug Facts for Your Personal Formulary: *Antiretroviral Agents and Treatment of HIV Infection (continued)*

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Protease Inhibitors: Active against HIV-1 and HIV-2; generally used as second-line agents in treatment-experienced patients		
Ritonavir	• Used only as a PK-boosting agent in combination with other PIs	• Commonly causes nausea • Associated with elevated cholesterol and triglycerides at higher doses • Potent inhibitor of CYP3A4 • Moderate hepatic enzyme inducer
Lopinavir	• Treatment-naïve or -experienced HIV-infected adults and children ≥14 days	• Must be combined with ritonavir • Commonly causes nausea and other GI toxicities • Associated with elevated cholesterol and triglycerides in adults with prolonged use
Atazanavir	• Treatment-naïve or -experienced HIV-infected adults and children ≥3 months	• Usually combined with ritonavir or cobicistat • Can be given without a PK booster at a higher dose of 400 mg • Absorption reduced with proton pump inhibitors and H_2 blockers • Commonly causes unconjugated hyperbilirubinemia • Can cause nephrolithiasis and cholelithiasis
Darunavir	• Treatment-naïve or -experienced HIV-infected adults and children ≥3 years	• Must be combined with ritonavir or cobicistat • May cause transient rash • Better tolerated than other PIs
Tipranavir	• Treatment-experienced HIV-infected adults and children ≥2 years, generally those who have failed all other PIs	• Toxicity: rare but potentially fatal hepatotoxicity; rare but potentially fatal bleeding diathesis, including intracranial hemorrhage • Rarely used because of the availability of better-tolerated PIs
Entry Inhibitors: Generally reserved for second-line or salvage therapy		
Maraviroc	• Treatment-naïve or -experienced HIV-infected adults who have evidence of predominantly CCR5-tropic virus	• CYP3A4 substrate susceptible to drug interactions with other antiretrovirals • Adverse effect: dose- and concentration-dependent orthostatic hypotension
Enfuvirtide	• Treatment-experienced HIV-infected adults and children weighing ≥11 kg • Generally reserved for those with no other treatment options	• Injected subcutaneously twice daily • Adverse effects: injection site reactions and subcutaneous nodules are common • Not active against HIV-2
Ibalizumab	• Heavily treatment-experienced adults with multidrug-resistant HIV-1 infection	• Intravenous infusion given every 2 weeks
Fostemsavir	• Heavily treatment-experienced adults with multidrug-resistant HIV-1 infection	• Oral drug that must be given twice daily • Can cause elevation of hepatic transaminases • May prolong QTc interval if given at higher than recommended doses
Integrase Inhibitors: Widely used in treatment-naïve patients because of excellent tolerability, safety, and antiretroviral activity		
Raltegravir	• HIV-infected adults and children weighing ≥2 kg	• Given once or twice daily without the need for a PK-boosting agent • Reduced bioavailability if given concurrently with divalent cations • Generally well tolerated
Elvitegravir	• HIV-infected adults and children >12 years of age	• Requires cobicistat as a PK booster • Should be taken with food • Reduced bioavailability if given concurrently with divalent cations • Generally well tolerated
Dolutegravir	• HIV-infected adults and children ≥4 weeks of age	• Given once daily without the need for a PK-boosting agent • Reduced bioavailability if given concurrently with divalent cations • May be associated with long-term weight gain • Generally well tolerated
Bictegravir	• HIV-infected adults and children weighing ≥25 kg	• Given once daily without the need for a PK-boosting agent • Reduced bioavailability if given concurrently with divalent cations • Generally well tolerated

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Chapter 65

Chemotherapy of Tuberculosis and Nontuberculous Mycobacteria, Including Leprosy

Elisa H. Ignatius and Kelly E. Dooley

INTRODUCTION

ANTIMYCOBACTERIAL DRUGS

DRUGS DEVELOPED AND USED PRIMARILY FOR MYCOBACTERIAL INFECTIONS

- First-Line Drugs
- Second-Line Drugs
- New Drugs

DRUGS REPURPOSED FOR USE IN MYCOBACTERIAL INFECTIONS

- Oxazolidinones: Linezolid, Tedizolid, and Sutezolid
- Fluoroquinolones
- β-Lactam Antibiotics for the Treatment of TB
- Macrolides
- Dapsone

PRINCIPLES OF ANTITUBERCULOSIS CHEMOTHERAPY

- Evolution and Pharmacology
- Antituberculosis Therapy
- Types of Antituberculosis Therapy

PRINCIPLES OF THERAPY FOR NONTUBERCULOUS MYCOBACTERIA

- Therapy of NTM Pulmonary Infection
- Therapy of Disseminated NTM

THERAPY FOR OTHER NTM, INCLUDING LEPROSY

- Types of Antileprosy Therapy
- Therapy of Other NTM

Introduction

Mycobacteria have caused epic diseases: Tuberculosis (TB) and leprosy have terrorized humankind since antiquity, and TB is thought to have killed one in seven of humans who have ever lived. Although the burden of leprosy has decreased, TB surpassed human immunodeficiency virus (HIV) as the leading infectious disease killer in 2014, and nontuberculous mycobacteria (NTM) are a growing threat in certain populations (Winthrop et al., 2020). *Mycobacterium abscessus,* a species of NTM, is especially devastating because of its tenacity, lack of response to combination antibiotics, and nearly universal propensity to develop acquired drug resistance. These distinct mycobacterial infections continue to be difficult to treat, owing mainly to three natural barriers:

- **Cell wall.** Mycobacteria are waxy in appearance, due to the composition of the cell walls. More than 60% of the cell wall is lipid, mainly mycolic acids composed of 2-branched, 3-hydroxy fatty acids with chains made of 76 to 90 carbon atoms. This extraordinary shield prevents many pharmacological compounds from getting to the bacterial cell membrane or inside the cytosol.
- **Efflux pumps.** A second layer of defense comes from an abundance of efflux pumps in the cell membrane. These transport proteins pump out potentially harmful chemicals from the bacterial cytoplasm back into the extracellular space and are responsible for the native resistance of mycobacteria to many standard antibiotics (Morris et al., 2005). As an example, ATP binding cassette (ABC) transporters, a group of permeases that transport across membranes, comprise a full 2.5% of the genome of *Mycobacterium tuberculosis* (Braibant et al., 2000).
- **Location in host.** Mycobacterial infections are intracellular and extracellular, with bacilli hiding both inside patients' cells and within necrotic, avascular areas of the lung. Antimicrobials must therefore penetrate the intracellular compartments and into the lesions where mycobacteria reside to be effective.

Summarizing antimycobacterial therapeutics is challenging, but we have taken the approach in this chapter of (1) organizing agents into those developed specifically for TB (or NTM) treatment versus those repurposed for that indication and (2) summarizing therapies for TB versus other types of mycobacteria. In presenting pharmacotherapies for TB, we periodically refer to drug groups A, B, and C, as delineated by the World Health Organization (WHO). This classification is explained fully later in the chapter (see the section on *Definitive Therapy of Drug-Resistant TB* and Table 65–6).

The first-line drugs we use for TB treatment were developed expressly for that purpose (see History). In fact, the first randomized controlled trial with concealed allocation in human history was for the treatment of TB—*streptomycin* versus bed rest. We divide mycobacteria into tuberculous versus nontuberculous mycobacteria; additionally, we often categorize them by their rate of growth on agar—as *rapid* and *slow* growers (see list in Table 65–1). Rapid growers are visible to the naked eye within 7 days; slow growers are visible later. The pharmacology

HISTORY

The first successful drug for treating TB was *para-aminobenzoic acid*, developed by Lehman in 1943. A more dramatic success came when Waksman and Schatz developed *streptomycin*. Further efforts led to development of *thioacetazone* by Domagk in 1946; *isoniazid* by Squibb, Hoffman La Roche, and Bayer in 1952; *pyrazinamide* by Kushner and colleagues in 1952; and rifamycins by Sensi and Margalith in 1957. *Ethambutol* was discovered at Lederle Laboratories in 1961. After a long pause, TB drug development experienced a "second wave" with *bedaquiline* licensed by the FDA in 2012 and *delamanid* receiving marketing approval the following year in Europe. The "third wave" is now here, with multiple compounds in preclinical and clinical development. In addition, pharmacophores in clinical use for other bacteria have been repurposed as antimycobacterial agents, including *moxifloxacin* and *levofloxacin*, oxazolidinones, and β-lactams.

Abbreviations

ABC: ATP binding cassette
ART: antiretroviral therapy
AUC: area under the curve
C_{Pmax}: maximal plasma concentration
CYP: cytochrome P450
ECG: electrocardiogram
FDA: U.S. Food and Drug Administration
FGD1: NADP-dependent glucose-6-phosphate dehydrogenase
GABA: γ-aminobutyric acid
GI: gastrointestinal
G6PD: glucose-6-phosphate dehydrogenase
HIV: human immunodeficiency virus
IC_{50}: concentration causing 50% inhibition
INH: isoniazid
InhA: enoyl acyl carrier protein reductase
KasA: β-ketoacyl-acyl carrier protein synthase
KatG: catalase-peroxidase
MAC: *Mycobacterium avium* complex
MDR-TB: multidrug-resistant TB, or TB resistant to isoniazid and rifampin
MIC: minimum inhibitory concentration
NAT2: *N*-acetyltransferase type 2
NO: nitric oxide
NRPB: nonreplicating persistent bacilli
NTM: nontuberculous mycobacteria
PABA: *para*-aminobenzoic acid
PAS: *para*-aminosalicylic acid
PK: pharmacokinetics
POA: pyrazinoic acid
RR-TB: rifampin-resistant TB
TB: tuberculosis
V_d: volume of distribution
WHO: World Health Organization
XDR-TB: extensively drug resistant (RR-TB or MDR-TB that is also resistant to fluoroquinolones and at least one Group A drug)

of drugs developed against slow growers is discussed in detail in this chapter as these drugs were mostly developed specifically for mycobacteria. Treatment of rapid growers often relies on repurposed drugs, so while this topic is addressed in this chapter, the pharmacology of many of these antibiotics (like macrolides, aminoglycosides, quinolones, and β-lactams) is described more fully elsewhere (see Chapters 57–60). The mechanisms of action of the antimycobacterial drugs are summarized in Figure 65–1. Pharmacokinetic (PK) parameter definitions are presented in Figure 56–1 and Equation 56–1.

Resistance in mycobacteria is chromosomally mediated, with preexisting mutants present in frequencies ranging from less than 1 in 10^6 (for *isoniazid* and nitroimidazoles) to 1 in 10^7 to 10^8 bacilli (for rifamycins). Development of drug resistance is determined by both epistasis, or the interaction between various mutations, and bacterial fitness (Trauner et al., 2014). The fitness cost of various mutations is not fixed, however, and subsequent mutations may compensate, thereby restoring bacterial fitness and potentially enhancing onward transmissibility. Subtherapeutic concentrations, promoted by interindividual variability in PK and suboptimal dosing practices (Pasipanodya et al., 2013; Srivastava et al., 2011), drive early induction of efflux pumps that leads to chromosomal mutations in drug target proteins and efflux pumps (Gumbo et al., 2014; Schmalstieg et al., 2012; Srivastava et al., 2010). Host genetics and immune response may also contribute to the development of drug resistance. The mycobacterial mechanisms of resistance are summarized in Figure 65–2.

TABLE 65–1 ■ PATHOGENIC MYCOBACTERIAL SLOW AND RAPID GROWERS (RUNYON CLASSIFICATION)

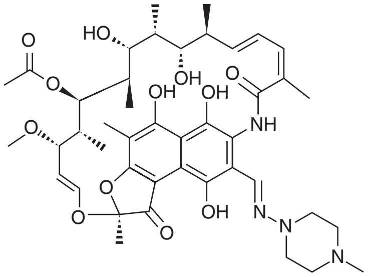
SLOW GROWERS
Runyon I: Photochromogens
Mycobacterium kansasii
Mycobacterium marinum
Runyon II: Scotochromogens
Mycobacterium scrofulaceum
Mycobacterium szulgai
Mycobacterium gordonae
Runyon III: Nonchromogens
Mycobacterium avium complex (*M. avium, M. intracellulare*)
Mycobacterium haemophilum
Mycobacterium xenopi
Mycobacterium ulcerans
RAPID GROWERS
Runyon IV
Mycobacterium fortuitum complex
Mycobacterium chelonae
Mycobacterium smegmatis group
Mycobacterium abscessus

Antimycobacterial Drugs

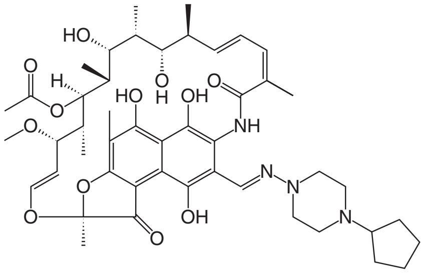
Drugs Developed and Used Primarily for Mycobacterial Infections

First-Line Drugs

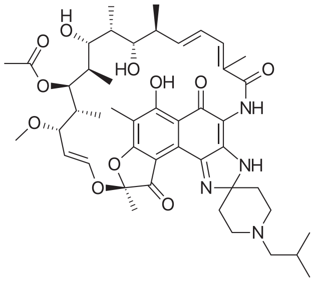
Rifamycins: Rifampin, Rifapentine, and Rifabutin



RIFAMPIN



RIFAPENTINE



RIFABUTIN

Rifamycins, which include *rifampin* (also known as *rifampicin*), *rifapentine*, and *rifabutin*, are macrocyclic antibiotics important in the treatment of most mycobacterial diseases. *Rifaximin*, a nonabsorbed rifamycin, is used for intestinal conditions and is discussed in Chapter 54.

Mechanism of Action. The mechanism of action for rifamycins is typified by *rifampin*'s action against *M. tuberculosis. Rifampin* enters bacilli and binds to the β subunit of DNA-dependent RNA polymerase (*rpoB*) to form a stable drug-enzyme complex (Gumbo et al., 2007a). Drug binding suppresses chain formation in RNA synthesis.

Antibacterial Activity. *Rifampin* inhibits the growth of most gram-positive bacteria as well as many gram-negative microorganisms, such as *Escherichia coli, Pseudomonas*, indole-positive and indole-negative *Proteus*, and *Klebsiella in vitro*. *Rifampin* is active against *Staphylococcus aureus,* coagulase-negative staphylococci, *Neisseria meningitidis, Haemophilus influenzae,* and *Legionella* species (Thornsberry et al., 1983).

Rifampin inhibits the growth of many mycobacteria, including *M. tuberculosis*, at concentrations of 0.06 to 0.25 mg/L (Heifets, 1991), *Mycobacterium leprae* at less than 1 μg/mL (Bullock, 1983), and *Mycobacterium kansasii* at 0.25 to 1 mg/L. Most strains of *Mycobacterium scrofulaceum, Mycobacterium intracellulare*, and *Mycobacterium avium* are suppressed by concentrations of 4 mg/L. *M. abscessus* harbors innate resistance to rifamycins—it inactivates *rifampin* via an ADP-ribosyltransferase and monooxygenase (Nessar et al., 2012). *Rifapentine* minimum inhibitory concentrations (MICs) are lower than those of *rifampin* (Mor et al., 1995). *Rifabutin* inhibits the growth of many strains of *M. tuberculosis* at concentrations of 0.125 mg/L or less. *Rifabutin* also inhibits the growth of most isolates of *Mycobacterium avium* complex (MAC) at concentrations ranging from 0.25 to 1 mg/L.

Bacterial Resistance. The prevalence of *rifampin*-resistant isolates (1 in every 10^7–10^8 bacilli) is due to an alteration of the target of this drug, *rpoB,* with resistance in 86% of cases due to mutations at codons 526 and 531 of the *rpoB* gene (Somoskovi et al., 2001). Rifamycin monoresistance occurs at higher rates when rifamycins are dosed intermittently, especially among patients with HIV, or when companion drugs are underdosed and are not present at high enough concentrations at the site of disease to protect against the emergence of rifamycin resistance (Burman et al., 2006). Efflux pump induction and mutations in efflux pumps have also been associated with rifamycin resistance (Li et al., 2015). Rifamycin resistance in TB has serious clinical consequences in TB given its unique role as a sterilizing agent; treatment of *rifampin*-monoresistant (RR-) or multidrug-resistant (MDR-) TB is, therefore, often prolonged to 9 to 12 months (World Health Organization, 2019).

ADME. After oral administration, rifamycins are absorbed to variable extents (Tables 65–2 and 65–3) (Burman et al., 2001). Food decreases the *rifampin* maximal plasma concentration (C_{Pmax}) by one-third; a high-fat meal increases the area under the concentration-time curve (AUC) of *rifapentine* by 50%. Food has no effect on *rifabutin* absorption. Thus, *rifampin* is best taken on an empty stomach, whereas *rifapentine* should be taken with food if possible.

Rifamycins are metabolized by microsomal β-esterases and cholinesterases. A major pathway for *rifabutin* elimination is CYP3A. Due to autoinduction, clearance of all three rifamycins increases with repeated administration (Table 65–3) (Dooley et al., 2012). Rifamycins penetrate well into many tissues, with *rifampin* appearing to display better penetration into lung lesions than *rifapentine*. CNS and pericardial fluid penetration are poor (5%–10%) in the absence of inflammation and have

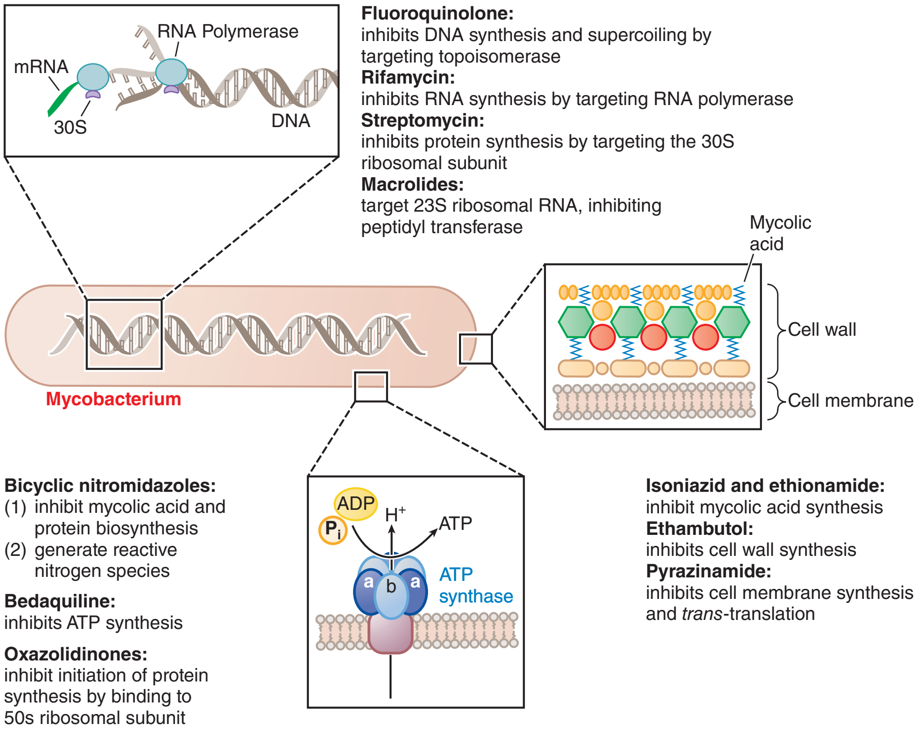

RNA Polymerase
mRNA
30S
DNA
Fluoroquinolone:
inhibits DNA synthesis and supercoiling by targeting topoisomerase
Rifamycin:
inhibits RNA synthesis by targeting RNA polymerase
Streptomycin:
inhibits protein synthesis by targeting the 30S ribosomal subunit
Macrolides:
target 23S ribosomal RNA, inhibiting peptidyl transferase
Mycolic acid
Cell wall
Cell membrane
Mycobacterium
Bicyclic nitromidazoles:
(1) inhibit mycolic acid and protein biosynthesis
(2) generate reactive nitrogen species
Bedaquiline:
inhibits ATP synthesis
Oxazolidinones:
inhibit initiation of protein synthesis by binding to 50s ribosomal subunit
ADP
P$_i$
H$^+$
ATP
a
b
a
ATP synthase
Isoniazid and ethionamide:
inhibit mycolic acid synthesis
Ethambutol:
inhibits cell wall synthesis
Pyrazinamide:
inhibits cell membrane synthesis and trans-translation

Figure 65–1 *Mechanisms of action of established and experimental drugs used for the chemotherapy of mycobacterial infections.* Approved drugs for the chemotherapy of mycobacterial diseases may be grouped according to the sites of action indicated by the pictures above that expand regions of the *Mycobacterium*: inhibitors of nucleic acid and protein synthesis; disruptors of cell wall and cell membrane synthesis; inhibitors of membrane transport. Specific antimycobacterial agents and their mechanisms of action are also listed. Rifamycin is used as a generic term for several drugs, of which *rifampin* is used most frequently. *Clofazimine*, whose mode of action is not understood, is omitted.

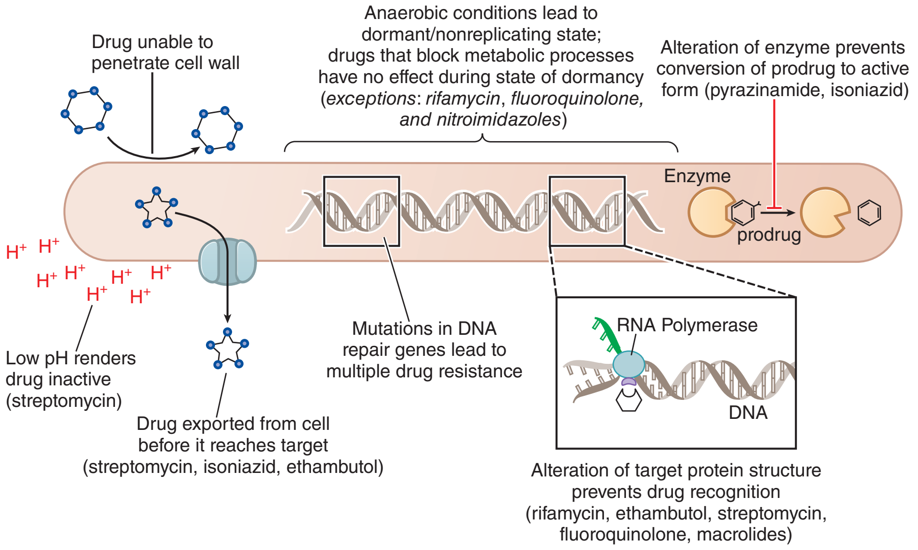

Drug unable to penetrate cell wall
Anaerobic conditions lead to dormant/nonreplicating state; drugs that block metabolic processes have no effect during state of dormancy (*exceptions*: *rifamycin*, *fluoroquinolone*, *and nitroimidazoles*)
Alteration of enzyme prevents conversion of prodrug to active form (pyrazinamide, isoniazid)
Enzyme
prodrug
H+ H+ H+ H+ H+ H+ H+ H+
Low pH renders drug inactive (streptomycin)
Drug exported from cell before it reaches target (streptomycin, isoniazid, ethambutol)
Mutations in DNA repair genes lead to multiple drug resistance
RNA Polymerase
DNA
Alteration of target protein structure prevents drug recognition (rifamycin, ethambutol, streptomycin, fluoroquinolone, macrolides)

Figure 65–2 *Mechanisms of resistance in mycobacteria.*

only been evaluated for *rifampin* (Nau et al., 1992; Shenje et al., 2015). Nonetheless, rifamycins are a recommended component of therapy for tuberculous meningitis, and *rifabutin* has been shown to be effective in a rabbit model of bacterial meningitis (Schmidt et al., 1997). Rifamycins and their metabolites are excreted by bile and eliminated via feces, with urine being a minor elimination pathway.

The population PK of *rifampin* are best described using a one-compartment model with transit compartment absorption (Wilkins et al., 2008). *Rifampin* displays concentration-dependent clearance and autoinduction of clearance, with dose increases producing more-than-proportional increases in exposures and prolonged dosing resulting in a shorter half-life (Abulfathi et al., 2019; Hibma et al., 2020; Jayaram et al., 2003; Svensson et al., 2018). *Rifapentine*'s PK are best described with a one-compartment model with first-order absorption and elimination (Langdon et al., 2005); this drug also displays autoinduction, but not saturable clearance. Other PK parameters are summarized in Tables 65–2 and 65–3.

In contrast, *rifabutin* disposition is biexponential, and its PK are best described by a two-compartment model with first-order absorption and elimination. *Rifabutin* concentrations are substantially higher in tissue than in plasma due to its lipophilic properties, leading to the very high apparent volumes of distribution (see Table 65–2). As a consequence, C_{Pmax} values for *rifabutin* are lower than other rifamycins. With concomitant *azithromycin* or protease inhibitor administration, central volume of distribution (divided by bioavailability, or V_{dc}/F) increases while peripheral volume of distribution (V_{dp}/F) decreases (Hennig et al., 2016); tobacco smoking increases the V_{dp}/F by 39% (Gatti et al., 1998).

The clearance of all rifamycins is affected by patient weight. A recent systematic review and pooled *rifapentine* PK analysis, however, found that body weight was not predictive of clearance (Hibma et al., 2020); flat

TABLE 65–2 ■ POPULATION PHARMACOKINETIC PARAMETER ESTIMATES FOR ANTIMYCOBACTERIAL DRUGS IN ADULT PATIENTS

	PARAMETER ESTIMATE		
	k_a (h^{-1})	SYSTEMIC CLEARANCE (L/h)	V_d (L)
Rifampin	1.15	19	53
Rifapentine	0.6	2.03	37.8
Rifabutin	0.2	61	231/1050[a]
Pyrazinamide	3.56	3.4	29.2
Isoniazid	2.3	22.1	35.2
Ethambutol	0.7	1.3[b]	6.0[b]
Clofazimine	0.209	11.5	262/10,500
Dapsone	1.04	1.83	69.6
Bedaquiline	0.128	2.62	198/8550[a]
Ethionamide	0.25	1.9[b]	3.2[b]
Para-aminosalicylic acid	0.4	0.3[b]	0.9[b]
Cycloserine	1.9	0.04[b]	0.5[b]
Pretomanid	1.38	3.30	90.4
Delamanid	0.397	37.1	655/870[a]

k_a is the absorption constant (see Chapter 56).
[a] Volume of central compartment/volume of peripheral compartment.
[b] Expressed per kilogram of body weight.

TABLE 65–3 ■ PHARMACOKINETIC PARAMETERS OF RIFAMPIN, RIFABUTIN, AND RIFAPENTINE

	RIFABUTIN	RIFAMPIN	RIFAPENTINE
Protein binding (%)	71	85	97
Oral bioavailability (%)	20	68	70
t_{max} (h)	2.5–4.0	1.5–2.0	5.0–6.0
C_{max} total (μg/mL)	0.2–0.6	8–20	8–30
C_{max} free drug (μg/mL)	0.1	1.5	0.5
$t_{1/2}$ (h)	32–67	2–5	14–18
Intracellular/extracellular penetration	9	5	24–60
Autoinduction (AUC decrease)	40%	38%	35%
CYP3A induction	Weak	Pronounced	Pronounced
CYP3A substrate	Yes	No	No

dosing of *rifapentine* has since been used in clinical trials (Dorman et al., 2020). For *rifampin*, while weight impacts clearance, its effect is modest, and strict milligram per kilogram dosing results in systematic underdosing of lower-weight patients (Court et al., 2018).

Microbial Pharmacokinetics-Pharmacodynamics. *Rifampin*'s bactericidal and sterilizing effect appears to be largely concentration-dependent, with high AUC or C_{max} associated with optimal sterilizing activity and treatment outcomes in mice and humans (Abulfathi et al., 2019; Jayaram et al., 2003). However, resistance suppression and *rifampin*'s enduring postantibiotic effect are best optimized by high C_{Pmax}/MIC. Recent clinical trials confirm that current dosing is on the steep part of the dose-response curve; better sputum bacillary decline is achieved with up to 3.5 times higher *rifampin* doses than currently used. Such increased doses would increase both AUC/MIC and C_{Pmax}/MIC nonlinearly (Boeree et al., 2015). The safety, tolerability, and efficacy of *rifampin* doses up to 35 mg/kg are being tested in phase III trials (Nabisere et al., 2020), with one recent trial demonstrating that a regimen containing *moxifloxacin* plus high-dose *rifapentine* successfully shortened treatment duration for TB from 6 months to 4 months (Dorman et al., 2021).

Therapeutic Uses. *Rifampin* for oral administration is available alone and as fixed-dose combinations together with *isoniazid*; *isoniazid* and *pyrazinamide*; or *isoniazid*, *pyrazinamide*, and *ethambutol* (not all fixed-dose combinations are available in all countries). A parenteral form of *rifampin* is also available. The standard dose of *rifampin* for treatment of TB disease in adults is 600 mg, given once daily, either at least 1 h before or 2 h after a meal. Children should receive 15 mg/kg (range 10–20 mg/kg), with a maximum dose of 600 mg/day, given in the same way. *Rifabutin* is administered at 300 mg daily. *Rifapentine* is not currently used for treatment of TB disease, but the dose used in the recent successful phase III trial was 1200 mg daily. *Rifampin* and *rifapentine* are used in the treatment of latent tuberculosis infection (see Table 65–5).

Rifampin is also useful for the prophylaxis of meningococcal disease and *H. influenzae* meningitis. To prevent meningococcal disease, adults may be treated with 600 mg twice daily for 2 days or 600 mg once daily for 4 days; children older than 1 month should receive 10 to 15 mg/kg, to a maximum of 600 mg. Combined with a second antibacterial, *rifampin* may be useful for therapy in selected cases of staphylococcal endocarditis or osteomyelitis, especially for infections associated with prosthetic materials. *Rifampin* may also be indicated for the eradication of the staphylococcal nasal carrier state in patients with chronic furunculosis. In the treatment of brucellosis, *rifampin* 900 mg daily can be combined with *doxycycline* for 6 weeks.

Untoward Effects. Rifamycins are generally well tolerated, although they frequently cause harmless red-orange discoloration of skin, urine, feces, saliva, tears, and contact lenses. Usual doses of *rifampin* result in less than 4% of patients with TB developing significant adverse reactions; the most common are rash (0.8%), fever (0.5%), and nausea and vomiting (1.5%). Rifamycins can cause liver disease (mostly cholestasis), with drug-induced liver injury or death due to liver failure rarely observed. Chronic liver disease, alcoholism, and older age appear to increase the incidence of clinically important hepatotoxicity. Gastrointestinal (GI) disturbances have occasionally required discontinuation of the drug.

Hypersensitivity reactions may be encountered. These present with a flu-like syndrome of fever, chills, and myalgias with rare cases progressing to include eosinophilia, interstitial nephritis, acute tubular necrosis, thrombocytopenia, hemolytic anemia, and shock. Hypersensitivity reaction is more common with intermittent dosing and among female and Asian patients (Yew and Leung, 2006). Light chain proteinuria, thrombocytopenia, transient leukopenia, and anemia have occurred during therapy. Although *rifampin* clearance is slightly reduced in pregnancy and does cross the placenta, it is still considered the treatment of choice in pregnancy (Denti et al., 2015). *Rifapentine* has a similar adverse event profile to *rifampin*, including rash, hepatitis, and flu-like symptoms.

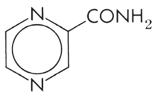
Primary reasons for discontinuation of *rifabutin* therapy include rash (4%), GI intolerance (3%), and neutropenia (2%) (Nightingale et al., 1993). Rarely, thrombocytopenia, flu-like symptoms, hemolysis, myositis, chest pain, and hepatitis develop in patients treated with *rifabutin*. Unique side effects for this rifamycin include polymyalgia, pseudojaundice, and anterior uveitis. Patients should be cautioned to discontinue the drug if visual symptoms (pain or blurred vision) occur.

Rifampin overdose is uncommon but can be life threatening. The most prominent symptoms are orange discoloration of skin, fluids, and mucosal surfaces. Treatment consists of supportive measures; there is no antidote.

Drug Interactions. *Rifampin* is a potent inducer of cytochrome P450 (CYP) and phase 2 metabolizing enzymes, as well as transporters such as P-glycoprotein, via its effects on pregnane X receptor (PXR) (see Table 3–3). As a result, its administration often results in higher clearance for concurrently administered medicines subject to these metabolic and transport pathways. The magnitude of the effect on concurrent medications can be large, and therapeutic failures may result. *Rifapentine* displays a similar effect on metabolizing enzymes. *Rifabutin* is a less-potent inducer of CYPs than the other rifamycins; however, *rifabutin* does induce hepatic microsomal enzymes modestly and decreases the $t_{1/2}$ of *zidovudine*, *prednisone*, *digitoxin*, *quinidine*, *ketoconazole*, *propranolol*, *phenytoin*, sulfonylureas, and *warfarin*. Unlike other rifamycins, *rifabutin* is a substrate of CYP3A, and so dose adjustments may be needed (e.g., to 150 mg daily) when this drug is given together with strong CYP3A inhibitors, such as protease inhibitors used to treat HIV.

Pyrazinamide

Pyrazinamide is the synthetic pyrazine analogue of nicotinamide. *Pyrazinamide* was first synthesized at Merck in 1936 in Germany but was first examined as an anti-TB agent in 1952.



PYRAZINAMIDE

Mechanism of Action. Despite clinical use of *pyrazinamide* for decades, only recently has its mechanism of action been elucidated. *Pyrazinamide* is well known to be active only in acidic conditions. The drug passively diffuses into mycobacterial cells, where *M. tuberculosis* pyrazinamidase (encoded by the *pncA* gene) deaminates *pyrazinamide* to pyrazinoic acid (POA^-, in its dissociated form) (Zhang et al., 1999). POA^- binds competitively to the enzyme PanD, essential for coenzyme A biosynthesis in *M. tuberculosis*; this triggers degradation of the protein by a protease called CLPC1-ClpP. An additional target of *pyrazinamide* may be ribosomal protein S1 (encoded by *RpsA*) in the trans-translation process. Blocking RpsA activity leads to accumulation of toxic proteins that kill the bacteria (Gopal et al., 2020; Shi et al., 2011; Sun et al., 2020).

Antibacterial Activity. *Pyrazinamide* exhibits antimicrobial activity *in vitro* only at acidic pH. At pH 5.9, 95% of *M. tuberculosis* clinical isolates have an MIC of 6.25 to 200 mg/L (Gumbo et al., 2014).

Mechanisms of Resistance. There are various mechanisms of drug resistance to *pyrazinamide*. First, mutations in several positions in the *pncA* gene lead to expression of pyrazinamidase with reduced affinity for *pyrazinamide*, leading to decreased conversion of *pyrazinamide* to its active form. In addition, mutations in *RpsA* or *panD* confer *pyrazinamide* resistance (Kuhlin et al., 2021; Zhang et al., 2014). Finally, mutations in *M. tuberculosis* efflux pumps can cause resistance to *pyrazinamide*, as well as other anti-TB drugs (Liu et al., 2019; Zimic et al., 2012).

ADME. The oral bioavailability of *pyrazinamide* exceeds 90%, although some patients absorb *pyrazinamide* more quickly than others (absorption rate constant of 3.56/h vs. 1.25/h) (Wilkins et al., 2006). *Pyrazinamide* is metabolized by microsomal deamidase to POA and subsequently hydroxylated to 5-hydroxy-POA, which is then excreted by the kidneys. Clearance and V_d increase with patient mass (0.5 L/h and 4.3 L for every 10 kg above 50 kg), and V_d is larger in males (by 4.5 L) (see Table 65–2). Weight-based dosing is typically employed for *pyrazinamide*, although fixed doses may be more practical, likely to achieve targets, and effective for low-weight individuals (Sahota and Della Pasqua, 2012). *Pyrazinamide* clearance is reduced in renal failure; therefore, the dosing frequency is reduced to three times a week at low glomerular filtration rates. Hemodialysis removes *pyrazinamide*, so the drug should be redosed after each session (Malone et al., 1999b).

Microbial Pharmacokinetics-Pharmacodynamics. The PK parameter that correlates most closely with *pyrazinamide*'s activity is AUC, followed closely by C_{max} (Chigutsa et al., 2015; Gumbo et al., 2009). Higher C_{max} or AUC and lack of genetic resistance markers are associated with shorter time to culture conversion and better clinical outcomes (Kuhlin et al., 2021; Zhang et al., 2021).

Therapeutic Uses. Adding *pyrazinamide* to *isoniazid*- and *rifampin*-containing regimens allows for shortening duration of treatment from 9–12 to 6 months, producing the current "short-course" chemotherapeutic regimen. *Pyrazinamide* is administered at an oral dose of 25 to 35 mg/kg per day.

Untoward Effects. Injury to the liver is the most serious side effect of *pyrazinamide*. Hepatotoxicity appears to be idiosyncratic, up to doses of at least 40 mg/kg, at which time an exposure-toxicity relationship is seen (Pasipanodya and Gumbo, 2010; Sahota and Della Pasqua, 2012; Zhang et al., 2021). At high doses, hepatic disease appears in about 15% of patients, with jaundice in 2% to 3% and death due to hepatic necrosis in rare instances; current dosing regimens are much safer. Prior to *pyrazinamide* administration, all patients should undergo studies of hepatic function, and these studies should be repeated during treatment. If evidence of significant hepatic damage becomes apparent, therapy must be stopped. *Pyrazinamide* should be avoided in individuals with baseline hepatic dysfunction. If *pyrazinamide* cannot be used, "short-course" treatment with *rifampin*, *isoniazid*, and *ethambutol* can still be successful provided treatment duration is prolonged to 9 months from 6 months.

In nearly all patients, *pyrazinamide* inhibits excretion of urate, resulting in hyperuricemia, which may cause acute episodes of gout. Other untoward effects observed with *pyrazinamide* include arthralgias, anorexia, nausea, vomiting, dysuria, malaise, and fever. Although the World Health Organization (WHO) recommends routine use of *pyrazinamide* in pregnancy, the use of *pyrazinamide* is not approved during pregnancy in the U.S. because of inadequate teratogenicity data.

Isoniazid

Isoniazid (*isoni*cotinic acid hydr*azide*), also called INH (Figure 65–3), is an important drug for the chemotherapy of drug-susceptible TB and may

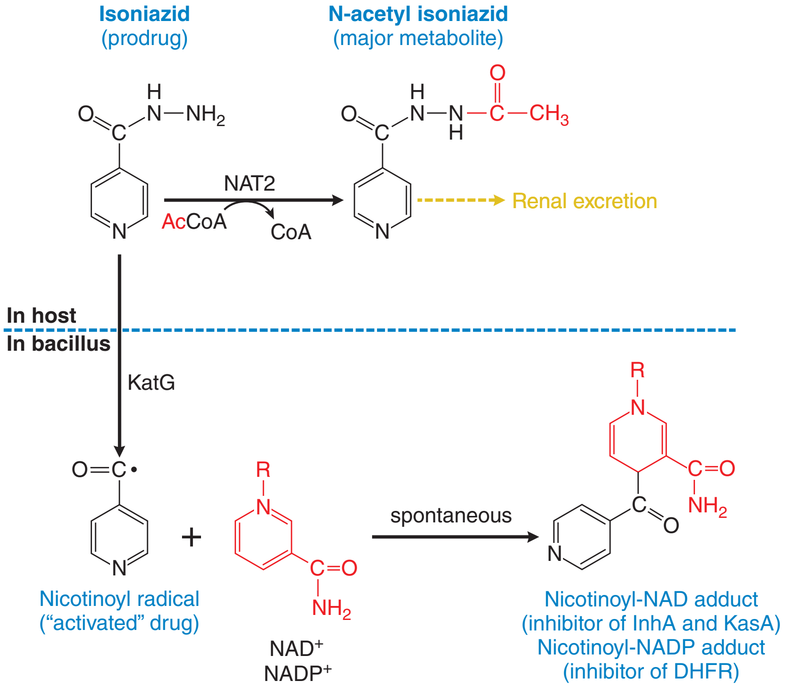


Figure 65–3 *Metabolism and activation of isoniazid.* The prodrug *isoniazid* is metabolized in humans by NAT2 isoforms to its principal metabolite, *N*-acetyl isoniazid, which is excreted by the kidney. *Isoniazid* diffuses into mycobacteria, where it is "activated" by KatG (oxidase/peroxidase) to the nicotinoyl radical. The nicotinoyl radical reacts spontaneously with NAD^+ to produce adducts that inhibit essential enzymes in synthesis of the cell wall and with $NADP^+$ to produce an inhibitor of nucleic acid synthesis.

have a role in some cases of drug-resistant TB. The use of combination therapy (INH + *pyrazinamide* + *rifampin*) provides the basis for short-course therapy and improved cure rates.

Mechanism of Action. *Isoniazid* enters bacilli by passive diffusion. The drug is not directly toxic to the bacillus and must be activated by KatG, a multifunctional catalase-peroxidase. KatG catalyzes the production of an isonicotinoyl radical that subsequently interacts with mycobacterial NAD and NAPD to produce a dozen adducts (Argyrou et al., 2007). One of these, a nicotinoyl-NAD isomer, inhibits the activities of enoyl acyl carrier protein reductase (InhA) and KasA (β-ketoacyl-acyl carrier protein synthase), blocking synthesis of mycolic acid, an essential component of the mycobacterial cell wall, leading to bacterial cell death. Another adduct, a nicotinoyl-NADP isomer, potently inhibits ($K_i < 1$ nM) mycobacterial dihydrofolate reductase, thereby interfering with nucleic acid synthesis (Argyrou et al., 2006) (see Figure 65–3).

Other products of KatG activation of INH include superoxide, H_2O_2, alkyl hydroperoxides, and the nitric oxide (NO) radical, which may also contribute to the mycobactericidal effects of INH (Timmins and Deretic, 2006). *M. tuberculosis* could be especially sensitive to damage from these radicals because the bacilli have a defect in the central regulator of the oxidative stress response, *oxyR*. Backup defense against radicals is provided by alkyl hydroperoxide reductase (encoded by *ahpC*), which detoxifies organic peroxides. Increased expression of *ahpC* reduces INH effectiveness.

Antibacterial Activity. The INH MICs with clinical *M. tuberculosis* strains vary from country to country. In the U.S., for example, MICs are typically 0.025 to 0.05 mg/L. INH is first-line therapy for *M. kansasii*, has moderate activity against *Mycobacterium bovis*, and poor activity against MAC. It has no activity against any other microbial genus.

Mechanisms of Resistance. The prevalence of INH-resistant mutants is about 1 in 10^6 bacilli. Because TB cavities may contain as many as 10^7 to 10^9 microorganisms, preexisting resistance can be expected in pulmonary TB cavities of untreated patients. These spontaneous mutants can be selected and amplified by INH monotherapy. Thus, two or more agents are used to treat active TB disease. Because the mutations resulting in drug resistance are independent events, the probability of resistance to two antimycobacterial agents is small, about 1 in 10^{12} ($1 \times 10^6 \times 10^6$), a low probability considering the number of bacilli present.

Resistance to INH is associated with mutation or deletion of KatG (generally conferring high-level resistance), overexpression of the genes for InhA (conferring low-level resistance to INH and some cross-resistance to *ethionamide*) and AhpC, and mutations in the *kasA* and *katG* genes. The most common mechanism of INH resistance in clinical isolates is due to single point mutations in the heme-binding catalytic domain of KatG, especially a serine-to-asparagine change at position 315. Although isolates with this mutation completely lose the ability to form nicotinoyl-NAD^+/$NADP^+$ adducts, they retain good catalase activity and maintain good biofitness. Compensatory mutations in the *ahpC* promoter occur and increase survival of *katG*-mutant strains under oxidative stress. Efflux pump induction by INH has been demonstrated and also confers resistance to *ethambutol* (Colangeli et al., 2005). Studies of the evolution of drug-resistant TB over time in human populations demonstrate that INH resistance has overwhelmingly been the initial acquired resistance mutation, followed by resistance to *rifampin* and other drugs (Cohen et al., 2015).

ADME. The bioavailability of orally administered INH is about 100% for the 300-mg dose. The PK of INH are best described by a two-compartment model, with the PK parameters in Table 65–2 (Pasipanodya and Gumbo, 2013). Approximately 10% of drug is bound to protein. From 75% to 95% of a dose of INH is excreted in the urine within 24 h, mostly as acetylisoniazid and isonicotinic acid.

Isoniazid is metabolized by hepatic arylamine NAT2 (*N*-acetyltransferase type 2), encoded by a variety of *NAT2** alleles (Figure 65–4). A pioneering report in pharmacogenetics elucidated differences in INH clearance by NAT2 acetylator status (Evans and Clarke, 1961). Patients who are slow acetylators of INH have concentrations of the drug that are three times higher than fast acetylators; fast acetylators are at risk for reduced microbial cure, increased relapse, and acquired drug resistance (Pasipanodya et al., 2012). Eighty-eight percent of the variability in INH clearance can be explained by NAT2 status, the distribution of which varies geographically.

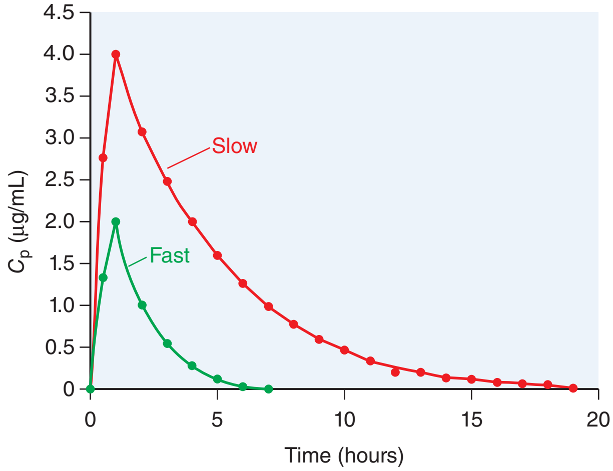


Figure 65–4 *Multimodal distribution of INH clearance due to NAT2 polymorphisms.* A group of matched male volunteers received INH (250 mg orally), and the time courses of plasma drug levels (C_p) were assessed. One-third of the subjects had INH elimination $t_{1/2}$ values less than 1.5 h; these were the *fast acetylators*. Two-thirds had $t_{1/2}$ values ranging from 2.1 to 4.0 h, with a suggestion of multiple groups; these were the *slow acetylators*. Plots of the mean data (C_p vs. time after administration) demonstrate the PK effects of acetylation rate. Both groups reached C_{Pmax} at 1 h. The slow acetylators (red line) achieved a higher C_p (4 µg/mL) with a mean elimination $t_{1/2}$ of 3.0 h; the fast acetylators (green line) reached a lower maximal C_p (2 µg/mL) with a mean elimination $t_{1/2}$ of 1.0 h. The acetylation rate reflects variable expression of active polymorphic forms of NAT2. Slow acetylators may be a greater risk for adverse effects from INH, sulfonamides, and procainamide; fast acetylators may have diminished responses to standard doses of these agents but greater risk from bioactivation by NAT2 of arylamine/hydrazine carcinogens. Recently, researchers have identified three elimination subgroups for INH metabolism: *fast*, *slow*, and *intermediate* (codominant fast and slow alleles).

Fast acetylation is found more commonly in Inuit and Japanese populations, while slow acetylation is the predominant phenotype in most Scandinavians, North African whites, and those of Jewish descent. The high acetyltransferase activity (fast acetylation) is inherited as an autosomal dominant trait; fast acetylators of INH are either heterozygous or homozygous.

Microbial Pharmacokinetics-Pharmacodynamics. *Isoniazid*'s microbial kill, as well as protection against emergence of resistance, is concentration dependent and correlates well with AUC_{0-24} or C_{Pmax} (Gumbo et al., 2007b; Jayaram et al., 2004; Pasipanodya et al., 2013).

Therapeutic Uses. *Isoniazid* is available as a pill, as an elixir, and for parenteral administration. The recommended total daily dose of INH is 5 mg/kg with a maximum of 300 mg administered daily.

Untoward Effects. After NAT2 converts INH to acetylisoniazid, it is either excreted by the kidney or is converted to acetylhydrazine (Roy et al., 2008) and then to hepatotoxic metabolites by CYP2E1. Alternatively, acetylhydrazine may be further acetylated by NAT2 to diacetylhydrazine, which is nontoxic. In this scenario, rapid acetylators will rapidly remove acetylhydrazine, while slower acetylation or induction of CYP2E1 or mutations in CYP2E1 that lead to increased enzyme activity may lead to more of the toxic metabolites (Sheng et al., 2014).

Elevated serum aspartate and alanine transaminases are encountered commonly in patients taking INH. However, the enzyme levels often normalize even when INH therapy is continued (Blumberg et al., 2003). Severe hepatic injury occurs in about 0.1% of all patients taking the drug. Hepatic damage is rare in patients less than 20 years old, and the incidence increases with age. Most cases of hepatitis occur 4 to 8 weeks after the start of therapy.

If *pyridoxine* is not given concurrently, peripheral neuritis (most commonly paresthesias of feet and hands) is encountered in about 2% of patients receiving 5 mg/kg INH daily. Neuropathy is more frequent in slow acetylators and in individuals with diabetes mellitus, poor nutrition, or anemia. Other neurological toxicities include convulsions in patients with seizure disorders, optic neuritis and atrophy, muscle twitching, dizziness, ataxia, paresthesias, stupor, and toxic encephalopathy. Mental abnormalities may appear during the use of this drug, including euphoria, transient impairment of memory, loss of self-control, and florid psychoses.

Patients may develop hypersensitivity to INH as well as rheumatological conditions. Vasculitis associated with antinuclear antibodies may appear during treatment but disappears when the drug is stopped. Arthritic symptoms have been attributed to this agent. A drug-induced syndrome resembling systemic lupus erythematosus has also been reported. Miscellaneous reactions associated with INH therapy include dryness of the mouth, epigastric distress, methemoglobinemia, tinnitus, and urinary retention. In persons predisposed to *pyridoxine* deficiency anemia, the administration of INH may result in dramatic anemia. Treatment of the anemia with large doses of vitamin B_6 gradually returns the blood count to normal.

As little as 1.5 g of INH can be toxic. INH overdose has been associated with the clinical triad of:

- Seizures refractory to treatment with *phenytoin* and barbiturates
- Anion gap metabolic acidosis that is refractory to treatment with sodium bicarbonate
- Coma

The common early symptoms appear within 0.5 to 3 h of ingestion and include ataxia, peripheral neuropathy, dizziness, and slurred speech. The most dangerous are grand mal seizures and coma, encountered when patients ingest 30 mg/kg or more of the drug. Mortality in these circumstances is as high as 20%. Intravenous *pyridoxine* is administered over 5 to 15 min on a gram-to-gram basis with the ingested INH. If the dose of ingested INH is unknown, then 70 mg/kg dose of *pyridoxine* is given. In patients with seizures, benzodiazepines are utilized.

Isoniazid binds to pyridoxal 5′-phosphate to form isoniazid-pyridoxal hydrazones, thereby depleting neuronal pyridoxal 5′-phosphate and interfering with pyridoxal phosphate–requiring reactions, including the synthesis of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Decreased levels of GABA lead to cerebral overexcitability and lowered seizure threshold. The antidote is replenishment of pyridoxal 5′-phosphate.

Drug Interactions. *Isoniazid* is a potent inhibitor of CYP2C19 and CYP3A, a weak inhibitor of CYP1A2, CYP2A6, and CYP2D6, and a weak inducer of CYP2E1 (Desta et al., 2001). Drugs that are metabolized by these enzymes will potentially be affected (Table 65–4).

Ethambutol

Ethambutol hydrochloride is a water-soluble and heat-stable compound. It was discovered in the 1960s and has demonstrated utility in protecting companion drugs against resistance in mice and humans and is generally used for that purpose in tuberculosis.

Mechanism of Action. *Ethambutol* inhibits arabinosyl transferase III, thereby disrupting the transfer of arabinose into arabinogalactan biosynthesis, which in turn disrupts assembly of the mycobacterial cell wall. The arabinosyl transferases are encoded by *embB* genes.

Antibacterial Activity. *Ethambutol* has activity against a wide range of mycobacteria but has no activity against any other genus. *Ethambutol* MICs are 0.5 to 2 mg/L in clinical isolates of *M. tuberculosis*, about 0.8 mg/L for *M. kansasii,* and 2 to 7.5 mg/L for *M. avium*. The following *Mycobacterium* species are also susceptible: *M. gordonae, M. marinum, M. scrofulaceum,* and *M. szulgai*. However, the majority of *M. xenopi*, *M. fortuitum*, *M. abscessus,* and *M. chelonae* have been reported as resistant.

TABLE 65–4 ■ SOME ISONIAZID-DRUG INTERACTIONS VIA INHIBITION AND INDUCTION OF CYPs

COADMINISTERED DRUG	CYP ISOFORM	ADVERSE EFFECTS
Acetaminophen	CYP2E1 induction	Hepatotoxicity
Carbamazepine	CYP3A inhibition	Neurological toxicity
Diazepam	CYP3A and CYP2C19 inhibition	Sedation and respiratory depression
Ethosuximide	CYP3A inhibition	Psychotic behaviors
Isoflurane and enflurane	CYP2E1 induction	Decreased effectiveness
Phenytoin and fosphenytoin	CYP2C19 inhibition	Neurological toxicity
Theophylline	CYP3A inhibition	Seizures, palpitation, nausea
Vincristine	CYP3A inhibition	Limb weakness and tingling
Warfarin	CYP2C9 inhibition	Possibility of increased bleeding (higher risk with isoniazid doses >300 mg/day)

Mechanisms of Resistance. *In vitro*, mycobacterial resistance to the drug develops via mutations in the *embB* gene that encodes arabinosyltransferases. In 30% to 70% of clinical isolates that are resistant to *ethambutol*, mutations are encountered in the *ethambutol* resistance–determining region of the *embB* gene. However, mutations in codon 306 (the most common mutation) are also encountered in *ethambutol*-susceptible mycobacteria; therefore, this mutation is necessary, but not sufficient, to confer *ethambutol* resistance (Safi et al., 2008). Enhanced efflux pump activity may induce resistance to both INH and *ethambutol in vitro*.

ADME. The oral bioavailability of *ethambutol* is about 80%. Approximately 10% to 40% of the drug is bound to plasma protein. *Ethambutol* drug concentrations have been modeled using a two-compartment model, with first-order absorption and elimination (Zhu et al., 2004). The decline in *ethambutol* is biexponential, with a $t_{1/2}$ of 3 h in the first 12 h and a $t_{1/2}$ of 9 h between 12 and 24 h due to redistribution of drug. Clearance and V_d are greater in children than in adults on a per-kilogram basis. Slow and incomplete absorption is common in children (Zhu et al., 2004). See Table 65–2 for PK data on this drug.

About 80% of the drug is not metabolized and is renally excreted as parent drug. Therefore, in renal failure, *ethambutol* should be dosed at 15 to 25 mg/kg three times a week instead of daily, including in patients on hemodialysis. The remainder of *ethambutol* (~20%) is oxidized by aldehyde dehydrogenase and excreted as aldehyde and dicarboxylic acid derivatives.

Microbial Pharmacokinetics-Pharmacodynamics. *Ethambutol*'s microbial kill of *M. tuberculosis* is optimized by AUC/MIC, while that against disseminated MAC is optimized by C_{max}/MIC (Deshpande et al., 2010) (see Chapter 56).

Therapeutic Uses. *Ethambutol* is available for oral administration in tablets containing the D-isomer. It is used for the treatment of TB, disseminated MAC, and in *M. kansasii* infection. *Ethambutol* is administered at 15 to 25 mg/kg per day for both adults and children.

Untoward Effects. At standard doses, *ethambutol* produces very few serious untoward reactions: About 1% of patients experience diminished visual acuity, 0.5% a rash, and 0.3% drug fever. Other side effects include pruritus, joint pain, GI upset, abdominal pain, malaise, headache, dizziness, mental confusion, disorientation, and possible hallucinations.

Therapy with *ethambutol* results in an increased concentration of urate in the blood in about 50% of patients, owing to decreased renal excretion of uric acid.

The most important side effect is optic neuritis, resulting in decreased visual acuity and loss of red-green discrimination. The incidence of this reaction is proportional to the dose and duration of *ethambutol* and is observed in 15% of patients receiving 50 mg/kg per day, in 5% of patients receiving 25 mg/kg per day, and in less than 1% of patients receiving daily doses of 15 mg/kg. While 15 mg/kg may be considered the minimal effective dose (Radenbach, 1973), this is the recommended dose for drug-sensitive TB given its narrow therapeutic window (higher doses are commonly used in drug-resistant TB). Severity of the visual loss is related to the duration of therapy after decreased visual acuity first becomes apparent and may be unilateral or bilateral. Tests of visual acuity and red-green discrimination prior to the start of therapy and periodically thereafter are thus recommended. Recovery usually occurs when *ethambutol* is withdrawn but in some cases can be permanent. There are no known clinically important drug interactions involving *ethambutol*.

Second-Line Drugs

Injectable Agents: Aminoglycosides (Streptomycin, Amikacin, Kanamycin) and Capreomycin

While *streptomycin* was commonly used as first-line therapy, and an injectable agent such as *amikacin*, *kanamycin*, or *capreomycin* was recommended as standard of care for MDR-TB until very recently, these drugs have largely been phased out of TB treatment regimens, owing to their dose- and duration-dependent toxicities, which includes irreversible hearing loss and vestibular damage. They are still used in hard-to-treat NTM infections, such as drug-resistant MAC or *M. abscessus*. The pharmacological properties and therapeutic uses of aminoglycosides are discussed in full in Chapter 59.

Capreomycin is an antimycobacterial cyclic peptide. It consists of four active components: capreomycins IA, IB, IIA, and IIB. The agent used clinically contains primarily IA and IB. Antimycobacterial activity is similar to that of aminoglycosides, as are adverse effects, and *capreomycin* should not be administered with other drugs that damage cranial nerve VIII. Adverse reactions include pain with injection, hearing loss, tinnitus, transient proteinuria, cylindruria, and nitrogen retention. Eosinophilia is common; leukocytosis, leukopenia, rash, and fever have been observed. Severe renal failure is rare.

Bacterial resistance to *capreomycin* develops when it is given alone; such microorganisms show cross-resistance with *kanamycin* and *neomycin*. The recommended daily dose is 1 g (no more than 20 mg/kg) per day for 60 to 120 days, followed by 1 g two or three times a week.

Clofazimine

Clofazimine is a fat-soluble riminophenazine dye initially evaluated as a treatment for TB in 1954, but owing to inconsistent results in early animal models, it was instead developed and first licensed for use in leprosy in 1969. It is now considered a Group A (standard of care, see Definitive Therapy of Drug-Resistant TB section below) drug for the treatment of RR- or MDR-TB in the WHO treatment guideline scheme, is increasingly used in NTM, and is useful for treatment of chronic skin ulcers (Buruli ulcer) produced by *Mycobacterium ulcerans*.

Mechanism of Action. The mechanism of antimicrobial activity of *clofazimine* against mycobacteria appears to be multifactorial and includes membrane disruption, inhibition of mycobacterial phospholipase A_2, inhibition of microbial K^+ transport, generation of hydrogen peroxide, interference with the bacterial electron transport chain, and efflux pump inhibition. *Clofazimine* has both antibacterial activity and anti-inflammatory effects via inhibition of macrophages, T cells, neutrophils, and complement.

Antibacterial Activity. The MICs for *M. tuberculosis* are 0.06 to 2 mg/L. The MICs for *M. avium* clinical isolates are 1 to 5 mg/L and for *M. abscessus* are 0.25 to 0.5 mg/L (Shen et al., 2010). It has activity against many gram-positive bacteria with an MIC of 1.0 mg/L or less against *S. aureus*, coagulase-negative staphylococci, *Streptococcus pyogenes*, and *Listeria monocytogenes*. Gram-negative bacteria have MICs greater than 32 mg/L.

Bacterial Resistance. Resistance has been associated with mutations in *Rv0678*, a repressor gene that affects the mmpS5-mmpL5 efflux pump; this is associated with cross-resistance to *bedaquiline* (Hartkoorn et al., 2014; Zhang S et al., 2015). Mutations in *pepQ* appear also to confer resistance to both *clofazimine* and *bedaquiline* via effects on drug efflux. Mutations in *Rv1979c* affect *clofazimine* but not *bedaquiline* (Kadura et al., 2020; Xu et al., 2017).

ADME. *Clofazimine* is administered orally at doses up to 300 mg a day. *Clofazimine*'s oral bioavailability is 45% to 60%; it is increased 2-fold by high-fat meals and decreased 30% by antacids (Nix et al., 2004). *Clofazimine*'s PK are best modeled using a three-compartment model. It has a prolonged absorption phase and large volume of distribution (10,000 L for a typical patient) (Abdelwahab et al., 2020). After sustained repeated dosing, the $t_{1/2}$ is about 35 days (see Table 65–2 for PK data). As a result of high penetration into many tissues, a reddish-black discoloration of skin and body secretions may occur and take months to years to resolve. Crystalline deposits of the drug have been encountered in many tissues at autopsy (TB Alliance, 2008). *Clofazimine* is metabolized in the liver in four steps: hydrolytic dehalogenation, hydrolytic deamination, glucuronidation, and hydroxylation.

Untoward Effects. Gastrointestinal problems are encountered in 40% to 50% of patients. In patients on *clofazimine* who died after complaints of abdominal pain, crystal deposition throughout the intestinal mucosa, liver, spleen, and abdominal lymph nodes was found (TB Alliance, 2008). Ichthyosis and discoloration of skin, eyes, and bodily secretions can be distressing or stigmatizing to patients. *Clofazimine* is also associated with prolongation of the QT interval on electrocardiogram (ECG).

Drug Interactions. The anti-inflammatory effects of *clofazimine* may be inhibited by *dapsone*.

Ethionamide

Ethionamide is a congener of thioisonicotinamide. It is currently considered a Group C drug for the treatment of MDR-TB (to be used only when a regimen cannot be constructed using Group A and B drugs).

Mechanism of Action. Mycobacterial EthA, an NADPH-specific, flavin adenine dinucleotide (FAD)-containing monooxygenase, converts *ethionamide* to a sulfoxide and then to 2-ethyl-4-aminopyridine (Vannelli et al., 2002). Although these products are not toxic to mycobacteria, it is believed that a closely related and transient intermediate is the active antibiotic. *Ethionamide* inhibits mycobacterial growth by inhibiting the activity of the *inhA* gene product, the enoyl-ACP (acyl carrier protein) reductase of fatty acid synthase II (Larsen et al., 2002). This is the same enzyme that activated INH inhibits. Although the exact mechanisms of inhibition may differ, the results are the same: inhibition of mycolic acid biosynthesis and consequent impairment of cell wall synthesis.

Antibacterial Activity. The multiplication of *M. tuberculosis* is suppressed by concentrations of *ethionamide* ranging from 0.6 to 2.5 mg/L. A concentration of 10 mg/L or less will inhibit about 75% of photochromogenic mycobacteria; the scotochromogens are more resistant.

Bacterial Resistance. Resistance occurs mainly via changes in the enzyme EthA that activates *ethionamide* or in a transcriptional repressor gene that controls its expression, *etaR*. Mutations in the *inhA* gene or in its promoter lead to resistance to both *ethionamide* and INH (Vilcheze and Jacobs, 2014).

ADME. The oral bioavailability of *ethionamide* approaches 100%. The PK are adequately explained by a one-compartment model with first-order absorption and elimination (Al-Shaer et al., 2020; Zhu et al., 2002) (see PK values in Table 65–2). After oral administration of 500 mg of *ethionamide*, a C_{max} of 1.4 mg/L is achieved in 2 h; $t_{1/2}$ is about 2 h. *Ethionamide* is cleared by hepatic metabolism. Metabolites are eliminated in the urine, with less than 1% of *ethionamide* excreted in an active form.

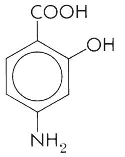
Therapeutic Uses. *Ethionamide* is administered orally. The initial dosage for adults is 250 mg twice daily; it is increased by 125 mg/day every

5 days until a dose of 15 to 20 mg/kg per day is achieved up to a daily maximal dose of 1 g. The drug is best taken with meals in divided doses to minimize gastric irritation. Children should receive 10 to 20 mg/kg per day in two divided doses, not to exceed 1 g/day.

Untoward Effects. *Ethionamide* is very poorly tolerated. Approximately 50% of patients are unable to tolerate a single dose larger than 500 mg because of GI upset. The most common reactions are anorexia, nausea and vomiting, gastric irritation, and a variety of neurological symptoms. Severe postural hypotension, mental depression, drowsiness, and asthenia are common. Other reactions referable to the nervous system include olfactory disturbances, blurred vision, diplopia, dizziness, paresthesias, headache, restlessness, and tremors. *Pyridoxine* (vitamin B_6) may relieve or prevent the neurological symptoms, and its concomitant administration is recommended. Severe allergic skin rashes, purpura, stomatitis, gynecomastia, impotence, menorrhagia, acne, metallic taste, and alopecia have also been observed. Hepatitis has been associated with the use of the *ethionamide* in about 5% of cases. Hepatic function should be assessed at regular intervals in patients receiving the drug.

Para-aminosalicylic Acid

Para-aminosalicylic acid (PAS), discovered by Lehman in 1943, was the second effective drug against TB. It is currently a Group C drug, to be used in MDR-TB only when regimens using Group A and B drugs are not possible.



AMINOSALICYLIC ACID

Mechanism of Action. *Para-aminosalicylic acid* is a structural analogue of *para*-aminobenzoic acid (PABA), the substrate of dihydropteroate synthase (*fol*P1/P2). It was previously believed that PAS was a competitive inhibitor of *fol*P1, but recent metabolomics work demonstrated that PAS is a prodrug whose active products block *M. tuberculosis* growth downstream of dihydropteroate synthase (DHPS) (Chakraborty et al., 2013). Mutation of the thymidylate synthase gene (*thyA*) results in resistance to PAS; however, only 37% of PAS-resistant clinical isolates or spontaneous mutants encode a mutation in the *thyA* gene or in any genes encoding enzymes in the folate pathway or biosynthesis of thymine nucleotides (Mathys et al., 2009).

Antibacterial Activity. *Para-aminosalicylic acid* is bacteriostatic. *In vitro*, most strains of *M. tuberculosis* are sensitive to a concentration of 1 mg/L. It has no activity against other bacteria.

Bacterial Resistance. Mutations in *thyA*, *folC*, and *ribD* lead to PAS resistance in up to 61% of resistant isolates (Zhang X et al., 2015). Recently, MDR-TB strains due to deletions of entire *dfrA* and *thyA* have been identified, a surprising finding given the conserved nature of folate synthesis (Moradigaravand et al., 2016).

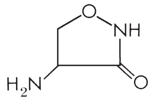
ADME. *Para-aminosalicylic acid* oral bioavailability is more than 90%. PAS PK are described by a one-compartment model (Peloquin et al., 2001) (see PK values in Table 65–2). The C_{max} increases 1.5-fold and AUC 1.7-fold with food compared to fasting (Peloquin et al., 2001). Administration with food also reduces gastric irritation. Protein binding is 50% to 60%. PAS is *N*-acetylated in the liver to *N*-acetyl PAS, a potential hepatotoxin. Over 80% of the drug is excreted in the urine; more than 50% is in the form of the acetylated compound. Excretion of PAS acid is reduced by renal dysfunction; thus, the dose must be reduced in renal dysfunction.

Therapeutic Uses. *Para-aminosalicylic acid* is administered orally in a total daily dose of 12 g. The drug is best administered after meals, with the daily dose divided into three equal portions. Children should receive 150 to 300 mg/kg per day in three or four divided doses.

Untoward Effects. The incidence of untoward effects associated with the use of PAS is about 10% to 30%. GI problems predominate and often limit patient adherence. In the days of TB sanatoria, it is rumored that one could tell which patients were on PAS because many patients threw it out the window, causing discoloration of the grass: the drug turns brown or purple in direct sunlight or if exposed to water. Hypersensitivity reactions to PAS are seen in 5% to 10% of patients and manifest as skin eruptions, fever, eosinophilia, and other hematological abnormalities. People with glucose-6-phosphate dehydrogenase (G6PD) deficiency should not take this drug, as it would cause hemolysis.

Cycloserine and Terizidone

Cycloserine is D-4-amino-3-isoxazolidone. It is a broad-spectrum antibiotic produced by *Streptococcus orchidaceous.* It is a Group B drug, used for MDR-TB.



CYCLOSERINE

Mechanism of Action. *Cycloserine* and D-alanine are structural analogues; thus, *cycloserine* inhibits alanine racemase, which converts L-alanine to D-alanine, and D-alanine–D-alanine ligase, thereby stopping reactions in which D-alanine is incorporated into bacterial cell wall synthesis. *Terizidone* is a structural analogue of *cycloserine*, and the two drugs are used interchangeably.

Antibacterial Activity. *Cycloserine* is a broad-spectrum antibiotic. It inhibits *M. tuberculosis* at concentrations of 5 to 20 mg/L. It has good activity against MAC, enterococci, *E. coli, S. aureus, Nocardia* species, and *Chlamydia*.

Mechanisms of Resistance. *Cycloserine* resistance in clinical isolates of *M. tuberculosis* results mostly from mutations in *alr*, which encodes alanine racemase or in the *alr* promoter (Chen et al., 2017; Desjardins et al., 2016).

ADME. Oral *cycloserine* is almost completely absorbed. The population PK are best described using a one-compartment model with first-order absorption and elimination. The drug's $t_{1/2}$ is 9 h. The C_{max} in plasma is reached in 45 min in fasting subjects but is delayed for up to 3.5 h with a high-fat meal. See Table 65–2 for PK values. *Cycloserine* is widely distributed throughout the body, including to the cerebrospinal fluid and brain. About 50% of *cycloserine* is excreted unchanged in the urine in the first 12 h; a total of 70% is recoverable in the active form over a period of 24 h. The drug may accumulate to toxic concentrations in patients with renal failure. About 60% of *cycloserine* is removed by hemodialysis, and the drug must be readministered after each hemodialysis session (Malone et al., 1999a).

Therapeutic Uses. *Cycloserine* is available for oral administration. The usual dose for adults is 250 to 500 mg twice daily.

Untoward Effects. Neuropsychiatric symptoms are common and occur in 50% of patients on 1 g/day. Because of the prevalence of these occurrences, the drug is sometimes referred to as "psycho-serine." Symptoms range from headache and somnolence to severe psychosis, seizures, and suicidal ideations. Large doses of *cycloserine* or the concomitant ingestion of alcohol increases the risk of seizures. *Cycloserine* is contraindicated in individuals with a history of epilepsy and should be used with caution in individuals with a history of depression.

Newer Drugs

Bedaquiline

Bedaquiline was discovered by Andries and colleagues in 2005 and was registered with the FDA in 2012 for the treatment of drug-resistant TB. Since then, it has been shown to have potent sterilizing activity and to reduce mortality in patients with MDR-TB (Ndjeka et al., 2018). It is now classified as a Group A drug and considered standard of care for patients with MDR-TB. *Bedaquiline* is a cationic amphiphilic drug, which may account for its high accumulation in tissues.

Mechanism of Action. *Bedaquiline* acts by targeting subunit *c* of the ATP synthase of *M. tuberculosis*, leading to inhibition of the proton pump activity of the ATP synthase, thereby targeting bacillary energy metabolism (Andries et al., 2005; Koul et al., 2007).

Antibacterial Activity. The *bedaquiline* MIC for *M. tuberculosis* is 0.03 to 0.12 mg/L. It has potent activity against MAC, *M. leprae, M. bovis, M. marinum, M. kansasii, M. ulcerans, M. fortuitum, M. szulgai,* and *M. abscessus* (Andries et al., 2005; Huitric et al., 2007).

Bacterial Resistance. The proportion of *M. tuberculosis* mutants resistant to four times the MIC is 5×10^{-7} to 2×10^{-8}. Resistance is associated with mutations in D32V and A63P in the region of the gene encoding the membrane-spanning domain of the ATP synthase *c* subunit. Resistance due to efflux pump mutations and their regulators also occurs and is associated with cross-resistance to *clofazimine*.

ADME. The population PK of *bedaquiline* are well characterized (Svensson et al., 2013, 2016). After oral ingestion, there is a large lag time in absorption, resulting in a t_{max} of 5 h. The PKs are best described using a three-compartment model, with a central compartment and two peripheral compartments. The drug has a very large volume of distribution (>10,000 L) (Svensson et al., 2016) and accumulates intracellularly. Food increases bioavailability 2-fold (van Heeswijk et al., 2014). Clearance is 52% higher in people of African descent and 16% lower in women compared to men. The terminal $t_{1/2}$ is about 5.5 months, mainly driven by redistribution from the tissues. *Bedaquiline* is metabolized by CY3A4 to M2, an *N*-monodesmethyl metabolite, which has approximately 20% of parent drug's activity against *M. tuberculosis*.

Microbial Pharmacokinetics-Pharmacodynamics. Microbial kill of *bedaquiline* is linked to AUC/MIC ratios (Rouan et al., 2012; Svensson and Karlsson, 2017). The PK-pharmacodynamic parameters linked to resistance suppression are not established.

Efficacy and Therapeutic Use. A regimen of *bedaquiline* 400 mg daily for 2 weeks followed by 200 mg three times per week to complete 24 weeks of therapy was added to a background second-line regimen of either *kanamycin* or *amikacin* and *ofloxacin* with or without *ethambutol* in patients with MDR-TB and led to an 8-week sputum conversion of about 50% with *bedaquiline* compared to 9% with background regimen alone (Diacon et al., 2009). This study led to licensing of the drug as part of multidrug therapy for MDR-TB. Subsequent studies confirmed the bacterial activity seen in the initial trial (Diacon et al., 2014). More recently, a 6-month regimen of *bedaquiline* combined with *pretomanid* and *linezolid* proved highly effective against extensively drug-resistant (XDR-) TB (Conradie et al., 2020).

Untoward Effects. *Bedaquiline* is generally well tolerated. The most common side effects include nausea in 26% of patients and diarrhea in 13% of patients, but these are rarely treatment-limiting. *Bedaquiline* causes prolongation of the QT interval, driven by its M2 metabolite; thus, serial ECG monitoring is recommended.

Drug Interactions. Bedaquiline is metabolized by CYP3A4; thus, it is prudent to avoid strong CYP3A4 inducers while taking *bedaquiline*. For patients taking a companion drug that inhibits CYP3A4, if substitution is not possible, then close ECG monitoring should be undertaken. The same is true if *bedaquiline* is taken with other drugs that prolong the QT interval.

Bicyclic Nitroimidazoles: Delamanid and Pretomanid

Pretomanid, discovered by Stover and coleagues in 2000, and *delamanid*, discovered by Matsumoto and colleagues in 2006, are bicyclic nitroimidazopyrans that are being used in the treatment of XDR- and MDR-TB. *Pretomanid* is FDA-approved for the treatment of XDR-TB in combination with *bedaquiline* and *linezolid*. *Delamanid* is licensed by the European Medicines Agency for the treatment of MDR-TB.

Mechanisms of Action. *Pretomanid* has two mechanisms of action. First, under aerobic conditions, it inhibits *M. tuberculosis* mycolic acid and protein synthesis at the step between hydroxymycolate and ketomycolate (Stover et al., 2000). Similar to the structurally related *metronidazole*, *pretomanid* requires activation by the bacteria via a nitroreduction step that requires, among other factors, a specific G6PD, FGD1 (NADP-dependent G6PD), and the reduced deazaflavin cofactor F_{420} encoded by Rv3547 (Bashiri et al., 2008). Second, in nonreplicating persistent bacilli (NRPB), it generates reactive nitrogen species such as NO via its des-nitro metabolite, which then augment the kill of intracellular NRPB by the innate immune system (Singh et al., 2008). In addition, direct poisoning of the respiratory complex in the NRPB leads to ATP depletion.

Delamanid is also a prodrug that is activated by the same enzyme encoded by Rv3547 (Xavier and Lakshmanan, 2014). Similarly, it also forms a reactive intermediate metabolite that inhibits mycolic acid production.

Antibacterial Activity. The MICs of *pretomanid* against *M. tuberculosis* range from 0.015 to 0.25 mg/L, but the drug lacks activity against other mycobacteria. Similarly, for *delamanid*, MICs range from 0.006 to 0.012 mg/L (Matsumoto et al., 2006).

Mechanism of Resistance. The proportion of mutants resistant to 5 mg/L of *pretomanid* is 1 in 10^{-6}. Resistance arises from changes in the structure of FGD, which is due to a variety of point mutations in the *fgd* gene. However, resistant isolates have also been identified that lack *fgd* mutations (Stover et al., 2000). Mutation frequencies for *delamanid* are unclear; however, resistance has also been shown to be due to *fgd1* and *fbiA* mutations (Bloemberg et al., 2015).

ADME. Systemic exposures of *pretomanid* and *delamanid* increase when taken with food. *Delamanid* metabolism involves albumin. *Delamanid* is not metabolized by CYP4s. *Pretomanid* is metabolized approximately 20% by CYP3A; coadministration of *rifampin* reduces *pretomanid* exposures (Dooley et al., 2014; Ignatius et al., 2021). PK parameters of *pretomanid* and *delamanid* are summarized in Table 65–2 (Ginsberg et al., 2009; Ignatius et al., 2021; Salinger et al., 2019; Wang et al., 2020).

Microbial Pharmacokinetics-Pharmacodynamics. Nitroimidazoles display time-dependent activity in mouse models of TB. There are no published analyses evaluating PK-pharmacodynamic relationships for nitroimidazoles in patients with TB.

Therapeutic Uses. *Delamanid* is currently dispensed in 50-mg tablets, and it is taken at a dose of 100 mg twice daily, with food; in the phase III trial among patients with pulmonary MDR-TB, the dose was switched to 200 mg once daily after the initial 2 months of treatment for ease of administration. *Pretomanid* is administered at 200 mg/day and has shown efficacy in phase III trials in combination with *bedaquiline* and *linezolid* for the treatment of MDR- and XDR-TB (Conradie et al., 2020).

Untoward Effects. *Delamanid* can cause headaches and insomnia. It has a modest effect on the QT interval on ECG. *Pretomanid* was registered based on data from just 100 patients, so the safety profile of this drug has not been fully elucidated. This drug has been associated with hepatotoxicity, but the magnitude of the risk and the role of companion drugs in this potential toxicity are still under investigation. Its effects on human male fertility have not been adequately evaluated, but studies are underway.

Drug interactions. *Pretomanid* is metabolized in part by CYP3A, so coadministration with strong or moderate CYP3A inducers should be avoided. *Delamanid* displays low bioavailability, so to maximize absorption, it should be taken with food and separated from dosing of other medications, if possible.

Drugs Repurposed for Use in Mycobacterial Infections

Oxazolidinones: Linezolid, Tedizolid, and Sutezolid

Oxazolidinones have been in clinical use for over a decade for the treatment of gram-positive cocci. *Linezolid* and *tedizolid* are highly efficacious in the treatment of TB (Sotgiu et al., 2012). Several studies have examined the utility of *linezolid* for the treatment of MDR-TB, finding that *linezolid* significantly increases sputum conversion but causes adverse responses in a large fraction of patients (see, for instance, Tang et al., 2015). *Linezolid* is now considered a Group A drug (standard of care) for MDR-TB by the WHO. It is also a required component of a novel 6-month, three-drug regimen that is FDA-approved for patients with XDR-TB, along with *pretomanid* and *bedaquiline* (Conradie et al., 2020). However, side effects related to mitochondrial toxicity (e.g., bone marrow suppression, peripheral neuropathy, optic neuropathy) are common with prolonged dosing, and so new oxazolidinones such as *sutezolid* are in the development pipeline.

Linezolid is also used in the treatment of more complex NTM cases when an effective regimen cannot be constructed owing to resistance or tolerability challenges, but advice regarding its use has not been incorporated into treatment guidelines.

The pharmacology of oxazolidinones is discussed in Chapter 60.

Fluoroquinolones

Fluoroquinolones are DNA gyrase inhibitors. Their chemistry, spectrum of activity, and pharmacology are discussed in detail in Chapter 57. Of the C8 methoxy quinolones, *moxifloxacin* and *levofloxacin* have been integrated into the treatment of pulmonary and meningeal TB. They are also sometimes used in the treatment of NTM infections. *Ofloxacin* and *ciprofloxacin* are no longer used for TB treatment.

Microbial Pharmacokinetics-Pharmacodynamics Relevant to TB

Fluoroquinolone microbial kill is best explained by the AUC_{0-24}/MIC ratio. In preclinical models of *M. tuberculosis*, *moxifloxacin* AUC_{0-24}/MIC exposures equivalent to those from the standard 400-mg dose were associated with good microbial kill, and in a recent trial, the addition of *moxifloxacin* at a dose of 400 mg daily to a *rifapentine*-based regimen led to enhanced activity that was sufficient to shorten treatment duration from 6 to 4 months (Dorman et al., 2021). This might be in part due to *moxifloxacin*'s high penetration into the lung lesions that characterize human TB and activity against mycobacteria in both aerobic and anaerobic conditions (Sarathy et al., 2019; Strydom et al., 2019). Whether *levofloxacin* has similar treatment-shortening properties is unknown. Clinical trial simulations have revealed that *moxifloxacin* doses greater than 400 mg daily might better achieve AUC/MIC targets for both bacterial killing and suppression of emergence of resistance (Ginsburg et al., 2003; Gumbo et al., 2004). Whether these benefits outweigh the increase in corrected QT interval on the ECG seen with higher-dose *moxifloxacin* remains to be seen.

Fluoroquinolones have very good penetration into the CNS (Thwaites et al., 2011), unlike *ethambutol* and *streptomycin*. In a phase III study, intensified anti-TB treatment with high-dose *rifampin* plus *levofloxacin* was associated with a higher rate of survival among patients with tuberculous meningitis whose *M. tuberculosis* isolates were isoniazid-resistant (but not among those whose isolates were isoniazid-sensitive) (Heemskerk et al., 2016). This suggests that fluoroquinolones may have a role as a replacement for the highly bactericidal INH in INH-monoresistant TB.

Currently, fluoroquinolones, especially *moxifloxacin*, continue to be standard of care as Group A drugs for the treatment of pulmonary MDR-TB (WHO, 2019), and their use is strongly associated with improvement in treatment outcomes.

Fluoroquinolones are not considered first-line treatment for any NTM but are used when other first-line drugs cannot be.

β-Lactam Antibiotics for the Treatment of TB

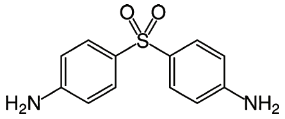
Until recently, it was assumed that β-lactam antibiotics lacked activity against mycobacteria because these bacteria have Ambler class A β-lactamases such as BlacC. Carbapenems, which are poorer substrates of these enzymes, in conjunction with inhibitors such as *clavulanate*, have demonstrated efficacy against *M. tuberculosis*. Compounds such as *faropenem*, which possess both *carbapenem* and *cephalosporin* structures, are effective against *M. tuberculosis* without a β-lactamase inhibitor. The general pharmacology of this class of compounds is discussed in Chapter 58. Patients with MDR-TB have reportedly responded to β-lactam antibiotics such as *ertapenem* (Tiberi et al., 2016). A meta-analysis has identified seven clinical studies in which *ertapenem*, *imipenem*, and *meropenem* were used to treat MDR-TB with sputum conversion rates exceeding 60% (Sotgiu et al., 2016). These data notwithstanding, the role and optimal dosing of β-lactams in the treatment of MDR-TB remain poorly defined.

Macrolides

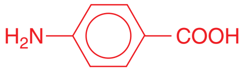
The pharmacology, bacterial activity, and resistance mechanisms of macrolides are discussed in Chapter 60. *Azithromycin* and *clarithromycin* are used for the treatment of MAC.

Dapsone

Dapsone (diamino-diphenylsulfone) is a broad-spectrum agent with antibacterial, antiprotozoal, and antifungal effects.



DAPSONE



PARA-AMINOBENZOIC ACID

Mechanism of Action

Dapsone is a structural analogue of PABA (similar to sulfonamides; see Chapter 57) and a competitive inhibitor of dihydropteroate synthase (*fol*P1/P2) in the folate pathway (Figure 65–5). The anti-inflammatory effects of *dapsone* occur via inhibition of tissue damage by neutrophils (Wolf et al., 2002). *Dapsone* inhibits neutrophil myeloperoxidase activity and respiratory burst, and it inhibits activity of neutrophil lysosomal enzymes. *Dapsone* may act as a scavenger of free radicals generated by neutrophils, and *dapsone* may inhibit migration of neutrophils to inflammatory lesions (Wolf et al., 2002).

Antimicrobial Effects

Antibacterial. *Dapsone* is bacteriostatic against *M. leprae* at concentrations of 1 to 10 mg/L. More than 90% of clinical isolates of MAC and *M. kansasii* have an MIC of 8 mg/L or lower, but the MICs for *M. tuberculosis* isolates are high. *Dapsone* has little activity against other bacteria.

Antiparasitic. *Dapsone* is also highly effective against *Plasmodium falciparum* with IC_{50} (concentration causing 50% inhibition) of 0.6 to 1.3 mg/L even in *sulfadoxine-pyrimethamine*–resistant strains. *Dapsone* has an IC_{50} of 0.55 mg/L against *Toxoplasma gondii* tachyzoites.

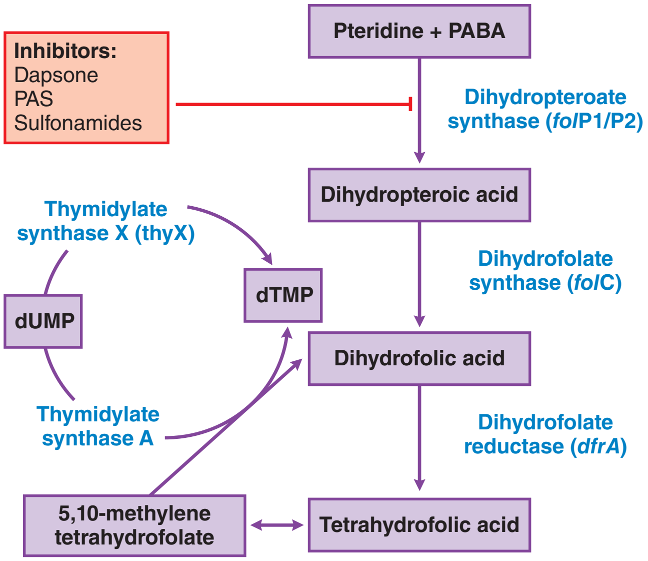

Inhibitors:
Dapsone
PAS
Sulfonamides
Pteridine + PABA
Dihydropteroate synthase (folP1/P2)
Dihydropteroic acid
Dihydrofolate synthase (folC)
Dihydrofolic acid
Dihydrofolate reductase (dfrA)
Tetrahydrofolic acid
5,10-methylene tetrahydrofolate
Thymidylate synthase X (thyX)
Thymidylate synthase A
dUMP
dTMP

Figure 65–5 *Effects of antimicrobials on folate metabolism and deoxynucleotide synthesis.* dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate.

Antifungal. *Dapsone* is effective at concentrations of 0.1 mg/L against the fungus *Pneumocystis jirovecii*.

Drug Resistance

Resistance to *dapsone* in *P. falciparum*, *P. jirovecii*, and *M. leprae* results primarily from mutations in genes encoding dihydropteroate synthase (see Figure 65–5).

ADME

After oral administration, absorption is complete; the elimination $t_{1/2}$ is 20 to 30 h. Table 65–2 shows the population PK of *dapsone* (Simpson et al., 2006). *Dapsone* undergoes *N*-acetylation by NAT2. *N*-Oxidation to dapsone hydroxylamine is via CYP2E1 and to a lesser extent by CYP2C. Dapsone hydroxylamine enters red blood cells, leading to methemoglobin formation. Sulfones tend to be retained for up to 3 weeks in skin and muscle and especially in liver and kidney. Intestinal reabsorption of sulfones excreted in the bile contributes to long-term retention in the bloodstream; periodic interruption of treatment is advisable for this reason. Epithelial lining fluid-to-plasma ratio is between 0.76 and 2.91; cerebrospinal fluid–to–plasma ratio is 0.21 to 2.01 (Gatti et al., 1997). Approximately 70% to 80% of a dose of *dapsone* is excreted in the urine as an acid-labile mono-*N*-glucuronide and mono-*N*-sulfamate.

Therapeutic Uses

Dapsone is administered as an oral agent. Therapeutic uses of *dapsone* in the treatment of leprosy are described later in this chapter. *Dapsone* is also used for the prophylaxis for *T. gondii* (see Chapter 67). It is no longer used for malaria. The anti-inflammatory effects are the basis for therapy of pemphigoid, dermatitis herpetiformis, linear immunoglobulin A bullous disease, relapsing chondritis, and ulcers caused by the brown recluse spider (Wolf et al., 2002).

Dapsone and Glucose-6-Phosphate Dehydrogenase Deficiency

Glucose-6-phosphate dehydrogenase protects red cells against oxidative damage. However, G6PD deficiency is encountered in nearly half a billion people worldwide, the most common of 100 variants being G6PD-A$^-$. *Dapsone*, an oxidant, causes severe hemolysis in patients with G6PD deficiency. Thus, G6PD deficiency testing should be performed prior to use of *dapsone* whenever possible.

Other Untoward Effects

Hemolysis develops in almost every individual treated with 200 to 300 mg of *dapsone* per day. Doses of 100 mg or less in healthy persons and 50 mg or less in healthy individuals with a G6PD deficiency do not cause hemolysis. Methemoglobinemia also is common. A genetic deficiency in NADH-dependent methemoglobin reductase can result in severe methemoglobinemia after administration of *dapsone*. Isolated instances of headache, nervousness, insomnia, blurred vision, paresthesias, reversible peripheral neuropathy (thought to be due to axonal degeneration), drug fever, hematuria, pruritus, psychosis, and a variety of skin rashes have been reported. An infectious mononucleosis–like syndrome, which may be fatal, occurs occasionally.

Principles of Antituberculosis Chemotherapy

Evolution and Pharmacology

The *M. tuberculosis* complex is comprised of several species with 99.9% similarity at the nucleotide level. The complex includes *M. tuberculosis (typus humanus), M. canettii, M. africanum, M. bovis,* and *M. microti*. They all cause TB, with *M. microti* responsible for only a handful of human cases.

Antituberculosis Therapy

Following a series of multiple, large, well-designed trials conducted from the 1940s to the 1970s, INH, *pyrazinamide*, *rifampin*, and *ethambutol* were established as first-line anti-TB agents that, when used together, could treat TB in 6 months, so-called "short-course" chemotherapy (Fox et al., 1999). Each drug in the regimen has a unique role. INH is highly bactericidal, resulting in rapid reductions in bacilli burden within just a few days of starting TB treatment. This results in rapid improvement in TB symptoms and reduces transmission risk. *Rifampin* is able to kill semi-dormant or dormant bacilli, so-called "persisters" (Dickinson and Mitchison, 1981), and it remains the best sterilizing agent available. It is unparalleled in its ability to kill persister organisms that must be eradicated to cure the patient. *Pyrazinamide* adds sterilizing activity to the first-line regimen, seeming to kill subpopulations of *M. tuberculosis* that reside in acidic conditions inside macrophages and in necrotic lesions; further, it is synergistic with other anti-TB drugs. Use of *pyrazinamide* allows for treatment shortening from 9 to 6 months (Hong Kong Chest Service, 1978). *Ethambutol* is given at its lowest effective dose and does not contribute meaningfully to the antibacterial effects of multidrug regimens; rather, its role is to protect companion drugs against emergence of resistance (Doster et al., 1973). As new drugs are developed for TB, it is critical to consider their roles in multidrug regimens and to ensure that new drug combinations have bactericidal activity, sterilizing activity, and robustness to emergence of resistance (Ignatius and Dooley, 2019). Other considerations include penetration into lung lesions, drug interactions, and overlapping toxicities. Second-line agents are used in case of poor tolerance or resistance to first-line agents.

We use multiple drugs to treat TB because each drug plays a specific role in the regimen. Combination therapy is also mandatory to prevent emergence of clinical resistance. Pretreatment mutation rates to anti-TB drugs are between 10^{-6} and 10^{-10}, so that the probability of resistance is high to any single anti-TB drug in patients with cavitary TB who have about 10^9 colony-forming units of bacilli in a 3-cm pulmonary lesion. However, the likelihood that bacilli will develop mutations to two or more different drugs is the product of two mutation rates (between 1 in 10^{14} and 1 in 10^{20}).

Types of Antituberculosis Therapy

Prophylaxis

After infection with *M. tuberculosis*, about 10% of people will develop active disease over a lifetime. The highest risk of reactivation TB is in patients with Mantoux tuberculin skin test reaction 5 mm or greater who also fall into one of the following categories: household contact of someone with pulmonary TB, HIV co-infection, lung fibrosis, or immunosuppressed due to treatment of rheumatological disease or cancer. If the tuberculin skin test is 10 mm or greater, those with a high risk of TB include recent (≤5 years) immigrants from areas of high TB prevalence, children younger than 4 years, children exposed to adults with TB, people who inject intravenous drugs, as well as residents and employees of high-risk congregate settings. Any person with a skin test greater than 15 mm is also at high risk of developing active TB disease.

In these higher-risk patients, prophylactic treatment with "TB preventive therapy" (TPT) is recommended to prevent active disease. There are now several TPT regimens (Table 65–5). The most tried-and-true regimen is oral INH 300 mg daily (or 10–15 mg/kg daily for children) for 6 to 9 months. While effective, treatment duration is long, and completion rates are poor. *Rifampin* 10 mg/kg daily for 4 months is an alternative regimen. Once-weekly *rifapentine* (900 mg) plus INH (900 mg) given for a total of 12 doses over 3 months (or 3HP) is highly effective, and adherence is generally excellent (Sterling et al., 2011). The newest (and shortest) TPT regimen is 1HP, or once-daily *rifapentine* (10 mg/kg) plus INH (300 mg) given for 28 days (Swindells et al., 2019). To date, published clinical data for this alternative regimen are only available for patients with HIV.

Definitive Therapy of Drug-Susceptible Active TB

All patients with active TB should have sputum collected for genotypic or phenotypic resistance testing. In adults, the current standard regimen for drug-susceptible pulmonary TB consists of INH (5 mg/kg, maximum 300 mg/day), *rifampin* (10 mg/kg, maximum 600 mg/day), *pyrazinamide*

TABLE 65–5 ■ WHO TREATMENT REGIMENS FOR LATENT TUBERCULOSIS INFECTION

REGIMEN	DOSE (PER kg BODY WEIGHT)	DURATION
Daily isoniazid	Adults: 5 mg Children: 10 mg (range 7–15 mg)	6–9 months
Daily rifampin	Adults: 10 mg Children: 15 mg (range 10–20 mg)	3–4 months
Daily isoniazid PLUS rifampin	Adults: 5 mg isoniazid, 10 mg rifampin Children: 10 mg isoniazid, 15 mg rifampin	3–4 months
Weekly rifapentine PLUS isoniazid	≥12 years: isoniazid 15 mg 2–11 years: isoniazid 25 mg Rifapentine: 10.0–14.0 kg = 300 mg 14.1–25.0 kg = 450 mg 25.1–32.0 kg = 600 mg 32.1–50.0 kg = 750 mg >50 kg = 900 mg	3 months (12 doses)

(15–30 mg/kg, maximum of 2 g/day), and *ethambutol* (15–20 mg/kg) given daily for 2 months, followed by 4 months of INH and *rifampin*, to complete 6 months of therapy. Daily therapy is preferred over intermittent dosing, as daily treatment gives the highest chance for cure without relapse (the current regimen is unforgiving to missed doses) (Imperial et al., 2018). Children should receive *rifampin* 15 to 20 mg/kg at a maximum dose of 600 mg/day, *pyrazinamide* 30 to 40 mg/kg per day, and INH 10 to 15 mg/kg at a maximum dose of 300 mg/day. *Rifabutin* can be substituted for *rifampin* in cases where there are drug interactions that preclude use of *rifampin*, for example, among patients with HIV taking second-line antiretroviral therapy containing protease inhibitors, but the *rifabutin* dose should be decreased to 150 mg daily during coadministration (Kendall et al., 2021). *Ethambutol* is only continued until INH susceptibility can be confirmed. Because monitoring of visual acuity is difficult in children younger than 5 years, caution should be exercised in using *ethambutol* in this group.

The first 2 months of the four-drug regimen compose the *initiation phase*, and the last 4 months compose the *continuation phase* of therapy. *Pyridoxine*, vitamin B_6 (10–50 mg/day), should be administered with INH to minimize the risks of neurological toxicity in patients predisposed to neuropathy (e.g., the malnourished, elderly, pregnant women, or patients with HIV, diabetes mellitus, high alcohol intake, or renal failure). To support adherence, therapy is typically administered as directly observed therapy (Volmink and Garner, 2007). More patient-centered strategies for adherence support are now endorsed by the WHO and offered as alternatives.

Most cases of extrapulmonary TB are treated for 6 months. TB meningitis is typically treated for longer, 9 to 12 months, although that duration is not evidence based (Jullien et al., 2016), and it is likely that using drugs at doses that reach effective concentrations in the brain and cerebrospinal fluid is more important than prolonging treatment duration. Patients with TB meningitis should also receive steroids as standard of care (Prasad et al., 2016).

The treatment of TB pericarditis is a special case in which the use of steroids has been advocated for many decades. The IMPI trial randomized 1400 patients to examine the effect of *prednisolone* on the composite outcome of death, cardiac tamponade requiring pericardiocentesis, or constrictive pericarditis. There was no significant difference in the primary outcome between patients who received *prednisolone* and those who received placebo, even though the corticosteroids were associated with a 44% decrease in the development of constrictive pericarditis (Mayosi et al., 2014). Free drug concentrations of *rifampin* in pericardial fluid are close to zero, *ethambutol* concentrations are low, and the pH in infected pericardial fluid is around 7.3, which is a pH at which *pyrazinamide* has no effect (Shenje et al., 2015). The optimal regimen for treatment of TB pericarditis thus remains to be identified.

TABLE 65–6 ■ WORLD HEALTH ORGANIZATION GROUPINGS OF DRUGS FOR MDR-/RR-TB

GROUP	DRUG	COMMENTS
A	Levofloxacin or moxifloxacin Bedaquiline Linezolid	Include all three (unless they cannot be used)
B	Clofazimine Cycloserine or terizidone	Add both, unless they cannot be used
C	Ethambutol Delamanid Pyrazinamide Meropenem (plus amoxicillin-clavulanate) Amikacin Ethionamide or prothionamide	Add to complete the regimen and when medicines from Groups A and B cannot be used

Definitive Therapy of Drug-Resistant TB

The WHO has laid out guidance for the treatment of MDR-TB—all three Group A drugs (a fluoroquinolone plus *bedaquiline* and *linezolid*), one or both Group B drugs (*clofazimine* and *cycloserine* or *terizidone*), and one or more Group C drugs (*ethambutol*, *delamanid*, *pyrazinamide*, β-lactam, *ethionamide*, *amikacin*, or PAS), for a total of at least four to five active drugs, should be given (Table 65–6). Treatment duration is 18 to 20 months. If a patient's TB isolate is resistant to one or more of these second-line agents or the patient cannot tolerate a recommended drug, then therapy should be based on evidence of susceptibility and should include at least four drugs to which the pathogen is susceptible. A standardized, shorter MDR-TB regimen of 9 to 12 months is possible for patients whose *M. tuberculosis* isolate is susceptible to fluoroquinolones and comprises *bedaquiline*, a fluoroquinolone, *clofazimine*, *pyrazinamide*, *ethambutol*, high-dose INH, and *ethionamide* (WHO, 2020).

Principles of Therapy for Nontuberculous Mycobacteria

NTM are composed of nearly 200 subspecies and cause both pulmonary and extrapulmonary disease among susceptible individuals. *In vitro* antimicrobial susceptibility can vary widely within and between species; therefore, drug susceptibility testing should be performed on isolates deemed to represent true infection (rather than colonization). The most common species causing human infection are MAC, *M. kansasii, M. xenopi*, and *M. abscessus* (Daley et al., 2020). MAC includes *M. intracellulare, M. avium,* and *M. chimaera,* among others. NTM are ubiquitous in the environment and can be encountered in water, food, and soil. Therefore, when NTM are isolated from a nonsterile site in a patient's body, one cannot assume they are causing an infection.

Therapy of NTM Pulmonary Infection

Although distinguishing colonization from disease can be challenging, the 2007 American Thoracic Society/Infectious Diseases Society of America guidelines provide clear diagnostic criteria for clinicians. These criteria include pulmonary or systemic symptoms, nodules or cavitations on chest imaging, exclusion of other diagnoses, and either two positive cultures from expectorated sputum or one from either bronchial wash/lavage or biopsy. Once the diagnosis is established, the decision to commence treatment can be complex, given that many patients experience mild chronic or waxing/waning symptoms and medications may be poorly tolerated (Griffith et al., 2007). Recently updated clinical guidelines, however, advocate treatment for those meeting diagnostic criteria for NTM pulmonary disease over watchful waiting, particularly when there is significant baseline mycobacterial burden (sputum is acid-fast bacilli smear positive) or cavitary disease (Daley et al., 2020).

In newly diagnosed patients with pulmonary MAC, triple-drug therapy is recommended. These drugs include a rifamycin, *ethambutol*, and a macrolide. Either oral *clarithromycin* or *azithromycin* may be used; however, *azithromycin* is favored in the updated 2020 guidelines as it is better tolerated, less frequently dosed, and less prone to drug interactions (Daley et al., 2020). *Rifampin* is often the rifamycin of choice, although *rifabutin* may be substituted to mitigate drug-drug interactions. *Clarithromycin* 1000 mg or *azithromycin* 500 mg is combined with *ethambutol* 25 mg/kg and *rifampin* 600 mg and administered three times a week for nodular and bronchiectatic disease. Therapy is continued for 12 months after the last negative culture. The same drugs are recommended for patients with cavitary disease, but the dosing is *azithromycin* 250 mg, *ethambutol* 15 mg/kg, and *rifampin* 600 mg administered daily. Parenteral *streptomycin* or *amikacin* at 15 mg/kg is recommended as a fourth drug for patients with severe disease or macrolide-resistant MAC. Inhaled *amikacin* liposome inhalation suspension is now recommended for add-on therapy for treatment-refractory MAC (Daley et al., 2020).

Given high rates of sustained cure for pulmonary *M. kansasii* infection, currently guidelines recommend that *rifampin*-sensitive *M. kansasii* be treated with *rifampin*, *ethambutol*, and either INH or a macrolide, without the routine addition of an aminoglycoside. For *rifampin*-resistant *M. kansasii*, a fluoroquinolone may be added in lieu of *rifampin*. Dosing frequency recommendations for *M. kansasii* treated with a macrolide are similar to pulmonary MAC, with daily dosing usually reserved for more severe or cavitary disease. For *M. kansasii* treated with INH (instead of a macrolide), daily triple therapy is recommended.

Current guidelines for treatment of *M. xenopi* recommend daily triple therapy with *rifampin*, *ethambutol*, and either *moxifloxacin* or a macrolide, with the addition of *amikacin* for more severe disease.

M. abscessus is an increasingly recognized and virulent pathogen among those with predisposing factors like structural lung disease, and treatment options are often limited by baseline resistance that is extensive, unpredictable, and, in many cases, identified weeks after the initial *M. abscessus* infection is diagnosed. Given the risk of inducible macrolide resistance caused by a functional *erm*(41) gene, prior guidelines recommended use of macrolides for *M. abscessus* only when there was demonstrable sensitivity; updated guidelines, however, recommend addition of a macrolide in all cases for the macrolide's immunomodulatory properties. Multidrug therapy with three active medications is recommended for *M. abscessus* disease.

Duration of therapy for these pulmonary NTM infections is usually a minimum of 12 months (and often 12 months from culture conversion) for both cavitary and nodular/bronchiectatic disease, although data to support precise treatment durations are scant. For both MAC and *M. abscessus*, macrolide sensitivity correlates with outcomes (Griffith et al., 2006; Jeon et al., 2009; Koh et al., 2011; Moon et al., 2016); therefore, use of a macrolide is highly encouraged, provided the patient can tolerate it. Patients at risk for treatment failure also include those with cavitary disease, presumably due to higher bacillary load. Even with these therapies, long-term success rates are low. Only half of patients have successful outcomes as defined by both culture conversion and clinical resolution.

Therapy of Disseminated NTM

Although various drugs and combination regimens have been used with success in the treatment of a variety of disseminated NTM infections, there are firm guidelines only for the treatment of disseminated MAC among patients with HIV. In this population, *M. avium* is the most common infecting species and tends to occur in patients with CD4 cell count less than 50/mm^3 who are not on antiretroviral therapy (ART).

The symptoms and laboratory findings of disseminated disease are nonspecific and include fever, night sweats, weight loss, diarrhea, elevated serum alkaline phosphatase, and anemia. However, when disease occurs in patients already on ART, it may manifest as focal disease of the lymph nodes, osteomyelitis, pneumonitis, pericarditis, skin or soft-tissue abscesses, genital ulcers, or CNS infection (Department of Health and Human Services, 2020). In addition to a compatible clinical picture, isolation of MAC from cultures of blood, lymph node, bone marrow, or other normally sterile tissue or body fluids is required for diagnosis.

Prophylaxis against disseminated MAC is no longer recommended for patients with HIV who are taking ART, even those patients newly initiating HIV treatment. For patients with CD4 count below 50 cells/mm^3 who cannot be on suppressive ART, prophylaxis with weekly *azithromycin* 1200 mg, twice-weekly *azithromycin* 600 mg, twice-daily *clarithromycin* 500 mg, or daily *rifabutin* 300 mg should be prescribed.

First-line treatment of disseminated MAC consists of either *clarithromycin* 500 mg twice daily or *azithromycin* 500 to 600 mg daily plus *ethambutol* 15 mg/kg. The addition of a third or fourth drug (*rifabutin* 300 mg, *levofloxacin* 500 mg, *moxifloxacin* 400 mg, *amikacin* 10–15 mg/kg, or *streptomycin* 1 mg IV/IM daily) is recommended for those with high baseline bacterial load (Department of Health and Human Services, 2021). Patients should be continued on multidrug suppressive therapy until all three of the following criteria are met:

- Therapy duration of at least 12 months
- CD4 count greater than 100/mm^3 for at least 6 months
- Asymptomatic from MAC infection

Therapy for Other NTM, Including Leprosy

The global prevalence of leprosy (Hansen disease) has markedly declined, largely due to the global initiative of the WHO to eliminate it as a public health problem by providing multidrug therapy (*rifampin*, *clofazimine*, and *dapsone*) free of charge. Prevalence of the disease has dropped by about 90% since 1985. Nevertheless, there are pockets of disease around the world, especially in Africa, Asia, and South America.

There are four major clinical types of leprosy, and clinical phenotype determines therapy. At one end of the spectrum is *tuberculoid leprosy*, also termed paucibacillary leprosy because the bacterial burden is low and *M. leprae* is rarely found on smears. On the other end of the spectrum is the *lepromatous* form of the disease (Levis and Ernst, 2005). This is characterized by disseminated infection and high bacillary burden. Two major intermediate forms of the disease are recognized: (1) borderline (dimorphous) tuberculoid disease, which has features of both tuberculoid and lepromatous leprosy; and (2) indeterminate disease, which has early hypopigmented lesions without features of the lepromatous and tuberculoid leprosy.

M. leprae was discovered by Armauer Hansen in 1873. The *M. leprae* genome has undergone reductive evolution and has radically downsized its genome (Cole et al., 2001). It has a long doubling time (14 days) and is an obligate intracellular pathogen. *M. leprae* is, therefore, difficult to culture on synthetic media, an impediment to basic research on the disease and to its clinical identification.

Types of Antileprosy Therapy

Leprosy is treated with multidrug therapy consisting of *rifampin*, *clofazimine*, and *dapsone*. Combination therapy reduces the development of resistance, overcomes preexisting primary resistance, and reduces the duration of therapy. The most bactericidal drug in current regimens is *rifampin*. Because of its high kill rate resulting in massive release of bacterial antigens, *rifampin* is not often given during a "reversal" reaction (see discussion below) or in patients with erythema nodosum leprosum. *Clofazimine* is only bacteriostatic against *M. leprae*. However, it also has anti-inflammatory effects and can treat reversal reactions and erythema

TABLE 65–7 ■ PHARMACOTHERAPY FOR SELECT NONTUBERCULOUS MYCOBACTERIA

MYCOBACTERIAL SPECIES	FIRST-LINE THERAPY	ALTERNATIVE AGENTS
M. Avium intracellulare	Macrolide + rifampin + ethambutol	Amikacin; clofazimine; moxifloxacin; linezolid
M. kansasii	Isoniazid + rifampin[a] + ethambutol	Trimethoprim-sulfamethoxazole; ethionamide; cycloserine; clarithromycin; amikacin; streptomycin; moxifloxacin
M. fortuitum complex	Amikacin + doxycycline	Cefoxitin; rifampin; a sulfonamide; moxifloxacin or gatifloxacin; clarithromycin; trimethoprim-sulfamethoxazole; imipenem
M. marinum	Rifampin + ethambutol	Trimethoprim-sulfamethoxazole; clarithromycin; minocycline; doxycycline
M. ulcerans	Rifampin + streptomycin[b]	Clarithromycin[c]; rifapentine[c]
M. abscessus	Cefoxitin (or imipenem) + amikacin + clarithromycin	Tigecycline; moxifloxacin
M. malmoense	Rifampin + ethambutol ± clarithromycin	Fluoroquinolone
M. haemophilum	Clarithromycin + rifampin + quinolone	—

[a] In HIV-infected patients, the substitution of rifabutin for rifampin minimizes drug interactions with the HIV protease inhibitors and nonnucleoside reverse transcriptase inhibitors.

[b] For *M. ulcerans*, surgery is the primary therapy.

[c] Based on animal models.

nodosum leprosum. The third major agent in the regimen is *dapsone*. The objective of administering these drugs is total cure.

Definitive Therapy; Standard Therapy

Paucibacillary Leprosy. The WHO regimen consists of once-monthly oral *rifampin* 600 mg, plus once-daily *dapsone* 100 mg for 6 months. In the U.S., the regimen consists of *dapsone*, 100 mg daily, and *rifampin*, 600 mg daily, for 12 months, and then therapy is discontinued (National Hansen's Disease Program, 2018).

Multibacillary Therapy. The WHO recommends the same regimen as for paucibacillary leprosy, with two major changes. First, *clofazimine* 300 mg daily is added for the entirety of therapy. Second, the regimen lasts 1 year instead of 6 months. In the U.S., the regimen is also the same as for paucibacillary leprosy, with the addition of *clofazimine* 50 mg daily. Treatment is given for 24 months and then discontinued.

Treatment of Reactions in Leprosy

Patients with tuberculoid leprosy may develop "reversal reactions," manifestations of delayed hypersensitivity to antigens of *M. leprae*. Cutaneous ulcerations and deficits of peripheral nerve function may occur. Early therapy with corticosteroids or *clofazimine* is effective. Reactions in the lepromatous form of the disease (erythema nodosum leprosum) are characterized by the appearance of raised, tender, intracutaneous nodules, severe constitutional symptoms, and high fever. This reaction is often associated with therapy and is thought to be an Arthus-type reaction related to release of microbial antigens in patients harboring large numbers of bacilli. Treatment with *clofazimine* or *thalidomide* is effective.

Therapy of Other NTM

Mycobacteria other than those already discussed can be recovered from a variety of lesions in humans. Because they frequently are resistant to many of the commonly used agents, they must be examined for sensitivity *in vitro* and drug therapy selected on this basis. Therapy of infections from these organisms is summarized in Table 65–7. In some instances, surgical removal of the infected tissue followed by long-term treatment with effective agents is necessary. One of the most exciting advances in mycobacterial therapeutics is the successful treatment of Buruli ulcer in mice with ultrashort courses of *telacebec*, an investigational agent (Almeida et al., 2020; Chauffour et al., 2020). *Telacebec* targets the respiratory cytochrome Bc1:aac complex, and unlike *M. tuberculosis*, *M. ulcerans* does not have an alternative pathway for maintaining the electron transport chain. If proven to work clinically, this will transform therapy for this hard-to-treat and disfiguring disease.

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Drug Facts for Your Personal Formulary: *Antimycobacterial Drugs*

Drug	Therapeutic Uses	Major Toxicity and Clinical Pearls
Rifamycins		
Rifampin	• Treatment of TB • Prophylaxis of TB disease • *M. kansasii* disease • Leprosy • *M. avium, M. marinum, M. uclerans, M. malmoense,* and *M. haemophilum* diseases • Prophylaxis of meningococcal disease and *Haemophilus influenzae* meningitis • Brucellosis • Combination therapy in selected cases of staphylococcal endocarditis, osteomyelitis, and infections associated with prosthetic material	• Peak concentration and AUC-driven efficacy • Rifampin potently induces CYPs and thus increases metabolism of many classes of drugs. Prior to putting a patient on rifampin, all the patient's medications and contraception should be examined for potential interactions • Hypersensitivity reactions, especially with high-dose intermittent therapy, including flu-like symptoms, eosinophilia, interstitial nephritis, acute tubular necrosis, thrombocytopenia, hemolytic anemia, and shock • Hepatitis, especially in combination with other anti-TB agents, in alcoholics, or with preexistent liver disease

Drug Facts for Your Personal Formulary: *Antimycobacterial Drugs (continued)*

Drug	Therapeutic Uses	Major Toxicity and Clinical Pearls
Rifamycins (cont.)		
Rifapentine	• Treatment of TB • Prophylaxis of TB disease	• 97% protein bound • Strong inducer of metabolizing enzymes and transporters
Rifabutin	• Used as rifampin replacement to avoid drug interactions of rifampin with other medications, especially in HIV co-infection • Treatment of disseminated MAC in patients with AIDS	• Weaker CYP3A induction than rifampin • Concentrations higher in tissue than plasma • $t_{1/2}$ ~45 h • Neutropenia in 25% of patients with HIV • Primary reasons for therapy discontinuation include rash, GI intolerance, and neutropenia • Uveitis is a unique but rare side effect for this rifamycin
Isoniazid		
Isoniazid	• *M. tuberculosis* infection • *M. kansasii* infection • Prophylaxis of TB disease	• Patients divided into slow, intermediate, and fast acetylators, which has consequences for efficacy and toxicity • Hepatotoxicity, increased above age of 60 years • Peripheral neuritis: should be administered with pyridoxine • Reversible vasculitis • Overdose associated with the clinical triad: (1) seizures refractory to treatment with phenytoin and barbiturates, (2) metabolic acidosis, and (3) coma • Several drug interactions via inhibition and induction of several CYPs
Pyrazinamide		
Pyrazinamide	• TB	• No activity against *M. bovis* • Activated under acidic conditions; synergizes with rifampin • Pyrazinamide clearance reduced in renal failure; reduce dosing frequency to 3 times per week at low glomerular filtration rate • Removed by hemodialysis; redose after each session • Adverse effects: hepatotoxicity and hyperuricemia
Ethambutol		
Ethambutol	• TB • MAC infections • *M. kansasii* infection • Activity against *M. gordonae, M. marinum, M. scrofulaceum,* and *M. szulgai*	• Incidence of optic neuritis leading to decreased visual acuity and loss of red-green discrimination. Test visual acuity and red-green discrimination prior to the start of therapy and periodically thereafter • In renal failure, ethambutol should be dosed at 15–25 mg/kg three times a week instead of daily, even in patients receiving hemodialysis
Bicyclic Nitroimidazoles		
Pretomanid, delamanid	• Treatment of MDR-TB; being tested for regimens used to treat drug-susceptible TB	• Kills both replicating and nonreplicating *M. tuberculosis* • Delamanid: modest QT interval prolongation
Riminophenazines		
Clofazimine	• Treatment of leprosy • Treatment of MDR-TB • Treatment of NTM infection	• GI problems are encountered in 40%–50% of patients • Abdominal pain due to crystal deposition in cavities and tissues • Body secretion, eye, and skin reddish-black discoloration occurs in most patients
Diarylquinoline		
Bedaquiline	• Treatment of MDR-TB; being tested for regimens used to treat drug-susceptible TB	• Apparent volume of distribution >10,000 L • 5-month terminal half-life • QT interval prolongation; ECG monitoring recommended • CYP3A substrate; avoid coadministration with CYP3A inducers
Ethionamide		
Ethionamide	• Treatment of MDR-TB and XDR-TB	• Same mutations in ethionamide-resistant bacteria as for isoniazid-resistant bacteria • 50% of patients are unable to tolerate a single dose larger than 500 mg because of GI toxicity • Adverse effects: postural hypotension, mental depression, drowsiness, asthenia; neurological toxicity • Concomitant administration with pyridoxine is recommended • Hepatitis in ~5% of cases

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Drug Facts for Your Personal Formulary: *Antimycobacterial Drugs (continued)*

Drug	Therapeutic Uses	Major Toxicity and Clinical Pearls
***Para*-aminobenzoic Acid Analogues**		
Dapsone	• Treatment of leprosy • Treatment of *Pneumocystis jirovecii* infection and prophylaxis • Prophylaxis of *Toxoplasma gondii* infection • Anti-inflammatory effects for treatment of pemphigoid, dermatitis herpetiformis, linear immunoglobulin A bullous disease, relapsing chondritis, and brown recluse spider bite ulcers	• Test for G6PD deficiency prior to use • NADH-dependent methemoglobin reductase deficiency–associated methemoglobinemia • Hemolysis at doses of 200–300 mg of dapsone per day • Used topically for acne
P-aminosalicylic acid	• Treatment of MDR-TB	• Should be administered with food • Dose must be reduced in renal dysfunction • Adverse events incidence is ~10%–30% • GI problems predominate • Hypersensitivity reactions in 5%–10% of patients
Cycloserine		
Cycloserine	• Treatment of MDR-TB	• Oral second-line drug • "Psycho-serine": 50% of patients develop neuropsychiatric symptoms; headache, somnolence, severe psychosis, seizures, and suicidal ideas • Must be redosed after dialysis

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Chapter 66

Chemotherapy of Malaria

Abdoulaye A. Djimde and Steve M. Taylor

GLOBAL IMPACT OF MALARIA

BIOLOGY OF MALARIAL INFECTION

CLINICAL MANIFESTATIONS OF MALARIA

CLASSIFICATION OF ANTIMALARIAL AGENTS

SPECIFIC ANTIMALARIAL AGENTS
- Artemisinin and Its Derivatives
- ACT Partner Drugs
- Atovaquone
- Diaminopyrimidines
- Proguanil
- Quinolines and Related Compounds
- Sulfonamides and Sulfones
- Tetracyclines and Clindamycin

ANTIMALARIALS IN DEVELOPMENT

PRINCIPLES AND GUIDELINES FOR CHEMOPROPHYLAXIS AND CHEMOTHERAPY OF MALARIA
- Malaria Chemoprophylaxis
- Self-Treatment of Presumptive Malaria for Travelers
- Diagnosis and Treatment of Malaria
- Chemoprophylaxis and Treatment During Pregnancy

TARGETING THE MOSQUITO RATHER THAN THE INFECTED HUMAN

Global Impact of Malaria

Malaria remains among the top five causes of death among children younger than 5 years, affects about a quarter of a billion people, and caused over 600,000 deaths in 2021 (World Health Organization, 2021). Malaria transmission occurs in regions of Africa, Latin and South America, Asia, the Middle East, the South Pacific, and the Caribbean (Figure 66–1). This disease is caused by infection with protozoan parasites of the genus *Plasmodium*, five species of which are known to infect humans: *P. falciparum, P. vivax, P. ovale, P. malariae,* and *P. knowlesi. P. falciparum* and *P. vivax* cause most malarial infections worldwide. On the basis of genetic data, *P. ovale* has recently been subdivided in two subspecies, *P. ovale wallikeri* and *P. ovale curtisi. P. falciparum* accounts for the majority of the burden of malaria in sub-Saharan Africa and causes most severe disease and deaths. *P. vivax* accounts for half of the malaria burden in South and East Asia and more than 80% of the malarial infections in the Americas and has been underappreciated as a cause of severe malaria (Baird, 2013).

Over the past half-century, malaria parasites worldwide—primarily *P. falciparum* and *P. vivax*—have become increasingly resistant to antimalarial drugs, including *chloroquine* (Djimde et al., 2001; Fidock et al., 2000; Warhurst, 2001), *mefloquine* (White et al., 2014), *quinine* (White et al., 2014), *sulfadoxine/pyrimethamine* (Artimovich et al., 2015; Plowe et al., 1995; Sibley et al., 2001), and *atovaquone* (Garcia-Bustos et al., 2013; Kessl et al., 2007). In response, new, multipronged international public-private partnerships as well as other funding agencies and sources have emerged to create new pipelines that advance drug candidates from discovery to clinical development (Hemingway et al., 2016; Wells et al., 2010, 2015).

Biology of Malarial Infection

The life cycle of malaria parasites comprises distinct morphological and functional stages. Because these stages have differential susceptibilities to antimalarial drugs, a basic understanding of the stages is critical to understand the chemotherapy for malaria (Figure 66–2). Malaria infections are initiated when an infected female anopheline mosquito injects *Plasmodium* sporozoites during a blood meal (Miller et al., 1998). After entering the dermis, sporozoites enter the bloodstream and, within minutes, arrive at the liver, where they infect individual hepatocytes via cell surface receptor-mediated events (Sinnis et al., 2013). This process initiates the *prepatent period* or *exoerythrocytic stage* of infection, which typically lasts about 1 week and is asymptomatic.

During this period, the parasite undergoes asexual replication within hepatocytes, resulting in production of liver-stage *schizonts*. When an infected hepatocyte ruptures, tens of thousands of *merozoites* are released into the bloodstream to infect red blood cells and initiate the *erythrocytic stage* of infection. During this stage, there are no residual *P. falciparum, P. malariae,* or *P. knowlesi* parasites in the liver. In contrast, *P. vivax* and *P. ovale* can, while establishing the erythrocytic stage, also maintain a quiescent hepatocyte infection as a dormant form of the parasite known as the *hypnozoite,* which can emerge to later cause symptomatic disease long after the initial malaria episode is recognized and treated. Erythrocytic forms cannot reestablish infection of hepatocytes. Transmission of human-infecting malarial parasites is maintained in human populations by the persistence of hypnozoites (several months to a few years for *P. vivax* and *P. ovale*), by antigenic variation in *P. falciparum* (probably months), by the putative antigen variation in *P. malariae* (for as long as several decades), and, in *P. knowlesi*, by the ability to infect nonhuman primates.

The *asexual erythrocytic stages* of malarial parasites are responsible for the clinical manifestations of malaria. This part of the *Plasmodium* life cycle is initiated by the recognition of red blood cells by merozoites by a process that is mediated by cell surface receptors that facilitate invasion of red blood cells.

Once inside a red blood cell, the merozoite develops into a ring form, which becomes a hemoglobin-metabolizing trophozoite (feeding stage) that matures into an asexually dividing blood-stage *schizont*. Schizont rupture at the end of the growth-and-division cycle releases 8 to 32 merozoites that invade new red blood cells. The erythrocytic replication cycle lasts for 24 h (for *P. knowlesi*), 48 h (for *P. falciparum, P. vivax,* and *P. ovale*), and 72 h (for *P. malariae*). Although most invading merozoites develop into schizonts, a small proportion becomes *gametocytes*, the form of the parasite that is infective to mosquitoes. These morphologically distinct and sexually dimorphic gametocytes are ingested by the mosquito

Abbreviations

ACT: artemisinin-based combination therapy
CDC: Centers for Disease Control and Prevention
cytbc$_1$: cytochrome bc$_1$
GI: gastrointestinal
G6PD: glucose-6-phosphate dehydrogenase
5HT: serotonin
SP: Sulfadoxine+Pyrimethamine
t_{max}: time to reach peak plasma drug concentration
WHO: World Health Organization

during an infectious blood meal; on reaching the midgut of the mosquito, the gametocytes transform into gametes that fertilize to become zygotes. Zygotes mature into ookinetes that invade the mosquito midgut wall and transform into oocysts. Numerous rounds of asexual replication occur in the oocyst to generate sporozoites over 10 to 14 days. Fully developed sporozoites rupture from oocysts and invade the mosquito salivary glands, from which they can initiate a new infection during subsequent mosquito blood meals (Figure 66–2). Thus, aside from *P. knowlesi*, which also infects macaques, the infection cycles from mosquito to human to mosquito.

Plasmodium falciparum has a family of binding proteins that recognize a variety of host cell molecules that this parasite species uses to invade all stages of erythrocytes (Lim et al., 2015; Weiss et al., 2016); high parasitemia may result from this mechanism. In contrast, *P. vivax* preferentially binds to the Duffy chemokine receptor protein as well as reticulocyte-specific proteins (Chitnis et al., 2008; Paul et al., 2015). *P. falciparum* assembles cytoadherence proteins (e.g., PfEMP1) (Weiss et al., 2016), encoded by a highly variable family of *var* genes into structures called knobs that are presented on the erythrocyte surface (Hviid et al., 2015; Ukaegbu et al., 2015). Knobs allow the *P. falciparum*–parasitized erythrocyte to bind to postcapillary vascular endothelium to avoid spleen-mediated clearance and allow the parasite to grow in a low-O_2, high-CO_2 microenvironment.

Clinical Manifestations of Malaria

The cardinal signs and symptoms of malaria are high, spiking fevers (with or without periodicity), chills, headaches, myalgias, malaise, and gastrointestinal (GI) symptoms (White et al., 2014). Severe headache, a characteristic early symptom in malaria caused by all *Plasmodium* spp., often heralds the onset of disease, even before fever and chills. *P. falciparum* causes the most severe disease and may lead to organ failure and death. Placental malaria, of particular danger for primigravidae, is due to *P. falciparum* adherence to CSA (chondroitin sulfate A) in the placenta. This often leads to severe complications, including miscarriage, stillbirth, and intrauterine growth retardation. When treated early, symptoms of malarial infection usually improve within 24 to 48 h. The nonspecific nature of the clinical presentation of malaria renders its signs and symptoms insufficient for accurate diagnosis (Taylor et al., 2010), underscoring the need for diagnostic testing in suspected cases, typically with light microscopy or rapid lateral-flow immunochromatographic tests.

Acute illness due to *P. vivax* infection may appear severe due to high fever and prostration. Indeed, the pyrogenic threshold of this parasite (i.e., "blood stage" parasite burden associated with fever) is lower than that of *P. falciparum*. Nonetheless, *P. vivax* malaria generally has a low mortality rate. *P. vivax* malaria is characterized by relapses caused by the reactivation of latent tissue forms. Clinical manifestations of relapse are the same as those of primary infection. In recent years, severe *P. vivax* malaria from Oceania (Papua New Guinea, Indonesia) and India has shown important similarities to severe malaria caused by *P. falciparum*. These include neurological symptoms (diminished consciousness, seizure) and pulmonary edema. Rare but life-threatening complications can occur, including splenic rupture, acute lung injury, and profound anemia.

Plasmodium ovale causes a clinical syndrome similar to that of *P. vivax* but may be milder with lower levels of parasitemia. It shares with *P. vivax* the ability to form the hypnozoite (dormant liver stage) that may relapse after months to 2 years later. *P. ovale* is more common in sub-Saharan Africa and some islands in Oceania.

Plasmodium malariae generally causes an indolent infection with very low levels of parasitemia and often does not produce clinical symptoms. This parasite can be found in all malaria-endemic areas but is most common in sub-Saharan Africa and the southwest Pacific. Interestingly, *P. malariae* prevalence increases during the dry season and can be found as a coinfection with *P. falciparum*. An uncommon but potentially fatal complication of *P. malariae* is a glomerulonephritis syndrome that does not respond to antimalarial treatment.

Plasmodium knowlesi, previously thought to be restricted to macaques, was often misdiagnosed as *P. malariae* by light microscopy owing to morphological similarity. *P. knowlesi* is distinguished by a shorter erythrocytic cycle (24 h compared with 72 h for *P. malariae*) and higher levels of parasitemia that account for its more severe clinical presentation. Like *P. malariae, P. knowlesi* is generally sensitive to chloroquine, but patients presenting with advanced disease nonetheless may progress to death despite adequate drug dosing.

Despite the above, asymptomatic infections with either *P. falciparum* or *P. vivax* are common in endemic regions and represent important potential reservoirs for malaria transmission. Although different studies are not entirely consistent in the definition of *asymptomatic*, generally this state implies a lack of fever, headache, and other systemic complaints, within a defined time period prior to a positive test for malaria parasitemia. Migration of asymptomatic individuals to areas where malaria is not present but vector mosquitoes are (i.e., anophelism without malaria) is an important mechanism for the introduction or reintroduction of malaria, in addition to facilitating the spread of drug-resistant isolates. Novel approaches to preventing transmission from asymptomatic reservoirs—whether through new drugs or vaccines—will be essential for future malaria control, elimination, and eradication strategies.

Classification of Antimalarial Agents

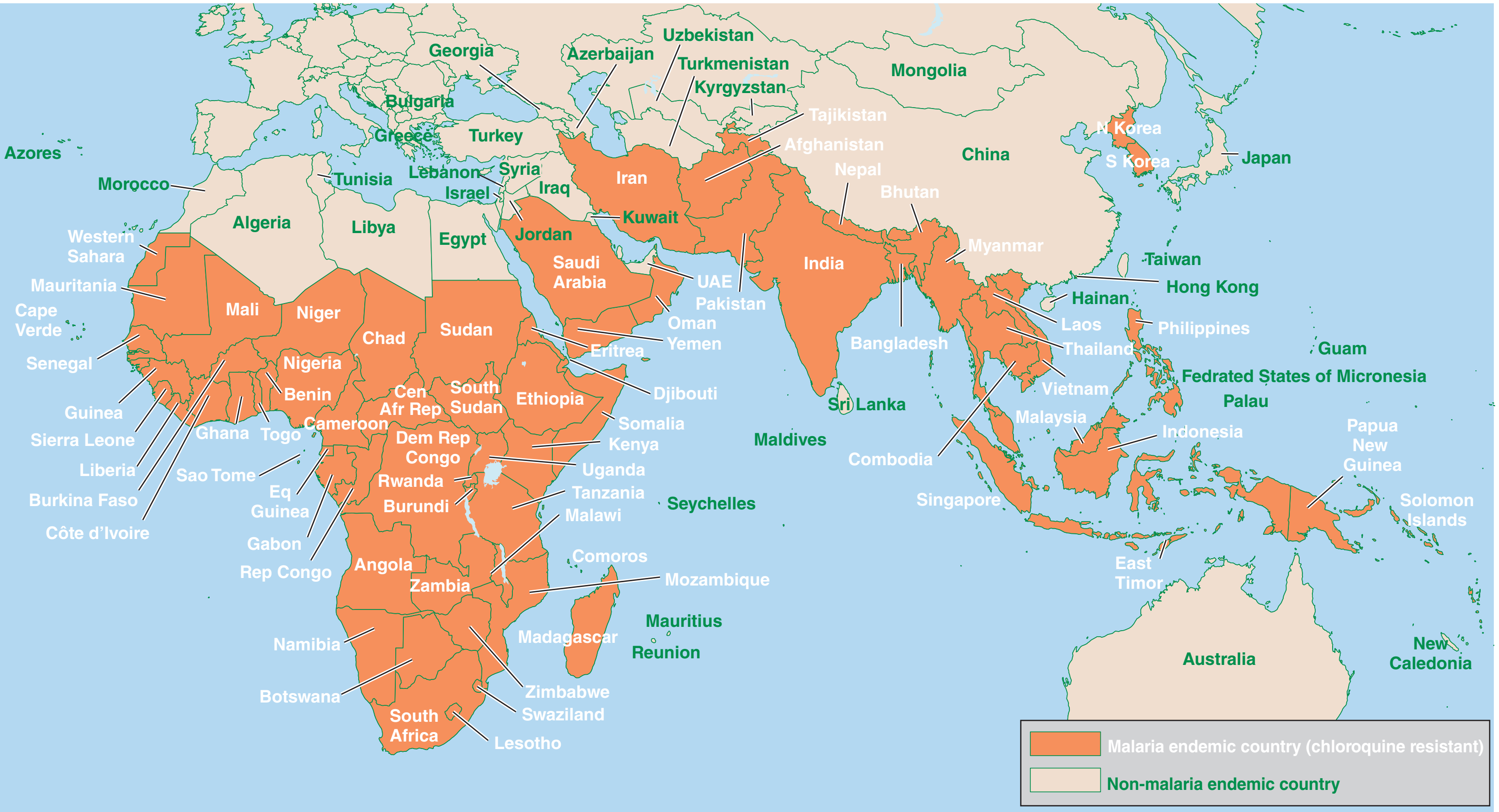
The various stages of the malarial parasite life cycle in humans differ in their drug sensitivity. Thus, antimalarial drugs can be classified based on their activities during this life cycle as well as by their intended use for either chemoprophylaxis or treatment. The spectrum of antimalarial drug activity leads to several generalizations.

The first relates to chemoprophylaxis: *Because no antimalarial drug kills sporozoites, it is not truly possible to prevent infection; drugs can only prevent the development of symptomatic malaria caused by the asexual erythrocytic forms, either in the bloodstream or as produced within and released by hepatocytes prior to erythrocyte invasion.*

The second relates to the treatment of an established infection: *No single antimalarial is effective against all hepatic and intraerythrocytic stages of the life cycle that may coexist in the same patient. Complete elimination of the parasite infection, therefore, may require more than one drug.*

The patterns of clinically useful antimalarial agents fall into three general categories (Table 66–1):

1. Agents that are not reliably effective against primary or latent liver stages: *artemisinins, chloroquine, mefloquine, quinine* and *quinidine, pyrimethamine, sulfadoxine,* and *tetracycline*. Instead, their action is directed against the asexual blood stages responsible for disease. These drugs will treat, or prevent, clinically symptomatic malaria.
2. Drugs (e.g., *atovaquone* and *proguanil*) that target not only the asexual erythrocytic forms but also the primary liver stages of *P. falciparum*. This additional activity shortens to several days the required period for postexposure chemoprophylaxis.
3. *Primaquine* and *tafenoquine,* 8-aminoquinolines that are effective against primary and latent liver stages as well as gametocytes. *Primaquine* is used most commonly to eradicate the intrahepatic hypnozoites of *P. vivax* and *P. ovale* that are responsible for relapsing infections. *Tafenoquine* is FDA-approved for similar uses.



A. Eastern Hemisphere

Azores
Morocco
Western Sahara
Mauritania
Cape Verde
Senegal
Guinea
Sierra Leone
Liberia
Burkina Faso
Côte d'Ivoire
Algeria
Tunisia
Libya
Egypt
Mali
Niger
Chad
Sudan
Nigeria
Benin
Ghana
Togo
Cameroon
Sao Tome
Eq Guinea
Gabon
Rep Congo
Cen Afr Rep
South Sudan
Ethiopia
Eritrea
Djibouti
Somalia
Kenya
Dem Rep Congo
Rwanda
Burundi
Uganda
Tanzania
Malawi
Angola
Zambia
Namibia
Botswana
South Africa
Lesotho
Swaziland
Zimbabwe
Mozambique
Comoros
Madagascar
Mauritius
Reunion
Seychelles
Maldives
Georgia
Bulgaria
Greece
Turkey
Azerbaijan
Lebanon
Syria
Israel
Iraq
Jordan
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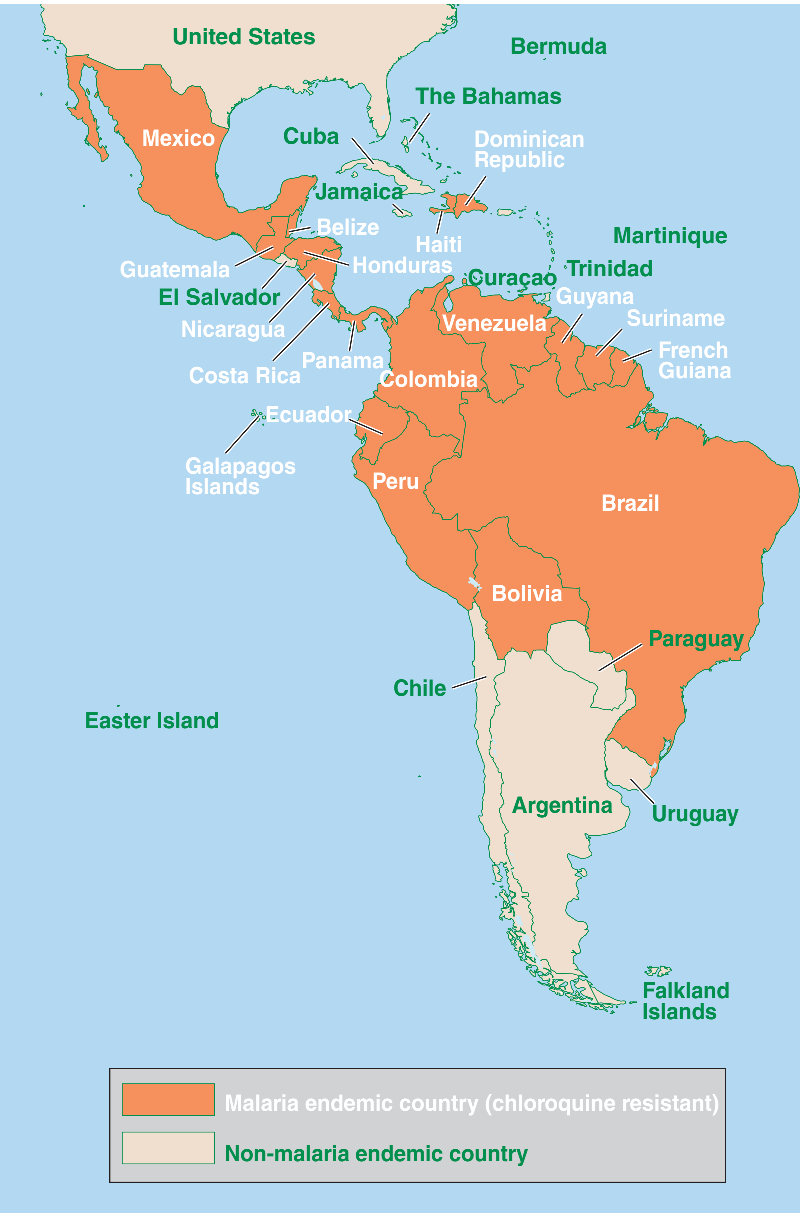
Uzbekistan
Turkmenistan
Kyrgyzstan
Tajikistan
Afghanistan
Pakistan
Nepal
Bhutan
India
Bangladesh
Sri Lanka
Mongolia
China
N Korea
S Korea
Japan
Myanmar
Taiwan
Hong Kong
Hainan
Laos
Thailand
Vietnam
Combodia
Malaysia
Singapore
Philippines
Guam
Fedrated States of Micronesia
Palau
Indonesia
Papua New Guinea
Solomon Islands
East Timor
Australia
New Caledonia
Malaria endemic country (chloroquine resistant)
Non-malaria endemic country

Figure 66–1 *Malaria-endemic countries.* **A.** Eastern Hemisphere. **B.** Western Hemisphere. A country is shaded orange even if malaria is endemic in just a portion of that country. Large regions not shown on the maps (e.g., Scandinavia, Russia, Canada, U.S., Tasmania, New Zealand) are nonendemic for malaria. In areas of endemic malaria, the disease is largely *chloroquine* resistant. For up-to-date information, consult the CDC's Malaria Information and Prophylaxis, by Country (https://www.cdc.gov/malaria/travelers/country_table/a.html; accessed January 12, 2022). (Reproduced from Centers for Disease Control and Prevention, https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/malaria; accessed January 12, 2022).



B. Western Hemisphere

United States
Bermuda
The Bahamas
Mexico
Cuba
Dominican Republic
Jamaica
Haiti
Belize
Martinique
Guatemala
Honduras
Curaçao
Trinidad
El Salvador
Guyana
Nicaragua
Suriname
Venezuela
French Guiana
Panama
Costa Rica
Colombia
Ecuador
Galapagos Islands
Peru
Brazil
Bolivia
Paraguay
Chile
Easter Island
Argentina
Uruguay
Falkland Islands
Malaria endemic country (chloroquine resistant)
Non-malaria endemic country

Figure 66–1 (*Continued*)

The relative utility of antimalarials for chemoprophylaxis or therapy depends on their pharmacokinetics, efficacy, and safety. *Quinine* and *primaquine*, which have significant toxicity and relatively short half-lives, generally are reserved for the treatment of established infections and are not used for chemoprophylaxis in a healthy traveler. By contrast, *chloroquine*, which is relatively free from toxicity and has a long $t_{1/2}$, is convenient for chemoprophylactic dosing, though there are few regions where *P. falciparum* remains reliably susceptible to chloroquine.

Specific Antimalarial Agents

For ease of reference, detailed information on the antimalarial drugs appears below in alphabetical order by drug name.

Artemisinin and Its Derivatives

Artemisinin and its three major semisynthetic derivatives in clinical use, *dihydroartemisinin*, *artemether*, and *artesunate*, are potent and fast-acting antimalarials. They are optimized for the treatment of severe *P. falciparum* malaria and are also effective against the asexual erythrocytic stages of *P. vivax*. For uncomplicated malaria, the standard treatment approach employs orally administered *artemisinin-based combination therapies* (*ACTs*), in which the rapid-acting and rapidly eliminated *artemisinin* component is paired with a slower-acting and slower-eliminated partner drug with a distinct antiparasitic mechanism of action. Such an approach is required because *artemisinins*, when administered alone, require more than 7 days of therapy to eliminate parasites, but when paired with an effective partner, antimalarial courses of treatment are commonly just 3 days for effective ACT.

The *artemisinin* drugs were discovered as potent antimalarials by Chinese scientists in the 1970s, garnering a Nobel Prize in Medicine/Physiology in 2015 for Tu Youyou "for her discoveries concerning a novel therapy against Malaria." By screening compounds described in ancient Chinese medical texts, an extract of sweet wormwood (*Artemisia annua*) was found to be effective in curing simian and murine *Plasmodium* infections. The pure ingredient in this extract was termed *qinghaosu*, or

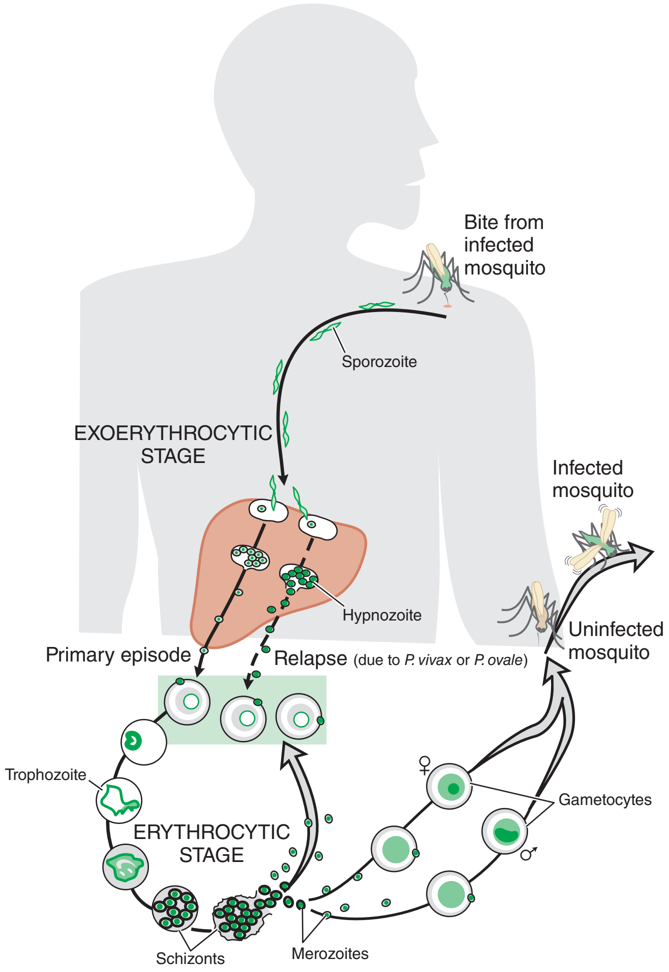
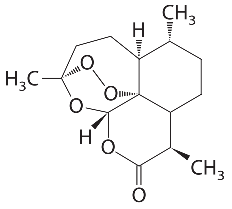


Figure 66–2 *Life cycle of malaria parasites.*

artemisinin (Tu, 2011). After refinement of the chemical structure, sourcing of natural supplies, and formulation of it as a pharmaceutical product, *artemisinin* therapies were successful against *chloroquine*-sensitive and -resistant *P. falciparum* infections in a wide variety of Chinese studies in the 1970s, prior to gaining broader recognition as a potent, novel class of antimalarial by Western science in the 1980s (Klayman, 1985).

The recent emergence of *P. falciparum artemisinin* "resistance" does not indicate classic resistance but rather reflects delayed parasite clearance time on the order of hours following the administration of ACT (Ashley et al., 2014; Huang et al., 2015); mutations in the *P. falciparum* gene *Pfk13* encoding the kelch13 propeller protein confer delayed parasite clearance times, possibly by rendering host hemoglobin inaccessible to *artemisinin* and thereby preventing its activation. True resistance to *artemisinin* has not been reported, and no infection from this parasite has been reported to survive ACT due to delayed clearance times (van Schalkwyk et al., 2015). Nevertheless, although the clinical significance of *P. falciparum artemisinin* resistance/delayed clearance remains unclear (Fairhurst, 2015), when coupled with widespread mutations that confer resistance to partner drugs (e.g., the ACT partner drug *piperaquine*), clinically significant ACT failure is substantial, with recrudescence rates reported to exceed 50% in parts of Asia (Amaratunga et al., 2016; Spring et al., 2015). For this reason, triple ACT strategies are being tested, in which *artemisinin* is paired with two partner drugs, an approach that remains in development. Resistance of non–*P. falciparum* malaria parasites to *artemisinin* class drugs has not been reported.



ARTEMISININ

Artemisinin is a revolutionary medicinal product. *Artemisinin* compounds cause a significant reduction of the parasite burden, with a 4-$\log_{10}$ reduction in the parasite population for each 48-h cycle of intraerythrocytic invasion, replication, and egress. Three to four cycles (6–8 days) of treatment are required to remove all the parasites from the blood. In addition, *artemisinin* treatment sharply decreases gametocyte carriage, which may possibly mitigate onward parasite transmission.

Mechanism of Action

Artemisinin and derivatives are activated by an interaction with intraparasitic heme, which is produced inside the highly acidic digestive vacuole of the parasite as it digests hemoglobin. This interaction cleaves the molecule's endoperoxide bridge to produce free radicals, which promiscuously alkylate a wide variety of parasite proteins (Meshnick, 1994). The site of action of the putatively toxic heme adducts is unclear. In addition, activated *artemisinin* might in turn generate free radicals that alkylate and oxidize macromolecules in the parasite.

TABLE 66–1 ■ SUSCEPTIBILITY TO DRUGS OF MALARIAL PARASITES AT VARIOUS DEVELOPMENTAL STAGES

			LIVER STAGES		BLOOD STAGES	
GROUP	DRUGS	SPOROZOITE	PRIMARY	HYPNOZOITE	ASEXUAL	GAMETOCYTE
1	Artemisinins	–	–	–	+	+
	Chloroquine	–	–	–	+	+/–
	Mefloquine	–	–	–	+	–
	Quinine, quinidine	–	–	–	+	+/–
	Pyrimethamine	–	–	–	+	–
	Sulfadoxine	–	–	–	+	–
	Tetracycline	–	–	–	+	–
2	Atovaquone/proguanil	–	+	–	+	+/–
3	Primaquine	–	+	+	–	+
	Tafenoquine	+	+	+	+	+

–, no activity; +/–, low to moderate activity; +, clinically important activity.

ADME

The *semisynthetic artemisinins* have been formulated for oral (*dihydroartemisinin*, *artesunate*, and *artemether*), intramuscular (*artesunate* and *artemether*), intravenous (*artesunate*), and rectal (*artesunate*) routes. Bioavailability after oral dosing typically is 30% or less. Peak serum levels occur rapidly with *artemisinins* and in 2 to 6 h with intramuscular *artemether*. Both *artesunate* and *artemether* have modest levels of plasma protein binding, ranging from 43% to 82%. These derivatives are extensively metabolized and converted to *dihydroartemisinin*, which has a plasma $t_{1/2}$ of 1 to 2 h. Drug bioavailability via rectal administration is highly variable among individual patients. With repeated dosing, *artemisinin* and *artesunate* induce their own CYP-mediated metabolism, primarily via CYPs 2B6 and 3A4, which may enhance clearance by as much as 5-fold.

Therapeutic Uses

Given their rapid and potent activity against even multidrug-resistant parasites, the *artemisinins* are invaluable for the treatment of severe *P. falciparum* malaria, for which parenteral *artesunate* was FDA-approved in 2020. Other than this use of parenteral *artesunate* for the initial treatment of severe malaria, as a rule, *artemisinins* are not used as monotherapy. When combined with partner drugs as ACT, *artemisinins* are highly effective for the first-line treatment of malaria. *Artemisinins* should not be used for chemoprophylaxis because of their short $t_{1/2}$ values.

Toxicity and Contraindications

In pregnant rats and rabbits, *artemisinins* can cause increased embryo lethality or malformations in the early postconception period. Preclinical toxicity studies have identified the brain (and brainstem), liver, and bone marrow as the principal target organs. However, no systematic neurological changes have been attributed to treatment in patients 5 years of age or older. Patients may develop dose-related and reversible decreases in reticulocyte and neutrophil counts and increases in transaminase levels. About 1 in 3000 patients develops an allergic reaction. Although studies of *artemisinin* treatment during the first trimester have found no evidence of adverse effects on fetal development, use of ACTs is not recommended during the first trimester of pregnancy or for the treatment of children weighing 5 kg or less. Accumulating clinical evidence for the safety of *artemether* (when combined with *lumefantrine*) in the first trimester may herald a broader use of this or other ACTs safely in early pregnancy.

ACT Partner Drugs

Partner drugs for ACT are chosen for potency and $t_{1/2}$ that substantially exceeds that of the *artemisinin* partner. The primary ACT regimens that are well tolerated in adults and children weighing 5 kg or more are *artemether-lumefantrine*, *artesunate-amodiaquine*, *artesunate-pyronaridine*, and *dihydroartemisinin-piperaquine*. In the U.S., *artemether-lumefantrine* is FDA-approved and is arguably the first choice for all malaria cases if oral drug treatment is appropriate (see Centers for Disease Control and Prevention [CDC] treatment tables at https://www.cdc.gov/malaria/resources/pdf/Malaria_Treatment_Table.pdf).

- ***Lumefantrine*** shares structural similarities with the arylamino alcohol drugs *mefloquine* and *halofantrine* and is formulated with *artemether*. *Artemether-lumefantrine* is highly effective for the treatment of uncomplicated malaria and is the most widely used first-line antimalarial across Africa. The pharmacokinetic properties of *lumefantrine* include a large apparent volume of distribution and a terminal elimination $t_{1/2}$ of 4 to 5 days. Administration with a high-fat meal is recommended because it significantly increases absorption. A sweetened dispersible formulation of *artemether-lumefantrine* has been approved for treatment of children.
- ***Amodiaquine*** is a congener of *chloroquine* that is no longer recommended in the U.S. for chemoprophylaxis of *P. falciparum* malaria because of toxicities (hepatic and agranulocytosis) associated with its prophylactic use. *Amodiaquine* is rapidly converted by hepatic CYPs into monodesethyl-amodiaquine. This metabolite, which retains substantial antimalarial activity, has a plasma $t_{1/2}$ of 9 to 18 days and reaches a peak concentration of about 500 nM 2 h after oral administration of the recommended dose. By contrast, *amodiaquine* has a $t_{1/2}$ of about 3 h, attaining a peak concentration of about 25 nM within 30 min of oral administration. Clearance rates of *amodiaquine* vary widely among individuals (78–943 mL/min/kg). The ACT *artesunate-amodiaquine* is a highly effective therapy for uncomplicated malaria and is first-line treatment in many African countries.
- ***Piperaquine*** is a potent and well-tolerated bisquinoline structurally related to *chloroquine*. It is rapidly absorbed, with a t_{max} (time to peak plasma drug concentration) of 2 h after a single dose. *Piperaquine* has a large volume of distribution and reduced rates of excretion after multiple doses. *Piperaquine* has the longest plasma $t_{1/2}$ (5 weeks) of all ACT partner drugs, a factor that could reduce the likelihood of reinfection following treatment. Reduced efficacy of *piperaquine* in combination with *dihydroartemisinin* in Cambodia has been reported, primarily associated with copy number amplifications of *P. falciparum* plasmepsin-2 (*pfpm2*) owing to unknown mechanisms. These mutations have undermined the efficacy of *dihydroartemisinin-piperaquine* in Southeast Asia, though its efficacy in African settings remains high (West African Network for Clinical Trials of Antimalarial Drugs, 2018).
- ***Pyronaridine***, an antimalarial structurally related to *amodiaquine*, is well tolerated and potent against both *P. falciparum* and *P. vivax*. *Pyronaridine* has a peak plasma concentration 2 to 8 h after administration, distributes widely to tissues, and is eliminated slowly with a $t_{1/2}$ in adults of 14 to 18 days. This drug, highly efficacious in clinical trials against *P. falciparum* or *P. vivax* when partnered as *pyronaridine-artesunate*, has not yet been licensed by the FDA but is available globally.

In addition, there are two antimalarials that are used both alone and in combination as ACT:

- ***Sulfadoxine+Pyrimethamine* (SP)**, partnered as *artesunate*-SP
- ***Mefloquine,*** partnered as *artesunate-mefloquine*

Details of these ACT partner drugs are in the alphabetical listing below.

Atovaquone

A fixed combination of *atovaquone* with *proguanil hydrochloride* is available in the U.S. for malaria chemoprophylaxis and for the treatment of uncomplicated *P. falciparum* malaria in adults and children.

Mechanism of Action, Selective Toxicity, Antimalarial Action, and Resistance

Atovaquone is a lipophilic analogue of ubiquinone (coenzyme Q), the electron acceptor for the parasite's cytochrome bc_1 ($cytbc_1$) complex. $Cytbc_1$, situated on the inner mitochondrial membrane, supplies oxidized ubiquinone for dihydroorotate dehydrogenase, an essential enzyme in pyrimidine biosynthesis in the parasite. In addition, $cytbc_1$ is part of the respiratory chain and transports H^+ into the intramembranous space of mitochondria. By binding at the Q_o site of $cytbc_1$, atovaquone inhibits electron transport, collapses the mitochondrial membrane potential, and inhibits regeneration of ubiquinone. The selective toxicity of *atovaquone* for the *Plasmodium* genus and not the human host may stem from structural differences in the amino terminal regions of plasmodial and human cytochrome b (Capper et al., 2015).

The drug is highly active against *P. falciparum* asexual blood-stage parasites and the liver stages but is not active against *P. vivax* liver-stage hypnozoites. Synergy between *proguanil* and *atovaquone* results from the ability of nonmetabolized *proguanil* to enhance the mitochondrial toxicity of *atovaquone*. Resistance to *atovaquone* alone in *P. falciparum* develops easily and is conferred by single, nonsynonymous nucleotide polymorphisms in the cytochrome b gene located in the mitochondrial genome. Addition of *proguanil* markedly reduces the frequency of appearance of *atovaquone* resistance. However, once *atovaquone* resistance is present, the synergy of the partner drug *proguanil* diminishes.

ADME

Atovaquone absorption is slow and variable after an oral dose; absorption improves when the drug is taken with a fatty meal. More than 99% of the drug is bound to plasma protein; levels in the cerebrospinal fluid are less than 1% of those in plasma. Profiles of drug concentration versus time often show a double peak, the first at 1 to 8 h and the second 1 to 4 days after a single dose; this pattern suggests enterohepatic circulation. Humans do not metabolize *atovaquone* significantly. The drug is excreted in bile, and more than 94% of the drug is recovered unchanged in feces. *Atovaquone* has a reported elimination $t_{1/2}$ from plasma of 2 to 3 days in adults and 1 to 2 days in children.

Therapeutic Uses

A tablet containing a fixed dose of 250 mg *atovaquone* and 100 mg *proguanil hydrochloride*, taken orally, is highly effective and safe in a 3-day regimen for treating mild-to-moderate infections due to *chloroquine*- or SP-resistant *P. falciparum* malaria. The same regimen followed by a *primaquine* course is effective in the treatment of *P. vivax* malaria. *Atovaquone-proguanil* is a standard agent for malaria chemoprophylaxis. Experience in prevention of non–*P. falciparum* malaria is limited. *P. vivax* infection may occur after drug discontinuation, indicating imperfect activity against exoerythrocytic stages of this parasite.

Toxicity

Atovaquone may cause side effects (abdominal pain, nausea, vomiting, diarrhea, headache, rash) that require cessation of therapy. Vomiting and diarrhea may decrease drug absorption, resulting in therapeutic failure. However, readministration of this drug within an hour of vomiting may still be effective in patients with *P. falciparum* malaria. *Atovaquone* occasionally causes transient elevations of serum transaminase or amylase.

Precautions and Contraindications

Although *atovaquone* is generally considered to be safe, it needs further evaluation in children weighing less than 11 kg, pregnant women, and lactating mothers. *Atovaquone* may compete with certain drugs for binding to plasma proteins. Therapy with *rifampin* reduces plasma levels of *atovaquone* substantially; the mechanism of this effect is not clear. Coadministration with *tetracycline* is associated with a 40% reduction in plasma concentration of *atovaquone*.

Diaminopyrimidines

Sulfadoxine+Pyrimethamine was a primary treatment of uncomplicated *P. falciparum* malaria, especially against *chloroquine*-resistant strains. Due to widespread resistance, it is no longer recommended for the treatment of uncomplicated malaria, except in some settings when paired with *artesunate*, as noted below (see Therapeutic Uses).

Antimalarial Action and Resistance

Pyrimethamine is a slow-acting blood *schizonticide* with antimalarial effects *in vivo* resulting from inhibition of *folate biosynthesis* in *Plasmodium,* similar to *proguanil*. The efficacy of *pyrimethamine* against hepatic forms of *P. falciparum* is less than that of *proguanil*. At therapeutic doses, *pyrimethamine* fails to eradicate *P. vivax* hypnozoites or gametocytes of any *Plasmodium* species. The drug increases the number of circulating *P. falciparum* mature infecting gametocytes, likely leading to increased transmission to mosquitoes during the period of treatment.

Synergy of *pyrimethamine* and the sulfonamides or sulfones results from inhibition of two metabolic steps in folate biosynthesis in the parasite:

- The utilization of *p*-aminobenzoic acid for the synthesis of dihydropteroic acid, which is catalyzed by dihydropteroate synthase and inhibited by sulfonamides
- The reduction of dihydrofolate to tetrahydrofolate, which is catalyzed by dihydrofolate reductase and inhibited by *pyrimethamine* (see Figure 57–2)

Dietary *p*-aminobenzoic acid or folate may affect the therapeutic response to antifolates. Resistance to *pyrimethamine* has developed in regions of prolonged or extensive drug use and can be attributed to mutations in dihydrofolate reductase that decrease the binding affinity of *pyrimethamine*.

ADME

Oral *pyrimethamine* is slowly but completely absorbed, reaching peak plasma levels in 2 to 6 h. The compound is significantly distributed in the tissues and is about 90% bound to plasma proteins. *Pyrimethamine* is slowly eliminated from plasma, with a $t_{1/2}$ of 85 to 100 h. Concentrations that are suppressive for responsive *Plasmodium* strains remain in the blood for about 2 weeks. *Pyrimethamine* also enters the milk of nursing mothers.

Therapeutic Uses

Due to increasing drug resistance, SP is no longer recommended for the treatment of uncomplicated malaria or for chemoprophylaxis. However, SP is still used as prevention in malaria-endemic areas, monthly during pregnancy starting in the second trimester as part of intermittent preventive treatment in pregnancy in parts of Africa, and paired with *amodiaquine* for monthly administration to children for seasonal malaria chemoprevention in the Sahel belt of Africa.

Toxicity, Precautions, and Contraindications

Antimalarial doses of *pyrimethamine* alone cause minimal toxicity except for occasional skin rashes and reduced hematopoiesis. Excessive doses can produce a megaloblastic anemia, resembling that of folate deficiency, which responds readily to drug withdrawal or treatment with folinic acid. At high doses, *pyrimethamine* is teratogenic in animals, and in humans, the related combination, *trimethoprim-sulfamethoxazole*, may cause birth defects.

Sulfonamides or sulfones, rather than *pyrimethamine*, usually account for the toxicity associated with coadministration of these antifolate drugs. The combination of *pyrimethamine* and *sulfadoxine* causes severe and even fatal cutaneous reactions, such as erythema multiforme, Stevens-Johnson syndrome, or toxic epidermal necrolysis. It has also been associated with serum sickness–type reactions, urticaria, exfoliative dermatitis, and hepatitis. SP is contraindicated for individuals with previous reactions to sulfonamides, for lactating mothers, and for infants less than 2 months of age. Administration of *pyrimethamine* with *dapsone*, a drug combination unavailable in the U.S., has occasionally been associated with agranulocytosis.

Proguanil

The antimalarial activity of *proguanil* (chloroguanide) is ascribed to *cycloguanil*, a cyclic triazine metabolite (structurally related to *pyrimethamine*) and selective inhibitor of the bifunctional plasmodial dihydrofolate reductase–thymidylate synthetase that is crucial for parasite *de novo* purine and pyrimidine synthesis.

Antimalarial Action and Resistance

In drug-sensitive *P. falciparum* malaria, *proguanil* exerts activity against both the primary liver stages and the asexual red blood cell stages, thus adequately controlling the acute episode and usually eradicating the infection. *Proguanil* is also active against acute *P. vivax* malaria, but because the latent tissue stages of *P. vivax* are unaffected, relapses may occur after the drug is withdrawn. *Proguanil* treatment does not destroy gametocytes, but oocysts in the gut of the mosquito can fail to develop normally.

Cycloguanil selectively inhibits the bifunctional dihydrofolate reductase–thymidylate synthetase of sensitive plasmodia, causing inhibition of DNA synthesis and depletion of folate cofactors. A series of amino acid changes near the dihydrofolate reductase–binding site have been identified that cause resistance to *cycloguanil*, *pyrimethamine*, or both. The presence of *Plasmodium* dihydrofolate reductase is not required for the intrinsic antimalarial activity of *proguanil* or *chlorproguanil*; however, the molecular basis for this alternative activity is unknown. *Proguanil* accentuates the mitochondrial membrane potential–collapsing action of *atovaquone* against *P. falciparum* but displays no such activity by itself. In contrast to *cycloguanil*, resistance to the parent drug, *proguanil*, either alone or in combination with *atovaquone*, is not well documented.

ADME

Proguanil is slowly but adequately absorbed from the GI tract. After a single oral dose, t_{max} is approximately 5 h. The mean plasma elimination $t_{1/2}$ is about 180 to 200 h or longer. The drug's activation and metabolism involve the CYP2C subfamily; about 3% of Caucasians are deficient in this oxidation phenotype, contrasted with about 20% of Asians and Kenyans. *Proguanil* is oxidized to two major metabolites, the active *cycloguanil* and an inactive 4-chlorophenyl biguanide. On a daily dosage regimen of 200 mg daily, plasma levels of *cycloguanil* in extensive metabolizers exceed the therapeutic range, whereas *cycloguanil* levels in poor metabolizers do not. *Proguanil* itself does not accumulate appreciably in tissues during long-term administration, except in red blood cells, where its concentration is about three times that in plasma. In humans, 40% to 60% of the absorbed *proguanil* is excreted in urine, either as the parent drug or as the active metabolite.

Therapeutic Uses

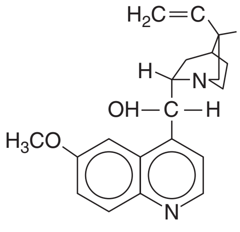
Proguanil as a single agent is not available in the U.S. but has been prescribed as chemoprophylaxis in England and Europe for individuals traveling to malarious areas in Africa. Strains of *P. falciparum* resistant to *proguanil* emerge rapidly in areas where the drug is used exclusively, but breakthrough infections may also result from deficient conversion of *proguanil* to its active antimalarial metabolite. *Proguanil* is effective and well tolerated when administered with *atovaquone* once daily for 3 days to treat drug-resistant strains of *P. falciparum* or *P. vivax* (see section on *atovaquone*). *P. falciparum* readily develops clinical resistance to monotherapy with either *proguanil* or *atovaquone*; however, resistance to the combination is uncommon unless the strain is initially resistant to *atovaquone*.

Toxicity and Side Effects

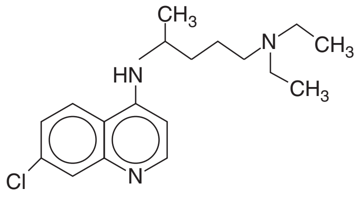
In chemoprophylactic doses of 200 to 300 mg daily, *proguanil* causes relatively few adverse effects, except occasional nausea and diarrhea. Large doses (≥1 g daily) may cause vomiting, abdominal pain, diarrhea, hematuria, and the transient appearance of epithelial cells and casts in the urine. Doses as high as 700 mg twice daily have been taken for more than 2 weeks without serious toxicity. *Proguanil* is safe for use during pregnancy. It is remarkably safe when used in conjunction with other antimalarial drugs.

Quinolines and Related Compounds

Quinine is the chief alkaloid of cinchona, the powdered bark of the South American cinchona tree. Structure-activity analysis of the cinchona alkaloids provided the basis for the discovery of more recent antimalarials, such as mefloquine.



QUININE



CHLOROQUINE

Antimalarial Action

Asexual malarial parasites flourish in host erythrocytes by digesting hemoglobin; this generates free radicals and iron-bound heme as highly reactive by-products. Heme is sequestered as an insoluble, chemically inert malarial pigment termed *hemozoin*. Quinolines interfere with heme sequestration. Failure to inactivate heme and drug-heme complexes is thought to kill the parasites via oxidative damage to membranes or other critical biomolecules.

Chloroquine and Hydroxychloroquine

Chloroquine is a 4-aminoquinoline derived from a precursor compound that was originally synthesized by German chemists in the 1930s. This precursor found purchase against a variety of *Plasmodium* species in early human studies during World War II. The compound and these data were delivered to Allied forces following the capture of Tunis in 1943, prompting American industrial-academic-military complex to synthesize and test additional 4-aminoquinolines. This process begat *chloroquine* as the most effective and safe antimalarial against *P. falciparum* and *P. vivax*. *Chloroquine*, a weak base, concentrates in the highly acidic digestive vacuoles of susceptible *Plasmodium*, where it binds to heme and disrupts its sequestration. *Hydroxychloroquine*, in which one of the *N*-ethyl substituents of *chloroquine* is β-hydroxylated, is essentially equivalent to *chloroquine* against *P. falciparum* malaria.

BOX 66–1 ■ Quinine: From Tree Bark to Tonic Water

The powdered bark of the South American cinchona tree was ingested by the Quechua people of Peru as a treatment for rigors. Jesuit missionaries in the 17th century adopted this practice and popularized the bark's use in Europe, where it became known as "Jesuit powder," among other names. For several centuries, cinchona was a widespread therapy for syndromes consistent with malaria, as well as a highly valued commodity, begetting plantations of cinchona plants to supply the global demand. It was not until the early 19th century that quinine was isolated from the bark, and only in the mid-20th century that industrial synthesis was established, although by this time, *quinine*'s routine use had been largely eclipsed by other antimalarials.

Quinine is now ingested socially as a carbonated beverage. The FDA has set an upper limit for the *quinine* content of tonic water: 83 mg/L.

Resistance. Resistance of erythrocytic asexual forms of *P. falciparum* to antimalarial quinolines, especially *chloroquine*, now is widespread. *Chloroquine* resistance results from mutations in the polymorphic gene *pfcrt* (*P. falciparum chloroquine* resistance transporter) that encodes a putative transporter that resides in the membrane of the acidic digestive vacuole, the site of hemoglobin degradation and *chloroquine* action. In addition to PfCRT, the P-glycoprotein transporter encoded by *pfmdr1,* and other transporters, including *P. falciparum* multidrug resistance-associated protein (PfMRP), may play a modulatory role in *chloroquine* resistance. *Chloroquine* resistance among *P. vivax* has also been reported; the resistance manifests clinically as early recurrences of infection following treatment and is associated mechanistically with overexpression of the *P. vivax chloroquine* resistance transporter (*pvcrt*).

ADME

Chloroquine is well absorbed from the GI tract and rapidly from intramuscular and subcutaneous sites. This drug extensively sequesters in tissues, particularly liver, spleen, kidney, lung, and to a lesser extent, brain and spinal cord. *Chloroquine* binds moderately (60%) to plasma proteins. The actions of hepatic CYPs produce two active metabolites, desethylchloroquine and bisdesethylchloroquine. Renal clearance of *chloroquine* is about half of its total systemic clearance. Unchanged *chloroquine* and desethylchloroquine account for more than 50% and 25% of the urinary drug products, respectively, and their renal excretion is increased by urine acidification. To avoid potentially lethal toxicity, parenteral *chloroquine* is given either slowly by constant intravenous infusion or in small divided doses by the subcutaneous or intramuscular route. *Chloroquine* is safer when given orally because the rates of absorption and distribution are more closely matched. Peak plasma levels are achieved in about 3 to 5 h. The $t_{1/2}$ of *chloroquine* increases from a few days to weeks as plasma levels decline. The terminal $t_{1/2}$ ranges from 30 to 60 days, and traces of the drug can be found in the urine for years after a therapeutic regimen.

Therapeutic Uses. *Chloroquine* is highly effective against the erythrocytic forms of *P. vivax* in most settings, *P. ovale, P. malariae*, *P. knowlesi*, and chloroquine-sensitive strains of *P. falciparum*. For infections caused by *P. ovale* and *P. malariae,* it remains the agent of choice for treatment. For *P. falciparum,* ACTs have largely replaced *chloroquine.*

The utility of *chloroquine* has declined across most malaria-endemic regions of the world because of the spread of *chloroquine*-resistant

P. falciparum. Except in areas where resistant strains of *P. vivax* are reported (e.g., Papua New Guinea and Indonesia), *chloroquine* is effective in chemoprophylaxis or treatment of acute attacks of malaria caused by *P. vivax, P. ovale*, and *P. malariae*. *Chloroquine* has no activity against primary or latent liver stages of the parasite. To prevent relapses in *P. vivax* and *P. ovale* infections, *primaquine* can either be given with *chloroquine* or used after a patient leaves an endemic area. *Chloroquine* rapidly controls the clinical symptoms and parasitemia of acute malaria. Most patients become completely afebrile within 24 to 48 h after receiving therapeutic doses. If patients fail to respond during the second day of *chloroquine* therapy, resistant strains should be suspected and therapy instituted with *quinine* plus *tetracycline* or *doxycycline* or with *atovaquone-proguanil*, *artemether-lumefantrine*, or *mefloquine* if the others are not available. In comatose children, *chloroquine* is well absorbed and effective when given through a nasogastric tube. Tables 66–2 and 66–3 provide information about recommended chemoprophylactic and therapeutic dosage regimens involving *chloroquine*. *Chloroquine* and its analogues are also used to treat certain nonmalarial conditions, including hepatic amebiasis.

Toxicity and Side Effects. Taken in proper doses and for recommended total durations, *chloroquine* is safe, but its margin of safety is narrow; a single dose of 30 mg/kg may be fatal. Acute *chloroquine* toxicity is encountered most frequently when therapeutic or high doses are administered too rapidly by parenteral routes. Cardiovascular effects include hypotension, vasodilation, suppressed myocardial function, cardiac arrhythmias, and eventual cardiac arrest. Confusion, convulsions, and coma may also result from overdose. *Chloroquine* doses of more than 5 g given parenterally usually are fatal. Prompt treatment with mechanical ventilation, *epinephrine*, and *diazepam* may be lifesaving.

Doses of *chloroquine* used for oral therapy of acute malaria may cause GI upset, headache, visual disturbances, and urticaria. Pruritus also occurs most commonly among dark-skinned persons. Prolonged treatment with suppressive doses occasionally causes side effects such as

TABLE 66–2 ■ CHEMOPROPHYLAXIS FOR PREVENTION OF MALARIA IN NONIMMUNE INDIVIDUALS

DRUG (USAGE)	ADULT DOSE	PEDIATRIC DOSE	COMMENTS
Atovaquone/proguanil (Prophylaxis in all areas)	Adult tablets contain 250 mg atovaquone and 100 mg proguanil hydrochloride; 1 adult tablet orally, daily	Pediatric tablets (62.5 mg atovaquone/25 mg proguanil HCl) 5–8 kg: 1/2 ped tab/day >8–10 kg: 3/4 ped tab/day >10–20 kg: 1 ped tab/day >20–30 kg: 2 ped tab/day >30–40 kg: 3 ped tab/day >40 kg: 1 adult tab daily	Begin 1–2 days before travel to malarious areas. Take daily at the same time each day while in the malarious area and for 7 days after leaving such areas. Contraindicated in persons with severe renal impairment (creatinine clearance <30 mL/min). Take with food or a milky drink. Not recommended for prophylaxis for children weighing less than 5 kg, pregnant women, and women breastfeeding infants weighing less than 5 kg.
Chloroquine phosphate (Prophylaxis in areas with chloroquine-sensitive malaria)	300 mg base (500 mg salt) orally, once/week	5 mg/kg base (8.3 mg/kg salt) orally, once/week, up to maximum adult dose (300 mg base)	Begin 1–2 weeks before travel to malarious areas. Take weekly on the same day of the week while in the malarious area and for 4 weeks after leaving such areas. May exacerbate psoriasis.
Doxycycline (Prophylaxis in all areas)	100 mg orally, daily	≥8 years of age: 2 mg/kg up to adult dose of 100 mg/day	Begin 1–2 days before travel to malarious areas. Take daily at the same time each day while in the malarious area and for 4 weeks after leaving such areas. Contraindicated in children less than 8 years of age and pregnant women.
Hydroxychloroquine sulfate (Alternative to chloroquine for prophylaxis in areas with chloroquine-sensitive malaria)	310 mg base (400 mg salt) orally, once/week	5 mg/kg base (6.5 mg/kg salt) orally, once/week, up to maximum adult dose (310 mg base)	Begin 1–2 weeks before travel to malarious areas. Take weekly on the same day of the week while in the malarious area and for 4 weeks after leaving such areas.
Mefloquine (Prophylaxis in areas with mefloquine-sensitive malaria)	228 mg base (250 mg salt) orally, once/week	≤9 kg: 4.6 mg/kg base (5 mg/kg salt) orally, once/week >9–19 kg: 1/4 tab weekly >19–30 kg: 1/2 tab weekly >31–45 kg: 3/4 tab weekly ≥45 kg: 1 tablet weekly	Begin 1–2 weeks before travel to malarious areas. Take weekly on same day of the week while in malarious area and for 4 weeks after leaving such areas. Contraindicated in persons allergic to mefloquine or related compounds (e.g., quinine, quinidine) and in persons with active depression, recent history of depression, generalized anxiety disorder, psychosis, schizophrenia, other major psychiatric disorders, or seizures. Use with caution in persons with psychiatric disturbances or a previous history of depression. Not recommended for persons with cardiac conduction abnormalities.
Primaquine (Prophylaxis for short-duration travel to areas with principally *P. vivax*)	30 mg base (52.6 mg salt) orally, daily	0.5 mg/kg base (0.8 mg/kg salt) up to adult dose orally, daily	Begin 1–2 days before travel to malarious areas. Take daily at same time each day while in malarious area and for 7 days after leaving such areas. Contraindicated in persons with G6PD[a] deficiency and during pregnancy and lactation (unless the infant being breastfed has documented normal G6PD level).

(Continued)

TABLE 66–2 ■ CHEMOPROPHYLAXIS FOR PREVENTION OF MALARIA IN NONIMMUNE INDIVIDUALS (*CONTINUED*)

DRUG (USAGE)	ADULT DOSE	PEDIATRIC DOSE	COMMENTS
Primaquine (For presumptive antirelapse therapy [terminal prophylaxis] to decrease the risk of relapses [*P. vivax*, *P. ovale*])	30 mg base (52.6 mg salt) orally, once/day for 14 days after departure from the malarious area	0.5 mg/kg base (0.8 mg/kg salt) up to adult dose orally, once/day for 14 days after departure from the malarious area	Indicated for persons who have had prolonged exposure to *P. vivax* and *P. ovale* or both. Contraindicated in persons with G6PD[a] deficiency and during pregnancy and lactation (unless the infant being breastfed has documented normal G6PD level).
Tafenoquine (Prophylaxis for travel to all areas)	200 mg daily (before travel) or weekly (during/after travel)	NA	Begin daily dosing for 3 days before travel to malarious areas. Then take weekly during travel, starting 1 week after last pretravel dose, then a final dose 1 week after travel. Contraindicated in people with G6PD[a] deficiency or unknown G6PD status, pregnant women, women breastfeeding an infant with G6PD deficiency or unknown G6PD status, people <18 years old, or people with history of psychotic disorder.
Tafenoquine (For antirelapse therapy [terminal prophylaxis] to reduce the risk of relapses [*P. vivax, P. ovale*])	300 mg, single dose	NA	Coadminister on the first or second day of therapy with the primary treatment for *P. vivax* or *P. ovale*. Contraindicated in people with G6PD[a] deficiency or unknown G6PD status, pregnant women, women breastfeeding an infant with G6PD deficiency or unknown G6PD status, people <16 years old. Use with caution in those with a history of a psychotic disorder.

NA, not applicable; ped, pediatric; tab, tablet.

These regimens are based on published recommendations of the U.S. Centers for Disease Control and Prevention. These recommendations may change over time. Up-to-date information should be obtained from https://wwwnc.cdc.gov/travel. Recommendations and available treatment differ among countries in the industrialized world, developing world, and malaria-endemic regions; in the last, some antimalarial treatments may be available without prescription, but the most effective drugs usually are controlled by governmental agencies.

[a] All persons who take primaquine or tafenoquine should have a documented normal G6PD level before starting the medication.

Source: https://www.cdc.gov/malaria/travelers/drugs.html; accessed June 21, 2022.

TABLE 66–3 ■ AGENTS FOR PRESUMPTIVE SELF-TREATMENT OF MALARIA[a]

DRUG	ADULT DOSE	PEDIATRIC DOSE	COMMENTS
Atovaquone-Proguanil			
Adult tablet: 250 mg atovaquone and 100 mg proguanil *Pediatric tablet*: 62.5 mg atovaquone and 25 mg proguanil	4 adult tablets, orally as a single daily dose for 3 consecutive days	Daily dose to be taken for 3 consecutive days: 5–8 kg: 2 pediatric tabs 9–10 kg: 3 pediatric tablets 11–20 kg: 1 adult tablet 21–30 kg: 2 adult tablets 31–40 kg: 3 adult tablets >41 kg: 4 adult tablets	Contraindicated in people with severe renal impairment (creatinine clearance <30 mL/min). Not recommended for people on atovaquone-proguanil prophylaxis. Not recommended for children weighing <5 kg, pregnant women, and women breastfeeding infants weighing <5 kg.

DRUG	DOSE	COMMENTS
Artemether-Lumefantrine		
A tablet contains 20 mg artemether and 120 mg lumefantrine	A 3-day treatment schedule with a total of 6 oral doses is recommended for both adult and pediatric patients *based on weight*. The patient should receive the initial dose, followed by the second dose 8 h later, then 1 dose twice daily for the following 2 days. 5 to <15 kg: 1 tablet per dose 15 to <25 kg: 2 tablets per dose 25 to <35 kg: 3 tablets per dose ≥35 kg: 4 tablets per dose	Not for people on mefloquine prophylaxis. Not recommended for children weighing <5 kg, pregnant women, and women breastfeeding infants weighing <5 kg.

[a] If used for presumptive self-treatment, medical care should be sought as soon as possible.

Source: Modified from https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/malaria; accessed June 21, 2022.

headache, blurring of vision, diplopia, confusion, convulsions, lichenoid skin eruptions, bleaching of hair, widening of the QRS interval, and T-wave abnormalities. These complications usually disappear soon after the drug is withheld. Rare instances of hemolysis and blood dyscrasias have been reported. *Chloroquine* may cause discoloration of nail beds and mucous membranes. This drug has also been reported to interfere with the immunogenicity of certain vaccines. Irreversible retinopathy and ototoxicity can result from high daily doses (>250 mg) of *chloroquine* or *hydroxychloroquine*, leading to cumulative total doses of more than 1 g/kg. Retinopathy presumably is related to drug accumulation in melanin-containing tissues and can be avoided if the daily dose is 250 mg or less. Prolonged therapy with high doses of *chloroquine* or *hydroxychloroquine* also can cause toxic myopathy, cardiopathy, and peripheral neuropathy. These reactions improve if the drug is withdrawn promptly. Rarely, neuropsychiatric disturbances, including suicide, may be related to overdose.

Precautions and Contraindications. *Chloroquine* is not recommended for treating individuals with epilepsy or myasthenia gravis and should be used cautiously, if at all, in the presence of advanced liver disease or severe GI, neurological, or blood disorders. The dose should be reduced in renal failure. In rare cases, *chloroquine* can cause hemolysis in patients with a deficiency of glucose-6-phosphate dehydrogenase (G6PD). *Chloroquine* should not be prescribed for patients with psoriasis or other exfoliative skin conditions. It should not be used to treat malaria in patients with porphyria cutanea tarda; however, it can be used in lower doses for treatment of manifestations of this form of porphyria.

Chloroquine inhibits CYP2D6 and thus can interact with a variety of different drugs. It attenuates the efficacy of the yellow fever vaccine when administered at the same time. It should not be given with *mefloquine* because of increased risk of seizures. *Chloroquine* opposes the action of anticonvulsants and increases the risk of ventricular arrhythmias when coadministered with *amiodarone* or *halofantrine*. By increasing plasma levels of *digoxin* and *cyclosporine*, *chloroquine* can increase the risk of toxicity from these agents. Patients receiving long-term, high-dose therapy should undergo ophthalmological and neurological evaluations every 3 to 6 months.

Quinine and Quinidine

Oral *quinine* is FDA-approved for the treatment of uncomplicated *P. falciparum* malaria. *Quinidine*, a stereoisomer of *quinine*, is more potent as an antimalarial and more toxic than *quinine*.

Antimalarial Action and Resistance. *Quinine* acts against asexual erythrocytic forms and has no significant effect on hepatic forms of malarial parasites. This drug is more toxic and less effective than *chloroquine* against malarial parasites susceptible to both drugs. Compared to *artemisinin* class therapy, *quinine* produces poorer clinical outcomes. However, *quinine*, along with its stereoisomer *quinidine*, is especially valuable for the parenteral treatment of severe illness owing to drug-resistant strains of *P. falciparum*. Because of its toxicity and short $t_{1/2}$, *quinine* is generally not used for chemoprophylaxis. The antimalarial mechanism of *quinine* is presumably similar to that of *chloroquine*. The basis of *P. falciparum* resistance to *quinine* is complex. Patterns of *P. falciparum* resistance to *quinine* correlate in some strains with resistance to *chloroquine* yet in others correlate more closely with resistance to *mefloquine* and *halofantrine*. A number of transporter genes may confer resistance to *quinine*.

Action on Skeletal Muscle. *Quinine* not only increases the tension response to a single maximal stimulus delivered to muscle directly or through nerves, but also increases the refractory period of muscle so that the response to tetanic stimulation is diminished. The excitability of the motor end-plate region decreases so that responses to repetitive nerve stimulation and to acetylcholine are reduced. *Quinine* can antagonize the actions of *physostigmine* on skeletal muscle. *Quinine* may also produce alarming respiratory distress and dysphagia in patients with myasthenia gravis.

ADME. *Quinine* is readily absorbed when given orally or intramuscularly. Oral absorption occurs mainly from the upper small intestine and is more than 80% complete, even in patients with marked diarrhea. After an oral dose, plasma levels reach a maximum in 3 to 8 h and, after distributing into an apparent volume of about 1.5 L/kg, decline with a $t_{1/2}$ of about 11 h. The pharmacokinetics of *quinine* may change with severe malarial infection: the apparent volume of distribution and the systemic clearance of *quinine* decrease, such that the average elimination $t_{1/2}$ increases to 18 h. The high levels of plasma α_1-acid glycoprotein produced in severe malaria may prevent toxicity by binding *quinine* and thereby reducing the free fraction of drug. Concentrations of *quinine* are lower in erythrocytes (33%–40%) and cerebrospinal fluid (2%–5%) than in plasma, and the drug readily reaches fetal tissues. The cinchona alkaloids are metabolized extensively, especially by hepatic CYP3A4; thus, only about 20% of an administered dose is excreted in an unaltered form in the urine. The major metabolite of *quinine*, 3-hydroxyquinine, retains some antimalarial activity and can accumulate and possibly cause toxicity in patients with renal failure. Renal excretion of *quinine* itself is more rapid when the urine is acidic.

Therapeutic Uses. Although *quinine* and *quinidine* have long been treatments of choice for drug-resistant and severe *P. falciparum* malaria, the advent of oral and intravenous *artemisinin* therapy has changed this situation. Standard of care for severe illness, and only until *artemisinin* therapy can be started, is the prompt use of loading doses of intravenous *quinine* (or *quinidine*, discontinued in the U.S. in 2017), which can be lifesaving. Oral medication to maintain therapeutic concentrations is then given as soon as tolerated and is continued for 5 to 7 days. Especially for treatment of infections with multidrug-resistant strains of *P. falciparum*, slower-acting blood schizonticides such as *tetracyclines* or *clindamycin* are given concurrently to enhance *quinine* efficacy. Formulations of *quinine* and *quinidine* and specific regimens for their use in the treatment of *P. falciparum* malaria are shown in the Drug Facts table. Note that in the U.S. *quinine* and *quinidine* have been eclipsed by parenteral *artesunate* for the treatment of severe malaria. Parenteral *artesunate* was FDA-approved in 2020 and is available both commercially and through an expanded access investigational new drug program accessible through the CDC Malaria Hotline (770-488-7788 or 855-856-4713).

The therapeutic range for "free" *quinine* is 0.2 and 2 mg/L. Regimens needed to achieve this target vary based on patient age, severity of illness, and the responsiveness of *P. falciparum* to the drug. Dosage regimens for *quinidine* are similar to those for *quinine*, although *quinidine* binds less to plasma proteins and has a larger apparent volume of distribution, greater systemic clearance, and shorter terminal elimination $t_{1/2}$ than *quinine*.

Nocturnal Leg Cramps. It is commonly believed that night cramps are relieved by *quinine* (200–300 mg) taken at bedtime. The FDA has required drug manufacturers to stop marketing over-the-counter *quinine* products for nocturnal leg cramps, stating that data supporting safety and efficacy of *quinine* for this indication were inadequate and that risks outweighed the potential benefits.

Toxicity and Side Effects. The fatal oral dose of *quinine* for adults is about 2 to 8 g. *Quinine* is associated with a triad of dose-related toxicities when given at full therapeutic or excessive doses: cinchonism, hypoglycemia, and hypotension. Mild forms of cinchonism (consisting of tinnitus, high-tone deafness, visual disturbances, headache, dysphoria, nausea, vomiting, and postural hypotension) occur frequently and disappear soon after the drug is withdrawn. Hypoglycemia is also common and can be life threatening if not treated promptly with intravenous glucose. Hypotension is rare and most often is associated with excessively rapid intravenous infusions of *quinine* or *quinidine*. Prolonged medication or high single doses also may produce GI, cardiovascular, and skin manifestations. GI symptoms (nausea, vomiting, abdominal pain, and diarrhea) result from the local irritant action of *quinine*, but the nausea and emesis also have a central basis. Cutaneous manifestations may include flushing, sweating, rash, and angioedema, especially of the face. *Quinine* and *quinidine*, even at therapeutic doses, may cause hyperinsulinemia and severe hypoglycemia through their powerful stimulatory effect on pancreatic beta cells.

Quinine rarely causes cardiac complications unless therapeutic plasma concentrations are exceeded. QTc prolongation is mild and does not appear to be affected by concurrent *mefloquine* treatment. Acute overdosage also may cause serious and even fatal cardiac dysrhythmias, such as sinus arrest, junctional rhythms, atrioventricular block, and ventricular tachycardia and fibrillation. *Quinidine* is even more cardiotoxic than *quinine*. Cardiac monitoring of patients on intravenous *quinidine* is advisable where possible.

Severe hemolysis can result from hypersensitivity to these cinchona alkaloids. Hemoglobinuria and asthma from *quinine* may occur more rarely. "Blackwater fever"—the triad of massive hemolysis, hemoglobinemia, and hemoglobinuria leading to anuria, renal failure, and in some instances death—is a rare hypersensitivity reaction to *quinine* therapy that can occur during treatment of malaria. *Quinine* occasionally may cause milder hemolysis, especially in people with G6PD deficiency. Thrombotic thrombocytopenic purpura also is rare but can occur even in response to ingestion of tonic water, which has about 4% of the therapeutic oral dose per 12 oz ("cocktail purpura"). Other rare adverse effects include hypoprothrombinemia, leukopenia, and agranulocytosis.

Research in model systems indicated that *quinine* can inhibit a number of transport proteins, including Tat2p, which transports tryptophan, the precursor of serotonin (5HT). *Quinine* also competitively inhibits the rate-limiting step in 5HT biosynthesis, tryptophan hydroxylase (Islahudin et al., 2014; Khozoie et al., 2009). Whether these data relate to adverse effects of *quinine* in humans remains to be determined.

Precautions, Contraindications, and Drug Interactions. *Quinine* must be used with considerable caution, if at all, in patients who manifest hypersensitivity. *Quinine* should be discontinued immediately if evidence of hemolysis appears. This drug should be avoided in patients with tinnitus or optic neuritis. In patients with cardiac dysrhythmias, the administration of *quinine* requires the same precautions as for *quinidine*. *Quinine* appears to be safe in pregnancy and is used commonly for the treatment of pregnancy-associated malaria. However, glucose levels must be monitored because of the increased risk of hypoglycemia.

Quinine and *quinidine* are highly irritating to tissues and should not be given subcutaneously. Concentrated solutions may cause abscesses when injected intramuscularly or thrombophlebitis when infused intravenously. Antacids that contain aluminum can delay absorption of *quinine* from the GI tract. *Quinine* and *quinidine* can delay the absorption and elevate plasma levels of cardiac glycosides, *warfarin*, and related anticoagulants. The action of *quinine* at neuromuscular junctions enhances the effect of neuromuscular blocking agents and opposes the action of acetylcholinesterase inhibitors. *Prochlorperazine* can amplify *quinine*'s cardiotoxicity, as can *halofantrine*. The renal clearance of *quinine* can be decreased by *cimetidine* and increased by urine acidification and by *rifampin*.

Mefloquine

Mefloquine was discovered through the Experimental Therapeutics Division of the U.S. Walter Reed Army Institute of Research in the 1970s and found to be safe and effective against drug-resistant strains of *P. falciparum*.

Mechanisms of Action and Resistance. *Mefloquine* is a highly effective blood schizonticide. *Mefloquine* associates with intraerythrocytic hemozoin, suggesting similarities to the mode of action of *chloroquine*. However, increased *pfmdr1* copy numbers are associated with both reduced parasite susceptibility to *mefloquine* and increased PfMDR1-mediated solute import into the digestive vacuole of intraerythrocytic parasites, suggesting that the drug's target resides outside this vacuolar compartment. The (–)-enantiomer is associated with adverse effects in the CNS (central nervous system); the (+)-enantiomer retains antimalarial activity with fewer side effects. *Mefloquine* can be paired with *artesunate* to reduce the selection pressure for resistance. This combination has proved efficacious for the treatment of *P. falciparum* malaria, even in regions with high prevalence of *mefloquine*-resistant parasites.

ADME. *Mefloquine* is taken orally because parenteral preparations cause severe local reactions. The drug is absorbed rapidly but with marked variability. Probably owing to extensive enterogastric and enterohepatic circulation, plasma levels of *mefloquine* rise in a biphasic manner to their peak in about 17 h. *Mefloquine* has a variable and long $t_{1/2}$ (13–24 days), reflecting its high lipophilicity, extensive tissue distribution, and extensive binding (~98%) to plasma proteins. The slow elimination of *mefloquine* fosters the emergence of drug-resistant parasites. *Mefloquine* is extensively metabolized in the liver by CYP3A4; this CYP can be inhibited by *ketoconazole* and induced by *rifampin*. Excretion of *mefloquine* is mainly by the fecal route; about 10% of *mefloquine* appears unchanged in the urine.

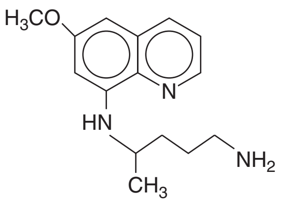
Therapeutic Uses. *Mefloquine* should be reserved for the prevention and treatment of malaria caused by known or suspected drug-resistant *P. falciparum* and *P. vivax*; it is no longer considered first-line treatment of malaria. The drug is especially useful as a chemoprophylactic agent for travelers spending weeks to years in areas where these infections are endemic and have not yet acquired significant resistance (see Table 66–2). In areas where drug-resistant strains of *P. falciparum* are circulating widely, *mefloquine* is more effective when used in combination with an *artemisinin* compound.

Toxicity and Side Effects. While oral *mefloquine* is generally well tolerated at chemoprophylactic dosages, the FDA in 2013 added a "black-box" warning to *mefloquine* labeling, noting the drug's potential to cause severe, possibly permanent, neurological and psychiatric adverse effects. Vivid dreams are common; significant neuropsychiatric signs and symptoms can occur in 10% or more of people receiving treatment doses, but serious adverse events (psychosis, seizures) are rare. Short-term adverse effects of treatment include nausea, vomiting, and dizziness. Dividing the dose improves tolerance. The full dose should be repeated if vomiting occurs within the first hour. After treatment of malaria with *mefloquine*, CNS toxicity can be as high as 0.5%; symptoms include seizures, confusion or decreased sensorium, acute psychosis, and disabling vertigo. Such symptoms are reversible on drug discontinuation. Mild-to-moderate toxicities (e.g., disturbed sleep, dysphoria, headache, GI disturbances, and dizziness) occur even at prophylactic dosages. Adverse effects usually manifest after the first to third doses and often abate even with continued treatment. Cardiac abnormalities, hemolysis, and agranulocytosis are rare.

Contraindications and Drug Interactions. At very high doses, *mefloquine* is teratogenic in rodents. Studies have suggested an increased rate of stillbirths with *mefloquine* use, especially during the first trimester. Pregnancy should be avoided for 3 months after *mefloquine* use because of the prolonged $t_{1/2}$ of this agent. This drug is contraindicated for patients with a history of seizures, depression, bipolar disorder, and other severe neuropsychiatric conditions, or adverse reactions to quinoline antimalarials. Although this drug can be taken safely 12 h after a last dose of *quinine*, taking *quinine* shortly after *mefloquine* can be hazardous because the latter is eliminated so slowly. Treatment with or after *halofantrine* or within 2 months of prior *mefloquine* administration is contraindicated. Controlled studies suggest that *mefloquine* does not impair performance in persons who tolerate the drug; nonetheless, some advise against the use of *mefloquine* for patients in occupations that require focused concentration, dexterity, and cognitive function.

Primaquine

Primaquine, in contrast to other antimalarials, acts on exoerythrocytic tissue stages of *Plasmodium* spp. in the liver to prevent and cure relapsing malaria. Patients should be screened for G6PD deficiency prior to therapy with this drug.



PRIMAQUINE

Antimalarial Action and Parasite Resistance. The mechanism of action of the 8-aminoquinolines has not been elucidated. *Primaquine* acts against primary and latent hepatic stages of *Plasmodium* spp. and prevents relapses in *P. vivax* and *P. ovale* infections. This drug and other 8-aminoquinolines also display gametocytocidal activity against *P. falciparum* and other *Plasmodium* species. However, *primaquine* is inactive against asexual blood-stage parasites, rendering it unsuitable as the principal treatment for an acute episode of malaria.

ADME. Absorption of *primaquine* from the GI tract approaches 100%. Peak plasma concentration occurs within 3 h and then falls with a variable $t_{1/2}$ averaging 7 h. *Primaquine* is metabolized rapidly; only a small fraction of a dose is excreted as the parent drug. Importantly, *primaquine* induces CYP1A2. The major metabolite, carboxyprimaquine, is inactive.

Therapeutic Uses. *Primaquine* is used primarily for terminal chemoprophylaxis and radical cure of *P. vivax* and *P. ovale* (relapsing) infections because of its high activity against the latent tissue forms (hypnozoites) of these *Plasmodium* species. The compound is given together with a blood schizonticide, usually *chloroquine*, to eradicate erythrocytic stages of these plasmodia and reduce the possibility of emerging drug resistance. For terminal chemoprophylaxis, *primaquine* regimens should be initiated shortly before or immediately after a subject leaves an endemic area (see Table 66–2). Radical cure of *P. vivax* or *P. ovale* malaria can be achieved if the drug is given either during an asymptomatic latent period of presumed infection or during an acute attack. Simultaneous administration of a schizonticidal drug plus *primaquine* is more effective than sequential treatment in promoting a radical cure. Limited studies demonstrated efficacy in prevention of *P. falciparum* and *P. vivax* malaria when *primaquine* was taken as chemoprophylaxis. *Primaquine* is generally well tolerated when taken for up to 1 year.

The role of *primaquine* has expanded owing to its unique gametocidal activity. Because most standard treatments for an episode of *P. falciparum* malaria effectively remove asexual blood stages but allow gametocytes to persist, treated patients may fuel onward transmission to mosquitos. For low transmission areas, the World Health Organization (WHO) recommends the use of single-dose *primaquine* at a dose of 0.25 mg/kg as adjunct to ACT in nonpregnant people over age 6 months with *P. falciparum* malaria. Owing to the use of a single dose, G6PD testing is not required.

Toxicity and Side Effects. *Primaquine* has few side effects when given in the usual therapeutic doses. *Primaquine* can cause mild-to-moderate abdominal distress in some individuals. Taking the drug at mealtime often alleviates these symptoms. Mild anemia, cyanosis (methemoglobinemia), and leukocytosis are less common. High doses (60–240 mg daily) worsen the abdominal symptoms. Methemoglobinemia can occur even with usual doses of *primaquine* and can be severe in individuals with congenital deficiency of NADH methemoglobin reductase (NADH-cytochrome b5 reductase [diaphorase 1]). *Chloroquine* and *dapsone* may synergize with *primaquine* to produce methemoglobinemia in these patients. Granulocytopenia and agranulocytosis are rare complications of therapy and usually are associated with overdosage. Other rare adverse reactions are hypertension, arrhythmias, and symptoms referable to the CNS.

Therapeutic or higher doses of primaquine may cause acute hemolysis and hemolytic anemia in humans with G6PD deficiency. Primaquine is the prototype of more than 50 drugs, including antimalarial tafenoquine and sulfonamides, that causes hemolysis in G6PD-deficient individuals.

Precautions and Contraindications. When used for terminal chemoprophylaxis or radical cure, G6PD deficiency should be ruled out prior to administration of *primaquine*. *Primaquine* has been used cautiously in subjects with the A form of G6PD deficiency, although benefits of treatment may not necessarily outweigh the risks, but should not be used in patients with more severe deficiency. If a daily dose of more than 30 mg *primaquine* base (>15 mg in potentially sensitive patients) is given, then blood counts should be followed carefully. Patients should be counseled to look for dark or blood-colored urine, which would indicate hemolysis. *Primaquine* should not be given to pregnant women; in treating lactating mothers, *primaquine* should be prescribed only after ascertaining that the breastfeeding infant has a normal G6PD level. *Primaquine* is contraindicated for acutely ill patients suffering from systemic disease characterized by a tendency to granulocytopenia (e.g., active forms of rheumatoid arthritis and lupus erythematosus). *Primaquine* should not be given to patients receiving drugs capable of causing hemolysis or depressing the myeloid elements of the bone marrow.

Tafenoquine

Tafenoquine is a derivative of *primaquine* that was discovered in the 1970s by the Walter Reed Army Institute of Research. As a derivative of *primaquine*, *tafenoquine* presumably has the same mechanism of action as *primaquine*; its reported toxicities and side effects are the same, particularly with relation to G6PD deficiency. Absorption after dosing is nearly complete but delayed over about 12 h in healthy volunteers (Charles et al., 2007). The main differences between *primaquine* and *tafenoquine* relate to ADME. There are no reported detectable QTc effects with *tafenoquine*. This agent has not been tested in pregnant women or children.

ADME. After oral administration (no parenteral formulation is available) *tafenoquine* is slowly absorbed, with maximum plasma concentrations occurring about 12 h after dosing in fasting healthy subjects; absorption and elimination are first order (Brueckner et al., 1998). The elimination $t_{1/2}$ of *tafenoquine* is about 14 days (Brueckner et al., 1998; Charles et al., 2007). The drug has a large volume of distribution and low clearance. *In vivo* metabolism of the parent drug and resultant metabolites is not well understood. Mild GI side effects include heartburn, gas, vomiting, and diarrhea. Methemoglobinemia, hemolytic anemia, thrombocytopenia, or changes in white blood cell counts or electrocardiograms are not observed in healthy fasting subjects without G6PD deficiency (Brueckner et al., 1998; Charles et al., 2007).

Therapeutic Uses. *Tafenoquine* is FDA-approved for the prevention of malaria and for the radical cure of *P. vivax* malaria. For the prevention of malaria, *tafenoquine* may be used for up to 6 months, with a loading regimen of 200 mg daily for 3 days prior to travel followed by 200 mg weekly thereafter. For the radical cure of *P. vivax* or *P. ovale*, a single dose of 300 mg is used, usually concomitantly with the therapy that is directed against the blood stage parasite.

Toxicity and Side Effects. The most common side effects in clinical trials of *tafenoquine* were headache, dizziness, back pain, diarrhea, nausea, motion sickness, depression, anxiety, abnormal dreams, and an elevated alanine aminotransferase level. Methemoglobinemia is a known side effect, and people with NADH-dependent methemoglobin reductase deficiency should undergo monitoring. Vortex keratopathy was reported at a high prevalence in two trials that included ophthalmic evaluations, although these were not accompanied by functional visual deficits or retinal changes.

Precautions and Contraindications. G6PD deficiency or unknown G6PD status is a contraindication to the use of *tafenoquine* owing to the risk of hemolytic anemia; therefore, G6PD testing is mandatory prior to *tafenoquine* administration. In males, qualitative G6PD tests are sufficient, although females often require quantitative G6PD testing. Psychosis or psychotic symptoms are also a contraindication, and their development while on preventive treatment should prompt evaluation and possible drug withdrawal. Because of the chemical similarity to *primaquine*, a hypersensitivity to *primaquine* is also a contraindication to *tafenoquine* use.

Sulfonamides and Sulfones

The sulfonamides and sulfones are slow-acting blood schizonticides and are more active against *P. falciparum* than *P. vivax*.

Mechanism of Action

Sulfonamides are *p*-aminobenzoic acid analogues that competitively inhibit *Plasmodium* dihydropteroate synthase. These agents are combined with an inhibitor of parasite dihydrofolate reductase to enhance their antimalarial action. See Figure 57–2 and neighboring text for details of these agents.

Drug Resistance

Sulfadoxine resistance is conferred by several point mutations in the dihydropteroate synthase gene. These *sulfadoxine* resistance mutations, when combined with mutations of dihydrofolate reductase that confer *pyrimethamine* resistance, greatly increase the likelihood of SP treatment failure. These mutations are widespread throughout global parasite populations, rendering SP unsuitable as a treatment for acute malaria. However, SP, given intermittently during the second and third trimesters of pregnancy as chemoprevention, is a routine component of antenatal care throughout Africa, an indication for which it retains a positive effect on newborn outcomes. Generally, one can anticipate that, in the absence of novel antifolates effective against existing drug-resistant strains, the use of these antimalarials for either prevention or treatment will continue to decline.

Tetracyclines and Clindamycin

Tetracycline and *doxycycline* are useful in malaria treatment, as is *clindamycin*. These agents are slow-acting blood schizonticides that can be used alone for short-term chemoprophylaxis in areas with *chloroquine*- and *mefloquine*-resistant malaria (only *doxycycline* is recommended for malaria chemoprophylaxis).

These antibiotics act via a delayed-death mechanism resulting from their inhibition of protein translation in the parasite apicoplast (an organelle evolutionarily derived from plant chloroplasts). This effect on malarial parasites manifests as death of the progeny of drug-treated parasites, resulting in slow onset of antimalarial activity. Their relatively slow mode of action makes these drugs ineffective as single agents for malaria treatment. Dosage regimens for tetracyclines and *clindamycin* are listed in the Drug Facts table. Because of their adverse effects on bones and teeth, tetracyclines should not be given to pregnant women or to children younger than 8 years. For details of these agents, see Chapter 60.

Antimalarials in Development

With resistance to *arteminisins* growing in Asia and appearing in sub-Saharan Africa, development of novel antimalarial drugs, ideally of a different class, is critical. Two such candidate drugs are currently the most advanced in clinical development: *ganaplacide* (KAF 156) and *cipargamin* (KAE 609). *Ganaplacide* is an imidazolopiperazine that is active on asexual forms as well as gametocytes. It is also active on *P. vivax* in addition to *P. falciparum*. It is currently in phase II clinical trials in several sites in combination with a new formulation of *lumefantrine* termed *lumefantrine* solid dispersible form (Ashley et al., 2018). *Cipargamin* (KAE 609) is a spiroindolone active as blood schizonticide on *P. falciparum*. It is under development as a single-dose single agent and is in field trials.

Ferroquine is a 4-aminoquinoline that retains *in vitro* efficacy against *chloroquine*-resistant and *piperaquine*-resistant *P. falciparum*. It is under clinical development in combination with *artefenomel*, which is a synthetic analogue of the *artemisinins* (Ashley et al., 2018).

Novel approaches using monoclonal antibodies are under development. Neutralizing human monoclonal antibody that targets epitopes in the *P. falciparum* circumsporozoite protein (PfCSP) were shown to be protective from malaria infection. This novel malaria prevention approach is now in phase I clinical trial Gaudinski et al., 2021).

Principles and Guidelines for Chemoprophylaxis and Chemotherapy of Malaria

Pharmacological prevention of malaria poses a difficult challenge because *P. falciparum*, which causes nearly all the human deaths from malaria, has become progressively more resistant to available antimalarial drugs. Oral *artemether-lumefantrine* is likely appropriate as first-line antimalarial treatment of uncomplicated malaria. *Chloroquine* remains effective against malaria caused by *P. ovale*, *P. malariae*, *P. knowlesi*, most strains of *P. vivax*, and chloroquine-sensitive strains of *P. falciparum*. However, chloroquine-resistant strains of *P. falciparum* are now the rule, not the exception, in most malaria-endemic regions (see Figure 66–1). Extensive geographic overlap also exists between *chloroquine* resistance and resistance to SP. Multidrug-resistant *P. falciparum* malaria is especially prevalent and severe in Southeast Asia and Oceania. These infections may not respond adequately even to *mefloquine* or *quinine*. The following section presents an overview of the chemoprophylaxis and chemotherapy of malaria. Current CDC recommendations for drugs and dosing regimens for the chemoprophylaxis and treatment of malaria in nonimmune individuals are provided in Tables 66–2 and 66–3.

Drugs should not replace simple, inexpensive measures for malaria prevention. Individuals visiting malarious areas should take appropriate steps to prevent mosquito bites. One such measure is to avoid exposure to mosquitoes at dusk and dawn, usually the times of maximal feeding of the anopheline species of mosquitos that transmit malaria parasites. Others include using insect repellents containing at least 30% DEET (*N,N′*-diethylmetatoluamide) and sleeping in well-screened rooms or under bed nets impregnated with a pyrethrin insecticide such as permethrin.

Malaria Chemoprophylaxis

Regimens for malaria chemoprophylaxis include primarily three drugs: *atovaquone-proguanil* and *doxycycline*, which can both be used in all areas, and *mefloquine*, which can be used in areas with *mefloquine*-sensitive malaria. Other available options are *chloroquine* or *hydroxychloroquine* (but their use is restricted to the few areas with *chloroquine*-sensitive malaria) and *primaquine* or *tafenoquine* (for short-duration travel to areas with principally *P. vivax*). In general, dosing should be started before exposure, ideally before the traveler leaves home (see Table 66–2).

In those few areas where *chloroquine*-sensitive strains of *P. falciparum* are found, *chloroquine* is still suitable for chemoprophylaxis. In areas where *chloroquine*-resistant malaria is endemic, *mefloquine* and *atovaquone-proguanil* are the regimens of choice for chemoprophylaxis. For chemoprophylaxis in long-term travelers, *chloroquine* is safe at the doses used, but some recommend yearly retinal examinations, and there is a finite dose limit for which chemoprophylaxis with *chloroquine* is recommended because of ocular toxicity. *Mefloquine* and *doxycycline* are well tolerated. *Mefloquine* is the best-documented drug for malaria prophylaxis in long-term travelers and, if well tolerated, can be used for prolonged periods. *Atovaquone-proguanil* has been studied for prophylactic use up to 20 weeks but probably is acceptable for years based on experience with the individual components.

Self-Treatment of Presumptive Malaria for Travelers

The CDC provides travelers' guidelines for self-treatment of presumptive malaria with appropriate drugs (*atovaquone-proguanil*, *artemether-lumefantrine*; as described in Table 66–3) when professional care is not available within 24 h. In such cases, medical care should be sought immediately after treatment. These recommendations may change over time and with specific locations. Consult the CDC Yellow Book (https://wwwnc.cdc.gov/travel/page/yellowbook-home).

Diagnosis and Treatment of Malaria

The diagnosis of malaria must be considered for patients presenting with acute febrile illness after returning from a malaria-endemic region. An organized, rational approach to diagnosis, parasite identification, and appropriate treatment is crucial. Guidelines for treatment of malaria in the U.S. are provided by the CDC and are shown in Figure 66–3, with details of the available agents summarized in Table 66–3. More information is available online (https://www.cdc.gov/malaria/resources/pdf/Malaria_Treatment_Table.pdf) and from the CDC Malaria Hotline (770-488-7100 and 855-856-4713).

Children and pregnant women are the most susceptible to severe malaria. The treatment of children generally is the same as for adults (pediatric dose should never exceed adult dose) (see the Drug Facts table

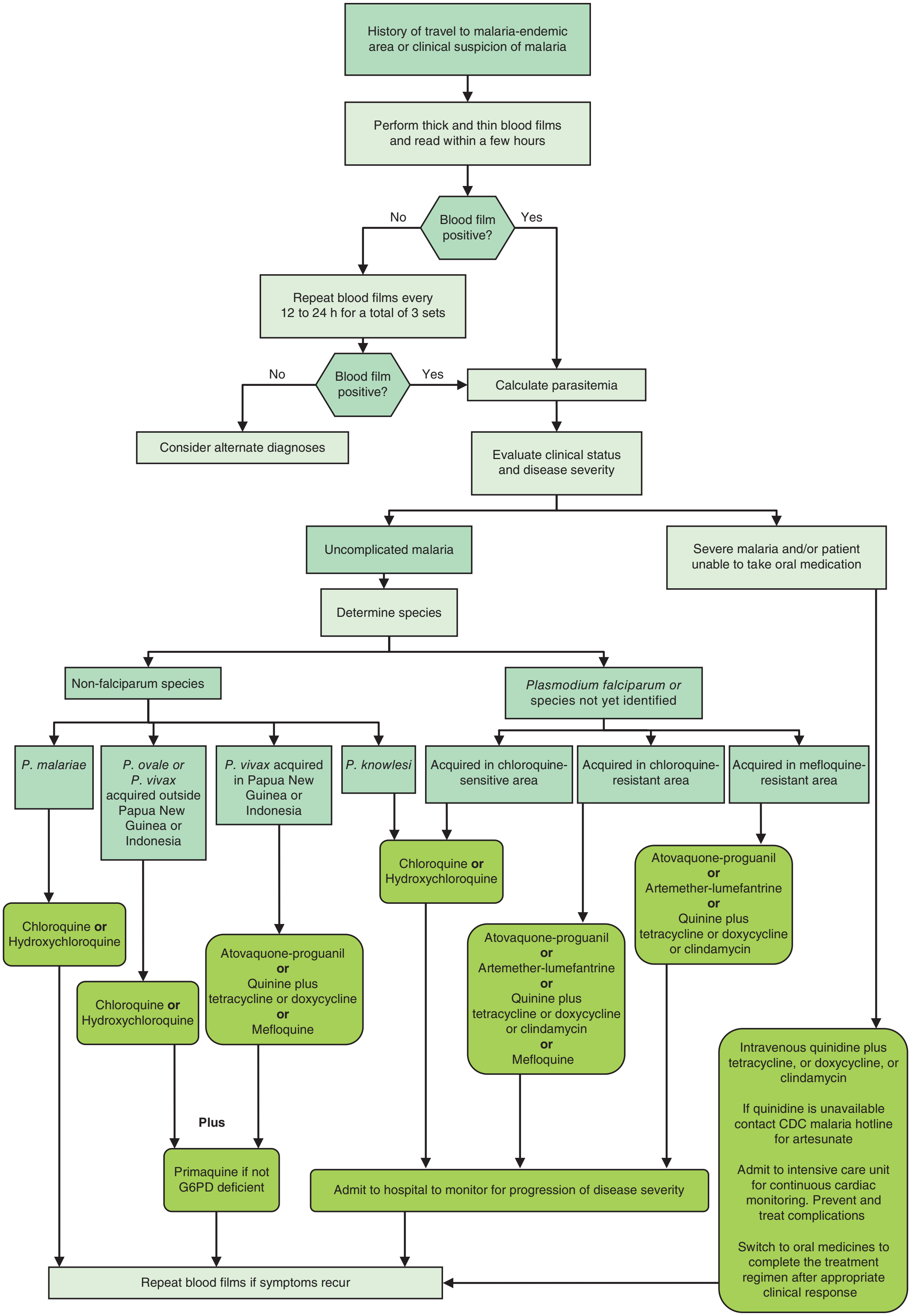

History of travel to malaria-endemic area or clinical suspicion of malaria
Perform thick and thin blood films and read within a few hours
Blood film positive?
No
Yes
Repeat blood films every 12 to 24 h for a total of 3 sets
Blood film positive?
No
Yes
Consider alternate diagnoses
Calculate parasitemia
Evaluate clinical status and disease severity
Uncomplicated malaria
Severe malaria and/or patient unable to take oral medication
Determine species
Non-falciparum species
Plasmodium falciparum or species not yet identified
P. malariae
P. ovale or P. vivax acquired outside Papua New Guinea or Indonesia
P. vivax acquired in Papua New Guinea or Indonesia
P. knowlesi
Acquired in chloroquine-sensitive area
Acquired in chloroquine-resistant area
Acquired in mefloquine-resistant area
Chloroquine **or** Hydroxychloroquine
Chloroquine **or** Hydroxychloroquine
Chloroquine **or** Hydroxychloroquine
Atovaquone-proguanil **or** Quinine plus tetracycline or doxycycline **or** Mefloquine
Atovaquone-proguanil **or** Artemether-lumefantrine **or** Quinine plus tetracycline or doxycycline or clindamycin **or** Mefloquine
Atovaquone-proguanil **or** Artemether-lumefantrine **or** Quinine plus tetracycline or doxycycline or clindamycin
Intravenous quinidine plus tetracycline, or doxycycline, or clindamycin
If quinidine is unavailable contact CDC malaria hotline for artesunate
Admit to intensive care unit for continuous cardiac monitoring. Prevent and treat complications
Switch to oral medicines to complete the treatment regimen after appropriate clinical response
Plus
Primaquine if not G6PD deficient
Admit to hospital to monitor for progression of disease severity
Repeat blood films if symptoms recur

Figure 66–3 *Decision algorithm for the treatment of malaria.* *Atovaquone-proguanil*, *mefloquine*, *artemether-lumefantrine*, *tetracycline*, and *doxycycline* are not indicated during pregnancy (pregnancy category C). *Tetracycline* and *doxycycline* are not indicated in children younger than 8 years. (Modified from Centers for Disease Control and Prevention. Available at: http://www.cdc.gov/malaria/resources/pdf/algorithm.pdf; accessed June 21, 2012.)

at the end of the chapter). However, tetracyclines should not be given to children less than 8 years of age except in an emergency, and *atovaquone-proguanil* as treatment has been approved only for children weighing more than 5 kg.

Chemoprophylaxis and Treatment During Pregnancy

Chemoprophylaxis during pregnancy is complex, and women should evaluate with expert medical staff the benefits and risks of different strategies with regard to their particular situations. Severe malaria during pregnancy should be treated with an intravenous antimalarial agent according to the general guidelines for severe malaria, taking into account the drugs that should be avoided during pregnancy. In lactating mothers, treatment with most compounds is acceptable, although *chloroquine* and *hydroxychloroquine* are the preferred agents. The use of *atovaquone-proguanil* is not recommended unless breastfeeding infants weigh more than 5 kg. Also, the breastfeeding infant should be shown to have a normal G6PD level before receiving *primaquine*.

Targeting the Mosquito Rather Than the Infected Human

The requisite transmission of parasites between human hosts by *Anopheles* spp. mosquitos renders sustained malaria transmission vulnerable to effective vector control. Controlling vectors through larval source management, indoor residual spraying of domiciles with mosquitocidal compounds, and use of long-lasting insecticide-treated bed nets are common vector-directed interventions that directly reduce the frequency of contact between humans and vectors.

A new approach to transmission reduction is the indirect targeting of mosquitos with the endectocide ivermectin. Ivermectin is a semisynthetic member of the avermectin class of macrocyclic lactones (see Chapter 68). The direct activity of ivermectin against *Plasmodium* spp. parasites is only mild; its application in malaria control results from its activity against the *Anopheles* spp. vectors when they take a blood meal from a host following the administration of ivermectin. The aspiration of serum ivermectin along with the blood meal delivers a potentially lethal dose to the vector and, in surviving mosquitos, renders them less efficient at supporting parasite development and less efficient at obtaining further blood meals. Given the vulnerability of malaria transmission to even slight reductions in vector efficiency, these effects have, in clinical trials of the administration of monthly doses of ivermectin to adult community members, reduced the incidence of *P. falciparum* malaria in children. Mathematical modeling supports the potential for this as a new tool to indirectly control malaria and sharply reduce transmission in some settings, and further trials of ivermectin delivery modes are underway.

In addition, antimalaria chemotherapies may be adapted to kill the parasite in the mosquito. In a proof-of-concept study, atovaquone, an inhibitor of parasite cytochrome B, was delivered to *Anopheles* spp. mosquitos across their cuticle when landing and inhibited the subsequent development of *P. falciparum* in the mosquito midgut. Should other conventional antiparasitics also be capable of incorporation into bednets and be successful at arresting parasite development in the mosquito, these compounds may find a new application beyond therapy in humans.

Recent technological developments seem likely to revolutionize mosquito control and mosquito susceptibility to malarial parasites. Isaacs et al. (2011) engineered resistance to infection by *P. falciparum* in mosquitoes by having the mosquitoes express single-chain antibodies that targeted antigens on the parasite's surface and inhibited the parasite's capacity to invade the midgut and salivary glands of the mosquito, effects that would reduce or eliminate the capacity of the mosquito to infect humans in the course of a blood meal.

The development of gene editing using CRISPR/cas9 (see Chapters 3 and 7) has opened up a new avenue for high-efficiency expression of resistance genes for treating the spread and prevalence of malaria. Gantz and Bier (2015) have described a "mutagenic chain reaction" based on CRISPR/cas9 that can spread a mutation from one chromosome to its homologous chromosome, converting heterozygous mutations to homozygosity in most germline and somatic cells in *Drosophila*. This gene drive system works in mosquitoes as well (Gantz et al., 2015), introducing anti-*Plasmodium* effector genes into the germline and thence into the progeny with very high frequency. Other CRISPR/cas9 endonuclease constructs have driven genes in the malarial vector *Anopheles gambiae,* targeting female reproduction and holding the promise of reducing the mosquito population in malarious areas to levels that will not support transmission of the disease (Hammond et al., 2016). Research on the use of gene-drive technology to control populations of mosquitoes continues to advance (Adolfi et al., 2020). The use of this technology among wild populations is not without concern, and the WHO has released revised guidance for the application of genetically modified mosquitoes into the wild (Benedict et al., 2021). As noted in a recent review, "These scientific advances, combined with ethical and social considerations, will facilitate the transparent and responsible advancement of these technologies towards field implementation" (Bier, 2022).

Drug Facts for Your Personal Formulary: *Regimens for Malaria Treatment*

Drug Indication	Adult Dosage	Pediatric Dosage[a]	Potential Adverse Effects	Comments
Artemether-lumefantrine *P. falciparum* from chloroquine-resistant or unknown areas	Tablet: 20 mg artemether, 120 mg lumefantrine. Dose: 4 tablets. Day 1: 2 doses separated by 8 h; thereafter 1 dose twice daily × 2 days	Weight (kg) 5–15: 1 tablet/dose 15–25: 2 tablets/doses 25–<35: 3 tables/doses >35: 4 tablets/doses Use same 3-day schedule as adults	Adults: headache, anorexia, dizziness, asthenia, arthralgia, myalgia Children: fever, cough, vomiting, loss of appetite, headache	Take with food or whole milk. If patient vomits within 30 min, repeat dose. Contraindicated in pregnancy.
Artesunate (IV) Severe malaria; see CDC guidelines	IV: 2.4 mg/kg doses at 0, 12, and 24 h each with reassessment for subsequent oral treatment with artemether-lumefantrine, atovaquone-proguanil, doxycycline, or mefloquine		See Artemether	See Artemether CDC guidelines (https://www.cdc.gov/malaria/diagnosis_treatment/artesunate.html).

Drug Facts for Your Personal Formulary: *Regimens for Malaria Treatment* (continued)

Drug Indication	Adult Dosage	Pediatric Dosage[a]	Potential Adverse Effects	Comments
Atovaquone-proguanil *P. falciparum* from chloroquine-resistant areas; *P. vivax*	Adult tablet: 250 mg atovaquone/100 mg proguanil; 4 adult tablets orally per day × 3 days	Pediatric tablet: 62.5 mg atovaquone/25 mg proguanil 5–8 kg: 2 pediatric tablets orally/day × 3 days >8–10 kg: 3 pediatric tablets daily × 3 days >10–20 kg: 1 adult tablet daily × 3 days >20–30 kg: 2 adult tablets daily × 3 days >30–40 kg: 3 adult tablets daily × 3 days >40 kg: 4 adult tablets daily × 3 days	Abdominal pain, nausea, vomiting, diarrhea, headache, rash, mild reversible elevations in liver aminotransferase levels	Not indicated for use in pregnant women due to limited data. Contraindicated if hypersensitivity to atovaquone or proguanil; severe renal impairment (creatinine clearance <30 mL/min). Should be taken with food to increase absorption of atovaquone.
Chloroquine phosphate *P. falciparum* from chloroquine-sensitive areas; *P. vivax* from chloroquine-sensitive areas; all *P. ovale*; all *P malariae*; all *P. knowlesi*	600 mg base (1000 mg salt) orally immediately, followed by 300 mg base (500 mg salt) orally at 6, 24, and 48 h Total dose: 1500 mg base (2500 mg salt)	10 mg base/kg orally immediately, followed by 5 mg base/kg orally at 6, 24, and 48 h Total dose: 25 mg base/kg	Nausea, vomiting, rash, headache, dizziness, urticaria, abdominal pain, pruritus	Safe in children and pregnant women. Give for chemoprophylaxis (500 mg salt orally every week) in pregnant women with chloroquine-sensitive *P. vivax*. Contraindicated if retinal or visual field change or hypersensitivity to 4-aminoquinolines. Use with caution in those with impaired liver function since the drug is concentrated in the liver.
Clindamycin (oral or IV) *P. falciparum* from chloroquine-resistant areas; *P. vivax* from chloroquine-resistant areas	Oral: 20 mg base/kg/day orally divided 3 times daily × 7 days IV: 10 mg base/kg loading dose IV followed by 5 mg base/kg IV every 8 h; switch to oral clindamycin (as above) as soon as patient can take oral meds; duration = 7 days	Oral: 20 mg base/kg/day orally divided 3 times daily × 7 days IV: 10 mg base/kg loading dose IV followed by 5 mg base/kg IV every 8 h; switch to oral clindamycin (oral dose as above) as soon as patient can take oral medication; treatment course = 7 days	Diarrhea, nausea, rash	Always use in combination with quinine-quinidine. Safe in children and pregnant women.
Doxycycline (oral or IV) *P. falciparum* and *P. vivax* from chloroquine-resistant areas	Oral: 100 mg orally twice daily × 7 days IV: 100 mg IV every 12 h and then switch to oral doxycycline (as above) as soon as patient can take oral medication; treatment course = 7 days	Oral: 2.2 mg/kg orally every 12 h × 7 days IV: Only if patient is not able to take oral medication; for children <45 kg, give 2.2 mg/kg IV every 12 h and then switch to oral doxycycline (dose as above) as soon as patient can take oral medication; for children >45 kg, use same dosing as for adults; duration = 7 days	Nausea, vomiting, diarrhea, abdominal pain, dizziness, photosensitivity, headache, esophagitis, odynophagia. Rarely hepatotoxicity, pancreatitis, and benign intracranial hypertension seen with tetracycline class of drugs.	Always use in combination with quinine or quinidine. Contraindicated in children <8 years old, pregnant women, and persons with known hypersensitivity to tetracyclines. Food, milk, and Ca^{2+} antacids decrease absorption and decrease GI disturbances. To prevent esophagitis, take tetracyclines with large amounts of fluids (patients should not lie down for 1 h after taking the drugs). Barbiturates, carbamazepine, or phenytoin may cause reduction in C_p of doxycycline.
Hydroxychloroquine (oral) Secondary alternative for treatment of *P. falciparum* and *P. vivax* from chloroquine-sensitive areas; all *P. ovale*; all *P. malariae*	620 mg base (800 mg salt) orally immediately, followed by 310 mg base (400 mg salt) orally at 6, 24, and 48 h Total dose: 1550 mg base (2000 mg salt)	10 mg base/kg orally immediately, followed by 5 mg base/kg orally at 6, 24, and 48 h Total dose: 25 mg base/kg	Nausea, vomiting, rash, headache, dizziness, urticaria, abdominal pain, pruritus	Safe in children and pregnant women. Contraindicated if retinal or visual field change; hypersensitivity to 4-aminoquinolines. Use with caution in those with impaired liver function.

Drug Facts for Your Personal Formulary: *Regimens for Malaria Treatment* (*continued*)

Drug Indication	Adult Dosage	Pediatric Dosage[a]	Potential Adverse Effects	Comments
Mefloquine[b] *P. falciparum* from chloroquine-resistant areas, except Thailand-Burmese and Thailand-Cambodian border regions; *P. vivax* from chloroquine-resistant areas	684 mg base (750 mg salt) orally as initial dose, followed by 456 mg base (500 mg salt) orally given 6–12 h after initial dose Total dose = 1250 mg salt	13.7 mg base/kg (15 mg salt/kg) orally as initial dose, followed by 9.1 mg base/kg (10 mg salt/kg) orally given 6–12 h after initial dose Total dose = 25 mg salt/kg	Nausea, vomiting, diarrhea, abdominal pain; dizziness, headache, somnolence, sleep disorders; myalgia, mild skin rash, and fatigue; moderate-to-severe neuropsychiatric reactions; electrocardiogram changes (sinus arrhythmia, sinus bradycardia, first-degree atrioventricular block, QTc prolongation, and abnormal T waves	Contraindicated if hypersensitive to the drug or to related compounds; cardiac conduction abnormalities; psychiatric disorders; and seizure disorders. Do not administer if patient has received related drugs (chloroquine, quinine, quinidine) less than 12 h ago.
Primaquine phosphate Radical cure of *P. vivax* and *P. ovale* (to eliminate hypnozoites)	30 mg base orally per day × 14 days	0.5 mg base/kg orally per day × 14 days	GI disturbances, methemoglobinemia (self-limited), hemolysis in persons with G6PD deficiency	Must screen for G6PD deficiency prior to use. Contraindicated in persons with G6PD deficiency; pregnant women. Should be taken with food to minimize GI adverse effects.
Quinine sulfate (oral) *P. falciparum* from chloroquine-resistant areas; *P. vivax* from chloroquine-resistant areas	542 mg base (650 mg salt)[d] orally 3 times daily × 3 days (infections acquired outside Southeast Asia) to 7 days (infections acquired in Southeast Asia)	8.3 mg base/kg (10 mg salt/kg) orally 3 times daily × 3 days (infections acquired outside Southeast Asia) to 7 days (infections acquired in Southeast Asia)	Cinchonism,[e] sinus arrhythmia, junctional rhythms, atrioventricular block, prolonged QT interval, ventricular tachycardia, ventricular fibrillation (these are rare and more commonly seen with quinidine), hypoglycemia	Combine with tetracycline, doxycycline, or clindamycin, except for *P. vivax* infections in children <8 years old or pregnant women. Contraindicated in hypersensitivity, including history of blackwater fever, thrombocytopenic purpura, or thrombocytopenia associated with quinine or quinidine use; many cardiac conduction defects and arrhythmias[f]; myasthenia gravis; optic neuritis.
Quinidine gluconate[c] (intravenous) Severe malaria (all species, independently of chloroquine resistance); patient unable to take oral medication; parasitemia >10%	6.25 mg base/kg (10 mg salt/kg) loading dose IV over 1–2 h, then 0.0125 mg base/kg/min (0.02 mg salt/kg/min) continuous infusion for at least 24 h Note alternative regimen[g]	Same as adult	Cinchonism, tachycardia, prolongation of QRS and QTc intervals, flattening of T wave (effects are often transient). Ventricular arrhythmias, hypotension, hypoglycemia	Combine with tetracycline, doxycycline, or clindamycin. Contraindicated in hypersensitivity, history of blackwater fever, thrombocytopenic purpura, or thrombocytopenia associated with quinine/quinidine use; many cardiac conduction defects and arrhythmias[f]; myasthenia gravis; optic neuritis.
Tafenoquine (oral) Radical cure of *P. vivax* and *P. ovale* (to eliminate hypnozoites)	300 mg single dose	Not defined	GI disturbances, methemoglobinemia (self-limited), hemolysis in persons with G6PD deficiency	Must screen for G6PD deficiency prior to use. Contraindicated in persons with G6PD deficiency; pregnant women.
Tetracycline (oral or IV) *P. falciparum* and *P. vivax* from chloroquine-resistant areas (with quinine/quinidine)	Oral: 250 mg 4 times daily × 7 days IV: dosage same as for oral	25 mg/kg/day orally, divided, 4 × daily × 7 days IV: dosage same as for oral	See doxycycline	See doxycycline.

These regimens are based on published recommendations of the U.S. CDC. Although current at the time of writing, these recommendations may change over time. Up-to-date information should be obtained from the CDC website at https://wwwnc.cdc.gov/travel. Recommendations and available treatment differ among countries in the industrialized world, developing world, and malaria-endemic regions; in the last, some antimalarial treatments may be available without prescription, but the most effective drugs usually are controlled by governmental agencies.

[a] Pediatric dosage should never exceed adult dosage.

[b] Mefloquine should not be used to treat *P. falciparum* infections acquired in the following areas: borders of Thailand with Burma (Myanmar) and Cambodia, western provinces of Cambodia, eastern states of Burma (Myanmar), border between Burma and China, Laos along borders of Laos and Burma (and adjacent parts of Thailand-Cambodia border), and southern Vietnam due to resistant strains.

[c] Extrapolated from chloroquine literature.

[d] Quinine sulfate capsule manufactured in the U.S. is in a 324-mg dose; therefore, two capsules should be sufficient for adult dosing.

[e] Nausea, vomiting, headache, tinnitus, deafness, dizziness, and visual disturbances.

[f] Refer to https://www.accessdata.fda.gov/drugsatfda_docs/label/2008/021799s008lbl.pdf; accessed January 12, 2022.

[g] Alternative dosing hypoglycemia optic neuritis regimen for quinidine gluconate (IV): 15 mg base/kg (24 mg salt/kg) loading dose IV infused over 4 h, followed by 7.5 mg base/kg (12 mg salt/kg) infused over 4 h every 8 h, starting 8 h after the loading dose (see package insert); once parasite density <1% and patient can take oral medication, complete treatment with oral quinine, dose as above. Quinidine or quinine course is 7 days in Southeast Asia (3 days in Africa or South America).

Sources: https://wwwnc.cdc.gov/travel/page/yellowbook-home-2020, accessed June 21, 2022;
and https://www.cdc.gov/malaria/resources/pdf/Malaria_Treatment_Table.pdf, accessed June 21, 2022.

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Chapter 67

Chemotherapy of Protozoal Infections: Amebiasis, Giardiasis, Trichomoniasis, Trypanosomiasis, Leishmaniasis, and Other Protozoal Infections

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PROTOZOAL INFECTIONS OF HUMANS

- Amebiasis
- Giardiasis
- Trichomoniasis
- Toxoplasmosis
- Cryptosporidiosis
- Trypanosomiasis
- Leishmaniasis
- Other Protozoal Infections

ANTIPROTOZOAL DRUGS

- Amphotericin B
- Eflornithine
- Fexinidazole
- 8-Hydroxyquinolines
- Melarsoprol
- Metronidazole and Tinidazole
- Miltefosine
- Nifurtimox and Benznidazole
- Nitazoxanide
- Paromomycin
- Pentamidine
- Sodium Stibogluconate
- Suramin

Humans host a wide variety of protozoal parasites that can be transmitted by insect vectors, directly from other mammalian reservoirs, or from one person to another. The immune system plays a crucial role in protecting against the pathological consequences of many protozoal infections. Thus, opportunistic infections with protozoa are prominent in patients with suppressed or underdeveloped immune systems, such as infants, individuals with cancer, transplant recipients, those receiving immunosuppressive drugs or extensive antibiotic therapy, and persons with advanced human immunodeficiency virus (HIV) infection. Because effective vaccines are unavailable, chemotherapy has been the only practical way to both treat infected individuals and reduce transmission. Satisfactory agents for treating important protozoal infections such as African trypanosomiasis (sleeping sickness) and chronic Chagas disease still are lacking. Many effective antiprotozoal drugs are toxic at therapeutic doses; this problem is exacerbated by increasing drug resistance (McCarthy et al., 2020). For a list of drugs and doses used to treat these diseases, see Kimberlin et al. (2018) and McCarthy et al. (2020).

Protozoal Infections of Humans

Amebiasis

Amebiasis affects about 10% of the world's population, causing invasive disease in about 50 million people and death in about 100,000 of these annually (Stanley, 2003). Amebiasis is seen most commonly among individuals living in poverty, crowded conditions, and areas with poor sanitation. Multiple morphologically identical but genetically distinct species of *Entamoeba*—such as *E. histolytica, E. dispar*, *E. bangladeshi*, and *E. moshkovskii*—exist (Petri et al., 2020). However, the major species that requires treatment is *E. histolytica*, a leading cause of mortality by parasitic infection (World Health Organization [WHO], 2020a).

Humans are the only known hosts for these protozoa, which are transmitted by the fecal-oral route. Ingested *E. histolytica* cysts survive acidic gastric contents and transform into *trophozoites* that reside in the large intestine (Petri et al., 2020). The outcome of *E. histolytica* infection is variable; both host and parasite factors influence the course and severity of the disease (Marie and Petri, 2014). Many individuals remain asymptomatic but excrete the infectious cyst form, making them a source for further infections. In other individuals, *E. histolytica* trophozoites invade into the colonic mucosa with resulting colitis and bloody diarrhea (amebic dysentery). This bloody diarrhea may result from a phenomenon termed trogocytosis ("trogo" means "nibble"), where the parasite acquires required nutrients by "nibbling" on human cells (Ralston and Petri, 2011). In a small proportion of patients, *E. histolytica* trophozoites invade through the colonic mucosa, reach the portal circulation, and travel to the liver, where they establish an amebic liver abscess (Haque et al., 2003).

The cornerstone of therapy for amebiasis causing colitis or liver abscess is metronidazole or its analogue tinidazole (Petri et al., 2020). Because *metronidazole* is so well absorbed in the gut, levels may not be therapeutic in the colonic lumen. The drug is also less effective against cysts. Therefore, patients with amebic colitis or amebic liver abscess should receive a luminal agent after the course of *metronidazole* has been completed to eradicate any *E. histolytica* trophozoites residing within the gut lumen (Kimberlin et al., 2018). Luminal agents are also used to treat asymptomatic individuals found to be infected with *E. histolytica*. The nonabsorbed aminoglycoside *paromomycin*, the 8-hydroxyquinoline compound *iodoquinol,* and *diloxanide furoate* are effective luminal agents (Haque et al., 2003). In addition, *nitazoxanide*, which is approved in the U.S. for treatment of cryptosporidiosis and giardiasis, has activity against *E. histolytica* (Petri et al., 2020). Since no treatment is 100% effective at eliminating infection from the intestinal tract, follow-up stool examination is recommended. In addition, all household members/close contacts of an index patient should have stool examinations performed, and they should be treated with a luminal agent if positive, even if they are asymptomatic (Kimberlin et al., 2018).

Giardiasis

Giardiasis, caused by the flagellated protozoan *Giardia intestinalis*, is prevalent worldwide and is the most commonly reported intestinal protozoal infection in the U.S. Infection results from ingestion of the cyst form of the parasite, which is found in fecally contaminated water or food. Cysts shed from animals or infected humans can contaminate

Abbreviations

AUC: area under the curve
CDC: U.S. Centers for Disease Control and Prevention
CSF: cerebrospinal fluid
DFMO: α-D,L-difluoromethylornithine
FDA: U.S. Food and Drug Administration
GI: gastrointestinal
HAT: Human African trypanosomiasis
HIV: human immunodeficiency virus
NECT: nifurtimox-eflornithine combination therapy
NTR: nitroreductase
PFOR: pyruvate-ferredoxin oxidoreductase
WBC: white blood cell
WHO: World Health Organization

recreational and drinking water supplies (Fletcher et al., 2012). Human-to-human transmission is common among children in day-care centers, institutionalized individuals, and men who have sex with men (Escobedo et al., 2014). Infection with *Giardia* results in one of three syndromes:

- An asymptomatic carrier state
- Acute self-limited diarrhea
- Chronic diarrhea, characterized by signs of malabsorption (steatorrhea) and weight loss (Nash and Bartlet, 2020)

Chemotherapy with a 5- to 7-day course of *metronidazole* usually is successful, although sometimes therapy may have to be repeated or prolonged, particularly in patients who are immunocompromised. A single dose of *tinidazole* may be superior to *metronidazole* (Kimberlin et al., 2018). *Paromomycin* can be used to treat pregnant women to avoid any possible mutagenic effects of the other drugs (Hill and Nash, 2014). *Nitazoxanide* is also approved for the treatment of giardiasis in adults and immune-competent children less than 12 years of age (Kimberlin et al., 2018).

Trichomoniasis

Trichomoniasis is caused by the flagellated protozoan *Trichomonas vaginalis* (Meites, 2013). This organism inhabits the human genitourinary tract, where it causes vaginitis in women and, less commonly, urethritis in men. Trichomoniasis essentially can only be transmitted by sexual contact; it is the most common nonviral sexually transmitted infection (Kimberlin et al., 2018). Infection with this organism is associated with an increased risk of acquiring HIV and adverse pregnancy outcomes in pregnant women (Kissinger, 2015). Only *trophozoite* forms of *T. vaginalis* have been identified in infected secretions. *Metronidazole* remains the drug of choice for treating trichomoniasis (Schwebke and Bachmann, 2020). *Tinidazole*, another nitroimidazole, appears to be better tolerated than *metronidazole* and has been used successfully to treat *metronidazole*-resistant *T. vaginalis* (Kimberlin et al., 2018). Because there is a high reinfection rate, retesting is recommended for all sexually active women within 3 months after initial treatment.

Toxoplasmosis

Toxoplasmosis is a zoonotic infection caused by the obligate intracellular protozoan *Toxoplasma gondii*. Although cats and other feline species are the natural hosts, tissue cysts (*bradyzoites*) have been recovered from all mammalian species examined. Common routes of infection in humans are as follows:

- Ingestion of undercooked meat containing tissue cysts
- Ingestion of contaminated vegetable matter containing infective *oocysts*
- Oral inoculation with feces of cats shedding oocysts
- Transplacental fetal infection with *tachyzoites* from acutely infected mothers (Woodhall et al., 2014)
- Donor-acquired infection and/or reactivation in transplant recipients (particularly cardiac transplant recipients) (Kimberlin et al., 2018)

The acute illness is usually self-limiting, and treatment rarely is required. However, individuals who are immunocompromised, such as patients with AIDS or transplants, are at risk of developing disseminated toxoplasmosis, as well as toxoplasmic encephalitis from reactivation of tissue cysts deposited in the brain (Montoya et al., 2020). Clinical manifestations of congenital toxoplasmosis vary, but classically, a triad of chorioretinitis, hydrocephalus, and cerebral calcifications is seen. Chorioretinitis, which may present decades after exposure, is the most common finding (Kieffer and Wallon, 2013).

The primary treatment of toxoplasmic encephalitis consists of the antifolates *pyrimethamine* and *sulfadiazine* along with *folinic acid* (*leucovorin*) (Montoya et al., 2020). Therapy must be discontinued in about 40% of cases because of toxicity, primarily due to the sulfa compound, and the cost of therapy is substantial (Dunay et al., 2018). *Pyrimethamine-clindamycin* appears to have comparable efficacy to *pyrimethamine-sulfadiazine* for treating toxoplasmosis in immunocompromised patients, but this combination also causes substantial toxicity. Alternative regimens combining *azithromycin*, *clarithromycin*, *atovaquone*, or *dapsone* with either *trimethoprim-sulfamethoxazole* or *pyrimethamine* are less toxic, but also less effective (Rajapakse et al., 2013). For severe, sight-threatening ocular toxoplasmosis, the addition of *prednisone* to decrease inflammation is recommended. *Spiramycin*, which concentrates in placental tissue, is used for the treatment of acute acquired toxoplasmosis in early pregnancy to prevent transmission to the fetus (Kieffer and Wallon, 2013). *Spiramycin* is available through the investigational new drug process at the U.S. Food and Drug Administration (FDA) (Kimberlin et al., 2018). If fetal infection occurs, the combination of *pyrimethamine*, *sulfadiazine*, and *folinic acid* is administered to the mother (only after the first 12–14 weeks of pregnancy) and to the newborn postnatally for 1 year (Kimberlin et al., 2018).

Cryptosporidiosis

Cryptosporidia are coccidian protozoan parasites that cause diarrhea. *Cryptosporidium parvum* and *Cryptosporidium hominis* appear to account for almost all infections in humans (White, 2020). Infectious oocysts in feces are tolerant to chlorination and may be spread either by direct human-to-human contact or by contaminated recreational or drinking water. Productive infection requires ingestion of as few as 10 oocysts (Wetzel et al., 2005). Groups at particular risk include travelers, children in day care, men who have sex with men, animal handlers, and veterinary or healthcare personnel. After ingestion, the mature oocyte is digested, releasing *sporozoites* that invade host epithelial cells (Wilhelm and Yarovinsky, 2014). In many individuals, infection is self-limited. However, in patients with AIDS and other immunocompromising conditions, such as patients with solid organ transplants or hyper-IgM syndrome, the severity of diarrhea or other complications may require hospitalization (White, 2020).

Nitazoxanide is used to treat cryptosporidiosis in immunocompetent children and adults (Kimberlin et al., 2018). It has questionable efficacy in children and adults with HIV/AIDS or other immunocompromising conditions, even when given for prolonged courses. The most effective therapy for cryptosporidiosis in immunocompromised patients is restoration of immune function (e.g., through initiation of antiretroviral therapy in patients with AIDS) (White, 2020).

Trypanosomiasis

African trypanosomiasis, or “sleeping sickness,” is caused by subspecies of the hemoflagellate *Trypanosoma brucei* that are transmitted by bloodsucking tsetse flies of the genus *Glossina* (Kennedy, 2019). Largely restricted to sub-Saharan Africa, the infection causes serious human illness and also threatens livestock (*nagana*), leading to protein malnutrition. In humans, the infection is almost always fatal unless treated. Sleeping sickness is found in 36 countries in Africa, but the caseload has dropped significantly due to renewed control efforts, and fewer than 1000 cases were reported in 2019 (Neau et al., 2020; WHO, 2020d).

The parasite is entirely extracellular, and early human infection is characterized by the finding of replicating parasites in the bloodstream or lymph without central nervous system (CNS) involvement (stage 1); stage 2 disease is characterized by involvement of the CNS (Kennedy, 2019; WHO, 2020d). Symptoms of early-stage disease include febrile illness, lymphadenopathy, splenomegaly, and occasional myocarditis that result from systemic dissemination of the parasites. There are two types of human African trypanosomiasis (HAT): The East African (Rhodesian; *T. brucei rhodesiense*) variety produces a progressive and rapidly fatal form of disease marked by early involvement of the CNS and frequent terminal cardiac failure; the West African type (Gambian; *T. brucei gambiense*) causes illness characterized by later involvement of the CNS and a more long-term course that progresses to the classical symptoms of sleeping sickness over months to years. Neurological symptoms include confusion, sensory deficits, psychiatric signs, disruption of the sleep cycle, and eventual progression to coma and death.

Treatments for Gambian HAT have evolved dramatically in recent years (Dickie et al., 2020; Neau et al., 2020). A new drug, *fexinidazole*, was added to the WHO list of essential medicines in 2019 and is now the recommended front-line treatment in Europe and Africa in adults for early-stage *T. brucei gambiense* and for less severe late-stage disease (disease with CNS involvement) (Lindner et al., 2020; WHO, 2019). *Nifurtimox-eflornithine* combination therapy (NECT) is still recommended for more severe late-stage disease, in the U.S., and for late-stage disease in children aged 6 years or less or with a body weight less than 20 kg. *Fexinidazole* replaces *pentamidine* for early-stage *T. brucei gambiense*, with the exception of the previously mentioned group of children. Importantly, the availability of both *fexinidazole* and NECT eliminates the need to treat the CNS phase of Gambian HAT with *melarsoprol*, a highly toxic agent that causes a fatal reactive encephalopathy in 2% to 10% of treated patients. Recommendations have not yet changed for *T. brucei rhodesiense* HAT, where *suramin* remains the drug of choice for early-stage disease and *melarsoprol* (available from the U.S. Centers for Disease Control and Prevention [CDC]) remains the only drug approved for the CNS phase of the disease.

Fexinidazole is a nitroheterocycle from the same drug class as *nifurtimox*, which is used as a combination agent (NECT treatment) for HAT and for treatment of *T. cruzi* as a single agent. *Fexinidazole* is the first orally available drug for the treatment of HAT. It is also the first that can be used for treatment of both early-stage and late-stage disease (Lindner et al., 2020; WHO, 2019). Its efficacy in treating both stages eliminates the need for lumbar puncture, except for severe late-stage disease. *Fexinidazole* is a prodrug that is activated by NADH-specific nitroreductase in a two-electron reduction of the NO_2 group, with the mechanism of action likely to be caused by the action of this highly reactive species on multiple cellular targets (Dickie et al., 2020). The antitrypanosomal activity of *fexinidazole* was first described in the 1980s, but concerns over toxicity of the drug class and lack of full efficacy in mouse models derailed its development. The success of *nifurtimox* as part of NECT therapy led to renewed interest, and after testing of a small library of nitroimidazoles, *fexinidazole* was selected as the most promising and was shown to be effective if used in more extended dosing schemes (Neau et al., 2020). It was incorporated into the WHO interim treatment guidelines in 2019 after having received a favorable European Medicines Agency scientific opinion (Lindner et al., 2020; WHO, 2019). It is not yet approved in the U.S. It is administered in tablets with food over 10 days, with a higher loading dose given for 4 days followed by a 6-day maintenance dose.

Eflornithine (available from the CDC), an inhibitor of ornithine decarboxylase, a key enzyme in polyamine metabolism, remains an important agent for the treatment of late-stage Gambian HAT (DeKoning, 2020; WHO, 2019). It has efficacy against both early and late-stages of human *T. brucei gambiense* infection; however, it is thought to be ineffective as monotherapy for infections of *T. brucei rhodesiense*. Notably, *eflornithine* has significantly fewer side effects than *melarsoprol* and is more effective than *melarsoprol* for treatment of late-stage Gambian trypanosomiasis. NECT allows shorter exposure to *eflornithine* with good efficacy and a reduction in adverse events; it remains the treatment of choice for severe infections of late-stage *T. brucei gambiense* in adults, for children under 6 years of age, or in cases where *fexinidazole* is not recommended (Lindner et al., 2020; WHO, 2019).

While drug therapies for HAT have notably improved in recent years, *fexinidazole* is not yet recommended for severe cases of late-stage CNS disease, NECT is difficult to administer in a rural setting, and the lack of an alternative to *melarsoprol* to treat *T. brucei rhodesiense* is concerning. One additional orally available agent, *acoziborole (SCYX-7158)*, has just completed a phase III human efficacy study. *Acoziborole*, which appears to target the RNA cleavage and polyadenylation specificity factor subunit 3 (CPSF3), has the potential to offer a single-dose cure for the treatment of both early- and late-stage Gambian trypanosomiasis (Dickie et al., 2020). If the safety and efficacy data from this study support authorization for clinical use, this compound has the potential to have a high impact on HAT treatment options.

American trypanosomiasis, or *Chagas disease*, is a zoonotic infection caused by *Trypanosoma cruzi* (Bern et al., 2019). The WHO estimates that Chagas affects about 6 to 7 million people worldwide (WHO, 2020b). The spread of Chagas disease is primarily confined to Latin America, but due to immigration, a number of cases are now seen outside that region. Blood-sucking triatomid bugs infesting poor rural dwellings most commonly transmit this infection to young children; transplacental transmission may also occur. Within the Western Hemisphere, the U.S. is estimated to have the sixth-highest caseload (~300,000 cases), representing a significant public health concern because the parasite can also be transmitted by blood transfusion and organ transplantation (Bern et al., 2019; Hotez et al., 2013). Although most cases in the U.S. arise through immigration, the parasite and its vector are endemic in the southern half of the U.S. and transmission within the U.S. has been documented (Bern et al., 2019). Seven states include Chagas disease as a reportable condition, and local transmission has been reported in Arizona, Louisiana, Mississippi, Tennessee, and Texas. While the U.S. blood supply is now being monitored, lack of awareness of the disease can lead to suboptimal care of those infected.

The clinical outcome of an infected patient can vary widely from asymptomatic to severe disease (Bern et al., 2019). The chronic form of the disease in adults is a major cause of cardiomyopathy, megaesophagus, megacolon, and death (Nunes et al., 2018). Common findings of Chagas heart disease include arrhythmias, myocardial abnormalities, aneurysms, and thromboembolism. End-stage heart failure is usually the result of progressive dilated cardiomyopathy. Chagas heart disease is typically managed in accordance with American College of Cardiology/American Heart Association guidelines for treatment of heart failure (Nunes et al., 2018).

Two nitroheterocyclic drugs, *benznidazole* and *nifurtimox* (both available from the CDC), are used to treat *T. cruzi* infection, and the better-tolerated *benznidazole* has recently been approved by the FDA for children 2 to 12 years of age (Kratz et al., 2018; Meymandi et al., 2018). Both agents suppress parasitemia and can cure the acute, congenital, and early chronic phases of Chagas disease. Treatment should also be offered to any patient with reactivated disease after immunosuppression. While clinical benefit of treatment has also been reported for chronic indeterminate cases, a recent study found no clinical benefit for treatment of patients with advanced cardiomyopathy, even though parasite levels were decreased (Morillo et al., 2015). Both *nifurtimox* and *benznidazole* are toxic and must be taken for long periods, though *benznidazole* is better tolerated in children than adults. Increased awareness among physicians, better drugs, and better diagnostic methods are badly needed to help combat this disease. *Fexinidazole* is currently under evaluation as a treatment for Chagas disease (Meymandi et al., 2018).

Leishmaniasis

Leishmaniasis is a complex vector-borne zoonosis caused by about 20 different species of intramacrophage protozoa of the genus *Leishmania*. Small mammals and canines generally serve as reservoirs for these pathogens, which can be transmitted to humans by the bites of female phlebotomine sandflies (WHO, 2020c). Various forms of leishmaniasis affect people in southern Europe and many tropical and subtropical regions throughout the world. The sandfly vector has also spread into the U.S.; there have been documented cases of endemic acquisition in humans in

Oklahoma and Texas, where it is a reportable condition (McIlwee et al., 2018). Flagellated extracellular free *promastigotes*, regurgitated by feeding flies, enter the host, where they attach to and become phagocytized by tissue macrophages. These transform into *amastigotes*, which reside and multiply within phagolysosomes. Amastigotes then propagate the infection by entering more macrophages. Amastigotes taken up by feeding sandflies then transform back into promastigotes (Aronson et al., 2017).

In increasing order of systemic involvement and clinical severity, major syndromes of human leishmaniasis are classified into *cutaneous, mucocutaneous, diffuse cutaneous*, and *visceral* (*kala azar*) forms (WHO, 2020c). The disease syndrome manifested depends on the species of infecting parasite, the distribution of infected macrophages, and the host immune response (Podinovskaia and Descoteaux, 2015). As such, leishmaniasis is recognized as an AIDS-associated opportunistic infection (van Griensven et al., 2014). Cutaneous forms of leishmaniasis generally are self-limiting, with cures occurring 3 to 18 months after infection, but can leave disfiguring scars. Mucocutaneous, diffuse cutaneous, and visceral leishmaniasis do not resolve without therapy. Visceral disease caused by *Leishmania donovani* is fatal unless treated (Sundar and Chakravarty, 2015).

The classic therapy for all species of *Leishmania* is with *pentavalent antimony compounds* such as *sodium stibogluconate* (sodium antimony gluconate); resistance is widespread, particularly in India (WHO, 2020c). Recently, treatment of leishmaniasis has undergone major changes owing to the success of the first orally active agent, *miltefosine,* which has been FDA-approved for cutaneous, mucocutaneous, and visceral disease (Aronson et al., 2017). *Miltefosine* also can be used to treat dogs, an important animal reservoir of the disease (Alvar et al., 2006). However, its teratogenic effects prevent its use in women of childbearing age (Aronson et al., 2017). As an alternative, *liposomal amphotericin B* is a highly effective agent for visceral leishmaniasis and is now recommended therapy in the U.S. in treatment guidelines (Aronson et al., 2017). In addition, *paromomycin* has been used with some success as a parenteral agent for visceral disease, and topical formulations of *paromomycin* have also been used for cutaneous disease (Aronson et al., 2020). Finally, azole antifungals such as *fluconazole* can be employed, but have only an approximate 50% success rate depending on species, thus limiting their utility to mild cases (Aronson et al., 2017).

Other Protozoal Infections

Just a few of the many less-common protozoal infections of humans are highlighted in this section.

Babesiosis

Babesiosis, caused by either *Babesia microti* or *B. divergens*, superficially resembles malaria in that the parasites invade erythrocytes and produce a febrile illness, hemolysis, and hemoglobinuria. This tick-borne zoonosis can be acquired in the upper Midwest and the Northeast in the U.S. or via blood transfusion. Although this infection usually is mild and self-limiting, it can be severe or even fatal in asplenic or severely immunocompromised individuals (Vannier and Gelfand, 2020). Therapy is with a combination of *clindamycin* and *quinine* for severe disease or the combination of *azithromycin* and *atovaquone* for mild or moderate infections (Kimberlin et al., 2018).

Balantidiasis

Balantidiasis, caused by the ciliated protozoan *Balantidium coli*, is an infection of the large intestine that may be confused with amebiasis. Unlike amebiasis, this infection usually responds to *tetracycline* therapy, although balantidiasis can also be treated with *metronidazole* (Suh et al., 2020).

Other Coccidia

Cyclospora cayetanensis (Szumowski and Troemel, 2015) causes self-limited diarrhea in normal hosts and can cause prolonged diarrhea in immunocompromised individuals. *Cystoisospora belli,* formerly known as *Isospora belli,* causes diarrhea in patients with AIDS. Both *Cyclospora* and *Cystoisospora* respond to *trimethoprim-sulfamethoxazole* (Suh et al., 2020).

Microsporidia

Microsporidia are spore-forming, unicellular, eukaryotic organisms that were once thought to be parasites but are now classified as fungi (Szumowski and Troemel, 2015). As such, treatments are discussed in Chapter 61 (Antifungal Agents).

Antiprotozoal Drugs

For ease of reference, the myriad agents used to treat nonmalarial protozoal diseases are presented alphabetically.

Amphotericin B

The pharmacology, formulation, and toxicology of *amphotericin B* are presented in Chapter 61.

Antiprotozoal Effects

Amphotericin B is a highly effective antileishmanial agent that cures more than 90% of visceral leishmaniasis cases and is the drug of choice for antimonial-resistant cases (WHO, 2020c). It is the recommended agent for visceral leishmaniasis in the U.S. (Aronson et al., 2017). *Amphotericin B* also treats cutaneous or mucosal leishmaniasis and is effective for immunocompromised patients (Aronson et al., 2020). Lipid preparations of the drug have reduced toxicity, but the cost of the drug and the difficulty of administration remain a problem in endemic regions (WHO, 2020c).

Mechanism of Action

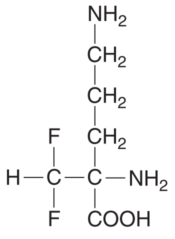
Amphotericin's activity against *Leishmania* is similar to its antifungal effects (see Figure 61–2). *Amphotericin* complexes with ergosterol precursors in the cell membrane, forming pores that allow ions to enter the cell (McCarthy et al., 2020). *Leishmania* has similar sterol composition to fungi, and *amphotericin* binds fungal sterols preferentially over host cholesterol (Moen et al., 2009).

Therapeutic Uses

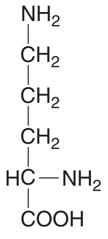
Typical regimens of 10 to 20 mg/kg total dose given in divided doses over 10 to 20 days by intravenous infusion have yielded cure rates of more than 95%. In the U.S., the FDA recommends 3 mg/kg intravenously on days 1 to 5, 14, and 21 for a total dose of 21 mg/kg to treat visceral leishmaniasis or 3 mg/kg per day for 7 to 10 days to treat cutaneous disease (Aronson et al., 2017). Shorter courses of the drug for treatment of visceral leishmaniasis have demonstrated good efficacy and provide a potential cost-saving alternative, although only a limited number of patients have been tested (Monge-Maillo and Lopez-Velez, 2013). In addition, combining antileishmanial drugs may be effective; further studies are still needed for such regimens (Sundar and Chakravarty, 2013).

Eflornithine

Eflornithine (DFMO, α-D,L-difluoromethylornithine) is an irreversible catalytic (suicide) inhibitor of ornithine decarboxylase, the enzyme that catalyzes the first step in the biosynthesis of polyamines (putrescine, spermidine, and spermine) that are required for cell division and for normal cell differentiation (Phillips, 2018). In trypanosomes, spermidine is required for the synthesis of trypanothione, a conjugate of spermidine and glutathione that replaces many of the functions of glutathione in the parasite. *Eflornithine* is transported into the cell via the amino acid transporter *Tb* AAT6.



EFLORNITHINE



ORNITHINE

Eflornithine in combination with *nifurtimox* (NECT) is currently the drug of choice for treatment of severe late-stage (CNS involvement) West African (Gambian) trypanosomiasis caused by *T. brucei gambiense* (Lindner et al., 2020). This includes patients with a white blood cell (WBC) count in the cerebrospinal fluid (CSF) of 100/μL or greater and patients whose clinical presentation meets criteria for evaluation by lumbar puncture. *Fexinidazole* has recently supplanted NECT as the front-line therapy for early-stage disease and for patients with noncomplicated presentations of the CNS stage. *Eflornithine* is thought to be less effective against East African trypanosomiasis and thus is not recommended for this application. *Eflornithine* is no longer available for systemic use in the U.S. but is available for treatment of Gambian trypanosomiasis by special request from the CDC.

Antitrypanosomal Effects

Eflornithine is a cytostatic agent that has multiple biochemical effects on trypanosomes, all of which are a consequence of polyamine depletion (Phillips, 2018). These include reduced levels of spermidine, which is required for hypusine modification of the essential eukaryotic translation factor eIF5A, and depletion of the trypanosomatid-specific redox cofactor trypanothione. The parasite and human enzymes are equally susceptible to inhibition by *eflornithine*; however, the mammalian enzyme turns over rapidly, whereas the parasite enzyme is stable, and this difference is thought to be one factor that contributes to selective toxicity.

ADME

Eflornithine is given by intravenous infusion. The drug does not bind to plasma proteins, is well distributed, and penetrates into the CSF, where estimated concentrations of at least 50 μM must be reached for parasite clearance (Burri and Brun, 2003). The mean $t_{1/2}$ is 3 to 4 h, and renal clearance after intravenous administration is rapid (2 mL/min/kg), with more than 80% of the drug cleared by the kidney largely in unchanged form (Sanderson et al., 2008).

Therapeutic Uses

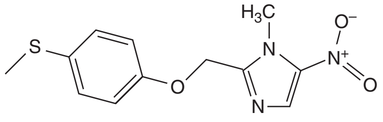
Eflornithine in combination with *nifurtimox* (NECT) is used for the treatment of late-stage West African trypanosomiasis caused by *T. brucei gambiense*. The combination is logistically easier to administer and better tolerated than *eflornithine* alone. NECT is safer and more efficacious than *melarsoprol* for late-stage gambiense sleeping sickness, and importantly, compared to *eflornithine* alone, NECT achieves a higher cure rate (96.5% vs. 91.5%) (Priotto G et al., 2009). NECT also showed better efficacy than *fexinidazole* in a randomized, phase II/III noninferiority trial (98% vs. 91% cure) of patients with late-stage Gambian HAT and had fewer side effects (Mesu et al., 2018). However, the need for lumbar puncture to evaluate the disease stage before use and the requirement for hospital administration and IV *eflornithine* dosing led to the recommendation that *fexinidazole* should become the front-line therapy with exceptions. NECT remains the recommended drug for severe cases of late-stage disease (WBCs in the cerebral spinal fluid of ≥100/μL) or for young children (<6 years or <20 kg) (Lindner et al., 2020; WHO, 2019). Dosing is as follows: 200 mg/kg IV every 12 h by 2-h infusion for 7 days plus *nifurtimox* (orally at 15 mg/kg per day in three divided doses [every 8 h]) for 10 days (Priotto S et al., 2009).

Toxicity and Side Effects

Eflornithine causes adverse reactions that are generally reversible on withdrawal of the drug. Abdominal pain and headache are the predominant complaints, followed by reactions at the injection sites. Tissue infections and pneumonia have also been reported. The most severe reactions for *eflornithine* alone were reported to include fever peaks (6%), seizures (4%), and diarrhea (2%) (Balasegaram et al., 2009; Priotto S et al., 2009). For NECT, severe adverse events were reduced compared to *eflornithine* alone (14% vs. 29%), and treatment-related deaths were also fewer (0.7% vs. 2%) (Priotto S et al., 2009). The case fatality rates for *eflornithine* (0.7%–1.2%) and for NECT (0.2%) are significantly lower than for *melarsoprol* (4.9%), and overall, either *eflornithine* alone or NECT is superior to *melarsoprol* with respect to both safety and efficacy. The more recent clinical study comparing NECT to *fexinidazole* found that NECT was well tolerated, with the most common side effects being vomiting (29%), nausea (19%), and headache (24%), and these occurred at a lower frequency than for *fexinidazole* (Mesu et al., 2018). Reversible hearing loss can occur after prolonged therapy with oral doses but was not reported in the NECT trials. Therapeutic doses of *eflornithine* are large and require coadministration of substantial volumes of intravenous fluid. This poses significant practical limitations in remote settings and can cause fluid overload in susceptible patients.

Fexinidazole

Fexinidazole is the first oral treatment for West African (Gambian) trypanosomiasis caused by *T. brucei gambiense,* and it is the first drug to be recommended for treatment of both early and late (CNS) stages of the disease (Lindner et al., 2020). The effectiveness of *fexinidazole* on both early- and late-stage disease alleviates the need to perform lumbar puncture to stage the disease, except in the most severe late-stage cases, and thus greatly simplifies HAT treatment. *Fexinidazole* received a positive review from the European Medicines Agency in 2018, and it was added to the WHO essential medicines list in 2019 (Neau et al., 2020).



FEXINIDAZOLE

Antitrypanosomal Effects

Mechanism of Action. *Fexinidazole* is a 5-nitroimidazole from the same drug class as *nifurtimox*. These compounds are prodrugs that are bioactivated by a bacterial-like type 1 nitroreductase (NTR1) in trypanosomatids that uses NADH as a reductive cofactor to perform $2e^-$ electron oxidation reactions (Patterson and Wyllie, 2014). This chemistry leads to the formation of a Michael acceptor in the form of an unsaturated open-chain nitrile. Cell toxicity is believed to result from the interaction of these reactive molecules with multiple targets in the cell including reports of DNA modification. This mechanism of activation provides a basis for species-selective toxicity as there is no mammalian homolog of this enzyme.

Parasites that were 6-fold resistant to *fexinidazole* could be selected *in vitro*, and this resistance was associated with mutations in the 3′ flanking region of the *NTR1* gene, which led to reduced expression, while knockout of a single NTR1 allele also led to partial resistance (Dickie et al., 2020). Overexpression of the *L. donovani* NTR led to 18-fold increased drug sensitivity (Wyllie et al., 2013). These studies show the importance of NTR1-mediated bioactivation in the mechanism of action of the 5-nitroimidazoles. The findings of both common mechanisms of bioactivation and drug resistance for *fexinidazole* and *nifurtimox* underscore a vulnerability in the current treatment strategy. Resistance generated to one in the field through changes in NTR1 expression levels could lead to clinical resistance to both compounds, thus putting at risk both front-line treatments for Gambian HAT.

ADME. *Fexinidazole* is given orally in tablet form and is a Biopharmaceutical Classification class III drug. Pharmacokinetic studies (Tarral et al., 2014) showed that absorption is rapid and the parent compound is quickly metabolized to the sulfoxide (t_{max} = 2–5 h) and the sulfone (t_{max} = 18–24 h). A fed state significantly increases plasma concentrations (C_{max} and area under the curve [AUC]) of the parent and the two metabolites by 4- to 5-fold. The two major metabolites (sulfoxide and sulfone) have been observed after oral dosing in both mice and humans, and the pharmacokinetic data in both species support the conclusion that they are responsible for most of the trypansomatid killing *in vivo* (Kaiser et al., 2011; Tarral et al., 2014). In humans under fed conditions, the two metabolites reach peak plasma concentrations and AUCs that are 5- to 7-fold higher than the parent drug, and exposure levels of the metabolites are sustained for significantly longer, with the sulfone providing the most prolonged exposure (Tarral et al., 2014). Accumulation over multiple dosing did not occur for the parent or the sulfoxide, but an accumulation ratio of approximately 7 was observed for the sulfone. *Fexinidazole* elimination was almost entirely extrarenal (renal clearance of 1.2–6.0 mL/h vs. oral clearance of 80 L/h).

Therapeutic Uses

Fexinidazole is the currently recommended drug of choice for early-stage Gambian HAT and for uncomplicated late-stage disease including in adults and children aged 6 years or older and weighing 20 kg or more (WHO, 2019). For children who do not meet these criteria, *pentamidine* should still be used for early-stage disease, while NECT is recommend for late-stage disease. NECT is also still recommended for adults with severe late-stage disease. A phase II/III, open-label, noninferiority trial was conducted with *fexinidazole* in comparison to NECT, and *fexinidazole* was curative in 91% of patients compared to a cure rate of 98% in patients who received NECT (Mesu et al., 2018). Follow-up studies demonstrated a 98.7% treatment success rate in early-stage Gambian HAT for adults aged 15 or older; the success rate in children aged 6 to 14 was 97.6% across all stages (Neau et al., 2020). *Fexinidazole* showed inferiority to NECT (86.9% vs. 98.7%, respectively) in adults with severe meningoencephalitic late-stage disease in which patients had a baseline WBC count in the CSF of 100/μL or greater. Thus for patients with severe late-stage disease who have WBC levels above this threshold, or if lumbar puncture is unavailable or inconclusive, NECT therapy is recommended (Lindner et al., 2020; WHO, 2019). Additional contraindications for treatment with *fexinidazole* include patients at risk for QT prolongation or those with jaundice (WHO, 2019).

The dose of *fexinidazole* in patients aged 15 years and older (and 35 kg) is 1800 mg (3 × 600 mg) on days 1 to 4, followed by 1200 mg (2 × 600 mg) on days 5 to 10, administered once daily as 600-mg oral tablets, with the initial higher dose providing a loading dose, followed by a lower maintenance dose (WHO, 2019). For body weight of 20 to 34 kg, the loading phase dose is reduced to 1200 mg (2 tablets) and the maintenance phase dose is 600 mg (1 tablet). The dose must be taken with food to increase drug exposure. The food effect on bioavailability is significant, and if a dose is taken without food, it should be considered as a missed dose.

Toxicity and Side Effects

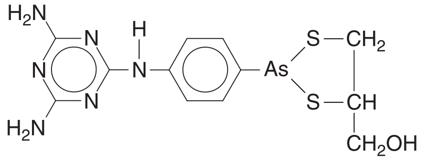
The most common reported side effects in the phase II/III study were nervous system (headache 35%, tremor 22%, and dizziness 19%) and gastrointestinal (GI) disorders (vomiting 28%, nausea 26%, and decreased appetite 21%) (Mesu et al., 2018; Tarral et al., 2014). Vomiting was more frequent in children than adults. Treatment-related adverse events were reported in this study at a similar rate for *fexinidazole* and NECT (81% vs. 79%), but the incidence of neuropsychiatric adverse reactions was higher for *fexinidazole* than for NECT.

8-Hydroxyquinolines

The halogenated 8-hydroxyquinolines *iodoquinol* (diiodohydroxyquin) and *clioquinol* (iodochlorhydroxyquin) can be used as luminal agents to eliminate intestinal colonization with *E. histolytica* and combined with *metronidazole* to treat amebic colitis or amebic liver abscess (WHO, 2020a). Because of its superior adverse event profile, *paromomycin* is preferred as the luminal agent for amebiasis (Petri et al., 2020). However, *iodoquinol*, the safer of the two 8-hydroxyquinolones, is available for use in the U.S. and is a reasonable alternative. When used at appropriate doses (never to exceed 2 g/day) for short periods of time (not greater than 20 days in adults), adverse effects are unusual (Haque et al., 2003). However, using these drugs at high doses for long periods carries significant risk. The most important toxic reaction, ascribed primarily to *clioquinol*, is subacute myelo-optic neuropathy (Meade, 1975). Administering *iodoquinol* in high doses to children with chronic diarrhea is associated with optic atrophy and permanent vision loss (Escobedo et al., 2009). Peripheral neuropathy is a less severe manifestation of neurotoxicity from these drugs (Haque et al., 2003). For adults, the recommended dose of *iodoquinol* is 650 mg orally three times daily for 20 days, whereas children receive 30 to 40 mg/kg body weight orally, divided three times a day (not to exceed 1.95 g/day) for 20 days (Kimberlin et al., 2018).

Melarsoprol

Despite the fact that it causes an often-fatal encephalopathy in 2% to 10% of the patients treated with it, *melarsoprol* is the only drug for the treatment of late (CNS) stages of East African trypanosomiasis caused by *T. brucei rhodesiense* (Kennedy, 2019). Although *melarsoprol* is also effective against late-stage West African trypanosomiasis caused by *T. brucei gambiense*, NECT and *fexinidazole* have become the first-line treatments of this disease (Lindner et al., 2020).



MELARSOPROL

Melarsoprol is supplied as a 3.6% (w/v) solution in propylene glycol for intravenous administration. It is available in the U.S. only from the CDC.

Mechanism of Action; Antiprotozoal Effects

Melarsoprol is metabolized to melarsen oxide, the active drug (Barrett et al., 2007; Barrett and Croft, 2012). Arsenoxides react avidly and reversibly with vicinal sulfhydryl groups and thereby inactivate many enzymes. *Melarsoprol* reacts with trypanothione, the spermidine-glutathione adduct that substitutes for glutathione in these parasites. Binding of *melarsoprol* to trypanothione results in a melarsen oxide–trypanothione adduct that inhibits trypanothione reductase. Treatment failure owing to resistance of trypanosomes to *melarsoprol* has risen sharply, and some of the resistant strains are an order of magnitude less sensitive to the drug. Resistance to *melarsoprol* arises due to transport defects linked to the aquaglyceroporin pore-forming protein (Munday et al., 2015).

ADME

Melarsoprol is always administered by slow intravenous injection, with care to avoid leakage into the surrounding tissues because the drug is intensely irritating. *Melarsoprol* is a prodrug and is metabolized rapidly (<30 min) to melarsen oxide, the active form of the drug (Barrett et al., 2007; Barrett and Croft, 2012). Bioassays show that the active metabolite has a terminal $t_{1/2}$ of 43 h. A small but therapeutically significant amount of the drug enters the CSF and clears trypanosomes infecting the CNS.

Therapeutic Uses

Melarsoprol remains the recommended drug for treatment of the late meningoencephalitic stage of East African (Rhodesian) trypanosomiasis, which is nearly 100% fatal if untreated (Kennedy, 2019). The drug is also effective in the early hemolymphatic stage of these infections, but because of its toxicity, it is reserved for therapy of late-stage infections. Patients infected with *T. brucei rhodesiense* who relapse after a course of *melarsoprol* usually respond to a second course of the drug. *Melarsoprol* is also effective against late-stage *T. brucei gambiense* but because both *fexinidazole* and NECT are safer and more effective, *melarsoprol* is no longer recommended for this application.

Dosing is 2.2 mg/kg per day IV for 10 days for both *T. brucei gambiense* (Pepin and Mpia, 2006) and *T. brucei rhodesiense* (Kuepfer et al., 2012).

Encephalopathy develops more frequently in patients with *T. brucei rhodesiense* compared to *T. brucei gambiense*. Concurrent administration of *prednisolone* is frequently employed throughout the treatment course to reduce the prevalence of encephalopathy.

Toxicity and Side Effects

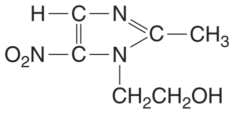
Treatment with *melarsoprol* is associated with significant toxicity and morbidity (Kennedy, 2019). A febrile reaction often occurs soon after drug injection, especially if parasitemia is high. The most serious complications involve the nervous system. A reactive encephalopathy occurs 9 to 11 days after treatment starts in about 5% to 10% of patients, leading to death in about half of these. Peripheral neuropathy occurs in about 10% of patients receiving *melarsoprol*. Hypertension and myocardial damage are not uncommon, although shock is rare. Albuminuria occurs frequently, and evidence of renal or hepatic damage may necessitate modification of treatment. Vomiting and abdominal colic also are common, but their incidence can be reduced by injecting *melarsoprol* slowly into the supine, fasting patient.

Precautions and Contraindications

Melarsoprol should be given only to patients under hospital supervision. Initiation of therapy during a febrile episode has been associated with an increased incidence of reactive encephalopathy. Administration of *melarsoprol* to leprous patients may precipitate erythema nodosum. Use of the drug is contraindicated during epidemics of influenza. Severe hemolytic reactions have been reported in patients with deficiency of glucose-6-phosphate dehydrogenase. The drug may be used safely in pregnancy.

Metronidazole and Tinidazole

Metronidazole is active *in vitro* against a wide variety of anaerobic protozoal parasites and anaerobic bacteria. Other clinically effective 5-nitroimidazoles closely related in structure and activity to *metronidazole* include *tinidazole*, *secnidazole,* and *ornidazole*. Among these, only *tinidazole* is available in the U.S. *Metronidazole* is clinically effective in trichomoniasis, amebiasis, and giardiasis. *Metronidazole* manifests antibacterial activity against all anaerobic cocci; anaerobic gram-negative bacilli, including *Bacteroides* spp.; anaerobic spore-forming, gram-positive bacilli such as *Clostridium*; and microaerophilic bacteria such as *Helicobacter* and *Campylobacter* spp. Nonsporulating gram-positive bacilli often are resistant, as are aerobic and facultatively anaerobic bacteria (Lofmark et al., 2010). Please refer to Chapter 57 for additional details about the use of *metronidazole* in bacterial infections.



METRONIDAZOLE

Mechanism of Action

Metronidazole is a prodrug requiring reductive activation of the nitro group by susceptible organisms. Unlike their aerobic counterparts, anaerobic and microaerophilic pathogens (e.g., the amitochondriate protozoa *T. vaginalis, E. histolytica*, and *G. lamblia* and various anaerobic bacteria) contain electron transport components that have a sufficiently negative redox potential to donate electrons to *metronidazole*. The single-electron transfer forms a highly reactive nitro radical anion that kills susceptible organisms by radical-mediated mechanisms that target DNA (McCarthy et al., 2020). *Metronidazole* is catalytically recycled; loss of the active metabolite's electron regenerates the parent compound. Increasing levels of O_2 inhibit *metronidazole*-induced cytotoxicity because O_2 competes with *metronidazole* for electrons. Thus, O_2 can both decrease reductive activation of *metronidazole* and increase recycling of the activated drug. Anaerobic or microaerophilic organisms susceptible to *metronidazole* derive energy from the oxidative fermentation of ketoacids such as pyruvate. Pyruvate decarboxylation, catalyzed by PFOR (pyruvate-ferredoxin oxidoreductase), produces electrons that reduce ferredoxin, which in turn catalytically donates its electrons to biological electron acceptors or to *metronidazole* (Lamp et al., 1999).

Resistance

Clinical resistance to *metronidazole* is well documented for *T. vaginalis, G. lamblia*, and a variety of anaerobic and microaerophilic bacteria. Resistance correlates with impaired oxygen-scavenging capabilities, leading to higher local O_2 concentrations, decreased activation of *metronidazole*, and futile recycling of the activated drug. Other resistant strains have lowered levels of PFOR and ferredoxin, perhaps explaining why they may still respond to higher doses of *metronidazole* (Townson et al., 1994).

ADME

Preparations of *metronidazole* are available for oral, intravenous, intravaginal, and topical administration. The drug usually is absorbed completely and promptly after oral intake and distributed to a volume approximating total body water; less than 20% of the drug is bound to plasma proteins. A linear relationship between dose and plasma concentration pertains for doses of 200 to 2000 mg. Repeated doses every 6 to 8 h result in some drug accumulation. The $t_{1/2}$ of *metronidazole* in plasma is about 8 h. With the exception of the placenta, *metronidazole* penetrates well into body tissues and fluids, including vaginal secretions, seminal fluid, saliva, breast milk, and CSF. After an oral dose, more than 75% of labeled *metronidazole* is eliminated in the urine, largely as metabolites formed by the liver from oxidation of the drug's side chains, a hydroxy derivative and an acid; about 10% is recovered as unchanged drug.

Two principal metabolites result. The hydroxy metabolite has a longer $t_{1/2}$ (~12 h) and has approximately 50% of the antitrichomonal activity of *metronidazole*. Small quantities of reduced metabolites are formed by the gut flora. The urine of some patients may be reddish brown owing to the presence of unidentified pigments derived from the drug. Oxidative metabolism of *metronidazole* is induced by *phenobarbital, prednisone, rifampin*, and possibly ethanol and is inhibited by *cimetidine* (Lamp et al., 1999; Martinez and Caumes, 2001).

Therapeutic Uses

Trichomoniasis. *Metronidazole* cures genital infections with *T. vaginalis* in more than 90% of cases. The preferred treatment regimen is 2 g *metronidazole* as a single oral dose for both males and females. *Tinidazole*, which has a longer $t_{1/2}$ than *metronidazole*, is also used as a 2-g single dose and appears to provide equivalent or better responses (Kimberlin et al., 2018). When repeated courses or higher doses of the drug are required, it is recommended that intervals of 4 to 6 weeks elapse between courses. Leukocyte counts should be obtained before, during, and after each course. Treatment failures owing to the presence of *metronidazole*-resistant strains of *T. vaginalis* are becoming increasingly common. Most of these cases can be treated successfully by giving a second 2-g dose to both patient and sexual partner (Schwebke and Bachmann, 2020). In addition to oral therapy, the use of a 500- to 1000-mg vaginal suppository may be beneficial in refractory cases (Muzny and Schwebke, 2013).

Amebiasis. *Metronidazole* is the agent of choice for the treatment of all symptomatic forms of amebiasis, including amebic colitis and amebic liver abscess. The recommended dose is 500 to 750 mg *metronidazole* taken orally three times daily for 7 to 10 days (WHO, 2020a) or, for children, 35 to 50 mg/kg per day given in three divided doses for 7 to 10 days (Kimberlin et al., 2018). Amebic liver abscess has been treated successfully by short courses of *metronidazole* or *tinidazole*. *E. histolytica* persists in most patients who recover from acute amebiasis after *metronidazole* therapy, so it is recommended that all such individuals also be treated with a luminal amebicide (Petri et al., 2020).

Giardiasis. *Tinidazole* is approved for the treatment of giardiasis as a single 2-g dose and is appropriate first-line therapy (Nash and Bartlet, 2020). Although *metronidazole* has never been FDA-approved for treatment of giardiasis in the U.S., there are many years of experience with its use (Kimberlin et al., 2018).

Toxicities and Contraindications

Metronidazole is intensely bitter, and the liquid formulation is often refused by pediatric patients (Kimberlin et al., 2018). Common side effects are headache, nausea, dry mouth, and a metallic taste. Vomiting, diarrhea, and abdominal distress are experienced occasionally. Dysuria, cystitis, and a sense of pelvic pressure have been reported. Dizziness, vertigo, and, very rarely, encephalopathy, convulsions, incoordination, and ataxia are neurotoxic effects that warrant drug discontinuation. *Metronidazole* also should be withdrawn if numbness or paresthesias of the extremities occur. Reversal of serious sensory neuropathies may be slow or incomplete (McCarthy et al., 2020).

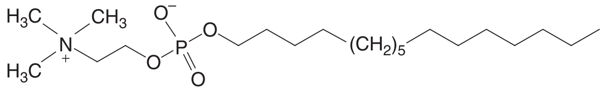
Urticaria, flushing, and pruritus indicate drug sensitivity and can require withdrawal of *metronidazole* (Lofmark et al., 2010). *Metronidazole* is a rare cause of Stevens-Johnson syndrome, which may be more common among individuals receiving high doses of *metronidazole* and concurrent therapy with the antihelminthic *mebendazole* (Chen et al., 2003).

Drug Interactions

Metronidazole has a *disulfiram*-like effect: some patients experience abdominal distress, vomiting, flushing, or headache if they drink alcoholic beverages during or within 3 days of therapy (Jang and Harris, 2007). *Metronidazole* and *disulfiram* or any *disulfiram*-like drug should not be taken together because confusional and psychotic states may occur. *Metronidazole* should be used cautiously in patients with active disease of the CNS because of potential neurotoxicity. The drug also may precipitate CNS signs of lithium toxicity in patients receiving high doses of *lithium*. *Metronidazole* can prolong the prothrombin time of patients receiving therapy with *warfarin* anticoagulants. The dosage of *metronidazole* should be reduced in patients with severe hepatic disease. *Metronidazole* use during the first trimester of pregnancy generally is not advised (Lofmark et al., 2010).

Miltefosine

Miltefosine is an alkylphosphocholine analogue developed originally as an anticancer agent. Its antiprotozoal activity was discovered in the 1980s as it was being evaluated for cancer chemotherapy. In 2002, it was approved in India as the first orally active treatment available for visceral leishmaniasis (WHO, 2020c). It is highly curative against visceral leishmaniasis and also is effective against the cutaneous forms of the disease (Aronson et al., 2020). Its main drawback is its teratogenicity; consequently, it must not be used in pregnant women (Aronson et al., 2017).



MILTEFOSINE

Antiprotozoal Effects

Miltefosine is the first consistently effective orally available therapy for leishmaniasis. It is a safe and effective treatment of visceral leishmaniasis and has shown greater than 90% efficacy against some species of cutaneous leishmaniasis, although significant strain variation has been noted in clinical trials (Sundar and Chakravarty, 2015). The mechanism of action of *miltefosine* is not understood. Studies suggest that the drug may alter ether-lipid metabolism, cell signaling, or glycosylphosphatidylinositol anchor biosynthesis (Dorlo et al., 2012). A transporter for *miltefosine* has been cloned by functional rescue of a laboratory-generated resistant strain of *L. donovani*. The transporter is a P-type ATPase that belongs to the aminophospholipid translocase subfamily. The basis for the drug resistance appears to be a point mutation in this transporter that leads to decreased drug uptake and thereby confers drug resistance (Sundar and Chakravarty, 2015).

ADME

Miltefosine is well absorbed orally and distributed throughout the human body. Detailed pharmacokinetic data are lacking, with the exception that *miltefosine* has a long $t_{1/2}$ (1–4 weeks). Plasma concentrations are proportional to the dose (Miltefosine [Impavido] for leishmaniasis, 2014).

Therapeutic Uses

In the U.S., the recommended dose for oral *miltefosine* for both visceral and cutaneous disease for adults over 45 kg is 150 mg/kg per day for 28 days, given in three divided doses, and for patients weighing 30 to 45 kg, the dose is 100 mg/kg per day, given in two divided doses (Aronson et al., 2017; Kimberlin et al., 2018). The compound cannot be given intravenously because it has hemolytic activity (Dorlo et al., 2012). It is also used to treat infections due to *Acanthamoeba, Balamuthia,* and *Naegleria*.

Toxicity and Side Effects

Vomiting and diarrhea are reported as frequent side effects, in up to 60% of patients. Elevations in hepatic transaminases and serum creatinine also have been reported. These effects are typically mild and reversible. Because of its teratogenic potential, *miltefosine* is contraindicated in pregnant women. In fact, guidelines for *miltefosine* use in the U.S. state that adequate contraception must be used during treatment and for 5 months after therapy is complete (Aronson et al., 2017).

Nifurtimox and Benznidazole

Nifurtimox and *benznidazole* are used to treat American trypanosomiasis caused by *T. cruzi*; *nifurtimox* is also used in combination with *eflornithine* for treatment of *T. brucei* (see previous discussion). *Nifurtimox*, a nitrofuran analogue, and *benznidazole*, a nitroimidazole analogue, can be obtained in the U.S. from the CDC.

Antiprotozoal Effects and Mechanisms of Action

Nifurtimox and *benznidazole* are trypanocidal against both the trypomastigote and amastigote forms of *T. cruzi* (Bern et al., 2019; Meymandi et al., 2018). *Nifurtimox* also has activity against *T. brucei* and can be curative against both early- and late-stage diseases (see previous discussion of NECT). The trypanocidal effects of *nifurtimox* and *benznidazole* derive from their activation by an NADH-dependent mitochondrial nitroreductase to nitro radical anions that are thought to account for the trypanocidal effects (Wilkinson et al., 2011). The generated nitro anion radicals form covalent attachments to macromolecules, leading to cellular damage that includes lipid peroxidation and membrane injury, enzyme inactivation, and damage to DNA. Reduced nitroreductase expression through single-allele gene knockout experiments or through drug selection leads to drug resistance (Wilkinson et al., 2011).

ADME

Nifurtimox is well absorbed after oral administration, with peak plasma levels observed after about 3.5 h. Less than 0.5% of the dose is excreted in urine (Paulos et al., 1989). The elimination $t_{1/2}$ is about 3 h. *Nifurtimox* undergoes rapid biotransformation, probably via a presystemic first-pass effect; high concentrations of several unidentified metabolites are found. *Nifurtimox* crosses the blood-brain barrier in mice (Jeganathan et al., 2011).

Benznidazole is absorbed rapidly and reaches peak plasma levels within 3 h; the terminal elimination half-life is 12 h (Raaflaub and Ziegler, 1979). Recent population studies suggest that, in adults, a dose of 2.5 mg/kg/24 h maintains the trough concentration above the therapeutic range of 3 to 6 mg/L, indicating that the current recommended dose of 2.5 mg/kg/12 h may be higher than needed (Soy et al., 2015). In children, drug levels were found to be lower than in adults, with excellent efficacy nonetheless (Altcheh et al., 2014).

Therapeutic Uses

Nifurtimox and *benznidazole* are employed in the treatment of American trypanosomiasis (Chagas disease) caused by *T. cruzi* (Bern et al., 2019; Meymandi et al., 2018). *Nifurtimox* is also used in combination with *eflornithine* (NECT) for treatment of late-stage African sleeping sickness (see *eflornithine* section for discussion) (Lindner et al., 2020). Because of toxicity concerns, *benznidazole* is the preferred treatment of Chagas disease. *Benznidazole* is FDA-approved for children 2 to 12 years old; *nifurtimox* is FDA-approved for children from birth to 18 years of age. In patients older than 50 years, the benefits of treatment are complicated by lowered drug tolerability. Treatment in all age groups is most effective if started early before the disease progresses to the chronic stage or to the point of organ damage. Both drugs markedly reduce the parasitemia, morbidity, and mortality of acute Chagas disease, with parasitological cures obtained in more than 80% of these cases, although the clinical response of the acute illness to drug therapy varies with geographic region (Messenger et al., 2015). In the chronic form of the disease, parasitological cures are still possible, but the drug is less effective than in the acute stage. In chronic Chagas patients treated with 150 mg *benznidazole* twice daily for 60 days, 94% remained polymerase chain reaction negative after 10 months (Molina et al., 2014a, 2014b). Current recommendations are that patients with acute or congenital disease should be treated (Pan American Health Organization, 2019). For patients with chronic disease, children and women of childbearing age should be treated (Kimberlin et al., 2018), whereas for adults with chronic disease and specific organ damage, treatment is not recommended (Pan American Health Organization, 2019). Therapy is encouraged for patients who will receive immunosuppressive therapy or who are HIV positive. Therapy with *nifurtimox* or *benznidazole* should start promptly after exposure for persons at risk of *T. cruzi* infection from laboratory accidents or from blood transfusions.

Both *nifurtimox* and *benznidazole* are given orally with doses as recommended by Kimberlin and colleagues (2018). For *nifurtimox,* adults (>17 years) with acute infection should receive 8 to 10 mg/kg per day in three to four divided doses for 90 days; children 1 to 10 years old should receive 15 to 20 mg/kg per day in three to four divided doses for 90 days; for individuals 11 to 16 years old, the daily dose is 12.5 to 15 mg/kg given according to the same schedule.

For *benznidazole*, the recommended treatment for adults (>13 years) is 5 to 7 mg/kg per day in two divided doses for 60 days, with children 2 to 12 years old receiving 5 to 8 mg/kg per day in two divided doses for 60 days. However, some studies have suggested that total doses exceeding 300 mg/day are less well tolerated. If gastric upset and weight loss occur during treatment, dosage should be reduced. The ingestion of alcohol should be avoided.

Toxicity and Side Effects

Side effects are common and range from hypersensitivity reactions (e.g., dermatitis, fever, icterus, pulmonary infiltrates, and anaphylaxis) to dose- and age-dependent complications referable to the GI tract and both the peripheral nervous system and CNS (Ribeiro et al., 2012). Nausea and vomiting are common, as are myalgia and weakness. For *benznidazole*, the most common adverse event (occurring in 30% of patients in the first week of treatment) is urticarial dermatitis, which can be treated with antihistamines or corticosteroids. However, treatment often has to be discontinued in patients with this reaction. Bone marrow suppression can occur early during therapy, so blood cell counts should be measured every 2 to 3 weeks, and treatment stopped if suppression is observed. Peripheral neuropathy and GI symptoms are especially common after prolonged treatment; the latter complication may lead to weight loss and preclude further therapy. *Benznidazole* should be given with food to minimize GI effects. Because of the seriousness of Chagas disease and the lack of superior drugs, there are few absolute contraindications to the use of these drugs.

Nitazoxanide

Nitazoxanide (*N*-[nitrothiazolyl] salicylamide) is an oral, synthetic, broad-spectrum antiparasitic agent (see Chapter 68). *Nitazoxanide* is FDA-approved for the treatment of cryptosporidiosis and giardiasis in adults and immunocompetent children (Kimberlin et al., 2018).

Antimicrobial Effects

Nitazoxanide and its active metabolite, tizoxanide (desacetyl-nitazoxanide), inhibit the growth of sporozoites and oocytes of *C. parvum*, as well as the trophozoites of *G. intestinalis*, *E. histolytica*, and *T. vaginalis in vitro* (McCarthy et al., 2020). *Nitazoxanide* also has activity against intestinal helminths (van den Enden, 2009). Although *nitazoxanide* is sometimes provided to immunocompromised and/or severely ill patients with GI viral infections, the mechanism by which it could be effective remains unclear, and there is minimal evidence to support its use under these circumstances.

Mechanism of Action

Nitazoxanide interferes with the PFOR enzyme-dependent electron-transfer reaction, which is essential to anaerobic metabolism in protozoan and bacterial species (Raether and Hanel, 2003).

ADME

Following oral administration, *nitazoxanide* is hydrolyzed rapidly to its active metabolite, tizoxanide, which undergoes conjugation to tizoxanide glucuronide. Bioavailability after an oral dose is excellent, and maximum plasma concentrations of the metabolites occur 1 to 4 h following administration. Tizoxanide is more than 99.9% bound to plasma proteins. Tizoxanide is excreted in the urine, bile, and feces; tizoxanide glucuronide is excreted in the urine and bile (Raether and Hanel, 2003).

Therapeutic Uses

In the U.S., *nitazoxanide* is approved for the treatment of *G. intestinalis* infection (therapeutic efficacy of 85%–90%) (Nash and Bartlet, 2020) and for the treatment of diarrhea caused by *Cryptosporidium* (therapeutic efficacy, 56%–88%) in adults and children more than 1 year of age (White, 2020). *Nitazoxanide* has decreased efficacy in immunocompromised patients with *Cryptosporidium* infection, leading to extension of treatment courses (Kimberlin et al., 2018).

Nitazoxanide has been used as a single agent to treat mixed infections with intestinal parasites (protozoa and helminths). Effective parasite clearance after *nitazoxanide* treatment was shown for *G. intestinalis, E. histolytica, Blastocystis hominis, C. parvum, C. cayetanensis, I. belli, Hymenolepis nana, Trichuris trichiura, Ascaris lumbricoides*, and *Enterobius vermicularis*, although more than one course of therapy was required in some cases. *Nitazoxanide* also has been used to treat infections with *G. intestinalis* resistant to *metronidazole* and *albendazole* (McCarthy et al., 2020).

To treat cryptosporidiosis, for children ages 12 to 47 months, the recommended dose is 100 mg *nitazoxanide* every 12 h for 3 days; for children ages 4 to 11 years, the dose is 200 mg *nitazoxanide* every 12 h for 3 days. A 500-mg tablet, suitable for adult dosing every 12 h for 3 days, is also available. Immunocompromised patients may require therapy for 2 weeks or longer (Kimberlin et al., 2018; McCarthy et al., 2020).

Toxicity and Side Effects

Adverse effects are rare with *nitazoxanide*. A greenish tint to the urine can be seen. *Nitazoxanide* is generally considered safe in pregnancy (former FDA pregnancy category B), based on animal teratogenicity and fertility studies (Anderson and Curran, 2007).

Paromomycin

Paromomycin (aminosidine) is an aminoglycoside of the neomycin/kanamycin family (see Chapter 59) that is used as an oral agent to treat *E. histolytica* infection, cryptosporidiosis, and giardiasis (McCarthy et al., 2020). Topical formulations have been used to treat trichomoniasis and cutaneous leishmaniasis; parenteral administration has been used to treat visceral leishmaniasis, both alone and in combination with *antimony compounds* (Sundar and Chakravarty, 2015). However, only oral *paromomycin* is available in the U.S. (Kimberlin et al., 2018).

Mechanism of Action; ADME

Paromomycin shares the same mechanism of action as *neomycin* and *kanamycin* (binding to the 30S ribosomal subunit) and has the same spectrum of antibacterial activity. The drug is not absorbed from the GI tract; thus, the actions of an oral dose are confined to the GI tract, with 100% of the oral dose recovered in the feces (Mishra et al., 2007).

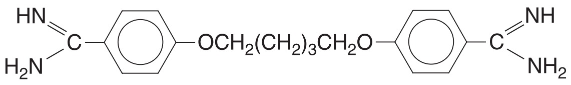
Antimicrobial Effects

Amebiasis. *Paromomycin* is the drug of choice for treating intestinal colonization with *E. histolytica* and is used in combination with *metronidazole* to treat amebic colitis and amebic liver abscess. Adverse effects are rare with oral usage but include abdominal pain and cramping, epigastric pain, nausea and vomiting, steatorrhea, and diarrhea. Rarely, rash and headache have been reported. Dosing for adults and children is 25 to 35 mg/kg per day in three divided oral doses (Kimberlin et al., 2018).

Giardiasis. *Paromomycin* has been advocated as a treatment of giardiasis when *metronidazole* is contraindicated. It is used in pregnant women and for *metronidazole*-resistant isolates (Wright, 2012).

Pentamidine

Pentamidine, a positively charged aromatic diamine, is a broad-spectrum agent with activity against several species of pathogenic protozoa and some fungi. The di-isethionate salt of *pentamidine* is marketed for injection or as an aerosol (De et al., 1986; Rex and Stevens, 2014).



PENTAMIDINE

Antiprotozoal and Antifungal Effects

Pentamidine is used for the treatment of early-stage *T. brucei gambiense* infection but is ineffective in the treatment of late-stage disease and has reduced efficacy against *T. brucei rhodesiense* (Kennedy, 2019; Lindner et al., 2020). *Fexinidazole* has superseded *pentamidine* as the front-line drug for early-stage Gambian HAT, but *pentamidine* remains the first-line treatment choice for children less than 6 years old or with a body weight of less than 20 kg, in whom insufficient data are currently available to support the use of *fexinidazole*.

Pentamidine is an alternative agent for the treatment of cutaneous leishmaniasis (Monge-Maillo and Lopez-Velez, 2013). *Pentamidine* is an alternative agent for the treatment and prophylaxis of pneumonia caused by *Pneumocystis jirovecii* (PJP), formerly known as *Pneumocystis carinii* (PCP). See Chapter 61 on antifungal agents for additional details.

Mechanism of Action and Resistance

The mechanism of action of the diamidines is unknown. The compounds display multiple effects on any given parasite and act by disparate mechanisms in different parasites. Multiple transporters contribute to *pentamidine* uptake. A single high-affinity transporter from the aquaglyceroporin gene family (TbAQP2) is responsible for cross-resistance between *pentamidine* and *melarsoprol* and represents the major route for the uptake of *pentamidine* (Munday et al., 2015).

ADME

Pentamidine isethionate is fairly well absorbed from parenteral sites of administration. Following a single intravenous dose, the drug disappears from plasma with an apparent $t_{1/2}$ of several minutes to a few hours; maximum plasma concentrations after intramuscular injection occur at 1 h. The $t_{1/2}$ of elimination is long (weeks to months); the drug is 70% bound to plasma proteins (Bronner et al., 1995). This highly charged compound is poorly absorbed orally and does not cross the blood-brain barrier, explaining its ineffectiveness against late-stage trypanosomiasis.

Therapeutic Uses

African Trypanosomiasis. *Pentamidine* isethionate is used for the treatment of early-stage *T. brucei gambiense* in children who do not fit criteria for treatment with *fexinidazole* and is given by intramuscular or intravenous injection in doses of 4 mg/kg daily for 7 days (Lindner et al., 2020; WHO, 2019).

Leishmaniasis. *Pentamidine* can be used in doses of 2 to 3 mg/kg IV or IM daily or every second day for four to seven doses to treat cutaneous leishmaniasis (Kimberlin et al., 2018). This compound provides an alternative to *antimonials*, lipid formulations of *amphotericin B*, or *miltefosine*, but it is overall the least well tolerated (Monge-Maillo and Lopez-Velez, 2013).

Toxicity and Side Effects

Approximately 50% of individuals receiving the drug at recommended doses show some adverse effect (Barrett et al., 2007; Barrett and Croft, 2012). Intravenous administration of *pentamidine* may be associated with hypotension, tachycardia, and headache. These effects can be ameliorated by slowing the infusion rate. Hypoglycemia, which can be life threatening, may occur at any time during *pentamidine* treatment. Careful monitoring of blood sugar is key. Paradoxically, pancreatitis, hyperglycemia, and the development of insulin-dependent diabetes have been seen in some patients. *Pentamidine* is nephrotoxic (~25% of treated patients show signs of renal dysfunction), and if the serum creatinine concentration rises, it may be necessary to withhold the drug temporarily or change to an alternative agent (Rex and Stevens, 2014). Other adverse effects include skin rashes, thrombophlebitis, anemia, neutropenia, and elevation of hepatic enzymes (Salamone and Cunha, 1988). Intramuscular administration of *pentamidine* is associated with the development of sterile abscesses at the injection site, which can become infected secondarily; most authorities recommend intravenous administration (Cheung et al., 1993).

Sodium Stibogluconate

Antimonials were introduced in 1945 and have been used for therapy of leishmaniasis and other protozoal infections since then. The first trivalent antimonial compound used to treat cutaneous leishmaniasis and kala azar was *antimony potassium tartrate* (tartar emetic), which was both toxic and difficult to administer. *Tartar emetic* and other trivalent arsenicals eventually were replaced by pentavalent antimonial derivatives of phenylstibonic acid. An early member of this family of compounds was *sodium stibogluconate* ($C_{12}H_{35}Na_3O_{26}Sb_2$; sodium antimony gluconate), a pentavalent antimonial compound that has been the mainstay of the treatment of leishmaniasis. Increasing resistance to antimonials has reduced their efficacy (WHO, 2020c). In the U.S., *sodium stibogluconate* can be obtained from the CDC (Aronson et al., 2016).

Mechanism of Action

Multiple mechanisms of action have been postulated. The most commonly invoked is that pentavalent antimonials act as prodrugs that are reduced to the more toxic Sb^{3+} species that kill amastigotes within the phagolysosomes of macrophages. Following reduction, the drugs seem to interfere with the trypanothione redox system. Sb^{3+} induces a rapid efflux of trypanothione and glutathione from the cells and also inhibits trypanothione reductase, thereby causing a significant loss of thiol reduction potential in the cells (Aronson et al., 2016).

ADME

The drug is given intravenously or intramuscularly; it is not active orally. The agent is absorbed rapidly and distributed in an apparent volume of about 0.22 L/kg. Elimination occurs in two phases, the first with a $t_{1/2}$ of about 2 h, the second with a much longer half-time (33–76 h). The prolonged terminal elimination phase may reflect conversion of the Sb^{5+} to the more toxic Sb^{3+} that is concentrated in and only slowly released from tissues. The drug is eliminated in the urine (Aronson et al., 2016).

Therapeutic Uses

The standard course is 20 mg/kg per day IV for 20 days for cutaneous disease and for 28 days for visceral leishmaniasis (Aronson et al., 2020). Increased resistance has greatly compromised the effectiveness of antimonials, and *sodium stibogluconate* is now obsolete in India. Previously, *liposomal amphotericin B* was the recommended alternative, but now the orally effective compound *miltefosine* is used (Sundar and Chakravarty, 2015). Intralesional treatment has also been advocated as a safer, alternative method for treating cutaneous disease (Monge-Maillo and Lopez-Velez, 2013). Patients who respond show clinical improvement within 1 to 2 weeks of initiating therapy. The drug may be given on alternate days or for longer intervals if unfavorable reactions occur in especially debilitated individuals (Sundar and Chakravarty, 2015). Patients infected with HIV often relapse after therapy (Sundar, 2015).

Toxicity and Side Effects

In general, regimens of *sodium stibogluconate* are tolerated; toxic reactions usually are reversible, and most subside despite continued therapy. Adverse effects include chemical pancreatitis in nearly all patients; elevation of serum hepatic transaminase levels; bone marrow suppression, manifested by decreased red cell, white cell, and platelet counts; muscle and joint pain; weakness and malaise; headache; nausea and abdominal pain; and skin rashes. Reversible polyneuropathy has been reported. Hemolytic anemia and renal damage are rare manifestations of antimonial toxicity, as are shock and sudden death (Aronson et al., 2016).

Suramin

Research into the trypanocidal activity of the dyes *trypan red, trypan blue*, and *afridol violet* led to the introduction of *suramin* into therapy in 1920. Today, the drug is used primarily for treatment of early-stage African trypanosomiasis caused by *T. brucei rhodesiense*, though it has been studied for a broad range of applications (Wiedemar et al., 2020); it has no clinical utility against American trypanosomiasis.

Suramin sodium is a water-soluble trypanocide; solutions deteriorate quickly in air, and only freshly prepared solutions should be used. In the U.S., *suramin* is available only from the CDC.

Antiparasitic Effects

Suramin is a relatively slow-acting trypanocide (>6 h *in vitro*) with high clinical activity against both *T. brucei gambiense* and *T. brucei rhodesiense*. Its mechanism of action is unknown (Wiedemar et al., 2020). *Suramin* inhibits many trypanosomal and mammalian enzymes and receptors, and the lack of any significant field resistance suggests multiple points of action. Selective toxicity is likely to result from selective uptake by the parasite.

ADME

Because it is not absorbed after oral intake, *suramin* is given intravenously to avoid local inflammation and necrosis associated with subcutaneous or intramuscular injections (Kaur et al., 2002). After its administration, the drug displays complex pharmacokinetics with marked interindividual variability. The drug is 99.7% serum protein bound and has a terminal elimination $t_{1/2}$ of 41 to 78 days. *Suramin* is not appreciably metabolized; renal clearance accounts for elimination of about 80% of the compound from the body. Very little *suramin* penetrates the CSF, consistent with its polar character and lack of efficacy once the CNS has been invaded by trypanosomes.

Therapeutic Uses

Suramin is the first-line therapy for early-stage *T. brucei rhodesiense* infection (Kennedy, 2019). Because only small amounts of the drug enter the brain, *suramin* is used only for the treatment of early-stage African trypanosomiasis (before CNS involvement). Treatment of active African trypanosomiasis should not be started until 24 h after diagnostic lumbar puncture to ensure no CNS involvement, and caution is required if the patient has onchocerciasis (river blindness) because of the potential for eliciting a Mazzotti reaction (i.e., pruritic rash, fever, malaise, lymph node swelling, eosinophilia, arthralgias, tachycardia, hypotension, and possibly permanent blindness). *Suramin* is given by slow intravenous injection as a 10% aqueous solution. The normal single dose for adults with *T. brucei rhodesiense* infection is 1 g. It is advisable to employ a test dose of 100 mg initially to detect sensitivity, after which the normal dose is given intravenously (e.g., on days 1, 3, 5, 14, and 21). The pediatric test dose is 2 mg/kg followed by a dose of 20 mg/kg, given according to the same schedule as adults. Patients in poor condition should be treated with lower doses during the first week. Patients who relapse after *suramin* therapy should be treated with *melarsoprol*.

Toxicity and Side Effects

The most serious immediate reaction, consisting of nausea, vomiting, shock, and loss of consciousness, is rare (~1 in 2000 patients) (Kaur et al., 2002). Malaise, nausea, and fatigue are also common immediate reactions. The most common problem encountered after several doses of *suramin* is renal toxicity, manifested by albuminuria, and delayed neurological complications, including headache, metallic taste, paresthesias, and peripheral neuropathy. These complications usually disappear spontaneously despite continued therapy. Other, less-prevalent reactions include vomiting, diarrhea, stomatitis, chills, abdominal pain, and edema. Patients receiving *suramin* should be followed closely. Therapy should not be continued in patients who show intolerance to initial doses, and the drug should be employed with great caution in individuals with renal insufficiency.

Drug Facts for Your Personal Formulary: *Antiparasitic Agents: Protozoal Infections Other Than Malaria*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Amebiasis		
Metronidazole	• Amoebic colitis and liver abscess	• Always followed by luminal agent • Orally administered ≥80% bioavailable • Common side effects = headache and metallic taste • Can have disulfiram-like effect
Tinidazole	• Amoebic colitis and liver abscess	• Always followed by luminal agent
Paromomycin	• Luminal agent (eradicates *E. histolytica* from gut)	• Drug of choice due to side effects of 8-hydroxyquinolones • Side effects of paromomycin: GI (nausea/vomiting/diarrhea)
Iodoquinol	• Luminal agent	• Use less than 2 g/day for less than 20 days to avoid neurotoxicity
Giardiasis		
Metronidazole	• Giardiasis	• 5-day course • Not FDA-approved for giardiasis but long experience supports its use
Tinidazole	• Giardiasis	• Single dose sufficient
Paromomycin	• Giardiasis	• Used in pregnancy
Nitazoxanide	• Giardiasis	• Orally bioavailable • Can treat resistant infections • Adverse events are rare
Trichomoniasis		
Metronidazole	• Trichomoniasis	• Drug of choice • 2 g once • If failure, give second dose in 4–6 weeks
Tinidazole	• Trichomoniasis	• 2 g once • Can be used for resistant infection

Drug Facts for Your Personal Formulary: *Antiparasitic Agents: Protozoal Infections Other Than Malaria (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Toxoplasmosis		
Pyrimethamine	• Acute or congenital toxoplasmosis	• Combine with sulfadiazine or clindamycin • Give with folinic acid (leucovorin) • Can cause marrow suppression
Sulfadiazine	• Acute or congenital toxoplasmosis	• Combine with pyrimethamine and folinic acid • Can cause bone marrow suppression
Clindamycin	• Acute toxoplasmosis	• Combine with pyrimethamine • Use if cannot tolerate sulfa agent
Spiramycin	• Acute toxoplasmosis during early pregnancy	• Prevent fetal transmission • Available through FDA investigational new drug process
Cryptosporidiosis		
Nitazoxanide	• Drug of choice for cryptosporidiosis	• Restore immune function in immunocompromised patients
Leishmaniasis		
Pentavalent antimony compounds (sodium stibogluconate)	• Cutaneous, mucocutaneous leishmaniasis • Visceral leishmaniasis (not in India)	• 20 days IV/IM for cutaneous disease • 28 days IV/IM for visceral disease • Side effects: pancreatitis, elevated hepatic transaminases, bone marrow suppression • Can cause hemolytic anemia and renal failure
Amphotericin B	• Visceral leishmaniasis • Second-line agent for cutaneous disease	• Used for antimony-resistant cases • Used during pregnancy • Side effects: renal toxicity, low potassium
Miltefosine	• Cutaneous leishmaniasis • Visceral leishmaniasis	• Only oral agent for leishmaniasis • GI side effects (vomiting/diarrhea) • Teratogenic—do not use in pregnancy
Trypanosomiasis—African sleeping sickness		
Suramin	• Early-stage *T. brucei rhodesiense* • Second-line agent for early-stage *T. brucei gambiense* (only if pentamidine and fexinidazole contraindicated)	• Immediate reactions: malaise, nausea, and fatigue • Side effects of multiple doses: renal toxicity, delayed neurological complications (headache, metallic taste, paresthesias, peripheral neuropathy)
Fexinidazole	• Early and late-stage *T. brucei gambiense* • First-line treatment for early-stage (before CNS involvement) • Late-stage *T. brucei gambiense* if noted criteria met	• Added to the WHO list of essential medicines in 2019 • Recommended for adults and children ≥6 years and ≥20 kg that can be managed without lumbar puncture (LP) or for patients in whom WBC count in CSF is <100/μL • Can be used for pregnant women after the first trimester • Most common side effects: vomiting/nausea, headache, insomnia, and anxiety. These were reported at a higher frequency than with NECT
Pentamidine	• Early-stage *T. brucei gambiense* ***before CNS involvement*** • Second-line to fexinidazole except for young children	• Remains front-line treatment for children <6 years or body weight <20 kg • IV administration associated with hypotension, tachycardia, and headache • Hypoglycemia occurs; monitor blood glucose • Nephrotoxic, can cause renal failure
Nifurtimox-eflornithine combination therapy (NECT)	• Late-stage *T. brucei gambiense* • Front-line treatment for severe late-stage disease and for other patients who do not meet criteria for fexinidazole	• Remains front-line treatment for patients in severe second stage with WBC in CSF ≥100/μL and for patients requiring stratification by LP who are unable to receive it or for whom results are unreliable • Remains front-line treatment for children <6 years or body weight <20 kg • Requires hospitalization and skilled healthcare professionals to administer • Safer and more effective than melarsoprol or eflornithine alone • Most common side effects: vomiting/nausea and headache
Melarsoprol	• Late-stage *T. brucei rhodesiense* • Last-line treatment for late-stage *T. brucei gambiense* (only if NECT and fexinidazole contraindicated)	• Fatal encephalopathy: 2%–10% of patients • Coadministration with prednisolone can reduce the prevalence of encephalopathy

Drug Facts for Your Personal Formulary: *Antiparasitic Agents: Protozoal Infections Other Than Malaria (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Trypanosomiasis—Chagas disease		
Benznidazole	• Drug of choice for Chagas	• Requires 60 days of treatment • Urticarial dermatitis in 30% of patients; coadministration of antihistamines or corticosteroids can help • Better tolerated in children, less well tolerated in adults >50 years • Most effective if administered early in the course of infection (acute stage) • Efficacy in chronic Chagas is lower • Give with food to minimize GI effects • Monitor blood cell counts
Nifurtimox	• Alternative treatment for Chagas	• Requires 60 days of treatment • Less well tolerated than benznidazole
Other Protozoal Infections		
Clindamycin and quinine	• Severe babesiosis	• Quinine—monitor for cardiac effects (prolonged QT interval)
Azithromycin and atovaquone	• Mild-moderate babesiosis	
Tetracycline	• Balatinidiasis	• Drug of choice
Trimethoprim-sulfamethoxazole	• Cyclosporiasis, isosporiasis	• Drug of choice

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Chapter 68

Chemotherapy of Helminth Infections

Jennifer Keiser, James McCarthy, and Peter Hotez

ANTHELMINTIC DRUGS

- Benzimidazoles
- Diethylcarbamazine
- Doxycycline
- Ivermectin
- Praziquantel
- Niclosamide
- Oxantel and Pyrantel Pamoate
- Tribendimidine
- Moxidectin
- Levamisole
- Nitazoxanide

Helminths are invertebrates featuring elongated, flat or round bodies. According to their morphology and the host organ they inhabit, they are classified as flatworms or platyhelminths, which include flukes (lung flukes [*Paragonimus* spp.], liver flukes [*Fasciola* spp., *Clonorchis sinensis*, *Opisthorchis* spp.], intestinal flukes, or blood flukes [*Schistosoma* spp.]), tapeworms (cestodes [including *Taenia solium*, *T. saginata*], *Diphyllobothrium latum*, *Hymenolepis nana*, and *Echinococcus* spp.), and roundworms (nematodes). The nematodes of major medical importance include the soil-transmitted helminths (STHs) (*Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, *Ancylostoma duodenale*, *Strongyloides stercoralis*) and the filarial worms. Lymphatic filariasis is caused by *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. Subcutaneous filariasis is caused by *Loa loa*, *Mansonella streptocerca*, and *Onchocerca volvulus*. *Mansonella perstans* and *Mansonella ozzardi* are the causative helminths for serous cavity filariasis.

The blood flukes and nematodes are bisexual, while all other flukes and tapeworm species infecting humans are hermaphroditic. The development of all helminths includes egg, larval (juvenile), and adult stages, but the life cycles greatly differ. The prevalence of helminth infection worldwide is quite extensive (Figure 68–1).

Anthelmintic Drugs

Although a large number of anthelmintic drugs have been approved for human use, only a small number are widely used. These include two drugs in the benzimidazole (BZ) class, *albendazole* and *mebendazole*, which are widely used in intestinal nematode and cestode infections; the macrocyclic lactone *ivermectin*, used to treat a variety of nematode and ectoparasite infections; and *praziquantel*, which is used to treat trematode and some cestode parasites. Because of their role in programs of mass drug administration (MDA), these drugs are among the most commonly used agents worldwide. The World Health Organization (WHO) estimated that through MDA approximately 1.24 billion people received one or more anthelmintic drugs in 2019 (WHO, 2020). In many resource-poor developing countries, several different anthelmintic drugs can be provided together through integrated programs of MDA in order to simultaneously target intestinal and filarial nematodes and trematodes (Webster et al., 2014). A number of clinical trials have been undertaken to ensure that there are not unknown pharmacokinetic interactions or toxicologic consequences of coadministration of these drugs. This is especially the case for *ivermectin*, *diethylcarbamazine*, and *albendazole*, which have been shown to have synergistic activity as treatment for lymphatic filariasis (King et al., 2018; Weil et al., 2019).

Benzimidazoles

Although a large number of drugs in the BZ class have been synthesized and several have undergone clinical development for treatment of parasitic infections of humans, only two are currently in wide use, namely *albendazole* and *mebendazole*, with *triclabendazole* being reserved for treatment of liver fluke infection caused by *Fasciola hepatica*. *Thiabendazole* was formerly recommended for treatment of strongyloidiasis, but *ivermectin* is more effective and better tolerated, and it is now of historical interest only.

Chemistry

Albendazole, *mebendazole*, and *triclabendazole* are all poorly water soluble and only slightly soluble in methanol. Figure 68–2 shows the chemical structures of these drugs.

Mechanism of Action

The primary mechanism of action of the BZs is thought to be inhibition of microtubule polymerization by binding to β-tubulin (Prichard, 1994). The selective toxicity of these agents against helminths results from their increased affinity for parasite β-tubulin than for the same target in higher eukaryotes. A range of other biochemical changes occur in nematodes following BZ exposure, including inhibition of mitochondrial fumarate reductase, reduced glucose transport, and uncoupling of oxidative phosphorylation.

ADME

Mebendazole. The low systemic bioavailability (22%) of *mebendazole* results from a combination of poor absorption and rapid first-pass metabolism at the intestinal wall and in the liver. Coadministration of *cimetidine* increases plasma levels of *mebendazole*, possibly due to inhibition of first-pass hepatic metabolism mediated by CYPs (cytochrome P450). The small proportion of *mebendazole* that is absorbed is approximately 95% bound to plasma proteins and is extensively metabolized. *Mebendazole*, rather than its metabolites, appears to be the active drug form (Gottschall et al., 1990). A new chewable formulation of *mebendazole* has recently been developed to facilitate use in pediatric populations (Friedman et al., 2012). Although this formulation is more palatable for administration to children and its systemic bioavailability may be higher (Friedman et al., 2012), its efficacy for treatment of STHs appears to be equivalent to the old formulation (Palmeirim et al., 2020). Conjugates of *mebendazole* and its metabolites have been found in bile, but little unchanged *mebendazole* appears in the urine.

Albendazole. *Albendazole* is variably and erratically absorbed after oral administration; absorption is enhanced by the presence of fatty foods

Abbreviations

AUC: area under the curve
BZ: benzimidazole
CDC: U.S. Centers for Disease Control and Prevention
CYP: cytochrome P450
dADT: p-(1-dimethylamino ethylimino) aniline
DEC: diethylcarbamazine
GABA: γ-aminobutyric acid
GI: gastrointestinal
MDA: mass drug administration
STH: soil-transmitted helminth
TPAC: terephalic acid
TPAL: terephthalaldehyde
WHO: World Health Organization

and possibly by bile salts. Administration following food, especially a fatty meal, enhances absorption by up to 5-fold in humans (Dayan, 2003). The activity of *albendazole* against tissue-dwelling helminths is attributable to its active metabolite *albendazole sulfoxide*. The better bioavailability of the parent drug and the activity of *albendazole sulfoxide* explain why *albendazole* is more active than *mebendazole* against tissue-dwelling helminths. The level of *albendazole sulfoxide* is enhanced 3.2-fold by ingestion of grapefruit juice. However, grapefruit juice shortens the drug's half-life by 46%. Data from indirect experiments suggest that *albendazole* is metabolized by CYP3A4 in the intestinal mucosa, a process that grapefruit juice inhibits (Nagy et al., 2002). In hookworm infected adolescents, *alebendazole* had a half-life of 1.5 h; the metabolites had a half-life of 7–8 h (Schultz et al. 2019).

Both the (+) and (−) enantiomers of *albendazole sulfoxide* are formed; the (+) enantiomer reaches much higher peak plasma concentrations in humans and is cleared much more slowly than the (−) form. *Albendazole sulfoxide* is approximately 70% bound to plasma proteins and has a highly variable plasma $t_{1/2}$ of 4 to 15 h (Marques et al., 1999). It is well distributed into various tissues including hydatid cysts, where it reaches a concentration of approximately 20% that in plasma (Morris et al., 1987). Oxidation of the sulfoxide derivatives to the nonchiral sulfone metabolite of *albendazole*, which is pharmacologically inactive, is probably rate limiting in determining the clearance and therefore the plasma half-life of the bioactive (+) sulfoxide metabolite. In animal models, BZs can induce their own metabolism. *Albendazole* metabolites are excreted mainly in the urine.

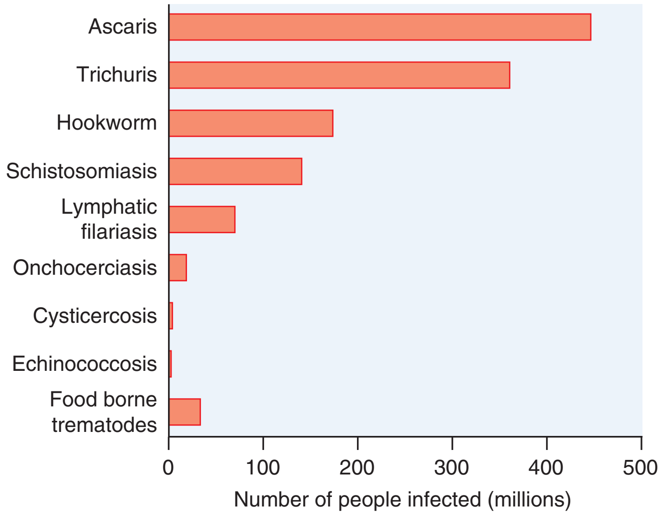


Figure 68–1 *Relative incidence of helminth infections worldwide.*

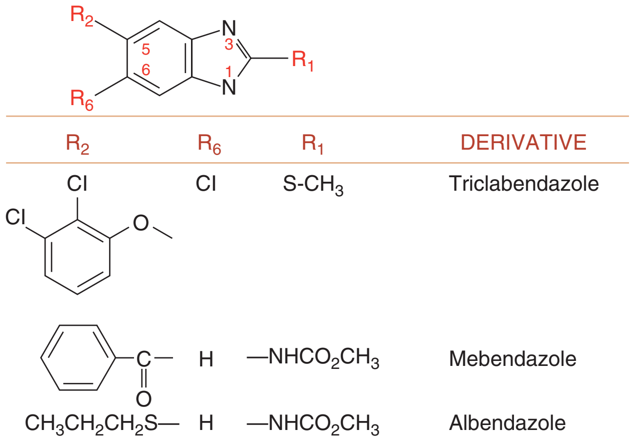


Figure 68–2 *Structure of benzimidazole anthelmintics.*

Triclabendazole. Administration of *triclabendazole* after food enhances its absorption, which might be due to the stimulation of gastric acid secretion, food-induced increase in drug solubility, and/or altered gastrointestinal (GI) motility and transit time. After oral administration, *triclabendazole* is rapidly oxidized into two major metabolites, *triclabendazole sulfoxide* and *triclabendazole sulfone* and only low concentrations of the parent drug can be detected in plasma. *Triclabendazole sulfoxide* is the metabolite active against *F. hepatica* (Keiser et al., 2005).

Therapeutic Uses

Albendazole is a safe and highly effective therapy for infections with GI nematodes, including *A. lumbricoides*, *T. trichiura*, and hookworms. For programmatic control of soil-transmitted helminth infections (enterobiasis, ascariasis, trichuriasis, and hookworm), *albendazole* is administered as a single oral 400-mg dose for adults and children greater than 2 years of age. Cure rates for light to moderate *Ascaris* infections typically are greater than 97%, although heavy infections may require therapy for 2 to 3 days. A 400-mg dose of *albendazole* appears to be superior to a 500-mg dose of *mebendazole* for curing hookworm infections and reducing egg counts (Keiser and Utzinger, 2008). A 3-day regimen of *albendazole* outperforms single-dose treatments against hookworm and *T. trichiura* infections. When administered at a dose of 400 mg daily for 3 days, *albendazole* shows some efficacy in treatment of strongyloidiasis but is less effective than *ivermectin* for treatment of this infection (Marti et al., 1996).

Albendazole is the drug of choice for chemotherapy of cystic hydatid disease caused by *Echinococcus granulosus*. Although prolonged treatment with the drug leads to only a modest cure rate, it is useful as adjunctive treatment in the perioperative period to reduce the risk of disseminated infection resulting from spillage of cyst contents at the time of surgery or with nonoperative puncture, aspiration, injection, and reaspiration procedures (Horton, 1997). A typical dosage regimen for adults is 400 mg given twice a day (for children, 15 mg/kg per day with a maximum of 800 mg) for 1 to 6 months. Although it is the only drug available with useful activity against alveolar echinococcosis caused by *Echinococcus multilocularis*, it is parasitostatic rather than parasiticidal, and lifelong therapy with or without surgical intervention is usually required to control this infection.

Albendazole also is the preferred treatment of neurocysticercosis caused by larval forms of *T. solium* (Garcia and Del Brutto, 2000).

The recommended dosage is 400 mg given twice a day for adults for 8 to 30 days, depending on the number, type, and location of the cysts. For children, the dose is 15 mg/kg per day (maximum: 800 mg) in two doses for 8 to 30 days. For both adults and children, the course can be repeated as necessary, as long as liver and bone marrow toxicities are monitored. Glucocorticoid therapy is usually begun before initiating *albendazole* therapy and continued for several days after commencement of therapy to reduce the incidence of side effects resulting from inflammatory reactions to dead and dying cysticerci. Glucocorticoids increase plasma levels of *albendazole sulfoxide*. Prior to initiating chemotherapy of neurocysticercosis, consideration should be given to close observation or the administration of presumptive anticonvulsant therapy. Possible complications include arachnoiditis, vasculitis, cerebral edema, damage to the orbit or spinal cord, and the need for surgical intervention should obstructive hydrocephalus occur. A randomized trial indicated that *albendazole* in combination with *praziquantel* showed superior efficacy against neurocysticercosis compared to *albendazole* alone, either in standard or high dose (Garcia et al., 2014).

Albendazole, 400 mg/day, also has shown efficacy for therapy of certain microsporidial intestinal infections in patients with AIDS. Infection with *Capillaria philippinensis* can be treated with a 10-day treatment regimen of *albendazole* (400 mg/day).

The combination of *ivermectin*, *diethylcarbamazine*, and *albendazole* is highly effective for treatment of lymphatic filariasis, with a trial in Papua New Guinea showing that a single dose of the combination cleared microfilaria from the blood for at least 3 years in 96% of 60 participants (King et al., 2018). To avoid serious reactions to dying microfilariae, the *ivermectin/diethylcarbamazine/albendazole* combination is not recommended in locations where filariasis coexists with either onchocerciasis or loiasis.

Mebendazole is an effective drug for treatment of some GI nematode infections. It is only administered orally, with the same dosage schedule applying to adults and to children greater than 2 years of age. For treatment of enterobiasis, a single 100-mg tablet is taken; a second dose should be given after 2 weeks. For control of ascariasis, trichuriasis, or hookworm infections, the recommended regimen is 100 mg of *mebendazole* taken in the morning and evening for 3 consecutive days (or a single 500-mg tablet administered once). If the patient is not cured 3 weeks after treatment, a second course should be given. A 3-day *mebendazole* regimen is more effective than single doses of either *mebendazole* (500 mg) or *albendazole* (400 mg).

Triclabendazole is used for the treatment of fascioliasis and represents an alternative to *praziquantel* for treatment of paragonimiasis. *Triclabendazole* is administered at 10 mg/kg with a repeated dose administered when patients have high infection intensities (Keiser et al., 2005).

Adverse Effects and Drug Interactions

Albendazole and *mebendazole* have very good safety profiles when administered in short courses. Overall, side effects, primarily mild GI symptoms, occur in only 1% of treated children. Side effects frequently encountered with therapeutic doses include anorexia, nausea, vomiting, and dizziness. *Mebendazole* does not cause significant systemic toxicity in routine clinical use due to its low systemic bioavailability. Transient symptoms of abdominal pain, distention, and diarrhea have occurred in cases of massive infection and expulsion of intestinal worms. *Albendazole* produces few side effects when used for short-term therapy of intestinal helminth infections, even in patients with heavy worm burdens. In long-term therapy of cystic hydatid disease and neurocysticercosis, *albendazole* is well tolerated by most patients. The most common side effect is liver dysfunction, generally manifested by an increase in serum transaminase levels; rarely, jaundice may be noted, but liver enzymes return to normal after therapy is completed. Liver function tests should be monitored during protracted *albendazole* therapy; the drug is not recommended for patients with cirrhosis. The safety of *albendazole* in children less than 2 years of age has not been established. Long-term *albendazole* therapy can occasionally cause marrow toxicity, so blood counts should be monitored in this setting as well.

The BZs as a group display few clinically significant interactions with other drugs. *Albendazole* may induce its own metabolism; plasma levels of its sulfoxide metabolites can be increased by coadministration of glucocorticoids and possibly *praziquantel*. Due to theoretical considerations, caution is advised when using high doses of *albendazole* together with drugs that inhibit the hepatic CYPs, such as *ritonavir*. Coadministration of *cimetidine* can increase the bioavailability of *mebendazole*.

Pediatric and Geriatric Indications and Problems

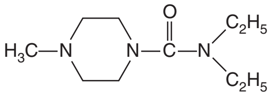
Although neither *albendazole* nor *mebendazole* is recommended for use in pregnancy, a review of the risk of congenital abnormalities from BZs concluded that their use during pregnancy was not associated with an increased risk of major congenital defects. Hookworm infections occur in many pregnant women in developing countries, including up to one-third of pregnant women in sub-Saharan Africa. Because of the increased morbidity conferred by iron deficiency anemia in pregnancy, monthly BZ treatment has been recommended by the WHO during the second and third trimesters of pregnancy on the basis that improved iron status due to eradication of hookworm infection has a demonstrable benefit for both mother and child. Nonetheless, it is recommended that treatment should be avoided during the first trimester of pregnancy. There is no evidence that maternal BZ therapy presents a risk to breastfed infants.

The BZs have not been extensively studied in children less than 2 years of age. The recommended dose is 200 mg of *albendazole* in children between the ages of 12 and 24 months. The WHO concluded that BZs may be used in children less than 1 year old if the risks from adverse consequences caused by STHs are justified.

Diethylcarbamazine

Chemistry

Diethylcarbamazine (DEC) (*N*,*N*-diethyl-4-methylpiperazine-1-carboxamide) is formulated as the water-soluble citrate salt containing 51% by weight of the active base. The drug is soluble in water. Because the compound is tasteless, odorless, and stable to heat, it also can be taken in the form of fortified table salt containing 0.2% to 0.4% by weight of the base. The drug is commercially available outside the U.S.; in the U.S., it is supplied by the U.S. Centers for Disease Control and Prevention (CDC).


DIETHYLCARBAMAZINE

Mechanism of Action

The mechanisms of action of DEC against filarial species are unknown. Microfilarial forms of susceptible filarial species are most affected by DEC. These developmental forms of *W. bancrofti*, *B. malayi*, and *L. loa* rapidly disappear from human blood after consumption of the drug. Microfilariae of *O. volvulus* rapidly disappear from skin after DEC administration, but the drug does not kill microfilariae in nodules that contain the adult (female) worms. The drug has some activity against the adult life cycle stages of *W. bancrofti*, *B. malayi,* and *L. loa* but negligible activity against adult *O. volvulus*.

ADME

DEC is absorbed rapidly from the GI tract. Peak plasma levels occur within 1 to 2 h; the plasma $t_{1/2}$ varies from 2 to 10 h, depending on the urinary pH. Alkalinizing the urine can elevate plasma levels, prolong the plasma $t_{1/2}$, and increase both the therapeutic effect and toxicity of DEC. Dosage reduction may be required for people with renal dysfunction. Metabolism is rapid and extensive; a major metabolite, DEC-*N*-oxide, is active.

Therapeutic Uses

Recommended regimens differ according to whether the drug is used for population-based chemotherapy, treatment of confirmed filarial infection, or prophylaxis against infection.

W. bancrofti, B. malayi, and B. timori. The standard regimen for the treatment of lymphatic filariasis traditionally has been a 12-day, 6 mg/kg per day course of DEC. In the U.S., it is common practice to administer small test doses of 50 to 100 mg (1–2 mg/kg for children) over a 3-day period prior to beginning the 12-day regimen. However, a single dose of 6 mg/kg reportedly has comparable macrofilaricidal and microfilaricidal efficacy to the standard regimen. Single-dose therapy may be repeated every 6 to 12 months, as necessary. Although DEC does not reverse existing lymphatic damage, early treatment of asymptomatic individuals may prevent progression of lymphatic damage. For mass treatment to interrupt transmission, effective strategies have included the introduction of DEC into table salt (0.2%–0.4% by weight of the base). As noted above, DEC, when given in combination with *albendazole* and *ivermectin*, is more effective for treatment of lymphatic filariasis than when given alone.

O. volvulus and L. loa. DEC is contraindicated for the treatment of onchocerciasis because it causes severe reactions related to microfilarial destruction, including worsening ocular lesions (Molyneux et al., 2003), and *ivermectin* is the preferred drug for this infection. DEC is the drug of choice for therapy of loiasis with some caveats (below). Treatment is initiated with test doses of 50 mg (1 mg/kg in children) daily for 2 to 3 days, escalating to maximally tolerated daily doses of 9 mg/kg in three doses for 2 to 3 weeks. In patients with high-grade microfilaremia, low test doses are used, often accompanied by pretreatment with glucocorticoids or antihistamines, to minimize reactions to dying microfilariae. *Albendazole* may be useful in patients who either fail therapy with DEC or who cannot tolerate the drug. DEC is clinically effective against microfilariae and adult worms of *M. streptocerca*. DEC is no longer recommended as a first-line drug for the treatment of toxocariasis.

Adverse Effects and Drug Interactions

Below a daily dose of 8 to 10 mg/kg, direct toxic reactions to DEC are rarely severe and usually disappear within a few days despite continuation of therapy. These reactions include anorexia, nausea, headache, and, at high doses, vomiting. Major adverse effects result directly or indirectly from the host response to destruction of parasites, primarily microfilariae. Delayed reactions to dying adult worms may result in lymphangitis, swelling, and lymphoid abscesses in bancroftian and brugian filariasis and small skin wheals in loiasis. The drug occasionally causes severe side effects in heavy *L. loa* infections, including retinal hemorrhage and life-threatening encephalopathy. In patients with onchocerciasis, the *Mazzotti* reaction, a florid hypersensitivity reaction to release of parasite antigen after mass death of worms, typically occurs within a few hours after the first dose. No significant drug interactions have been reported with DEC.

Precautions and Contraindications

Population-based therapy with DEC should be avoided where onchocerciasis or loiasis may be endemic in sub-Saharan Africa, although the drug can be used to protect foreign travelers from these infections. Pretreatment with glucocorticoids and antihistamines often is undertaken to minimize indirect reactions to DEC that result from release of antigen by dying microfilariae. Dosage reduction may be appropriate for patients with impaired renal function or persistent alkaline urine. DEC appears to be safe for use during pregnancy.

Doxycycline

Filarial parasites, including *W. bancrofti* and *O. volvulus,* harbor bacterial symbionts of the genus *Wolbachia*, against which long courses of *doxycycline* (see Chapter 60 for full discussion of *doxycycline*) (≥6 weeks) in bancroftian filariasis and onchocerciasis are effective. A 6-week regimen of *doxycycline* (100 mg daily), by killing the *Wolbachia,* leads to sterility of adult female *Onchocerca* worms.

Ivermectin

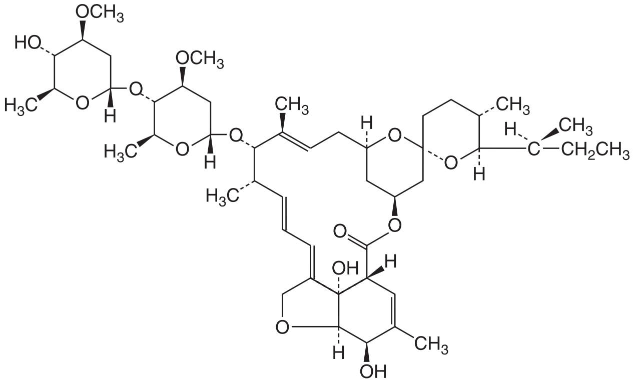
HISTORY

THE DISCOVERY OF IVERMECTIN: OMURA AND CAMPBELL

In the mid-1970s, surveys of natural products revealed that a fermentation broth of the soil actinomycete *Streptomyces avermitilis* ameliorated infection with *Nematospiroides dubius* in mice. Isolation of the anthelmintic components from cultures of this organism led to the discovery of the avermectins, a novel class of 16-membered macrocyclic lactones. *Ivermectin* (mectizan; stromectol; 22,23-dihydroavermectin B1a) is a semisynthetic analogue of avermectin B1a (abamectin), an insecticide developed for crop management. *Ivermectin* now is used to treat a broad spectrum of infections caused by parasitic nematodes (roundworms) and arthropods (insects, ticks, and mites) that plague livestock and domestic animals (Campbell, 1993). The discoverers of *ivermectin*, Satoshi Omura and William Campbell, won the 2015 Nobel Prize in Physiology/Medicine "for their discoveries concerning a novel therapy against infections caused by roundworm parasites," an award shared with Tu Youyou for "for her discoveries concerning a novel therapy against Malaria."

Chemistry

Ivermectin exists as an odorless off-white powder with high lipid solubility but poor solubility in water. It is a mixture of at least 80% 22,23-dihydroavermectin B1a and no more than 20% 22,23-dihydroavermectin B1b. B1a and B1b have nearly identical antiparasitic activities.



IVERMECTIN

Mechanism of Action

Ivermectin immobilizes affected organisms by inducing a tonic paralysis of the musculature. Avermectins (of which *ivermectin* is a member) induce paralysis by activating a family of ligand-gated Cl^- channels, particularly glutamate-gated Cl^- channels found only in invertebrates. *Ivermectin* probably binds to glutamate-activated Cl^- channels found in nematode nerve or muscle cells and causes hyperpolarization by increasing intracellular chloride concentration, resulting in paralysis. Glutamate-gated Cl^- channels probably are one of several sites of *ivermectin* action among invertebrates. Avermectins also bind with high affinity to γ-aminobutyric acid (GABA)-gated and other ligand-gated Cl^- channels in nematodes such as *Ascaris* and in insects, but the physiological consequences are less well defined. Lack of high-affinity avermectin receptors in cestodes and trematodes may explain why these helminths are not sensitive to *ivermectin* (Shoop et al., 1995). Avermectins also interact with GABA receptors in mammalian brain, but their affinity for invertebrate receptors is approximately 100-fold higher. *Ivermectin* interacts with a number of ion channels, Cys-loop receptors, the $P2X_4$ receptor, farnesoid X receptor, and G protein-gated K^+ channel (Chen and Kubo, 2018; Lynagh and Lynch, 2012).

ADME

Peak levels of *ivermectin* in plasma are achieved within 4 to 5 h after oral administration. The long $t_{1/2}$ (~57 h in adults) primarily reflects a low systemic clearance (~1-2 L/h) and a large apparent volume of distribution. *Ivermectin* is approximately 93% bound to plasma proteins. The drug is extensively metabolized by hepatic CYP3A4 (Zeng et al., 1998). Virtually no *ivermectin* appears in human urine in either unchanged or conjugated form. Although the drug causes significant neurotoxicity in some species such as the beagle dog, in humans, its penetration across the blood-brain barrier is prevented by an efflux pump of the ATP-binding cassette subfamily B member 1 (ABCB1), also known as MDR1 and P-glycoprotein. There is limited experience in the use of a veterinary parenteral preparation of *ivermectin* for treatment of *Strongyloides* hyperinfection.

Therapeutic Uses

Onchocerciasis. *Ivermectin*, administered as a single oral dose (150–200 μg/kg) given every 6 to 12 months, is the drug of choice for onchocerciasis in adults and children 5 years of age or older. Marked reduction of microfilariae in the skin results in major relief of the intense pruritus that is a feature of onchocerciasis. Clearance of microfilariae from skin and ocular tissues occurs within a few days and lasts for 6 to 12 months; the dose then should be repeated. However, the drug is not curative, because *ivermectin* has little effect on adult *O. volvulus.* Annual doses of the drug are quite safe and substantially reduce transmission of this infection.

Lymphatic Filariasis. *Ivermectin* is as effective as DEC for controlling lymphatic filariasis. As noted above, the combination of *albendazole*, DEC, and *ivermectin* is more efficacious than any of the drugs alone for treatment of lymphatic filariasis. The duration of treatment is at least 5 years, based on the estimated fecundity of the adult worms.

Strongyloidiasis. *Ivermectin*, administered as a single dose of 150 to 200 μg/kg, is the drug of choice for human strongyloidiasis (Marti et al., 1996). Although it has been recommended that a second dose be administered a week following the first dose, this has not been demonstrated to improve parasitological response (Buonfrate et al., 2019). *Ivermectin* is more efficacious than a 3-day course of *albendazole*.

Infections With Other Intestinal Nematodes. *Ivermectin* is more effective in ascariasis and enterobiasis than in trichuriasis or hookworm infection. In the latter two infections, although it is not curative, it significantly reduces the intensity of infection. It also shows efficacy against the zoonotic filarial species *Mansonella ozzardi* when given as a single dose (de Almeida Basano et al., 2018).

Since 2017, *ivermectin* (200 μg/kg) in combination with *albendazole* (400 mg) is on the WHO essential medicine list for the treatment of soil-transmitted helminthiasis (WHO, 2019).

Other Indications. Taken as a single 200-μg/kg oral dose, *ivermectin* is a first-line drug for treatment of cutaneous larva migrans caused by dog or cat hookworms and is an option for treatment of scabies and head lice. In uncomplicated scabies, two doses should be administered 1 to 2 weeks apart. In severe (crusted) scabies, *ivermectin* should be used in repeated doses, with one recommended regimen entailing seven doses of 200 μg/kg given with food on days 1, 2, 8, 9, 15, 22, and 29. The drug formulated as a topical 0.2% lotion is active against human head lice (Pariser et al., 2012).

Adverse Effects and Drug Interactions

Ivermectin is well tolerated by uninfected humans. In filarial infection, *ivermectin* therapy frequently causes a Mazzotti-like reaction to dying microfilariae. The intensity and nature of these reactions relate to the microfilarial burden. After treatment of *O. volvulus* infections, these side effects usually are limited to mild itching and swollen, tender lymph nodes, which occur in 5% to 35% of people, last just a few days, and are relieved by *aspirin* and antihistamines. Rarely, more severe reactions occur that include high fever, tachycardia, hypotension, dizziness, headache, myalgia, arthralgia, diarrhea, and facial and peripheral edema; these may respond to glucocorticoid therapy. *Ivermectin* induces milder side effects than does DEC, and unlike DEC, *ivermectin* seldom exacerbates ocular lesions in onchocerciasis. The drug can cause rare but serious side effects, occasionally resulting in permanent disability and encephalopathies in patients with heavy *L. loa* microfilaria. *Loa* encephalopathy is associated with *ivermectin* treatment of individuals with *Loa* microfilaremia levels of greater than or equal to 30,000 microfilariae per milliliter of blood. *Ivermectin* interactions with concurrently administered drugs can occur. For example, increased plasma levels of *ivermectin* have been observed in patients treated concurrently with *ivermectin* and *levamisole* (González Canga, 2008). *Ivermectin* may have significant neurotoxicity in certain susceptible individuals who have specific mutations in the *ABC1* gene conferring loss of activity of the efflux pump (Baudou et al., 2020).

Precautions and Contraindications

Because of its effects on GABA receptors in the CNS, *ivermectin* is contraindicated in conditions associated with an impaired blood-brain barrier (e.g., African trypanosomiasis and meningitis). In programs of MDA where loiasis is coendemic with either onchocerciasis or lymphatic filariasis, *ivermectin* should be used with caution and in consultation with local or international experts.

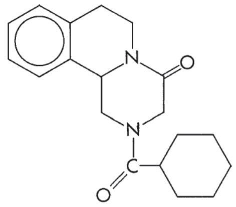
Pediatric and Geriatric Indications and Problems

Ivermectin is not approved for use in children who weigh less than 15 kg or in pregnant or lactating women (low levels of the drug appear in the mother's milk). This is principally due to concerns about the passage of the drug across the immature blood-brain barrier. A recent systematic review and individual patient data analysis showed that *ivermectin* can be safely administered to children who weigh less than 15 kg (Jittamala et al., 2021). Simulations accounting for the higher clearance rates in youth suggest that doses of 300 μg/kg (for ages 2 to 5) and 250μg/kg (for ages 6-12) will achieve ivermectin exposure in children equivalent to that in adults at 200 μg/kg. (Brussee et al., 2019). The safety of *ivermectin* in pregnancy remains to be determined (Westlake and Aronoff, 2021).

Praziquantel

Chemistry

Praziquantel, a pyrazinoisoquinoline derivative, is a racemate, consisting of the biologically active enantiomer, *R-praziquantel* and the inactive distomer *S-praziquantel*. The white crystalline powder is very slightly soluble in water and freely soluble in alcohol and in methylene chloride.



PRAZIQUANTEL

Mechanism of Action

Praziquantel causes Ca^{2+} influx and spastic paralysis of adult worms and rapid vacuolization of the worm surface, activating a schistosome transient receptor potential ion channel (Bais and Greenberg, 2020). The drug is inactive against juvenile schistosomes and therefore is relatively ineffective in early infection.

ADME

Praziquantel is readily absorbed after oral administration (<80%), reaching maximal levels in human plasma in approximately 2 h. The drug is highly protein bound (~80%, nearly exclusive to albumin). *Praziquantel* distributes throughout the body, with highest concentrations measured in the liver and kidneys. It crosses the blood-brain barrier. Breast milk concentrations are approximately 25% of plasma concentrations (Olliaro et al., 2014).

Extensive stereoselective first-pass metabolism takes place. The main metabolite in man is trans-4-hydroxypraziquantel. Plasma $t_{1/2}$ is 2.2 to 8.9 h following 40 mg/kg in healthy fasted adults. In patients with severe liver disease, including those with hepatosplenic schistosomiasis, pharmacokinetic parameters might be altered (Olliaro et al., 2014). About

70% of an oral dose is recovered as metabolites in the urine within 24 h; most of the remainder is eliminated in the bile.

Therapeutic Uses

Praziquantel is the drug of choice for treating schistosomiasis caused by all *Schistosoma* species that infect humans. A single oral dose of 40 mg/kg or three doses of 20 mg/kg each, given 4 to 6 h apart, generally produce cure rates of 70% to 95% and consistently high reductions (>85%) in egg counts (Utzinger and Keiser, 2004). Three doses of 25 mg/kg taken 4 to 8 h apart result in high rates of cure for infections with the liver flukes *C. sinensis* and *Opisthorchis viverrini* or the intestinal flukes *Fasciolopsis buski, Heterophyes heterophyes*, and *Metagonimus yokogawai*. The same three-dose regimen, used over 2 days, is highly effective against infections with the lung fluke, *Paragonimus westermani*. The liver fluke *F. hepatica* is resistant to *praziquantel* and should be treated with *triclabendazole* (Keiser et al., 2005). Low doses of *praziquantel* can be used to treat intestinal infections with adult cestodes (a single oral dose of 25 mg/kg for *H. nana* and 10–20 mg/kg for *D. latum, T. saginata*, or *T. solium*). Retreatment after 7 to 10 days is advisable for individuals heavily infected with *H. nana*. Although *albendazole* is preferred for therapy of human cysticercosis, *praziquantel* represents an alternative agent; its use for this indication is hampered by the important pharmacokinetic interaction with *dexamethasone* and other corticosteroids that should be coadministered in this condition (Evans et al., 1997).

Adverse Effects and Drug Interactions

Abdominal discomfort and drowsiness may occur shortly after taking *praziquantel*; these direct effects are transient and dose related. Indirect effects such as fever, pruritus, urticaria, rashes, arthralgia, and myalgia are noted occasionally. Such side effects and increases in eosinophilia often relate to parasite burden and may be a consequence of parasite killing and antigen release. In neurocysticercosis, inflammatory reactions to praziquantel may produce meningismus, seizures, and cerebrospinal fluid pleocytosis. These effects usually are delayed in onset, last 2 to 3 days, and respond to analgesics and anticonvulsants. *Praziquantel* is contraindicated in ocular cysticercosis because the host response can irreversibly damage the eye. Driving and other tasks requiring mental alertness should be avoided. Severe hepatic disease can prolong the $t_{1/2}$, requiring dosage adjustment. The bioavailability of *praziquantel* is reduced by inducers of hepatic CYPs such as *carbamazepine* and *phenobarbital*; predictably, coadministration of the CYP inhibitor *cimetidine* has the opposite effect (Dachman et al., 1994). *Dexamethasone* reduces the bioavailability of *praziquantel*. Under certain conditions, *praziquantel* may increase the bioavailability of *albendazole* (Homeida et al., 1994).

Pediatric and Geriatric Indications and Problems

Praziquantel is considered safe in children greater than 4 years old. A pediatric formulation, an oral dispersible tablet based *R-praziquantel*, is under development (Bagchus et al., 2019).

Niclosamide

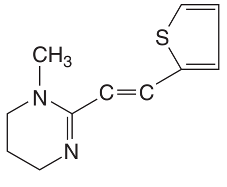
Niclosamide, a halogenated salicylanilide derivative, was introduced for human use as a taeniacide. Niclosamide is no longer approved for use in the U.S. It has some use in intestinal *T. solium* infection where neurocysticercosis is present or cannot be excluded.

Oxantel and Pyrantel Pamoate

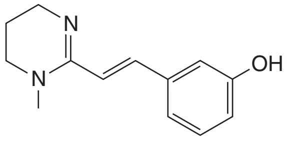
Pyrantel pamoate is a WHO-recommended anthelminthic and widely available; however, *oxantel pamoate* is marketed only in Asia and South America. Efforts are ongoing to register it at a stringent regulatory authority (Palmeirim et al., 2021).

Chemistry

The tetrahydropyrimidine analogue *pyrantel pamoate* and the *m*-oxyphenol analogue of pyrantel, *oxantel pamoate*, are practically insoluble in water and alcohol.



PYRANTEL



OXANTEL

Mechanism of Action

Pyrantel and its analogues are depolarizing neuromuscular blocking agents. They open nonselective cation channels and induce persistent activation of nicotinic acetylcholine receptors and spastic paralysis of the worm. *Pyrantel pamoate* is active at the L type, while *oxantel pamoate* is active at the N subtype, of nicotinic acetylcholine receptors.

Pyrantel also inhibits cholinesterases. It causes a slowly developing contracture of isolated preparations of *Ascaris* at 1% of the concentration of acetylcholine required to produce the same effect. *Pyrantel* exposure leads to depolarization and increased spike-discharge frequency, accompanied by increases in tension, in isolated helminth muscle preparations (Kopp and Keiser, 2017).

ADME

Pyrantel pamoate and *oxantel pamoate* are poorly absorbed from the GI tract, a property that confines their action to intraluminal GI nematodes. Less than 15% of *pyrantel pamoate* is excreted in the urine as parent drug and metabolites. The major proportion of an administered dose is recovered in the feces.

Therapeutic Uses

Pyrantel pamoate is an alternative to *mebendazole* or *albendazole* for treatment of ascariasis and enterobiasis. High cure rates are achieved after a single oral dose of 11 mg/kg, to a maximum of 1 g. *Pyrantel* also is effective against hookworm infections caused by *A. duodenale* and *N. americanus*, although repeated doses are needed to cure heavy infections by *N. americanus*. High efficacy against hookworm infections was also observed using a triple combination of *albendazole*, *oxantel pamoate*, and *pyrantel pamoate* (Moser et al., 2018). The drug should be used in combination with *oxantel pamoate* for mixed infections with *T. trichiura*. Indeed, *oxantel pamoate* was shown to have a higher efficacy than the BZs *mebendazole* and *albendazole* against infections with *T. trichiura* (Speich et al., 2016). *Oxantel pamoate* combined with *albendazole* is a highly effective combination in the treatment of ascariasis, trichuriasis, and hookworm infections. For pinworm infections, repeat the treatment after an interval of 2 weeks.

Adverse Events and Drug Interactions

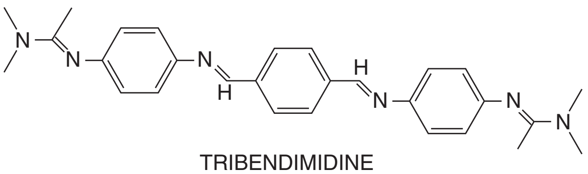
Transient and mild GI symptoms occasionally occur, as do headache, dizziness, rash, and fever. *Pyrantel* and *oxantel pamoate* have not been studied in pregnant women.

Tribendimidine

Tribendimidine is a drug that has been marketed in China for nearly two decades. Given the efforts to introduce the drug to Western markets, it is included in this chapter.

Chemistry

Tribendimidine (a symmetrical diamidine derivative) is a yellow crystalline powder that is soluble in chloroform. It does not dissolve in water and dissolves only marginally in anhydrous ethanol, methanol, or acetone.



TRIBENDIMIDINE

Mechanism of Action

Tribendimidine is an agonist of muscle nicotinic acetylcholine receptors of parasitic nematodes. The drug is more selective for the B-subtype nicotinic acetylcholine receptor (preferentially activated by *bephenium*) than the L-subtype nicotinic acetylcholine receptor (preferentially activated by *levamisole*); thus, *tribendimidine* can activate a specific subset of nematode parasite nicotinic acetylcholine receptors. This relative selectivity might explain why *tribendimidine* exhibits activity on a *levamisole*-resistant isolate of *Oesophagostomum dentatum* and why the spectrum of action of *tribendimidine* is broader (covering also the trematodes *C. sinensis* and *O. viverrini* and cestodes) to that of other cholinergic anthelmintics like *levamisole* (Robertson et al., 2015).

ADME

Tribendimidine pharmacokinetics have been studied in healthy Chinese volunteers (Yuan et al., 2010) and in *O. viverrini*-infected patients (Duthaler et al., 2015). *Tribendimidine* cannot be detected in plasma. It is rapidly and completely broken down to p-(1-dimethylamino ethylimino) aniline (dADT), which is the active metabolite, and to terephthalaldehyde (TPAL). Furthermore, dADT undergoes metabolism to acetylated dADT, and TPAL is transformed into terephalic acid (TPAC). The $t_{1/2}$ of dADT was 4.7 h in healthy volunteers following 400 mg of *tribendimidine*. *Tribendimidine* metabolites are mainly excreted via the urine.

Therapeutic Uses

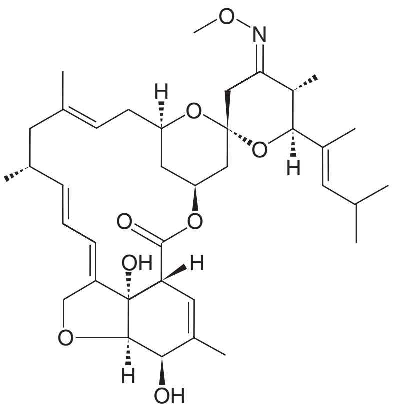
Tribendimidine is given to children younger than 15 years and to adults at doses of 200 mg and 400 mg, respectively. With regard to STH infections, *tribendimidine* has a similar activity profile as *albendazole*: *Tribendimidine* shows high cure and egg reduction rates against *A. lumbricoides*, moderate to good efficacy against hookworm, and low cure rate but moderate egg reduction rates against *T. trichiura*. Against pinworm, infection cure rates with *tribendimidine* were 74.1% (single dose) and 97.1% (two doses) (Xiao et al., 2013). *Tribendimidine* has high activity against *C. sinensis* and *O. viverrini*: Single dosages of *tribendimidine* (200 and 400 mg) show similar efficacy to multiple treatments with *praziquantel* (Qian et al., 2013; Soukhathammavong et al., 2011). The efficacy of *tribendimidine* against *Strongyloides* and cestode infections remains to be studied.

Adverse Events and Drug Interactions

Tribendimidine shows a good safety profile. Adverse events (e.g., dizziness, vertigo, headache, nausea, vomiting, fatigue) are mainly mild and self-limiting. Drug interactions have not yet been studied thoroughly.

Moxidectin

Moxidectin, a macrocyclic lactone related to *ivermectin*, is FDA-approved for the treatment of onchocerciasis due to *O. volvulus* in patients aged 12 years and older.



MOXIDECTIN

Chemistry

Moxidectin, a white or pale-yellow powder, is slightly soluble in water but readily soluble in organic solvents.

Mechanism of Action

The mechanism of action of the avermectins has been described above in the section on *ivermectin*. A different binding activity has been demonstrated between *ivermectin* and *moxidectin* for GABA-gated Cl^- channels. In contrast to *ivermectin*, *moxidectin* is a poor substrate for P-glycoproteins, suggesting a different mechanism or susceptibility to resistance (Cobb and Boeckh, 2009).

ADME

Pharmacokinetic parameters were dose proportional at dosages from 3 to 36 mg. Peak plasma concentrations occur 2 to 6 h after dosing. Administration with food resulted in an increase of over 30% in C_{max} and AUC (integrated area under curve of drug level versus time). In patients with onchocerciasis, *moxidectin* has a very long elimination half-life (mean: 23.3 days) and very large volume of distribution. Following a single dose, *moxidectin* was observed in the breast milk of lactating women (Korth-Bradley et al., 2011). Several hydroxy and oxidative metabolites have been identified *in vitro*; however, in animals, the level of metabolites is low.

Therapeutic Uses

Moxidectin is an effective treatment for *O. volvulus* infections with greater activity than *ivermectin* (Opoku et al., 2018). It also has useful activity in trichuriasis when given with *albendazole* (Keller et al., 2020) and excellent activity in strongyloidiasis (Hofmann et al., 2021) and in an animal model of scabies. The recommended dosage is a single dose of 8 mg (four tablets, each containing 2 mg of *moxidectin*).

Adverse Events and Drug Interactions

Mazzotti reactions, including pruritus, rash, increased pulse rate, and decreased mean arterial pressure, were commonly observed after *moxidectin* treatment (Awadzi et al., 2014). *Moxidectin* can be coadministered with CYP3A4 substrates.

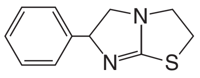
Pediatric and Geriatric Indications and Problems

Moxidectin has not been used in children less than 12 years of age; however, pediatric trials are ongoing.

Levamisole

Chemistry

Levamisole, the levorotatory isomer of the racemic molecule tetramisole, belongs to the imidazole derivatives. The hydrochloride salt is a white powder soluble in water and methanol.



LEVAMISOLE

Mechanism of Action

Levamisole is a cholinergic anthelminthic. The drug is a potent agonist of the L-type nematode nicotinic acetylcholine receptor. Activation of the receptor opens the central channel (see Figures 13–1 and 13–2), producing depolarization, calcium entry, and an increase in sarcoplasmic calcium; this results in spastic muscle contraction, causing the passive elimination of the worms (Martin et al., 2012). *Levamisole* can also inhibit fumarate reductase and hence succinate production, the main source of ATP, which is key for the survival of worms. With regard to mammalian cells, *levamisole* inhibits alkaline phosphatases in most tissues. The drug's immunomodulatory activity has been explained as a stimulation of antibody formation and enhancement of T-cell response by stimulating T-cell activation and proliferation.

ADME

Levamisole is quickly absorbed from the GI tract and C_{Pmax} levels are reached within 2 h. The drug is extensively metabolized in the liver, and its half-life is approximately 4 h (Janssen, 1976).

Therapeutic Uses

Levamisole has excellent activity against *A. lumbricoides* but low to moderate efficacy against *T. trichiura* and hookworm infections (Keiser and Utzinger, 2008). *Levamisole* has also been used for its immunomodulatory effects in cancer. Due to adverse effects (see below), the drug has been withdrawn for human use.

Adverse Events and Drug Interactions

Adverse events mostly occur at high dosages used for immunotherapy. At the single low dosages for anthelminthic therapy, adverse events are minor and include nausea, vomiting, headache, dizziness, or abdominal pain. Severe adverse events such as agranulocytosis have been described following the use of high dosages. In healthy volunteers, increased plasma levels of *ivermectin* and decreased plasma concentrations of *albendazole sulfoxide* were observed when these agents were coadministered with *levamisole* (Awadzi et al., 2004). *Levamisole* has been used as an adulterant in cocaine. *Levamisole*-adulterated cocaine use has been reported to cause neutropenia and agranulocytosis, vasculitis, skin necrosis, and arthralgia, adverse effects that contributed to the withdrawal of *levamisole* for human use (Conrad et al., 2021).

Nitazoxanide

Nitazoxanide (*N*-[nitrothiazolyl] salicylamide) is an oral synthetic broad-spectrum antiparasitic agent that has been used as a single agent to treat mixed infections with intestinal parasites (protozoa and helminths). *Nitazoxanide* is approved in the U.S. for treatment of cryptosporidiosis and giardiasis and has activity against *Entamoeba histolytica* (see Chapter 67 for details). In clinical trials, the drug was reported to have activity against a range of STHs, including *A. lumbricoides*, hookworms, *T. trichiura*, *Enterobius vermicularis*, *S. stercoralis*, *H. nana*, and *F. hepatica* (Fox and Saravolatz, 2005). However, subsequent *in vitro* studies and clinical trials have led to some reconsideration of its utility in whipworm and hookworm infection (Speich et al., 2012).

Drug Facts for Your Personal Formulary: *Anthelmintics*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Benzimidazoles: β-Tubulin inhibitors		
Albendazole	• Intestinal nematode infections • Cysticercosis • Cutaneous larva migrans • Toxocariasis • Echinococcosis	• Monitor for liver and hematological toxicity in long-term therapy • Absorption improved with fatty food
Mebendazole	• Intestinal nematode infections	• Poorly absorbed
Triclabendazole	• Fascioliasis	• Available from the CDC under an investigational new drug protocol
Macrocyclic Lactones: Glutamate-gated chloride channel blockers		
Ivermectin	• Onchocerciasis • Lymphatic filariasis • Scabies and head lice • Intestinal nematodes • Strongyloidiasis	• Safety in pregnancy and children <15 kg is uncertain
Moxidectin	• Onchocerciasis • Intestinal nematodes • Strongyloidiasis • Scabies and head lice	• Very long half-life (23 days)
Praziquantel		
	• Schistosomiasis • Food-borne trematode infections (opisthorchiasis and paragonimiasis) • Intestinal tapeworm infections	• Dizziness is a common adverse effect • May impair mental alertness; avoid tasks such as driving
Miscellaneous Anthelmintics		
Diethylcarbamazine	• Lymphatic filariasis	• Contraindicated in onchocerciasis • Available from CDC under an investigational new drug protocol
Niclosamide	• Intestinal tapeworm infection	• Discontinued in the U.S.
Oxantel and pyrantel pamoate	• Pyrantel pamoate is a second-line drug for intestinal nematode infection	• Oxantel pamoate is not licensed for use in the U.S. • Pyrantel pamoate is sold over the counter to treat pinworm infections
Doxycycline	• Filarial infection	• 6-week course of therapy advised
Levamisole		• May cause agranulocytosis at high doses • Discontinued in the U.S.
Nitazoxanide	• Moderately effective against intestinal nematode infection • Antiprotozoal and antiviral activity	• Broad-spectrum antiparasitic agent • Side effects are rare

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Section VIII

Pharmacotherapy of Neoplastic Disease

Section Editor: Anton Wellstein

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Chapter 69

General Principles in the Pharmacotherapy of Cancer

Anton Wellstein

THE CELL CYCLE

CANCER EVOLUTION AND DRUG DISCOVERY

DRUG RESISTANCE

MOLECULAR TESTING TO SELECT APPROPRIATE DRUGS
- Molecular Analysis and Tumor Heterogeneity
- Liquid Biopsies

ACHIEVING THERAPEUTIC INTEGRATION AND EFFICACY

A CAUTIONARY NOTE

Cancer pharmacology has changed dramatically during the recent past, with the improved understanding of cancer biology and an ever-expanding set of new drugs that target vulnerabilities in specific cancers. Effective treatments had been developed earlier for some fatal malignancies, including testicular cancer, lymphomas, and leukemia. Adjuvant chemotherapy and hormonal therapy can extend overall survival and prevent disease recurrence following surgical resection of localized breast, colorectal, and lung cancers. Chemotherapy is also employed as part of the multimodal treatment of locally advanced head and neck, breast, lung, and esophageal cancers; soft-tissue sarcomas; and pediatric solid tumors, thereby allowing for neoadjuvant surgery that is more limited and results in favorable outcomes (Chabner and Roberts, 2005).

In the past 10 years, the ability to harness the power of the immune system in the treatment of cancer has brought about a paradigm shift whereby some of the most feared diseases, such as melanoma and lung cancer and even late-stage metastatic disease, can be eradicated. For some cancers, response rates are surprisingly high: 87% in Hodgkin lymphoma even in heavily pretreated patients (Ansell et al., 2015), and 50% in patients with metastatic melanoma treated with combinations of anti-PD-1 and -CTLA4 immune checkpoint antibodies. Immune checkpoint inhibitors are currently approved for the treatment of over a dozen different cancers that include melanoma and cancers of the bladder, kidneys, liver, lungs as well as Hodgkin lymphoma. In addition to these histologically defined malignancies, any cancers with deficient DNA mismatch repair (e.g., colorectal, ovarian, pancreatic, endometrial cancers) were also approved for treatment.

Despite these major therapeutic successes, few categories of medication have a narrower therapeutic index and greater potential for causing harmful effects than anticancer drugs. A thorough understanding of their mechanisms of action, including clinical pharmacokinetics, drug interactions, and adverse effects, is essential for their safe and effective use. Anticancer drugs are quite varied in structure and mechanism of action. The group includes alkylating agents; antimetabolite analogues of folic acid, pyrimidine, and purine; natural products; hormones and hormone antagonists; and a variety of small-molecule drugs and antibodies directed at specific molecular targets, such as extracellular receptors, intracellular kinases, or the checkpoints of immune surveillance. Figure 69–1 depicts the cellular targets of these classes of drugs, and Chapters 70 to 73 provide information about them.

Anticancer drugs are increasingly used in a variety of nonmalignant diseases and have become treatment standards, for example, for autoimmune diseases (*rituximab*); rheumatoid arthritis (*methotrexate* and *cyclophosphamide*); Crohn's disease (*6-mercaptopurine*); organ transplantation (*methotrexate* and *azathioprine*); sickle cell anemia (*hydroxyurea*); psoriasis (*methotrexate*); and wet macular degeneration (*ranibizumab* and *aflibercept*).

The Cell Cycle

An understanding of the cell cycle is essential for the rational use of cytotoxic anticancer drugs that target proliferating cells (Figure 69–2). Many cytotoxic agents act by damaging DNA. Their efficacy is thus greatest during S phase, the DNA synthetic phase of the cell cycle. Other agents, such as the vinca alkaloids and taxanes, block the formation of a functional mitotic spindle in the M phase. These agents are most effective on cells entering mitosis, the most vulnerable phase of the cell cycle. Accordingly, human cancers most susceptible to chemotherapy are those having a high percentage of proliferating cells. However, normal tissues that proliferate rapidly (bone marrow, hair follicles, and intestinal epithelium) are thus also susceptible to damage from cytotoxic drugs. In addition, slowly growing tumors with a small growth fraction (e.g., carcinomas of the colon or NSCLC) are less responsive to cell cycle–specific drugs.

Although cells from different tumors display differences in the duration of their transit through the cell cycle and in the fraction of cells in active proliferation, all cells display a similar pattern of cell cycle progression (Figure 69–2):

- A phase that precedes DNA synthesis (G_1)
- A DNA synthesis phase (S)
- An interval following the termination of DNA synthesis (G_2)
- The mitotic phase (M) in which the cell, containing a double complement of DNA, divides into two daughter G_1 cells
- A probability of moving into a quiescent state (G_0) for long periods of time

Some anticancer drugs act at specific phases in the cell cycle, mainly at the S and M phases; other drugs are cytotoxic at any point in the cell cycle and are termed *cell cycle phase nonspecific*.

Each transition point in the cell cycle requires the activation of specific cyclin-dependent kinases (*CDKs*), which, in their active forms, couple with corresponding regulatory proteins called *cyclins*. The proliferative impact of CDKs is, in turn, dampened by inhibitory proteins such as $p16^{INK4A}$, a tumor suppressor named for its molecular mass (*p*rotein of *16* kDa) and *in*hibition of CD*K4*. Tumor cells often exhibit changes in cell cycle regulation that lead to relentless proliferation (e.g., mutations or loss of $p16^{INK4A}$ or of other inhibitory components of the so-called retinoblastoma pathway, enhanced cyclin, or enhanced CDK activity).

The CDK family consists of over 20 serine/threonine protein kinases that have been among the first pathway targets pursued for the treatment of cancer. However, different tissue selectivities and distinct cell cycle–specific activity periods of the various CDKs provide a challenge for the development of CDK inhibitors. CDK4/6 have become attractive targets because they control cell cycle progression from the G_1 to the

Abbreviations

ABL: Abelson murine leukemia viral oncogene homolog
ALK: anaplastic lymphoma kinase
BCR: breakpoint cluster region
BRAF: B-Raf proto-oncogene ser/thr protein kinase
CAR(T): chimeric antigen receptor T cell
CDK: cyclin-dependent kinase
ctDNA: circulating, cell-free mutant tumor DNA
CTLA4: cytotoxic T lymphocyte–associated protein 4
EGF(R): epidermal growth factor (receptor) [HER1, ErbB-1]
ER: estrogen receptor
FDA: Food and Drug Administration
GI: gastrointestinal
HER1: human EGFR (ErbB-1)
HER2: human EGFR 2 (ErbB-2)
MEK: mitogen-activated protein kinase kinase
MHC: major histocompatibility class (protein)
NCCN: National Comprehensive Cancer Network
NSCLC: non–small cell lung cancer
PARP: poly(ADP-ribose) polymerase
PD-1: programmed cell death 1
PD-L1: programmed cell death ligand 1
SARS-CoV-2: severe acute respiratory syndrome–coronavirus 2
TMB: tumor mutational burden

S phase. Interaction of cyclin D with CDK4/6 enhances phosphorylation and inactivation of the retinoblastoma (Rb) protein, followed by the transcription of factors that control transition into the S phase. CDK4/6 inhibition will thus cause a G_1 arrest in susceptible cells that utilize this pathway. CDK4/6 inhibitors were recently approved for the treatment of breast cancer (see Chapter 71).

Because of the central importance of DNA to the identity and functionality of a cell, elaborate cellular mechanisms ("cell cycle checkpoints") have evolved to monitor DNA integrity. At each transition point in the cell cycle, specific proteins, such as p53 and chk-1 and-2, monitor the integrity of DNA and, upon detection of DNA damage, may initiate DNA repair processes, or in the presence of massive damage, direct cells down a pathway of cell death (apoptosis). If a cell possesses normal checkpoint function, drug-induced DNA damage will activate apoptosis when the cell reaches the G_1/S or G_2/M boundary. However, if the p53 gene product or other checkpoint proteins are mutated or absent or the checkpoint function fails, damaged cells will not divert to the apoptotic pathway but will proceed through the S phase and mitosis. The cell progeny will then emerge as a mutated and potentially drug-resistant subpopulation (see Figure 69–3A).

Cancer Evolution and Drug Discovery

The rapidly expanding knowledge of cancer biology and the ability to analyze cancer genome alterations in thousands of patient samples have led to a better understanding of the molecular evolution of cancer and the discovery of cancer-specific drug targets: growth factor receptors, intracellular signaling pathways, epigenetic processes, tumor vascularity,

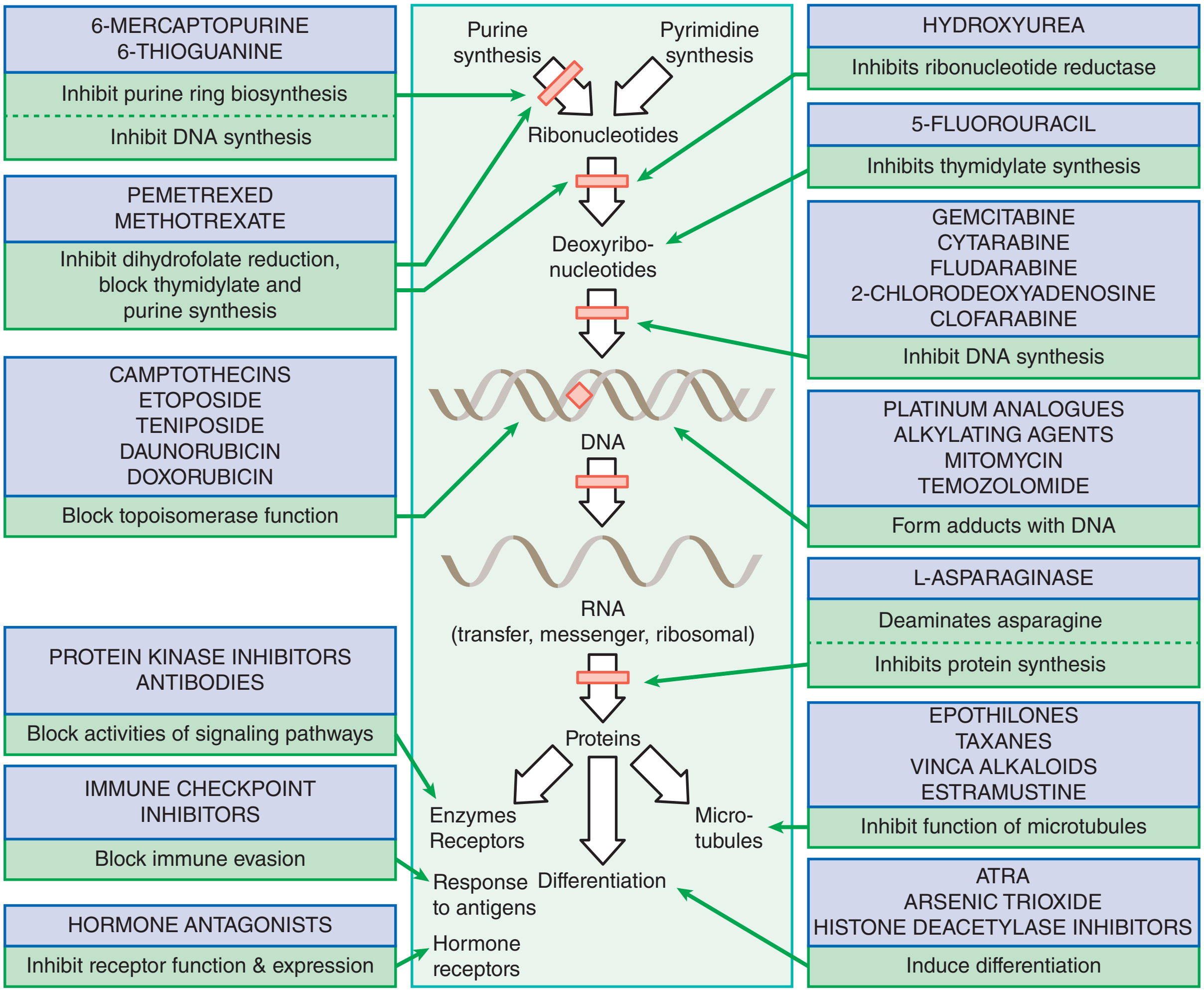
6-MERCAPTOPURINE
6-THIOGUANINE
Inhibit purine ring biosynthesis
Inhibit DNA synthesis
PEMETREXED
METHOTREXATE
Inhibit dihydrofolate reduction, block thymidylate and purine synthesis
CAMPTOTHECINS
ETOPOSIDE
TENIPOSIDE
DAUNORUBICIN
DOXORUBICIN
Block topoisomerase function
PROTEIN KINASE INHIBITORS
ANTIBODIES
Block activities of signaling pathways
IMMUNE CHECKPOINT INHIBITORS
Block immune evasion
HORMONE ANTAGONISTS
Inhibit receptor function & expression
Purine synthesis
Pyrimidine synthesis
Ribonucleotides
Deoxyribo-nucleotides
DNA
RNA
(transfer, messenger, ribosomal)
Proteins
Enzymes
Receptors
Micro-tubules
Response to antigens
Differentiation
Hormone receptors
HYDROXYUREA
Inhibits ribonucleotide reductase
5-FLUOROURACIL
Inhibits thymidylate synthesis
GEMCITABINE
CYTARABINE
FLUDARABINE
2-CHLORODEOXYADENOSINE
CLOFARABINE
Inhibit DNA synthesis
PLATINUM ANALOGUES
ALKYLATING AGENTS
MITOMYCIN
TEMOZOLOMIDE
Form adducts with DNA
L-ASPARAGINASE
Deaminates asparagine
Inhibits protein synthesis
EPOTHILONES
TAXANES
VINCA ALKALOIDS
ESTRAMUSTINE
Inhibit function of microtubules
ATRA
ARSENIC TRIOXIDE
HISTONE DEACETYLASE INHIBITORS
Induce differentiation

Figure 69–1 *Mechanisms and sites of action of some of the drugs used in the treatment of cancer.*

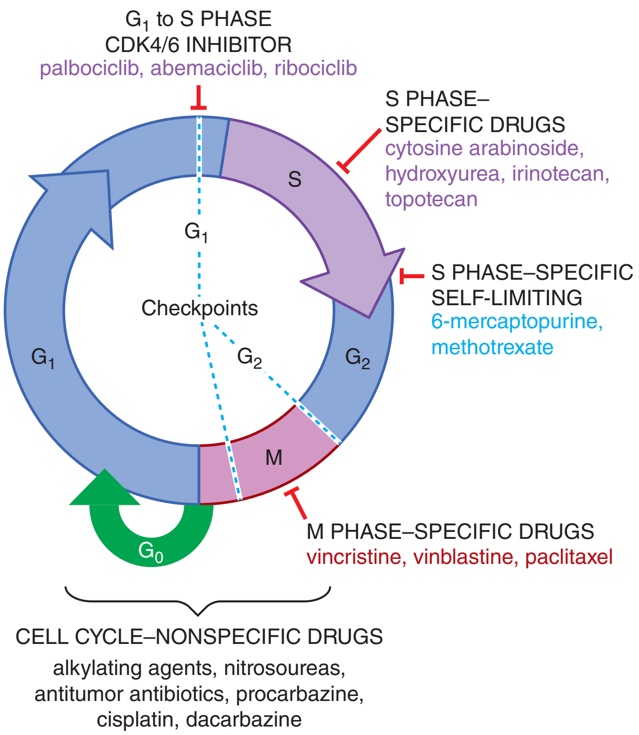

Figure 69–2 *Cell cycle specificity of drugs used in the treatment of cancer.*

DNA repair defects, cell death pathways, and immune escape mechanisms (Hanahan and Weinberg, 2011). Human malignancies are a highly diverse group of diseases that vary even within defined classifications such as organ of origin (lung, breast, prostate, colon, etc.), histology, or molecular marker. Also, the tumor cell population constituting a given cancer at the time of diagnosis has evolved over many years from a few precursor cells that accumulated mutations over time, generating heterogeneity within the primary tumor and at metastatic sites (Figure 69–3).

Clinically detectable cancer lesions represent approximately 1 g of tumor tissue or 10^9 cells and can contain a multitude of subpopulations and a wide variety of genetic alterations (see legend of Figure 69–3). The dynamic evolution of individual cancer genomes and its implications for the development of therapies were established from the analyses of specimens from diverse cancers (Yates and Campbell, 2012). This dynamic was exemplified in a detailed analysis of a series of multiple parallel biopsies from different sites in patients with metastatic melanoma during treatment with inhibitors of mutant BRAF. The genomic analysis of the biopsies revealed complex and distinct evolutionary branching architectures due to the selection of drug-resistant subpopulations during treatment (Shi et al., 2014).

Nevertheless, in many tumors, proliferation and survival of the majority of subpopulations depend on a shared (ancestral) constitutive activity of a single growth factor pathway, or so-called oncogene addiction. Inhibition of that pathway leads to cell death of the sensitive populations. Thus, *imatinib* attacks the unique and specific *bcr-abl* translocation in chronic myelocytic leukemia. *Imatinib* also inhibits *c-kit* and produces extended control of GI stromal tumors that express a mutated and constitutively activated form of *c-kit*. Monoclonal antibodies effectively target tumor-associated antigens such as the amplified *HER2* receptor in breast cancer cells (Slamon et al., 2001). Protein kinase inhibitors targeting mutant EGFR or mutant ALK in lung cancers improve disease outcomes over the use of conventional chemotherapy.

These examples emphasize that new strategies for drug discovery and development, and advances in patient care, will result from new knowledge of cancer biology. One response to the oncogene addiction paradigm has been to group cancers by shared vulnerabilities, include patients in so-called basket trials that evaluate a drug based on its target rather than particular disease entities, and consider sensitivity and resistance to treatments in that context.

However, in the foreseeable future, targeted drugs and cytotoxics will continue to be used in combination. For instance, cytotoxics in combination with monoclonal antibodies such as *trastuzumab* or *bevacizumab* improve efficacy. At the same time, the toxicities of cytotoxic drugs have become more manageable with the development of better antinausea

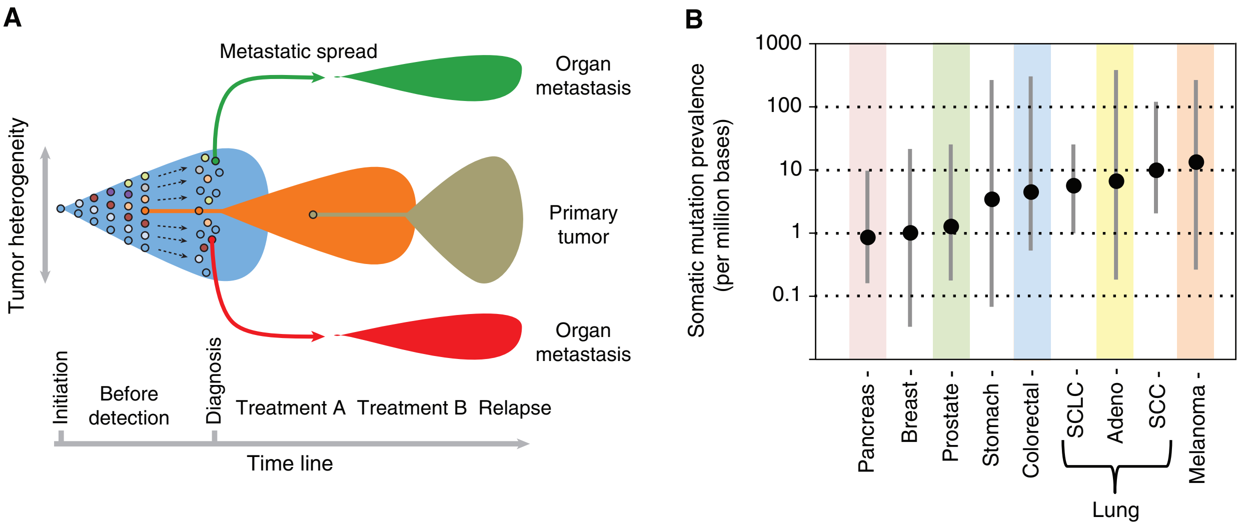

Figure 69–3 *Evolution of treatment resistance; mutational load of human cancers.* **A.** *Treatment resistance.* Cancers accumulate mutations during their evolution. Cancer cell subpopulations are selected based on their growth capacity, adaptation to the tumor microenvironment at the primary or metastatic site, and the evasion of immune surveillance. Drug treatment adds evolutionary pressure and selects for resistant subpopulations. Differently colored dots indicate tumor subpopulations of different genetic or epigenetic makeup. **B.** *Mutational load.* Data are median values ± range of numbers of somatic mutations per million bases observed for some major cancers. Note that the ordinate is a logarithmic scale. In 7042 cancer specimens, between 100 and 1,000,000 mutations were detected per tumor specimen, with a 30- to 1000-fold range among individual specimens from a single cancer type (see original data in Alexandrov et al., 2013). A tumor mutational burden (TMB) of 10 somatic mutations per megabase (= 30,000 mutations in the human genome of 3 × 10^9 base pairs) results in approximately 150 mutations in amino acid sequences that can alter protein function, drug sensitivity, and antigenicity. In many cancer types, higher TMB is associated with poorer survival. On the other hand, the formation of tumor-specific neoantigens due to DNA mutations permits the immune system to distinguish between tumor and normal cells and contributes to the efficacy of cancer immunotherapy (Schumacher and Schreiber, 2015). Thus, in patients treated with immune checkpoint inhibitors, higher TMB is associated with longer survival. Adeno, adenocarcinoma; SCC, squamous cell carcinoma; SCLC, small cell lung cancer.

medications (see Chapter 54) and with granulocyte colony-stimulating factor to restore bone marrow function (see Chapters 45 and 71).

Finally, targeted drugs are helping to overcome resistance to chemotherapeutic agents by normalizing blood flow, promoting apoptosis, and inhibiting prosurvival signals from growth factor pathways. Tumor angiogenesis leads to increased interstitial pressure and diminishes delivery of drugs to tumor cells; inhibitors of angiogenesis (e.g., *bevacizumab*) normalize blood flow and interstitial pressure, improve drug delivery, and can thus synergize with cytotoxic drugs in the treatment of lung, colon, and other cancers. It is also thought that the combination of cytotoxic drugs or pathway inhibitors can induce tumor cell death and antigen release and thus enhance responses to immune checkpoint inhibitors or other immune modulators. This concept is part of a recommendation for the treatment of patients with melanoma (Kaufman et al., 2013) and should be relevant for a range of cancers (Sharma and Allison, 2015). The ongoing development of activating and inhibitory drugs for additional immune checkpoint pathways (Anderson et al., 2016) will provide new options for drug combinations.

Drug Resistance

Resistance remains the major obstacle to successful cancer treatment. Resistance results from a variety of molecular changes acquired during evolution of a given cancer that can defeat the best-designed treatments. Mechanisms of drug resistance include poor drug absorption and delivery; genetically determined variability in drug transport, activation, and clearance; and mutations, amplifications, or deletions in drug targets (Holohan et al., 2013). The processes of resistance are best understood for pathway-targeted drugs. Tumors developing resistance to *bcr-abl* inhibitors and to inhibitors of the EGFR typically express mutations in the target enzyme. Cells exhibiting drug-resistant mutations preexist in a patient's tumor prior to drug treatment and are selected by drug exposure (see Figure 69–3A). Resistance to inhibitors of the EGFR may also develop through expression of an alternative receptor, *c-met*, which bypasses EGFR blockade and stimulates proliferation (Engelman et al., 2007). Defects in recognition of DNA breaks and overexpression of specific repair enzymes may contribute to resistance to cytotoxic drugs, and a loss of apoptotic pathways can lead to resistance to both cytotoxic and pathway-targeted drugs.

Resistance to immune checkpoint inhibitory drugs appears to follow patterns that are distinct from those of other anticancer drugs, as evidenced by their efficacy in some heavily pretreated patients. One recognized tumor variable of treatment sensitivity is the >30-fold range of the tumor mutational burden (TMB) among patient specimens from a given cancer type (Figure 69–3); TMB is thus used as one marker to assess the likelihood of response or resistance to immunotherapy (Table 69–1). Surprisingly, the composition of the gut microbiome as well as alterations by antibiotic treatment were found to be related to the response of different cancers to immune checkpoint inhibitory drugs, suggesting a potential vulnerability (Finlay et al., 2020; see also Chapter 6). Indeed, recent studies in patients with melanoma suggest that fecal microbiota transplant from appropriate donors may overcome resistance to immunotherapy (Woelk and Snyder, 2021). A major challenge in immunotherapy is the

TABLE 69–1 ■ DIAGNOSTIC TESTS OF CANCER SPECIMENS TO GUIDE TREATMENT DECISIONS

INDIVIDUAL MOLECULAR MARKER (*DNA, mRNA,* PROTEIN)	TARGET: drugs	CANCER INDICATION	CHAPTER
ALK translocation	ALK: alectinib, ceritinib, crizotinib	Lung NSCLC	71
BRAF V600 mutation	BRAF: dabrafenib, vemurafenib	Melanoma	71
BRAF V600 mutation	MEK: trametinib	Melanoma	71
BRCA mutation	PARP: olaparib, rucaparib, talazoparib	Breast, Ovarian, Pancreatic, Prostate	71
EGFR deletion of exon 19 or L858R mutation[a]	EGFR: afatinib, dacomitinib, erlotinib, gefitinib	Lung NSCLC	71
EGFR T790M mutation[a]	EGFR: osimertinib	Lung NSCLC	71
Estrogen receptor (ER) expression	antiestrogens (tamoxifen, raloxifene, fulvestrant) or aromatase inhibitors (anastrozole, letrozole, exemestane)	Breast	73
HER2 amplification; HER2 overexpression	HER2: trastuzumab, ado-trastuzumab emtansine, pertuzumab	Breast, Gastric	71
KRAS wild type	EGFR: cetuximab, panitumumab	Colorectal	71
PD-L1 expression	PD-1, PD-L1: pembrolizumab	Lung NSCLC, etc.	72
Panels of markers			
MSI-H (microsatellite instability high) or dMMR (deficient mismatch repair)	PD-1: pembrolizumab	Solid tumors	72
Tumor mutational burden (TMB) >10 DNA mutations per million bases[b]	PD-1: pembrolizumab	Solid tumors	72
mRNA expression of a panel of genes (Risk of recurrence score)	Chemotherapy	Breast	73
Next Generation DNA sequencing of tumor samples for the presence of gene variants (substitution, insertion, deletion), copy number, rearrangement	Genes sequenced include the above-named targets and their alterations. The respective drugs are listed above	Solid tumors	71

FDA-approved tests continuously updated: www.fda.gov "List of cleared or approved companion diagnostic devices." (Accessed 19 March 2022)

[a] Also detectable as circulating tumor DNA (ctDNA) in blood samples.

[b] See Figure 69–3.

poor predictability, in individual patients, of the immune responses that govern the success of the treatment. mRNA-based vaccines that contain the coding sequences of antigenic mutations detected in a given tumor are the most recently developed approach to elicit an immune response and enhance the efficacy of immune checkpoint inhibitor treatments (Sahin and Türeci, 2018; Sahin et al., 2020). It is noteworthy that this mRNA-based vaccine approach was also used to generate the first successful vaccines against SARS-CoV-2 (COVID-19).

Finally, T cells carrying chimeric antigen receptors (CARs) can be directed against cancer cells that express specific antigens. CARs are engineered to contain an antigen recognition domain of a monoclonal antibody in the extracellular portion and intracellular signaling domains capable of activating T cells independently of the physiological pathway of antigen presentation by an MHC molecule (see Figures 72–6, 38–2, and 38–4). CD19-targeted CAR T cells reached a 70% to 90% response rate in patients with previously treated, relapsed B-cell leukemias (Khalil et al., 2016), indicating a lack of cross-resistance with conventional therapies. This result is consistent with the requirement for effective combination therapies, that is, complementary mechanisms of action and no overlap in major toxicities. Combinations of immune modulating therapies with pathway-targeted drugs and cytotoxic agents are currently evaluated in national and international trials to generate effective treatment combinations (Hughes et al., 2016).

Molecular Testing to Select Appropriate Drugs

Clinical trials and patient treatments increasingly employ results from biomarker analysis to identify patients likely to benefit from particular treatments and individuals at the greatest risk of toxicity. Some of the tests have been FDA-approved as "companion diagnostics" in conjunction with specific drug therapies (see Table 69–1). Pretreatment testing of tumor specimens is standard practice in selecting patients for antihormonal therapy of breast cancer and for treatment with antibodies such as *trastuzumab* (anti-HER2). Detection of a mutated *KRAS* gene indicates that the tumor of a patient with colorectal cancer will not respond to anti-EGFR antibodies; in patients with lung cancers and EGFR mutations, treatment with *erlotinib*, *gefitinib*, or *afatinib* results in response rates of 70%, and in patients with ALK translocations, the response rates are similar for treatment with the ALK inhibitors *crizotinib* and *ceritinib*. A T790M "gatekeeper" mutation in EGFR (Kobayashi et al., 2005) accounts for about 60% of acquired resistance to first- and second-generation inhibitors but is sensitive to *osimertinib*, a third-generation EGFR inhibitor (Thomas et al., 2015). Overall, introduction of molecular analysis and appropriate choice of pathway-targeted inhibitors in the treatment of NSCLC has increased median survival of patients from less than 1 year to over 3 years and the response rate from 30% to 80% (Ke and Wu, 2016).

Inherited differences in protein sequence polymorphisms or levels of RNA expression can also influence toxicity and antitumor response. For example, tandem repeats in the promoter region of the gene encoding thymidylate synthase, the target of *5-fluorouracil*, determine the level of expression of the enzyme. Increased numbers of repeats are associated with increased gene expression, a lower incidence of toxicity, and a decreased rate of response in patients with colorectal cancer (Pullarkat et al., 2001). Polymorphisms of the dihydropyrimidine dehydrogenase gene, the product of which is responsible for degradation of *5-fluorouracil*, are associated with decreased enzyme activity and a significant risk of overwhelming drug toxicity, particularly in the rare individual that is homozygous for the polymorphic genes (Van Kuilenburg et al., 2002).

Gene expression profiling, in which the levels of messenger RNA from thousands of genes are surveyed using gene arrays, has revealed tumor profiles that are highly associated with poor outcomes and that warrant adjuvant chemotherapy (Sotiriou and Pusztai, 2009). As an alternative to this broad analysis, small sets of informative genes can be identified and used clinically. One example is a set of 21 genes used in the analysis of samples from patients with early-stage breast cancer. Based on the known association between the expression pattern of the 21 genes and disease outcomes, the analysis of patient samples can predict the risk of disease relapse. Thus, one can identify the patients at a high risk who will benefit from adjuvant chemotherapy (Paik et al., 2004).

Molecular Analysis and Tumor Heterogeneity

One of the caveats to conclusions drawn from the molecular analysis of tumor tissue specimens is the dynamic evolution of cancers noted above (see Figure 69–3). Clinically important mutations in subclones may be missed due to geographically inadequate sampling and may provide the wrong guidance to treatment decisions. Treatment responses of different subpopulations in a tumor or in different metastatic lesions present a further challenge and would require multiple-site tissue biopsies (Shi et al., 2014). The molecular analysis of serially collected blood samples ("liquid biopsies") provides an alternative approach for treatment monitoring.

Liquid Biopsies

More recent technology advances have made it possible to sequence and quantitate circulating, tumor-derived DNA (ctDNA) in blood samples from patients with cancer ("liquid biopsies"), as first demonstrated for the changing abundance of mutant *KRAS* during colon cancer treatment (Diehl et al., 2008). The analysis of ctDNA has shown that during antiestrogen therapy of breast cancer the appearance of mutant estrogen receptor coincides with subsequent resistance to aromatase inhibitor treatment (Schiavon et al., 2015). Furthermore, mutant *KRAS* DNA in the circulation was increased during the treatment of patients with colon cancer with EGFR antibodies but surprisingly reverted to baseline values after cessation of the treatment. This observation demonstrates the dynamic evolution of cancer subpopulations during drug treatment, as indicated in Figure 69–3 (Siravegna et al., 2015). As a consequence of these technological developments, the FDA approved a test for the presence of mutant *EGFR* DNA in blood samples of patients with NSCLC to select candidates for treatment with *erlotinib* or *osimertinib* and thus circumvent the need for a tissue biopsy. The incorporation of liquid biopsies into treatment monitoring can provide additional molecular insights into drug efficacy and adverse effects and reveal the onset of resistance to a chosen treatment (Kilgour et al., 2020).

Achieving Therapeutic Integration and Efficacy

The clinical benefit of cytotoxic drugs has primarily been measured by radiological assessment of drug effects on tumor size. Pathway-targeted agents, however, may only slow or halt tumor growth, so their effects may be measured in the assessment of time to disease progression; however, for some immune checkpoint inhibitors, tumor lesions may initially increase in size due to cytotoxic lymphocyte infiltration, so called pseudo-progression. Thus, one of the great challenges is to assess efficacy and adjust drug regimens to achieve a therapeutic but nontoxic outcome. Treatment of patients with cancer requires a skillful interdigitation of pharmacotherapy with other modalities of treatment (e.g., surgery and radiation). Each treatment modality carries its own risks and benefits, with the potential for both antagonistic and synergistic interactions between modalities, particularly between drug treatment and radiation.

Individual patient characteristics determine the choice of modalities. Not all patients can tolerate drugs of the primary choice, and not all drug regimens are appropriate for a given patient. Renal and hepatic function, bone marrow reserve, general performance status, and concurrent medical problems all come into consideration in making a therapeutic plan. Other less-quantifiable considerations, such as the natural history of the tumor, the patient's willingness to undergo difficult and potentially dangerous treatments, and the patient's physical and emotional tolerance for adverse effects enter the equation, with the goal of balancing the likely long-term gains and risks in the individual patient. In particular, the long-term adverse effects of cytotoxic drugs have been related to the induction of cellular senescence in different organs that resemble symptoms of premature aging and can adversely affect organ function and the overall well-being of patients long after completion of the treatments (Childs et al., 2015; Couzin-Frankel, 2019). The choice of treatment regimen should take all of this into account. Finally, in terminally ill patients, the treatment choices must be weighed carefully; the maximal length and highest quality of life may be achieved with palliative care and may reduce the need for chemotherapy (Temel et al., 2010).

A Cautionary Note

Although advances in drug discovery and molecular profiling of tumors offer great promise for improving the outcomes of cancer treatment, a final word of caution regarding all treatment regimen deserves emphasis: *The pharmacokinetics and toxicities of cancer drugs vary amongst individual patients.* It is imperative to *recognize toxicities early*, to *alter doses or discontinue offending medication* to relieve symptoms and reduce risk, and to *provide vigorous supportive care*. Toxicities affecting the heart, lungs, nervous system, or kidneys may be irreversible if recognized late in their course, leading to permanent organ damage or death. Fortunately, such toxicities can be minimized by early recognition and by adherence to standardized protocols and to the guidelines for each drug's use.

A Note on Treatment Regimens

Cancer treatment regimens change to reflect continuous advances in basic and clinical science: new drugs, both small molecules and biologicals; improved methods of targeting and timing of drug delivery; agents with altered pharmacokinetic properties and selectivities; the use of rational multidrug combinations; and greater knowledge of the basic cell biology of tumorigenesis, metastasis, and immune function, among other advances. As a consequence, this chapter and the four that follow present relatively few detailed treatment regimens; rather, we refer the reader to the web-based resources of the U.S. FDA and the National Comprehensive Cancer Network (NCCN). Table 71–1 provides examples that illustrate the complexities of current therapeutic regimens for two cancers.

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Chapter 70

Cytotoxics and Antimetabolites

Anton Wellstein and Edward A. Sausville

I. ALKYLATING AGENTS AND PLATINUM COORDINATION COMPLEXES

ACTIONS COMMON TO ALKYLATING DRUGS
- Structure-Activity Relationships
- General Pharmacological Actions
- Mechanisms of Resistance to Alkylating Drugs
- Adverse Effects of Alkylating Drugs

CLINICAL PHARMACOLOGY OF NITROGEN MUSTARDS
- Mechlorethamine
- Cyclophosphamide
- Ifosfamide
- Melphalan
- Chlorambucil
- Bendamustine

ETHYLENEIMINES AND METHYLMELAMINES
- Altretamine
- Thiotepa

ALKYL SULFONATES
- Busulfan

NITROSOUREAS
- Carmustine (BCNU) and Lomustine (CCNU)
- Streptozocin

TRIAZENES
- Dacarbazine (DTIC)
- Temozolomide

METHYLHYDRAZINES
- Procarbazine

PLATINUM COORDINATION COMPLEXES
- Mechanisms of Action
- Resistance to Platinum Analogues
- Cisplatin
- Carboplatin
- Oxaliplatin

II. ANTIMETABOLITES

FOLIC ACID ANALOGUES
- Mechanism of Action
- Selective Toxicity; Rescue
- Cellular Entry and Retention
- Newer Congeners
- Mechanisms of Resistance to Antifolates
- Therapeutic Uses
- Adverse Effects

PYRIMIDINE ANALOGUES
- Cellular Actions of Pyrimidine Antimetabolites
- Fluorouracil, Floxuridine, Capecitabine
- Trifluridine

CYTIDINE ANALOGUES
- Cytarabine (Cytosine Arabinoside; Ara-C)
- Azacitidine (5-Azacytidine); Decitabine
- Gemcitabine

PURINE ANALOGUES
- 6-Thiopurine Analogues
- Fludarabine Phosphate
- Cladribine
- Clofarabine (2-Chloro-2′-fluoro-arabinosyladenine)
- Nelarabine (6-Methoxy-arabinosyl-guanine)
- Pentostatin (2′-Deoxycoformycin)

III. NATURAL PRODUCTS

MICROTUBULE-DAMAGING AGENTS
- Vinca Alkaloids
- Eribulin
- Taxanes
- Estramustine
- Epothilones

CAMPTOTHECIN ANALOGUES
- Chemistry
- Mechanism of Action
- Mechanisms of Resistance
- Topotecan
- Irinotecan

ANTIBIOTICS
- Dactinomycin (Actinomycin D)
- Anthracyclines and Anthracenediones

EPIPODOPHYLLOTOXINS
- Podophyllotoxin Derivatives
- Etoposide
- Teniposide

TRABECTEDIN ANALOGUES
- Trabectedin
- Lurbinectedin

DRUGS OF DIVERSE MECHANISMS OF ACTION
- Bleomycin
- Mitomycin
- Mitotane
- L-Asparaginase
- Hydroxyurea
- Retinoids
- Arsenic Trioxide

Abbreviations

ABVD: ***a***driamycin (doxorubicin), ***b***leomycin, ***v***inblastine, ***d***acarbazine
ADA: adenosine deaminase
ALL: acute lymphoblastic leukemia
AML: acute myeloid leukemia; acute myelocytic leukemia
APL: acute promyelocytic leukemia
Ara-C: cytarabine, cytosine arabinoside
Ara-U: ara-uridine
L-ASP: L-asparaginase
ATO: arsenic trioxide
ATRA: all-*trans* retinoic acid
AUC: area under the curve
BCNU: carmustine [1,3-*bis*-(2-chloroethyl)-1-nitrosourea]
BCRP: breast cancer resistance protein
CCNU: 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (lomustine)
CDK: cyclin-dependent kinase
CHOP: ***c***yclophosphamide, doxorubicin (***H***), vincristine (***O***), and ***p***rednisone
CL_{Cr}: creatinine clearance
CLL: chronic lymphocytic leukemia
CML: chronic myelocytic leukemia; chronic myelogenous leukemia
CSF: cerebrospinal fluid
CTCL: cutaneous T-cell lymphoma
dCK: deoxycytidine kinase
dFdC: 2′,2′-difluorodeoxycytidine, gemcitabine
5′-dFdU: 5′-deoxyfluorodeoxyuridine
DHFR: dihydrofolate reductase
DPD: dihydropyrimidine dehydrogenase
FdUMP: fluorodeoxyuridine monophosphate
FOLFIRINOX: FOLFOX plus irinotecan
FOLFOX: *fol*inic acid, 5*F*U and *ox*aliplatin
5FU: 5-fluorouracil
FUdR: fluorodeoxyuridine or floxuridine or 5-FUdR
GART: glycinamide ribonucleotide transformylase
G-CSF: granulocyte colony-stimulating factor
HbF: hemoglobin (fetal)
HDM-L: high-dose MTX with leucovorin rescue
HGPRT: hypoxanthine guanine phosphoribosyl transferase
HMG: high-mobility group
HRD: homologous recombination DNA repair
HU: hydroxyurea
IMP: inosine monophosphate
MESNA: 2-***m***ercapto***e***thane***s***ulfonate-***Na***$^+$
MGMT: ***O***^{6}-alkyl, ***m***ethyl ***g***uanine ***m***ethyl***t***ransferase
MMR: mismatch repair
MOPP: nitrogen ***m***ustard, ***o***ncovin (vincristine), ***p***rocarbazine, and ***p***rednisone
6MP: 6-mercaptopurine
MRP: multidrug resistance-associated protein
MTIC: methyl-triazeno-imidazole-carboxamide
MTX: methotrexate
Nab-paclitaxel: albumin-bound nanoparticle solution of paclitaxel
NER: nucleotide excision repair
PG: polyglutamate
Pgp: P-glycoprotein
PML: promyelocytic leukemia
PRPP: 5-phosphoribosyl-1-pyrophosphate
RAR: retinoic acid receptor
RNR: ribonucleoside diphosphate reductase
ROS: reactive oxygen species
TEM: triethylenemelamine
TEPA: triethylenephosphoramide
6TG: 6-thioguanine
Thiotepa: triethylenethiophosphoramide
TPMT: thiopurine methyltransferase
TS: thymidylate synthase
TTP: thymidine triphosphate
VOD: veno-occlusive disease

A Note on Treatment Regimens

Cancer treatment regimens change to reflect continuous advances in basic and clinical science: new drugs, both small molecules and biologicals; improved methods of targeting and timing of drug delivery; agents with altered pharmacokinetic properties and selectivities; the use of rational multidrug combinations; and greater knowledge of the basic cell biology of tumorigenesis, metastasis, and immune function, among other advances. As a consequence, this chapter presents relatively few detailed treatment regimens; rather, we refer the reader to the web-based resources of the U.S. FDA and the National Comprehensive Cancer Network (NCCN.org). Table 71–1 provides two examples of therapeutic regimens that illustrate the complexity of current cancer drug therapy.

I. Alkylating Agents and Platinum Coordination Complexes

HISTORY OF ALKYLATING ANTICANCER DRUGS

The discovery and initial development of alkylating anticancer drugs are based on observations of the effects of chemical warfare in World War I (Chabner and Roberts, 2005). The pervasively toxic sulfur mustard gas that caused topical burns to skin, eyes, lungs, and mucosa also caused aplasia of the bone marrow and lymphoid tissue and ulceration of the GI tract (Krumbhaar, 1919). In 1942, Louis Goodman and Alfred Gilman, the originators of this text, demonstrated the activity of nitrogen mustards against mouse lymphoma. Their clinical studies of intravenous nitrogen mustards in patients with lymphoma launched the modern era of cancer chemotherapy (Gilman and Philips, 1946).

Actions Common to Alkylating Drugs

Six major types of alkylating agents are used in the chemotherapy of cancer: *nitrogen mustards*, *ethyleneimines*, *alkyl sulfonates*, *nitrosoureas*, *triazenes*, and *methylhydrazines*. Because of similarities in their mechanisms of action and resistance, *platinum complexes* are also discussed with classical alkylating agents, even though they do not alkylate DNA but instead form covalent metal adducts with DNA.

The chemotherapeutic alkylating agents have in common the property of forming highly reactive carbonium ion intermediates. These reactive intermediates covalently link to sites of high electron density, such as phosphates, amines, sulfhydryl, and hydroxyl groups. Their chemotherapeutic and cytotoxic effects are directly related to the alkylation of reactive amines, oxygens, or phosphates on DNA. The N7 atom of guanine is particularly susceptible to the formation of a covalent bond with bifunctional alkylating agents and may represent the key target that determines

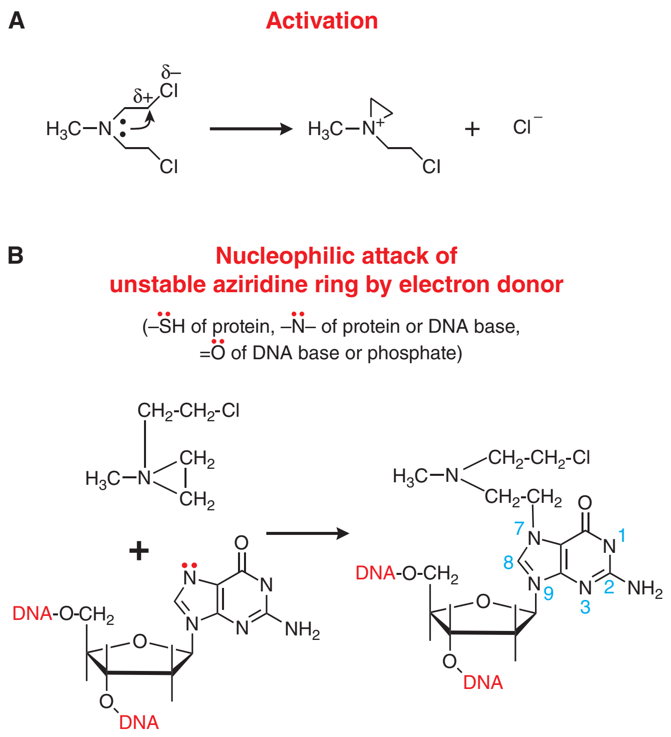

A
Activation
δ–
δ+
Cl
H_3C-N
Cl
H_3C-N^+
Cl
+
Cl^-
B
Nucleophilic attack of unstable aziridine ring by electron donor
(–SH of protein, –N– of protein or DNA base, =O of DNA base or phosphate)
CH_2-CH_2-Cl
H_3C-N
CH_2
CH_2
+
DNA-O-CH_2
O
DNA
N
N
N
N
O
NH_2
H_3C-N
CH_2-CH_2-Cl
CH_2-CH_2
7
8
9
1
2
3
NH_2
DNA-O-CH_2
O
DNA

Figure 70–1 *Mechanism of action of alkylating agents of mechlorethamine, a prototypic nitrogen mustard.* **A.** *Activation reaction.* **B.** *Alkylation of N7 of guanine.* With bifunctional drugs such as nitrogen mustards (see Figure 70–2), the second 2-chloroethyl side chain can undergo a similar activation cross-link of two nucleic acid chains or a nucleic acid to a protein.

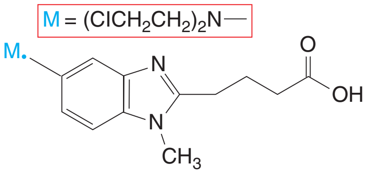
their biological effects (Figure 70–1). Other atoms in the purine and pyrimidine bases of DNA, including N1 and N3 of the adenine ring, N3 of cytosine, and O6 of guanine, react with these agents, as do the amino and sulfhydryl groups of proteins and the sulfhydryls of glutathione. The general mechanisms of alkylating agents on DNA are illustrated in Figure 70–1 using *mechlorethamine* (nitrogen mustard).

With the bifunctional alkylating agents such as nitrogen mustard and its derivatives that are used therapeutically (Figure 70–2), the second 2-chloroethyl side chain can undergo a similar cyclization reaction and alkylate a second guanine residue or another nucleophilic moiety, resulting in the cross-linking of two nucleic acid chains or the linking of a nucleic acid to a protein, alterations that cause a major disruption in nucleic acid function. Any of these effects can contribute to both the mutagenic and the cytotoxic effects of alkylating agents. The extreme cytotoxicity of the bifunctional alkylators correlates closely with interstrand cross-linkage of DNA.

The ultimate cause of cancer cell death related to drug-induced DNA damage is not known. However, cancer cells depend on mitogenic lesions that drive uncontrolled cell cycle progression but also bring them substantially closer to the threshold at which apoptosis can be triggered by cytotoxic drugs. This contrasts with normal cells that receive their growth and survival cues from their orthotopic environment keeping them far above the apoptosis threshold (Lowe et al., 2004). Genomic instability and the consequent acquisition of tumor mutations are recognized as "enablers" of the neoplastic phenotype (Hanahan and Weinberg, 2011). Such defects in DNA repair defects can render cancer cells more susceptible to death after exposure to DNA-damaging drugs (Costa de Almeida et al., 2021). Specific cellular responses to DNA-damaging agents include cell-cycle arrest and attempts to repair DNA. For example, faulty DNA damage repair mechanisms and mutations of p53 can lead to resistance to alkylating drugs (Kastan, 1999). The process of recognizing and repairing DNA generally requires an intact nucleotide excision repair (NER) complex but may differ with each drug and with each tumor. The specific repair enzyme complex utilized will depend on two factors: the chemistry of the adduct formed and the repair capacity of the cell involved. For example, defects in homologous recombination DNA repair (HRD) can convey relative sensitivity to platinum agents (Stover et al., 2020), and characterization of HRD present in clinical tumors has entered clinical practice as a means of selecting patients who might benefit from specific chemotherapeutic agents.

Structure-Activity Relationships

Although alkylating agents share the capacity to alkylate biologically important molecules, modification of the basic backbone structure changes reactivity, lipophilicity, active transport across biological membranes, sites of macromolecular attack, and mechanisms of DNA repair, all of which determine drug activity *in vivo*. Several of the most used agents (e.g., *cyclophosphamide* and ifosfamides) are prodrugs, and the active alkylating moieties are generated *in vivo* through hepatic metabolism (Figure 70–3) (Arnold et al., 1958; Wagner, 1994).


M = $(ClCH_2CH_2)_2N$—
M.
N
N
CH_3
O
OH

BENDAMUSTINE

The more recently approved alkylating agent, ***bendamustine***, differs from the other cross-linking agents by having the typical chloroethyl reactive groups in nitrogen mustard (M) attached to a benzimidazole backbone. The unique properties and activity of this drug may derive from this purine-like structure; the agent produces slowly repaired DNA cross-links, lacks cross-resistance with other classical alkylators (Leoni et al., 2008), and is approved for treatment of chronic lymphocytic leukemia (CLL) and large-cell lymphomas refractory to standard alkylators. Newer investigational alkylating agents include *fotemustine*, which is approved in Europe, and *evofosfamide,* a hypoxia-activated prodrug (Yan et al., 2020).

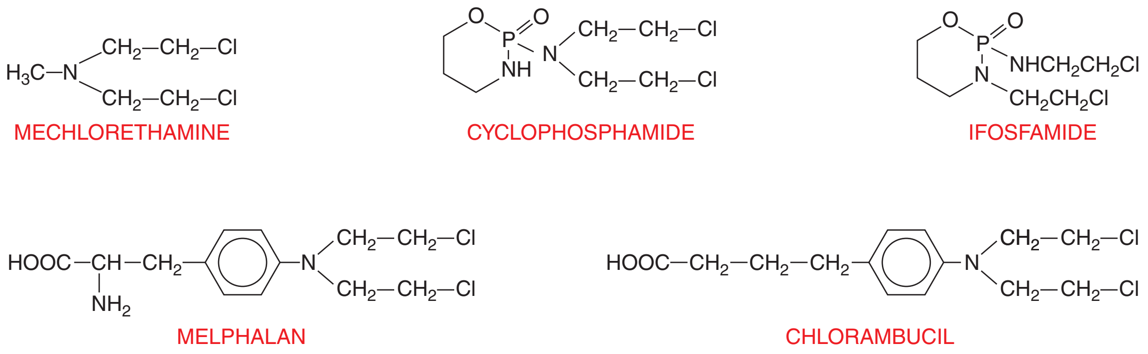

H_3C-N
CH_2-CH_2-Cl
CH_2-CH_2-Cl
MECHLORETHAMINE
O
P
O
NH
N
CH_2-CH_2-Cl
CH_2-CH_2-Cl
CYCLOPHOSPHAMIDE
O
P
O
N
$NHCH_2CH_2Cl$
CH_2CH_2Cl
IFOSFAMIDE
HOOC—CH—CH_2
NH_2
N
CH_2-CH_2-Cl
CH_2-CH_2-Cl
MELPHALAN
HOOC—$CH_2-CH_2-CH_2$
N
CH_2-CH_2-Cl
CH_2-CH_2-Cl
CHLORAMBUCIL

Figure 70–2 *Nitrogen mustards employed in therapy.*

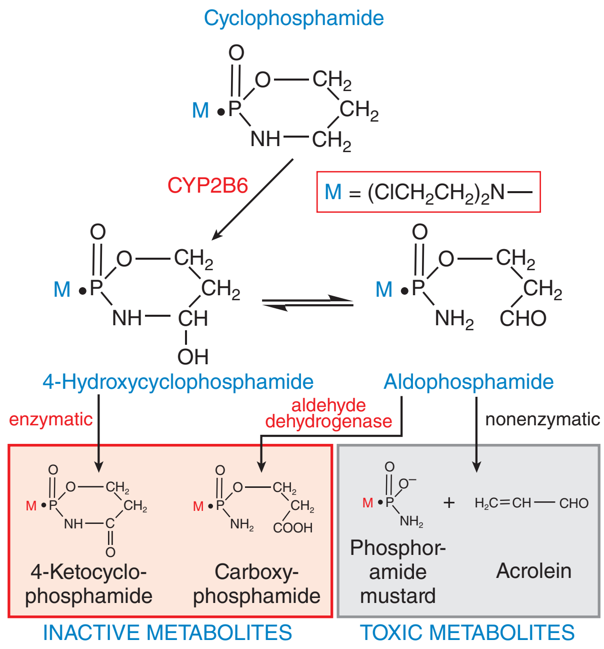


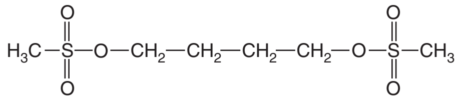
Figure 70–3 *Activation and metabolism of cyclophosphamide.* *Cyclophosphamide* is activated (hydroxylated) by CYP2B6, with subsequent transport of the activated intermediate to the sites of action. The selectivity of *cyclophosphamide* for some cancer types results from the capacity of normal tissues to degrade the activated intermediates via aldehyde dehydrogenase, glutathione transferase, and other pathways and the lack thereof in cancer cells. *Ifosfamide* is structurally similar to *cyclophosphamide* (see Figure 70–2); whereas *cyclophosphamide* has two chloroethyl groups on the exocyclic nitrogen atom, one of the 2-chloroethyl groups of *ifosfamide* is on the cyclic phosphoramide nitrogen of the oxazaphosphorine ring. *Ifosfamide* is activated by hepatic CYP3A4 and proceeds more slowly than activation of *cyclophosphamide*, with greater production of dechlorinated metabolites and chloroacetaldehyde. These differences in metabolism likely account for the higher doses of *ifosfamide* required for equitoxic effects, the greater neurotoxicity of *ifosfamide*, and perhaps differences in the antitumor spectra of *cyclophosphamide* and *ifosfamide*.

One class of alkylating agents transfers methyl rather than ethyl groups to DNA: The *triazene* derivative 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide, usually called *dacarbazine* or DTIC, is prototypical of methylating agents. *Dacarbazine* requires initial activation by hepatic CYPs through an *N*-demethylation reaction. In the target cell, spontaneous cleavage of the metabolite, methyl-triazeno-imidazole-carboxamide (MTIC), yields an alkylating moiety, a methyl diazonium ion. A related triazene, *temozolomide*, undergoes spontaneous, nonenzymatic activation to MTIC. It is able to cross the blood-brain barrier and has been shown to have significant activity against gliomas.

The *nitrosoureas,* which include compounds such as 1,3-*bis*-(2-chloroethyl)-1-nitrosourea (*carmustine* [BCNU]), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (*lomustine* [CCNU]), and its methyl derivative (*semustine* [methyl-CCNU]), as well as the antibiotic *streptozocin*, exert their cytotoxicity through the spontaneous breakdown to an alkylating intermediate, the 2-chloroethyl diazonium ion. As with the nitrogen mustards, interstrand DNA cross-linking appears to be the primary lesion responsible for the cytotoxicity of nitrosoureas. The reactions of the nitrosoureas with macromolecules are shown in Figure 70–4. The use of nitrosoureas is presently limited to brain tumors due to their lipophilicity, except for *streptozocin*, which is uniquely useful in pancreatic islet cell tumors, perhaps reflecting the selective uptake of the carbohydrate portion of the drug into these tumor cells.

Stable ethyleneimine derivatives have antitumor activity; *triethylenemelamine* (TEM) and *thiotepa* have been used clinically. In standard doses, *thiotepa* produces mostly myelosuppression; it is used for high-dose chemotherapy regimens in transplants for hematological malignancies, in which it causes both mucosal and CNS toxicity. *Altretamine* (hexamethylmelamine, HMM) is chemically similar to TEM and has been used to treat ovarian cancer. The methylmelamines are *N*-demethylated by hepatic microsomes with the release of formaldehyde; there is a direct relationship between the degree of the demethylation and their antitumor activity in model systems.

Esters of alkane sulfonic acids alkylate DNA through the release of methyl radicals. Busulfan is of value in high-dose chemotherapy.



$$H_3C-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-O-CH_2-CH_2-CH_2-CH_2-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-CH_3$$

BUSULFAN

General Pharmacological Actions

Cytotoxic Actions

The capacity of alkylating agents to interfere with DNA integrity and function and to induce cell death in rapidly proliferating tissues provides the basis for their therapeutic and toxic properties. Acute effects manifest primarily against rapidly proliferating tissues; however, certain alkylating agents may have damaging effects on tissues with normally low mitotic indices (e.g., liver, kidney, and mature lymphocytes); effects in these tissues usually are delayed. Lethality of DNA alkylation depends on the recognition of the adduct, the creation of DNA strand breaks by repair enzymes, and an intact apoptotic response. In nondividing cells, DNA damage activates a checkpoint that depends on the presence of a normal *p53* gene. Cells thus blocked in the G_1/S interface either repair DNA alkylation or undergo apoptosis. Malignant cells with mutant or absent *p53* fail to suspend cell-cycle progression, do not undergo apoptosis, and can exhibit resistance to these drugs.

Distinction Between Mono- and Bifunctional Agents

Although DNA is the ultimate target of all alkylating agents, there is a crucial distinction between the bifunctional agents, in which cytotoxic

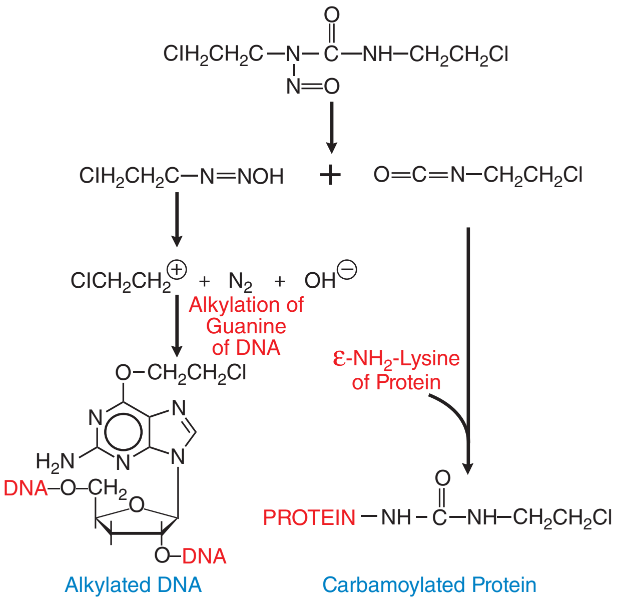


Figure 70–4 *Generation of alkylating and carbamylating intermediates from carmustine (BCNU).* The 2-chloroethyl diazonium ion, a strong electrophile, can alkylate guanine, cytidine, and adenine bases. Displacement of the halogen atom can then lead to interstrand or intrastrand cross-linking of DNA. The formation of cross-links after the initial alkylation reaction proceeds slowly and can be reversed by the DNA repair enzyme MGMT, which displaces the chloroethyl adduct from its binding to guanine in a suicide reaction. The same enzyme, when expressed in human gliomas, produces resistance to nitrosoureas and to other methylating agents, including *dacarbazine*, *temozolomide*, and *procarbazine*.

effects predominate, and the monofunctional methylating agents (*procarbazine*, *temozolomide*), which have greater capacity for mutagenesis and carcinogenesis. This suggests that the cross-linking of DNA strands represents a much greater threat to cellular survival than do other effects, such as single-base alkylation and the resulting depurination and single-chain scission. Conversely, simple methylation may be bypassed by DNA polymerases, leading to mispairing reactions that permanently modify a DNA sequence. These new sequences are transmitted to subsequent generations and may result in mutagenesis or carcinogenesis. Some methylating agents, such as *procarbazine*, are highly carcinogenic.

Adduct recognition systems and DNA repair systems play important roles in removing adducts and thereby determine the selectivity of action against particular cell types and the acquisition of resistance to alkylating agents. Alkylation of a single strand of DNA (monoadducts) is repaired by the NER pathway. The less frequent cross-links require participation of nonhomologous end joining, an error-prone pathway, or the potentially error-free HRD pathway. After drug infusion in humans, monoadducts appear rapidly and peak within 2 h of drug exposure, while cross-links peak at 8 h. The $t_{1/2}$ for repair of adducts varies amongst normal tissues and tumors; in peripheral blood mononuclear cells, both monoadducts and cross-links disappear with a $t_{1/2}$ of 12 to 16 h.

The repair process depends on the presence and accurate functioning of multiple proteins. Their absence or mutation, as in Fanconi anemia or ataxia telangiectasia, leads to extreme sensitivity to DNA cross-linking agents such as *mitomycin*, *cisplatin*, or classical alkylators. Other repair enzymes are specific for removing methyl and ethyl adducts from the O6 of guanine (methyl guanine methyltransferase [MGMT]) and for repair of alkylation of the N3 of adenine and N7 of guanine (3-methyladenine-DNA glycosylase). High expression of MGMT protects cells from cytotoxic effects of nitrosoureas and methylating agents and confers drug resistance, while methylation and silencing of the MGMT gene in brain tumors are associated with clinical response to BCNU and *temozolomide* (Hegi et al., 2008). *Bendamustine* differs from classical chloroethyl alkylators in activating base excision repair, rather than the more complex double-strand break repair or MGMT. It impairs physiological arrest of adduct-containing cells at mitotic checkpoints, leads to mitotic catastrophe rather than apoptosis, and does not require an intact p53 to cause cytotoxicity.

Recognition of DNA adducts is an essential step in promoting attempts at repair and ultimately leading to apoptosis. The Fanconi pathway, consisting of 12 proteins, recognizes adducts and signals the need for repair of a broad array of DNA-damaging drugs and irradiation. Absence or inactivation of components of this pathway leads to increased sensitivity to DNA damage. Conversely, for the methylating drugs, nitrosoureas, *cisplatin* and *carboplatin*, and thiopurine analogues, the mismatch repair (MMR) pathway is essential for cytotoxicity, causing strand breaks at sites of adduct formation, creating mispairing of thymine residues, and triggering apoptosis.

Mechanisms of Resistance to Alkylating Drugs

Resistance to an alkylating agent develops rapidly when it is used as a single agent. Specific biochemical changes implicated in the development of resistance include the following:

- Decreased permeation of actively transported drugs (e.g., *mechlorethamine* and *melphalan*)
- Increased intracellular concentrations of nucleophilic substances, principally thiols such as glutathione, which can conjugate with and detoxify electrophilic intermediates
- Increased activity of DNA repair pathways, which may differ for the various alkylating agents
- Increased rates of metabolic degradation of the activated forms of *cyclophosphamide* and *ifosfamide* to their inactive keto and carboxy metabolites by aldehyde dehydrogenase (see Figure 70–3) and detoxification of most alkylating intermediates by glutathione transferases
- Loss of ability to recognize adducts formed by nitrosoureas and methylating agents, as the result of defective MMR protein capability, confers resistance, as does defective cell-cycle checkpoint function, for virtually all alkylating drugs
- Impaired apoptotic pathways, with overexpression of bcl-2 as an example, confer resistance

Adverse Effects of Alkylating Drugs

Alkylating agents differ in their patterns of the tissue specificity and severity of their adverse effects, which are summarized next.

Bone Marrow

Most alkylating agents cause dose-limiting toxicity to marrow cells and, to a lesser extent, intestinal mucosa. Most alkylating agents (i.e., *melphalan*, *chlorambucil*, *cyclophosphamide*, and *ifosfamide*) cause myelosuppression, with a nadir of the peripheral blood granulocyte count at 6 to 10 days and recovery in 14 to 21 days. *Cyclophosphamide* has lesser effects on peripheral blood platelet counts than do the other agents. *Busulfan* suppresses all blood elements, particularly stem cells, and may produce a prolonged and cumulative myelosuppression lasting months or even years. For this reason, it is used as a preparative regimen in allogenic bone marrow transplantation. *Temozolomide*, *carmustine*, and other chloroethylnitrosoureas cause delayed and prolonged suppression of both platelets and granulocytes, reaching a nadir 4 to 6 weeks after drug administration and reversing slowly thereafter. Both cellular and humoral immunity are suppressed by alkylating agents, which have, therefore, been used to treat various autoimmune diseases. Immunosuppression is reversible at usual doses, but opportunistic infections may occur with extended treatment.

Mucosa, Hair Follicles

Alkylating agents are highly toxic to dividing mucosal cells and to hair follicles, leading to oral mucosal ulceration, intestinal denudation, and alopecia. Mucosal effects are particularly damaging in high-dose chemotherapy protocols associated with bone marrow transplantation, as they predispose to bacterial sepsis arising from the GI tract. In these protocols, *cyclophosphamide*, *melphalan*, and *thiotepa* have the advantage of causing less mucosal damage than the other agents. In high-dose protocols, however, other toxicities become limiting (Table 70–1).

Nervous System

Nausea and vomiting commonly follow administration of nitrogen mustard or BCNU. *Ifosfamide* is the most neurotoxic of the alkylating agents

TABLE 70–1 ■ DOSE-LIMITING EXTRAMEDULLARY TOXICITIES OF SINGLE ALKYLATING AGENTS

DRUG	MTD[a] mg/m^2	FOLD INCREASE OVER STANDARD DOSE	MAJOR ORGAN TOXICITIES
Cyclophosphamide	7000	7	Cardiac, hepatic VOD
Ifosfamide	16,000	2.7	Renal, CNS, hepatic VOD
Thiotepa	1000	18	GI, CNS, hepatic VOD
Melphalan	180	5.6	GI, hepatic VOD
Busulfan	640	9	GI, hepatic VOD
Carmustine (BCNU)	1050	5.3	Lung, hepatic VOD
Cisplatin	200	2	PN, renal
Carboplatin	2000	5	Renal, PN, hepatic VOD

GI, gastrointestinal; PN, peripheral neuropathy; VOD, veno-occlusive disease.

[a] Maximum tolerated dose (cumulative) in treatment protocols.

and may produce altered mental status, coma, generalized seizures, and cerebellar ataxia. These side effects result from the release of chloroacetaldehyde from the phosphate-linked chloroethyl side chain of *ifosfamide*. High-dose *busulfan* can cause seizures in up to 10% of patients without prophylaxis.

Other Organs

All alkylating agents, including *temozolomide*, have caused pulmonary fibrosis, usually several months after treatment. In high-dose regimens, particularly those employing *busulfan* or BCNU, vascular endothelial damage may precipitate veno-occlusive disease (VOD) of the liver (see Table 70–1), an often-fatal side effect that is successfully reversed by *defibrotide*. *Defibrotide* is an oligonucleotide mixture with profibrinolytic properties that is FDA-approved for the treatment of adult and pediatric patients with hepatic VOD but may cause hemorrhage as an adverse effect. The nitrosoureas and *ifosfamide*, after multiple cycles of therapy, may lead to renal failure. *Cyclophosphamide* and *ifosfamide* release a nephrotoxic and urotoxic metabolite, acrolein, which causes severe hemorrhagic cystitis in high-dose regimens. This adverse effect can be prevented by coadministration of MESNA (2-mercaptoethanesulfonate-Na^+), which conjugates acrolein in urine. *Ifosfamide* in high doses for hematopoietic stem cell transplant causes a chronic, and often irreversible, renal toxicity; its nephrotoxicity correlates with the total dose of drug received and increases in frequency in children younger than 5 years. The syndrome may be due to chloroacetaldehyde or acrolein excreted in the urine.

The more unstable alkylating agents (e.g., *mechlorethamine* and the nitrosoureas) have strong vesicant properties, damage veins with repeated use, and, if extravasated, produce ulceration. All alkylating agents have toxic effects on the male and female reproductive systems, causing an often-permanent amenorrhea, particularly in perimenopausal women, and an irreversible azoospermia in men.

Leukemogenesis

Alkylating agents have a high capacity to induce leukemia. Acute nonlymphocytic leukemia, induced by treatment, is often associated with a higher incidence of p53 mutations, partial or total deletions of chromosome 5 or 7, and reduced response to chemotherapy. Incidence peaks about 4 years after therapy and may affect up to 5% of patients treated on regimens containing alkylating drugs. Leukemia often is preceded by a period of neutropenia or anemia and by bone marrow morphology consistent with myelodysplasia. *Melphalan*, the nitrosoureas, and the methylating agent *procarbazine* have the greatest propensity to cause leukemia, which is less common after *cyclophosphamide*.

Clinical Pharmacology of Nitrogen Mustards

Mechlorethamine

Mechlorethamine HCl was the first clinically used nitrogen mustard and is the most reactive of the drugs in this class. It is used topically for treatment of cutaneous T-cell lymphoma (CTCL) as a solution that is rapidly mixed and applied to affected areas. It has been largely replaced by *cyclophosphamide*, *melphalan*, and other more stable alkylating agents.

Cyclophosphamide

ADME

Cyclophosphamide is well absorbed orally and is activated to the 4-hydroxy intermediate (see Figure 70–3). Its rate of metabolic activation exhibits significant interpatient variability and increases with successive doses in high-dose regimens but appears to be saturable at concentrations of the parent compound greater than 150 μM. 4-Hydroxycyclophosphamide may be oxidized further by aldehyde oxidase, either in liver or in tumor tissue, to inactive metabolites. 4-Hydroxycyclophosphamide and its tautomer, aldophosphamide, travel in the circulation to tumor cells, where aldophosphamide cleaves spontaneously, generating stoichiometric amounts of phosphoramide mustard and acrolein. Phosphoramide mustard is responsible for antitumor effects, while acrolein causes hemorrhagic cystitis often seen during therapy with *cyclophosphamide*.

Pretreatment with CYP inducers such as *phenobarbital* enhances the rate of activation of the azoxyphosphorenes but does not alter total exposure to active metabolites over time and does not affect toxicity or efficacy. *Cyclophosphamide* can be used in full doses in patients with renal dysfunction because it is eliminated by hepatic metabolism. Patients with significant hepatic dysfunction should receive reduced doses. Maximal plasma concentrations are achieved about 1 h after oral administration; the $t_{1/2}$ of parent drug in plasma is about 7 h.

Therapeutic Uses

Cyclophosphamide is administered orally or intravenously. Recommended doses vary widely, and standard protocols for determining the schedule and doses of *cyclophosphamide* in combination with other chemotherapeutic agents should be consulted. The neutrophil nadir of 500 to 1000 cells/mm^3 generally serves as a lower limit for dosage adjustments in prolonged therapy.

The clinical spectrum of activity for *cyclophosphamide* is broad. It is an essential component of many effective drug combinations for non-Hodgkin lymphomas, other lymphoid malignancies, breast and ovarian cancers, and solid tumors in children. Complete remissions have been reported when *cyclophosphamide* was given as a single agent for Burkitt lymphoma. It frequently is used in combination with *doxorubicin* and a taxane as adjuvant therapy after surgery for breast cancer. Because of its potent immunosuppressive properties, *cyclophosphamide* has been used to treat autoimmune disorders, including Wegener granulomatosis, rheumatoid arthritis, and the nephrotic syndrome. Caution is advised when the drug is considered for nonneoplastic conditions, not only because of its toxic effects but also because of its potential for inducing premature menopause, sterility, teratogenic effects, and leukemia.

Adverse Effects

Phosphoramide mustard is responsible for antitumor effects, while acrolein causes hemorrhagic cystitis often seen during therapy with *cyclophosphamide*. Patients should receive vigorous intravenous hydration during high-dose treatment. Hematuria in a patient receiving daily oral therapy should lead to immediate drug discontinuation. Refractory bladder hemorrhage can become life-threatening and may require cystectomy for control of bleeding. Inappropriate secretion of antidiuretic hormone has been observed (usually at doses greater than 50 mg/kg) and can lead to water intoxication because these patients usually are vigorously hydrated. In addition to the cystitis (counteracted by MESNA and diuresis), GI, pulmonary, renal, hepatic, and cardiac toxicities (hemorrhagic myocardial necrosis) may occur after high-dose therapy with total doses greater than 200 mg/kg.

Ifosfamide

Ifosfamide is an analogue of cyclophosphamide. Severe urinary tract and CNS toxicity initially limited the use of *ifosfamide*, but adequate hydration and coadministration of MESNA have reduced its bladder toxicity. CNS toxicity is handled with methylene blue (Pelgrims et al., 2000).

ADME

Ifosfamide has a plasma elimination $t_{1/2}$ of approximately 1.5 h after doses of 3.8 to 5 g/m^2 and a somewhat shorter $t_{1/2}$ at lower doses; its pharmacokinetics are highly variable due to variable rates of hepatic metabolism (see legend to Figure 70–3).

Therapeutic Uses

Ifosfamide is approved for treatment of patients with relapsed germ cell testicular cancer and is frequently used for first-time treatment of pediatric or adult patients with sarcomas. It is a common component of high-dose chemotherapy regimens with bone marrow or stem cell rescue. In nonmyeloablative regimens, *ifosfamide* is infused intravenously over at least 30 min at a dose of 1.2 g/m^2 per day or less for 5 days. Intravenous MESNA is given as bolus injections in a dose equal to 20% of the *ifosfamide* dose concomitantly and an additional 20% again 4 and 8 h later, for a total MESNA dose of 60% of the *ifosfamide* dose. Alternatively, MESNA may be given concomitantly in a single dose equal to the *ifosfamide* dose. Patients also should receive at least 2 L of oral or intravenous fluid daily. Treatment cycles are repeated every 3 to 4 weeks.

Adverse Effects

Ifosfamide has a similar toxicity profile as *cyclophosphamide*, although it causes greater platelet suppression, neurotoxicity, nephrotoxicity, and urothelial damage. In high-dose regimens (i.e., total doses of 12–14 g/m^2), it may cause severe neurological toxicity, including hallucinations, coma, and death, with symptoms appearing 12 h to 7 days after beginning the *ifosfamide* infusion. This toxicity may result from a metabolite, chloroacetaldehyde. *Ifosfamide* also causes nausea, vomiting, anorexia, leukopenia, nephrotoxicity, and VOD of the liver.

Melphalan

The alkylating agent *melphalan* primarily is used to treat multiple myeloma and, less commonly, in high-dose chemotherapy with marrow transplantation. The general pharmacological and cytotoxic actions of *melphalan* are similar to those of other bifunctional alkylators.

ADME

Oral *melphalan* is absorbed in an inconsistent manner and, for most indications, is given as an intravenous infusion. The drug has a plasma $t_{1/2}$ of about 45 to 90 min; 10% to 15% of an administered dose is excreted unchanged in the urine. Patients with decreased renal function may develop unexpectedly severe myelosuppression.

Therapeutic Uses and Adverse Effects

Melphalan for multiple myeloma is administered orally for 4 to 7 days every 28 days, with *dexamethasone* or *thalidomide*. Treatment is repeated at 4-week intervals based on response and tolerance. Dosage adjustments should be based on blood cell counts. *Melphalan* also may be used in myeloablative regimens followed by bone marrow or peripheral blood stem cell reconstitution. The toxicity of *melphalan* is mostly hematological and is similar to that of other alkylating agents. Nausea and vomiting are less frequent. The drug causes less alopecia and, rarely, renal or hepatic dysfunction. The drug is not a vesicant.

Chlorambucil

The cytotoxic effects of *chlorambucil* on the bone marrow, lymphoid organs, and epithelial tissues are similar to those observed with other nitrogen mustards. As an orally administered agent, *chlorambucil* is well tolerated in small daily doses and provides flexible titration of blood counts. Nausea and vomiting may result from single oral doses of 20 mg or greater.

ADME

Oral absorption of *chlorambucil* is adequate and reliable. The drug has a $t_{1/2}$ in plasma of about 1.5 h and is hydrolyzed to inactive products.

Therapeutic Uses and Adverse Effects

Chlorambucil historically was used almost exclusively in treating CLL; modern approaches to CLL frequently employ as first-line treatments noncytotoxic approaches such as modulators of B-cell antigen receptor signaling (see Chapter 71). When these agents are not available or not tolerated or as treatment for relapsed disease, *chlorambucil* may be given once daily and continued for 3 to 6 weeks. With a fall in the peripheral total leukocyte count or clinical improvement, the dosage is titrated to maintain neutrophils and platelets at acceptable levels. Maintenance therapy often is required to maintain clinical response. *Chlorambucil* treatment may continue for months or years, achieving its effects gradually and often without significant toxicity to a compromised bone marrow. Marked hypoplasia of the bone marrow may be induced with excessive doses, but the myelosuppressive effects are moderate, gradual, and rapidly reversible. GI discomfort, azoospermia, amenorrhea, pulmonary fibrosis, seizures, dermatitis, and hepatotoxicity rarely may be encountered. A marked increase in the incidence of acute myeloid leukemia (AML) and other tumors was noted when *chlorambucil* was used historically in the treatment of patients with polycythemia vera and as adjuvant chemotherapy after primary removal of breast cancers.

Bendamustine

Bendamustine is approved for treatment of CLL and non-Hodgkin lymphoma. *Bendamustine* is given as a 30-min intravenous infusion on days 1 and 2 of a 28-day cycle. Lower doses may be indicated in heavily pretreated patients. *Bendamustine* is rapidly degraded through sulfhydryl interaction and adduct formation with macromolecules; less than 5% of the parent drug is excreted in the urine intact. *N*-Demethylation and oxidation produce metabolites that have antitumor activity, but less than that of the parent molecule. The parent drug has a plasma $t_{1/2}$ of about 30 min. The clinical toxicity pattern of *bendamustine* is typical of alkylators, with a rapidly reversible myelosuppression and mucositis, both generally tolerable.

Ethyleneimines and Methylmelamines

Although nitrogen mustards containing chloroethyl groups constitute the most widely used class of alkylating agents, other alkylators such as ethyleneimines with greater chemical stability and well-defined activity in specific types of cancer have value in clinical practice.

Altretamine

Altretamine is structurally similar to TEM. Its precise mechanism of cytotoxicity is unknown, although it can alkylate DNA and proteins. It is a palliative treatment of patients with persistent or recurrent ovarian cancer following *cisplatin*-based combination therapy. The usual dosage of *altretamine* as a single agent in ovarian cancer is 260 mg/m^2 per day in four divided doses, for 14 or 21 consecutive days out of a 28-day cycle, for up to 12 cycles.

ADME

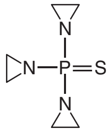
Altretamine is well absorbed from the GI tract; its elimination $t_{1/2}$ is 4 to 10 h. The drug undergoes rapid demethylation in the liver; the principal metabolites are pentamethylmelamine and tetramethylmelamine.

Adverse Effects

The main toxicities of *altretamine* are myelosuppression and neurotoxicity. *Altretamine* causes both peripheral and central neurotoxicity (ataxia, depression, confusion, drowsiness, hallucinations, dizziness, and vertigo). Neurological symptoms abate on discontinuation of therapy. Peripheral blood counts and a neurological examination should be performed prior to the initiation of each course of therapy. Therapy should be interrupted for at least 14 days and subsequently restarted at a lower dosage if the white cell count falls to less than 2000 cells/mm^3 or the platelet count falls to less than 75,000 cells/mm^3 or if neurotoxic or intolerable GI symptoms occur. If neurological symptoms fail to stabilize on the reduced-dose schedule, *altretamine* should be discontinued. Nausea and vomiting also are common side effects and may be dose limiting. Renal dysfunction may necessitate discontinuing the drug. Other rare adverse effects include rashes, alopecia, and hepatic toxicity. Severe, life-threatening orthostatic hypotension may develop in patients who receive monoamine oxidase inhibitors, *amitriptyline*, *imipramine*, or *phenelzine* concurrently with *altretamine*.

Thiotepa

Thiotepa consists of three ethyleneimine groups stabilized by attachment to the nucleophilic thiophosphoryl base. Its current use is primarily for high-dose chemotherapy regimens. Hepatic CYPs rapidly convert *thiotepa* to its desulfurated primary metabolite, triethylenephosphoramide (TEPA). Both *thiotepa* and TEPA form DNA cross-links.



ADME

Within hours of *thiotepa* administration, TEPA becomes the predominant form of the drug present in plasma. The parent compound has a plasma $t_{1/2}$ of 1.2 to 2 h; TEPA has a longer $t_{1/2}$, 3 to 24 h. *Thiotepa* pharmacokinetics essentially are the same in children as in adults at conventional doses (≤80 mg/m^2), and drug and metabolite $t_{1/2}$ are unchanged in children receiving high-dose therapy of 300 mg/m^2 per day for 3 days. Less than 10% of the administered drug appears in urine as the parent drug or the primary metabolite.

Adverse Effects

Toxicities include myelosuppression and, to a lesser extent, mucositis. Myelosuppression tends to develop somewhat later than with *cyclophosphamide*, with leukopenic nadirs at 2 weeks and platelet nadirs at 3 weeks. In high doses, *thiotepa* may cause neurotoxic symptoms, including coma and seizures.

Alkyl Sulfonates

Busulfan

Busulfan exerts few pharmacological actions other than myelosuppression at conventional doses and, prior to the advent of *imatinib mesylate*, was a standard agent for patients in the chronic phase of myelocytic leukemia and caused a severe and prolonged pancytopenia in some patients. *Busulfan* now is primarily used in high-dose regimens, in which pulmonary fibrosis, GI mucosal damage, and hepatic VOD are important toxicities.

ADME

Busulfan is well absorbed after oral administration and has a plasma $t_{1/2}$ of 2 to 3 h. The drug is conjugated to glutathione by GSTA1A and further metabolized by CYP-dependent pathways; its major urinary metabolite is methane sulfonic acid. In high doses, children less than 18 years of age clear the drug two to four times faster than adults and tolerate higher doses. *Busulfan* clearance varies considerably among patients. VOD is associated with high area under the curve (AUC; >1500 μM × min) and slow clearance, leading to recommendations for dose adjustment based on drug-level monitoring. A target steady-state concentration of 600 to 900 ng/mL in plasma in adults or AUC of less than 1000 μM × min in children achieves an appropriate balance between toxicity and therapeutic benefit.

Therapeutic Uses

The use of *busulfan* in initially treating Philadelphia chromosome–positive chronic myelocytic leukemia (CML) has been essentially supplanted by inhibitors of the oncogenic protein tyrosine kinase bcr-abl (see Chapter 71). However, where those agents are not available or not tolerated, the initial oral dose of *busulfan* varies with the total leukocyte count and the severity of the disease; daily doses are adjusted to subsequent hematological and clinical responses, with the aim of reducing the total leukocyte count to 10,000 cells/mm^3 or less. A decrease in the leukocyte count usually is not seen during the first 10 to 15 days of treatment, and the leukocyte count may actually increase during this period. Because the leukocyte count may fall for more than 1 month after discontinuing the drug, it is recommended that *busulfan* be withdrawn when the total leukocyte count has declined to about 15,000 cells/mm^3. A normal leukocyte count usually is achieved within 12 to 20 weeks. During remission, daily treatment resumes when the total leukocyte count reaches about 50,000 cells/mm^3.

In high-dose therapy as preparation for stem cell transplants, anticonvulsants must be used concomitantly with busulfan to protect against acute CNS toxicities, including tonic-clonic seizures that may occur several hours after each dose. Although *phenytoin* is a frequent choice, *phenytoin* induces the glutathione S-transferases that metabolize *busulfan*, reducing its AUC by about 20%. In patients requiring concomitant antiseizure medication, non–enzyme-inducing benzodiazepines such as *lorazepam* and *clonazepam* are recommended as an alternative to *phenytoin*. If *phenytoin* is used concurrently, plasma *busulfan* levels should be monitored and the *busulfan* dose adjusted accordingly (Salinger et al., 2010).

Adverse Effects

The toxic effects of *busulfan* are related to its myelosuppressive properties; prolonged thrombocytopenia may occur. Occasionally, patients experience nausea, vomiting, and diarrhea. Long-term use is associated with pulmonary fibrosis (*busulfan* lung) and leads to impotence, sterility, amenorrhea, and fetal malformation. Rarely, patients develop asthenia and hypotension. High-dose *busulfan* causes VOD of the liver in 10% or fewer of patients, as well as seizures, hemorrhagic cystitis, permanent alopecia, and cataracts. The coincidence of VOD and hepatotoxicity is increased by its coadministration with drugs that inhibit CYPs, including imidazoles and *metronidazole*, possibly through inhibition of the clearance of *busulfan* or its toxic metabolites.

Nitrosoureas

The nitrosoureas, except *streptozocin*, have an important role in the treatment of brain tumors and find occasional use in treating lymphomas and in high-dose regimens with bone marrow reconstitution. They function as bifunctional alkylating agents but differ from conventional nitrogen mustards in both pharmacological and toxicological properties.

Carmustine (BCNU) and *lomustine* (CCNU, not discussed in detail) are highly lipophilic and thus readily cross the blood-brain barrier, an important property in the treatment of brain tumors. Unfortunately, with the exception of *streptozocin*, nitrosoureas cause profound and delayed myelosuppression, with recovery 4 to 6 weeks after a single dose. Long-term treatment with the nitrosoureas, especially *semustine* (methyl-CCNU), has resulted in renal failure. As with other alkylating agents, the nitrosoureas are highly carcinogenic and mutagenic. They generate both alkylating and carbamylating moieties (see Figure 70–4).

Carmustine (BCNU) and Lomustine (CCNU)

Carmustine's major action is its alkylation of DNA at the O6-guanine position, an adduct repaired by MGMT. Methylation of the MGMT gene promoter inhibits its expression in about 30% of primary gliomas and is associated with sensitivity to nitrosoureas. In high doses with bone marrow rescue, *carmustine* (BCNU) produces hepatic VOD, pulmonary fibrosis, renal failure, and secondary leukemia. With repeated treatment courses at even conventional doses, its orally administered monochloroethyl analog *lomustine* (CCNU), can be associated with pulmonary dysfunction and prolonged bone marrow toxicity.

ADME

Carmustine (BCNU) is unstable in aqueous solution and in body fluids. After intravenous infusion, it disappears from the plasma with a highly variable $t_{1/2}$ of 15 to 90 min or more. Approximately 30% to 80% of the drug appears in the urine within 24 h as degradation products. The alkylating metabolites enter rapidly into the CSF, and their concentrations in the CSF reach 15% to 30% of the concurrent plasma values.

Therapeutic Uses

Carmustine (BCNU) is administered intravenously over 1 to 2 h and repeated every 6 weeks. *Lomustine* (CCNU) is orally bioavailable. Because of their ability to cross the blood-brain barrier, BCNU and CCNU have been used in the treatment of malignant gliomas. An implantable *carmustine* (BCNU) wafer is available for use as an adjunct to surgery for recurrent glioblastoma multiforme.

Streptozocin

Streptozocin (or streptozotocin) has a methylnitrosourea moiety attached to the C2 of glucose. The drug has a high affinity for cells of the islets of Langerhans and causes diabetes in experimental animals.

ADME

Streptozocin is rapidly degraded following intravenous administration. The $t_{1/2}$ of the drug is about 15 min. Only 10% to 20% of a dose is recovered intact in the urine.

Therapeutic Uses

Streptozocin is used in the treatment of human pancreatic islet cell carcinoma and carcinoid tumors. It is administered intravenously once daily for 5 days; this course is repeated every 6 weeks. Alternatively, a higher dose can be given weekly for 2 weeks, and the weekly dose then can be increased as tolerated.

Adverse Effects

Nausea is frequent. Mild, reversible renal or hepatic toxicity occurs in approximately two-thirds of cases; in fewer than 10% of patients, renal toxicity may be cumulative with each dose and may lead to irreversible renal failure. *Streptozocin* should not be given with other nephrotoxic drugs. Hematological toxicities (anemia, leukopenia, thrombocytopenia) occur in 20% of patients.

Triazenes

Dacarbazine (DTIC)

Dacarbazine functions as a methylating agent after metabolic activation in the liver to the monomethyl triazeno metabolite MTIC. It kills cells in all phases of the cell cycle. Resistance has been ascribed to the removal of methyl groups from the O6-guanine bases in DNA by MGMT activity.

ADME

Dacarbazine is administered intravenously. After an initial rapid phase ($t_{1/2}$ of ~20 min), *dacarbazine* is cleared from plasma with a terminal $t_{1/2}$ of about 5 h. The $t_{1/2}$ is prolonged in the presence of hepatic or renal disease. Almost 50% of the compound is excreted intact in the urine by tubular secretion.

Therapeutic Uses

The primary clinical indication for *dacarbazine* is in the chemotherapy of Hodgkin disease. In combination with other drugs for Hodgkin disease, *dacarbazine* is given on days 1 and 15 and repeated every 4 weeks for up to six cycles. It is modestly effective against malignant melanoma and adult sarcomas. *Dacarbazine* for malignant melanoma is given for a 10-day period, repeated every 28 days; alternatively, it can be given daily for 5 days and repeated every 3 weeks. Extravasation of the drug may cause tissue damage and severe pain. Its use in patients with melanoma has largely been replaced by immune checkpoint inhibitors and agents targeting the typically activated mitogen-activated protein kinase pathway (see Chapters 71 and 72).

Adverse Effects

Dacarbazine induces nausea and vomiting in more than 90% of patients; vomiting usually develops 1 to 3 h after treatment and may last up to 12 h. Myelosuppression, with both leukopenia and thrombocytopenia, is mild and readily reversible within 1 to 2 weeks. A flu-like syndrome may occur. Hepatotoxicity, alopecia, facial flushing, neurotoxicity, and dermatological reactions are less common adverse effects.

Temozolomide

Temozolomide is the standard agent, in combination with radiation therapy, for initial treatment of patients with high-grade malignant gliomas. *Temozolomide*, like *dacarbazine*, forms the methylating metabolite MTIC, but without the need for hepatic metabolism, and kills cells in all phases of the cell cycle. It is more active in MGMT-deficient tumors.

ADME

Temozolomide is administered orally and has a bioavailability approaching 100%. Plasma levels of the parent drug decline with a $t_{1/2}$ of 1 to 2 h. The primary active metabolite MTIC reaches a maximum plasma concentration (150 ng/mL) 90 min after a dose and declines with a $t_{1/2}$ of 2 h. Little intact drug is recovered in the urine, the primary urinary metabolite being the inactive imidazole carboxamide.

Adverse Effects

The toxicities of *temozolomide* mirror those of DTIC. Hematological monitoring is necessary to guide dosing adjustments.

Methylhydrazines

Procarbazine

Procarbazine is used in malignant brain tumors and in combination regimens for patients with Hodgkin disease.

Mechanisms of Action

The antineoplastic activity of *procarbazine* results from its conversion by CYP-mediated hepatic oxidative metabolism to highly reactive alkylating species that methylate DNA. Activated *procarbazine* can produce chromosomal damage, including chromatid breaks and translocations, consistent with its mutagenic and carcinogenic actions. Resistance to *procarbazine* develops rapidly when it is used as a single agent; one mechanism of resistance results from increased expression of MGMT, which repairs methylation of guanine.

ADME

The pharmacokinetic behavior of *procarbazine* has not been thoroughly defined. The drug is extensively metabolized by CYPs to azo, methylazoxy, and benzylazoxy intermediates, which are found in the plasma and yield the alkylating metabolites in tumor cells. In patients with brain cancer, the concurrent use of antiseizure drugs that induce hepatic CYPs does not significantly alter the pharmacokinetics of the parent drug.

Therapeutic Uses

Procarbazine is used in combination regimens such as MOPP (*nitrogen mustard*, Oncovin [*vincristine*], *procarbazine*, and *prednisone*) for Hodgkin disease. It is also used in treating gliomas as part of the PVC (*procarbazine*, *vincristine*, and CCNU) regimen.

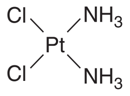
Adverse Effects

The most common toxic effects include leukopenia and thrombocytopenia, which begin during the second week of therapy and reverse within 2 weeks off treatment. GI symptoms such as mild nausea and vomiting occur in most patients; diarrhea and rash are noted in 5% to 10% of cases. Behavioral disturbances also have been reported. Because *procarbazine* augments sedative effects, the concomitant use of CNS depressants should be avoided. The drug is a weak monoamine oxidase inhibitor; it blocks the metabolism of catecholamines, sympathomimetics, and dietary tyramine and thus may provoke hypertension in patients concurrently exposed to these. *Procarbazine* has *disulfiram*-like actions; therefore, the ingestion of alcohol should be avoided. The drug is highly carcinogenic, mutagenic, and teratogenic and is associated with a 5% to 10% risk of acute leukemia in patients treated with MOPP. The highest risk is for patients who also receive radiation therapy. *Procarbazine* is a potent immunosuppressive agent. It causes infertility, particularly in males.

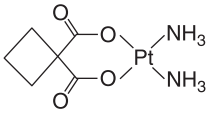
Platinum Coordination Complexes

The growth-inhibitory effect of *cisplatin* was discovered during studies of bacterial growth in electrical fields that were generated by platinum electrodes; *cisplatin* generated from corroding platinum electrodes was then found to be an effective anticancer drug in mouse models

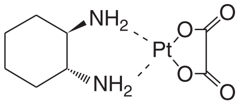
(Rosenberg et al., 1969) and gained FDA approval for cancer treatment in 1978. Platinum coordination complexes have broad antineoplastic activity and have become the foundation for treatment of ovarian, head and neck, bladder, esophagus, lung, and colon cancers. Although *cisplatin* and other platinum complexes do not form carbonium ion intermediates like other alkylating agents or formally alkylate DNA, they covalently bind to nucleophilic sites on DNA and share many pharmacological attributes with alkylators.



CISPLATIN



CARBOPLATIN



OXALIPLATIN

Mechanisms of Action

Cisplatin, *carboplatin*, and *oxaliplatin* enter cells by the high-affinity Cu^{2+} transporter CTR1 (SLC31A1, a member of the solute carrier family; see Chapter 5) and in doing so contribute to the degradation of the transporter. The compounds are actively extruded from cells by ATP7A and ATP7B copper exporters and by MRP1; variable expression of these transporters may contribute to clinical resistance, along with a variety of other proteins and epigenetic phenomena (Shen et al., 2012). Inside the cell, the chloride, cyclohexane, or oxalate ligands of the analogues are displaced by water molecules, yielding a positively charged molecule that reacts with nucleophilic sites on DNA and proteins.

Aquation of *cisplatin* is favored at the low concentrations of Cl$^-$ inside the cell and in the urine. High concentrations of Cl$^-$ stabilize the drug, explaining the effectiveness of Cl$^-$ diuresis in preventing nephrotoxicity. The activated platinum complexes can react with electron-rich regions, such as sulfhydryls, and with various sites on DNA, forming both intrastrand and interstrand cross-links. The DNA-platinum adducts inhibit replication and transcription, lead to single- and double-stranded breaks and miscoding, and, if recognized by p53 and other cell-cycle checkpoint proteins, cause induction of apoptosis. Adduct formation is an important predictor of clinical response (Reed, 1998). The analogues differ in the conformation of their adducts and the effects of adduct on DNA structure and function. *Oxaliplatin* and *carboplatin* are slower to form adducts. The *oxaliplatin* adducts are bulkier and less readily repaired, create a different pattern of distortion of the DNA helix, and differ from *cisplatin* adducts in the pattern of hydrogen bonding to adjacent segments of DNA (Sharma et al., 2007).

Unlike the other platinum analogues, *oxaliplatin* exhibits a cytotoxicity that does not depend on an active MMR system, which may explain its greater activity in colorectal cancer. It also seems less dependent on the presence of high-mobility group (HMG) proteins that are required by the other platinum derivatives. Testicular cancers have a high concentration of HMG proteins and are quite sensitive to *cisplatin*. Basal-type breast cancers, such as those with *BRCA1* and *BRCA2* mutations, lack *HER2* amplification and hormone-receptor expression and appear to be uniquely susceptible to *cisplatin* both through their upregulation of apoptotic pathways governed by p63 and p73 (Deyoung and Ellisen, 2007) and defective DNA repair mechanisms including those directed at double-strand breaks, interstrand cross-links, and replication gaps (Li and Heyer, 2008; Panzarino et al., 2021). The cell-cycle specificity of *cisplatin* differs among cell types; the effects of cross-linking are most pronounced during the S phase. The platinum analogues are mutagenic, teratogenic, and carcinogenic. *Cisplatin*- or *carboplatin*-based chemotherapy for ovarian cancer is associated with a 4-fold increased risk of developing secondary leukemia.

Resistance to Platinum Analogues

Resistance to the platinum analogues likely is multifactorial; the compounds differ in their degree of cross-resistance. *Carboplatin* shares cross-resistance with *cisplatin* in most experimental tumors, while *oxaliplatin* does not.

A number of factors influence sensitivity to platinum analogues, including intracellular drug accumulation and intracellular levels of glutathione and other sulfhydryls, such as metallothionein, that bind to and inactivate the drug and rates of repair of DNA adducts. Repair of platinum-DNA adducts requires participation of the NER pathway. Inhibition or loss of NER increases sensitivity to *cisplatin* in patients with ovarian cancer, while overexpression of NER components is associated with poor response to *cisplatin*- or *oxaliplatin*-based therapy in lung, colon, and gastric cancer (Paré et al., 2008).

Resistance to *cisplatin*, but not *oxaliplatin*, appears to be partly mediated through loss of function in the MMR proteins. In the absence of effective repair of DNA-platinum adducts, sensitive cells cannot replicate or transcribe affected portions of the DNA strand. Some DNA polymerases can bypass adducts, possibly contributing to resistance. Overexpression of copper efflux transporters ATP7A and ATP7B correlates with poor survival after *cisplatin*-based therapy for ovarian cancer (Shen et al., 2012).

Cisplatin

ADME

Cisplatin is given only intravenously. To prevent renal toxicity, it is important to establish a chloride diuresis by the infusion of 1 to 2 L of normal saline prior to treatment. The appropriate amount of *cisplatin* then is diluted in a solution containing dextrose, saline, and *mannitol* and administered intravenously over 4 to 6 h. Because aluminum inactivates *cisplatin*, the drug should not come in contact with needles or other infusion equipment that contain aluminum during its preparation or administration. After intravenous administration, *cisplatin* has an initial plasma elimination $t_{1/2}$ of 25 to 50 min; concentrations of total (bound and unbound) drug fall thereafter, with a $t_{1/2}$ of 24 h or more. More than 90% of the platinum in the blood is covalently bound to plasma proteins. High concentrations of *cisplatin* are found in the kidney, liver, intestine, and testes; *cisplatin* penetrates poorly into the CNS. Only a small portion of the drug is excreted by the kidney during the first 6 h; by 24 h, up to 25% is excreted, and by 5 days, up to 43% of the administered dose is recovered in the urine, mostly covalently bound to protein and peptides. Biliary or intestinal excretion of *cisplatin* is minimal.

Therapeutic Uses

Cisplatin, in combination with *bleomycin*, *etoposide*, or with *ifosfamide* and *vinblastine*, cures 90% of patients with testicular cancer. Used with *paclitaxel*, *cisplatin* or *carboplatin* induces complete responses in the majority of patients with carcinoma of the ovary. *Cisplatin* produces responses in cancers of the bladder, head and neck, cervix, and endometrium; all forms of carcinoma of the lung; anal and rectal carcinomas; and neoplasms of childhood. The drug also sensitizes cells to radiation therapy and enhances control of locally advanced lung, esophageal, and head and neck tumors when given with radiation therapy.

Adverse Effects

Cisplatin-induced nephrotoxicity can be ameliorated by forced pretreatment hydration and chloride diuresis. *Amifostine*, a thiophosphate cytoprotective agent, reduces renal toxicity associated with repeated administration of *cisplatin*. Ototoxicity caused by *cisplatin* is unaffected by diuresis and is manifested by tinnitus and high-frequency hearing loss. Marked nausea and vomiting occur in almost all patients and usually can be controlled with serotonin (5HT$_3$) receptor antagonists, neurokinin (NK$_1$) receptor antagonists (e.g., *aprepitant*), and high-dose corticosteroids (see Chapters 50 and 54).

At higher doses or after multiple cycles of treatment, *cisplatin* causes a progressive peripheral motor and sensory neuropathy that may worsen after discontinuation of the drug and may be aggravated by subsequent or simultaneous treatment with taxanes or other neurotoxic drugs. *Cisplatin* causes mild-to-moderate myelosuppression, with transient leukopenia and thrombocytopenia. Anemia may become prominent after multiple cycles of treatment. Electrolyte disturbances, including hypomagnesemia, hypocalcemia, hypokalemia, and hypophosphatemia, are common. Hypocalcemia and hypomagnesemia secondary to tubular damage and renal electrolyte wasting may produce tetany if untreated. Routine measurement of Mg^{2+} concentrations in plasma is recommended. Hyperuricemia, hemolytic anemia, and cardiac abnormalities are rare side effects. Anaphylactic-like reactions, characterized by facial edema, bronchoconstriction, tachycardia, and hypotension, may occur within minutes after administration and should be treated by intravenous injection of *epinephrine* and with corticosteroids or antihistamines. *Cisplatin* has been associated with the development of AML, usually 4 years or more after treatment.

Carboplatin

The mechanisms of action and resistance and the spectrum of clinical activity of *carboplatin* are similar to *cisplatin*. However, the two drugs differ significantly in their chemical, pharmacokinetic, and toxicological properties.

ADME

Because *carboplatin* is much less reactive than *cisplatin*, the majority of drug in plasma remains in its parent form, unbound to proteins. Most drug is eliminated via renal excretion, with a $t_{1/2}$ of about 2 h. A small fraction of platinum binds irreversibly to plasma proteins and disappears slowly, with a $t_{1/2}$ of 5 days or more.

Therapeutic Uses

Carboplatin and *cisplatin* are equally effective in the treatment of patients with suboptimally debulked ovarian cancer, non–small cell lung cancer, and extensive-stage small cell lung cancer; however, *carboplatin* may be less effective than *cisplatin* in the treatment of patients with germ cell, head and neck, and esophageal cancers. *Carboplatin* is an effective alternative for responsive tumors in patients unable to tolerate *cisplatin* because of impaired renal function, refractory nausea, significant hearing impairment, or neuropathy, but doses must be adjusted for renal function. In addition, it may be used in high-dose therapy with bone marrow or peripheral stem cell rescue. *Carboplatin* is administered as an intravenous infusion over at least 15 min and is given once every 21 to 28 days; current approaches to defining the overall dose of *carboplatin* use a regimen-specific target AUC (area under the concentration × time elimination curve) that takes into account the patient's creatinine clearance (CL_{Cr}) (Calvert et al., 1989).

Adverse Effects

Carboplatin is relatively well tolerated clinically, causing less nausea, neurotoxicity, ototoxicity, and nephrotoxicity than *cisplatin*. The dose-limiting toxicity of *carboplatin* is myelosuppression, primarily thrombocytopenia. It may cause a hypersensitivity reaction; in patients with a mild reaction, premedication, graded doses of drug, and more prolonged infusion lead to desensitization.

Oxaliplatin

ADME

Oxaliplatin has a short $t_{1/2}$ in plasma, probably as a result of its rapid uptake by tissues and its reactivity; the initial $t_{1/2}$ is about 17 min. No dose adjustment is required for hepatic dysfunction or for patients with a CL_{Cr} greater than or equal to 20 mL/min.

Therapeutic Uses

Oxaliplatin exhibits a range of antitumor activity (colorectal and gastric cancer) that differs from other platinum agents. *Oxaliplatin's* effectiveness in colorectal cancer is perhaps due to its MMR- and HMG-independent effects. It also suppresses expression of thymidylate synthase (TS), the target enzyme of *5-fluorouracil* (5FU) action, which may promote synergy of these two drugs (Fischel et al., 2002). In combination with 5FU, it is approved for treatment of patients with colorectal cancer.

Adverse Effects

The dose-limiting toxicity of *oxaliplatin* is peripheral neuropathy. An acute form, often triggered by exposure to cold liquids, manifests as paresthesias or dysesthesias in the upper and lower extremities, mouth, and throat. A second type relates to cumulative dose and has features similar to *cisplatin* neuropathy; 75% of patients receiving a cumulative dose of 1560 mg/m^2 experience some progressive sensory neurotoxicity, with dysesthesias, ataxia, and numbness of the extremities. Hematological toxicity is mild to moderate, except for rare immune-mediated cytopenias; nausea is well controlled with serotonin ($5HT_3$) receptor antagonists. *Oxaliplatin* may cause leukemia and pulmonary fibrosis months to years after administration. *Oxaliplatin* may cause an acute allergic response with urticaria, hypotension, and bronchoconstriction.

II. Antimetabolites

Folic Acid Analogues

Folic acid is an essential dietary factor that is converted by enzymatic reduction to FH_4 cofactors that provide methyl groups for the synthesis of precursors of DNA (thymidylate and purines) and RNA (purines) (Wilson et al., 2014). Folic acid analogues such as *methotrexate* (MTX) interfere with FH_4 metabolism (Figure 70–5), reducing the cellular capacity for one-carbon transfer and methylation reactions in the synthesis of purine ribonucleotides and TMP (thymidine monophosphate), thereby inhibiting DNA replication.

HISTORY OF ANTIFOLATE CHEMOTHERAPY

Antifolate chemotherapy produced the first striking, although temporary, remissions in leukemia (Farber et al., 1948) and the first cure of a solid tumor, choriocarcinoma (Berlin et al., 1963). Interest in folate antagonists further increased with the development of curative combination therapy for childhood acute lymphocytic leukemia; in this therapy, MTX played a critical role in both systemic treatment and intrathecal therapy. Introduction of high-dose regimens with "rescue" of host toxicity by the reduced folate *leucovorin* (folinic acid, citrovorum factor, 5-formyl tetrahydrofolate, N^5-formyl FH_4) further extended the effectiveness of this drug to both systemic and CNS lymphomas, osteogenic sarcoma, and leukemias. Recognition that MTX, an inhibitor of dihydrofolate reductase (DHFR), also directly inhibits the folate-dependent enzymes of *de novo* purine synthesis and thymidylate synthesis led to development of antifolate analogues that specifically target these other folate-dependent enzymes. New congeners have greater capacity for transport into tumor cells (*pralatrexate*) and exert their primary inhibitory effect on TS (*raltitrexed*), early steps in purine biosynthesis (*lometrexol*), or both (the multitargeted antifolate *pemetrexed*).

Mechanism of Action

The primary target of folic acid analogues, such as MTX, is the enzyme DHFR (Figure 70–6). To function as a cofactor in one-carbon transfer reactions, folate must be reduced by DHFR to FH_4. Inhibitors such as MTX, with a high affinity for DHFR (K_i ~0.01–0.2 nM), cause partial depletion of N^{5-10} methylene FH_4 and N^{10} formyl FH_4 cofactors that are required for the synthesis of thymidylate and purines. In addition, MTX, like cellular folates, undergoes addition of a series of polyglutamates (MTX-PGs) in both normal and tumor cells (see Figure 70–5). These PGs

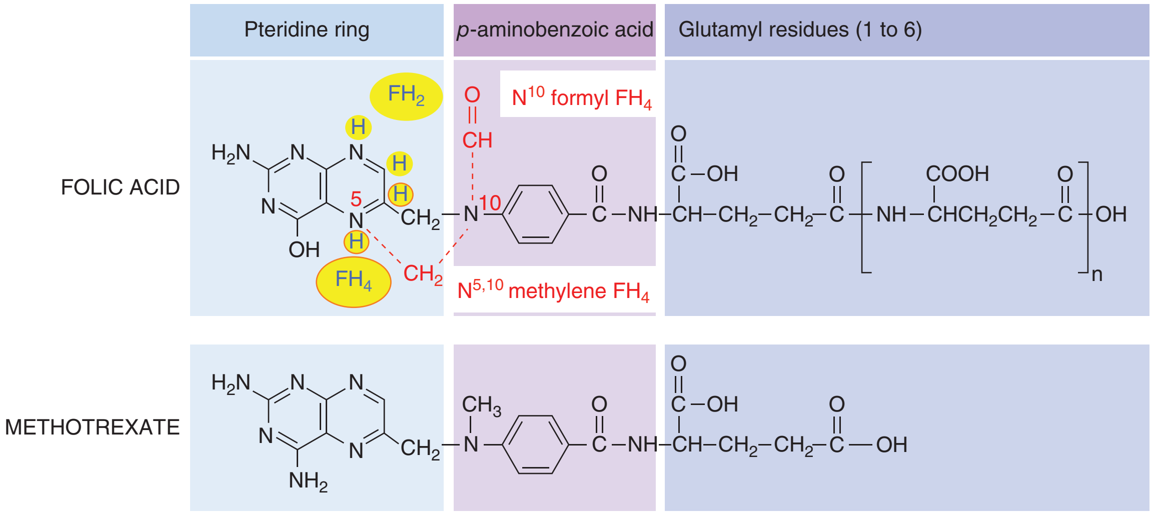

Pteridine ring
p-aminobenzoic acid
Glutamyl residues (1 to 6)
FOLIC ACID
FH2
H
N10 formyl FH4
5
10
FH4
N5,10 methylene FH4
COOH
n
METHOTREXATE
CH3

Figure 70–5 *Folic acid, metabolic intermediates, and MTX.* The shading identifies common structural features and areas of modification. Yellow highlights indicate sites modified by DHFR to FH_2 (DHF) and to FH_4 (THF, tetrahydrofolate); red atoms and bonds indicate metabolic intermediates that serve as single-carbon donors (see pathways in Figure 70–6).

constitute intracellular storage forms of folates and folate analogues that dramatically increase inhibitory potency of the analogue for additional targets, including TS and two early enzymes in the purine biosynthetic pathway. The FH_2 PGs that accumulate in cells behind the blocked DHFR reaction also act as inhibitors of TS and other enzymes (see Figure 70–6) (Allegra et al., 1987b).

Selective Toxicity; Rescue

As with most antimetabolites, MTX is only partially selective for tumor cells and kills rapidly dividing normal cells, such as those of the intestinal epithelium and bone marrow. Folate antagonists kill cells during the S phase of the cell cycle and are most effective when cells are proliferating rapidly. The toxic effects of MTX may be terminated by administering *leucovorin*, a fully reduced folate coenzyme that repletes the intracellular pool of FH_4 cofactors (see Figure 70–6). *Levoleucovorin,* approved in 2018, contains the active isomer and shows similar efficacy and adverse effects as racemic *leucovorin*.

Cellular Entry and Retention

Because folic acid and many of its analogues are polar, they cross the blood-brain barrier poorly and require specific transport mechanisms to enter mammalian cells. Three inward folate transport systems are found on mammalian cells:

1. A folate receptor, which has high affinity for folic acid but much lower ability to transport MTX and other analogues
2. The reduced folate transporter, the major transit protein for MTX, *raltitrexed*, *pemetrexed*, and most analogues
3. A transporter that is active at low pH

The reduced folate transporter is highly expressed in the hyperdiploid subtype of ALL, which has extreme sensitivity to MTX (Pui et al., 2004). Once in the cell, additional glutamyl residues are added to the molecule by the enzyme folylpolyglutamate synthase. Because these higher PGs are strongly charged and cross cellular membranes poorly, polyglutamation serves as a mechanism of ion trapping within

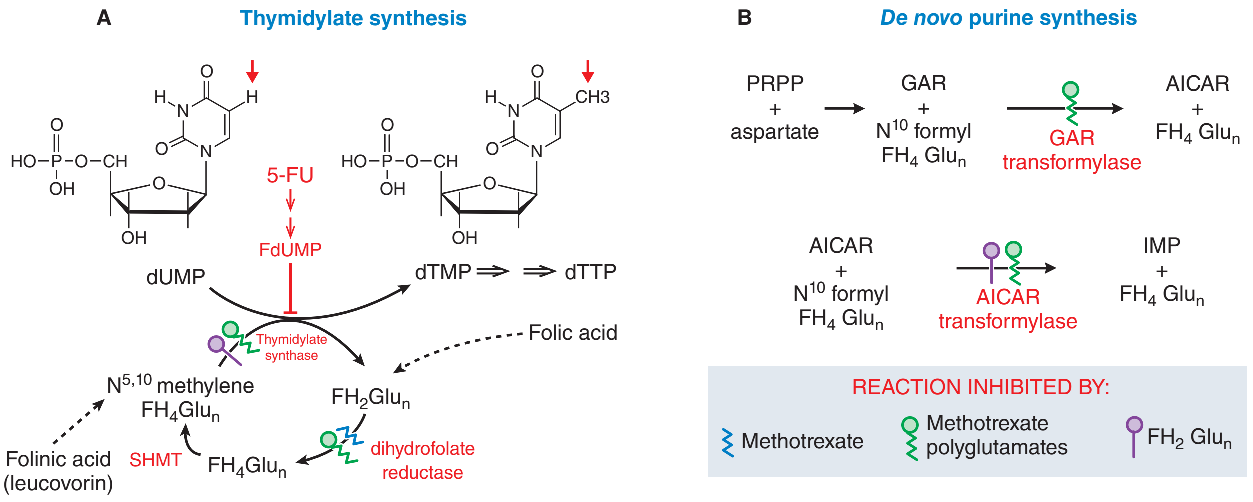

A
Thymidylate synthesis
5-FU
FdUMP
dUMP
dTMP
dTTP
CH3
Thymidylate synthase
Folic acid
N5,10 methylene FH4Glun
FH2Glun
Folinic acid (leucovorin)
SHMT
FH4Glun
dihydrofolate reductase
B
De novo purine synthesis
PRPP + aspartate
GAR + N10 formyl FH4 Glun
GAR transformylase
AICAR + FH4 Glun
AICAR + N10 formyl FH4 Glun
AICAR transformylase
IMP + FH4 Glun
REACTION INHIBITED BY:
Methotrexate
Methotrexate polyglutamates
FH2 Glun

Figure 70–6 *Folate metabolism and the actions of 5FU, MTX, and MTX polyglutamates.* **A.** *Thymidylate synthesis* (for composition of folates, see Figure 70–5). **B.** *De novo purine synthesis.* GAR transformylase provides the C8 and AICAR transformylase the C2 in the purine ring biosynthesis (see Figure 70–1 for numbering of purine ring atoms). AICAR, aminoimidazole carboxamide ribonucleotide is an intermediate in the generation of inosine monophosphate (IMP); dUMP, deoxyuridine monophosphate; FH_2Glu_n, dihydrofolate polyglutamate; FH_4Glu_n, tetrahydrofolate polyglutamate; GAR, glycinamide ribonucleotide; IMP, inosine monophosphate; PRPP, 5-phosphoribosyl-1-pyrophosphate; TMP, thymidine monophosphate.

the cell. The polyglutamation may also account for the prolonged retention of MTX in chorionic epithelium (where it is a potent abortifacient); in tumors derived from this tissue, such as choriocarcinoma cells; and in normal tissues subject to cumulative drug toxicity, such as liver. Polyglutamyl folates and analogues have substantially greater affinity than the monoglutamate form for folate-dependent enzymes that are required for purine and thymidylate synthesis and have at least equal affinity for DHFR.

Newer Congeners

New folate antagonists that are better substrates for the reduced folate carrier have been identified. In efforts to bypass the obligatory membrane transport system and to facilitate penetration of the blood-brain barrier, lipid-soluble folate antagonists also have been synthesized. *Trimetrexate*, a lipid-soluble analogue that lacks a terminal glutamate, has modest antitumor activity, primarily in combination with *leucovorin* rescue. However, it is beneficial in the treatment of *Pneumocystis jirovecii* (*Pneumocystis carinii*) pneumonia, for which *leucovorin* provides differential rescue of the host but not the parasite (Allegra et al., 1987a). The folate analogue, MTA (multitargeted antifolate) or *pemetrexed*, is a pyrrole-pyrimidine structure. It is avidly transported into cells via the reduced folate carrier and is converted to PGs that inhibit TS and glycinamide ribonucleotide transformylase (GART), as well as DHFR (see Figure 70–6). It has activity against ovarian cancer, mesothelioma, and adenocarcinomas of the lung. *Pemetrexed* and its PGs have a somewhat different spectrum of biochemical actions. Like MTX, *pemetrexed* inhibits DHFR, but as a PG, it even more potently inhibits TS and GART. Unlike MTX, it produces little change in the pool of reduced folates, indicating that the distal sites of inhibition (TS and GART) predominate. Its pattern of deoxynucleotide depletion also differs; it causes a greater fall in thymidine triphosphate (TTP) than in other triphosphates. Like MTX, it induces p53 and cell-cycle arrest, but this effect does not depend on induction of p21. A newer congener, *pralatrexate*, is more effectively taken up and polyglutamated than MTX and is approved for treatment of CTCL.

Mechanisms of Resistance to Antifolates

Resistance to MTX can involve alterations in each known step in MTX action, including

- Impaired transport of MTX into cells
- Production of altered forms of DHFR that have decreased affinity for the inhibitor
- Increased concentrations of intracellular DHFR through gene amplification or altered gene regulation
- Decreased ability to synthesize MTX-PGs
- Increased expression of a drug efflux transporter of the MRP class (see Chapter 5)

The DHFR levels in leukemic cells increase within 24 h after treatment of patients with MTX, probably as a result of induction of DHFR synthesis. Unbound DHFR protein may bind to its own message and reduce its own translation, while the DHFR-MTX complex is ineffective in blocking DHFR translation. With longer periods of drug exposure, tumor cell populations emerge that contain markedly increased levels of DHFR. These cells contain multiple gene copies of DHFR either in mitotically unstable double-minute chromosomes (extrachromosomal elements) or in stably integrated, homogeneously staining chromosomal regions or amplicons (Schimke et al., 1978). Similar gene amplification target proteins have been implicated in the resistance to other antitumor agents, including 5FU and *pentostatin* (2′-deoxycoformycin), and observed in patients with lung cancer (Curt et al., 1985).

High doses of MTX may permit entry of the drug into transport-defective cells and may permit the intracellular accumulation of MTX in concentrations that inactivate high levels of DHFR. The understanding of resistance to *pemetrexed* is incomplete. In various cell lines, resistance seems to arise from loss of influx transport, TS amplification, changes in purine biosynthetic pathways, or loss of polyglutamation.

ADME

Methotrexate is readily absorbed from the GI tract at doses of less than 25 mg/m^2; larger doses are absorbed incompletely and are routinely administered intravenously. After intravenous administration, the drug disappears from plasma in a triphasic fashion. The rapid distribution phase is followed by a second phase, which reflects renal clearance ($t_{1/2}$ of about 2–3 h). A third phase has a $t_{1/2}$ of about 8 to 10 h. This terminal phase of disappearance, if prolonged by renal failure, may be responsible for major toxic effects of the drug on the bone marrow, GI epithelium, and skin. Distribution of MTX into body spaces, such as the pleural or peritoneal cavity, occurs slowly. However, if such spaces are expanded (e.g., by ascites or pleural effusion), they may act as a site of storage and slow release of the drug, resulting in prolonged elevation of plasma concentrations and more severe bone marrow toxicity.

Approximately 50% of MTX binds to plasma proteins and may be displaced from plasma albumin by myriad drugs, including sulfonamides, salicylates, *tetracycline*, *chloramphenicol*, and *phenytoin*; caution should be used if these drugs are given concomitantly. Up to 90% of a given dose of MTX is excreted unchanged in the urine, mostly within the first 8 to 12 h. Metabolism of MTX usually is minimal. After high doses, however, metabolites are readily detectable; these include 7-hydroxy-MTX, which is potentially nephrotoxic. Renal excretion of MTX occurs through a combination of glomerular filtration and active tubular secretion. Therefore, the concurrent use of drugs that reduce renal blood flow (e.g., nonsteroidal anti-inflammatory drugs), that are nephrotoxic (e.g., *cisplatin*), or that are weak organic acids (e.g., *aspirin*) can delay drug excretion and lead to severe myelosuppression. In patients with renal insufficiency, the dose should be adjusted in proportion to decreases in renal function, and high-dose regimens should be avoided. Concentrations of MTX in CSF are only 3% of those in the systemic circulation at steady state; hence, neoplastic cells in the CNS probably are not killed by standard dosage regimens. When high doses of MTX are given, cytotoxic concentrations of MTX reach the CNS beyond the blood-brain barrier. MTX is retained in the form of PGs for long periods (e.g., weeks in the kidneys, several months in the liver).

Pharmacogenetics may influence the response to antifolates and their toxicity. The C677T substitution in methylenetetrahydrofolate reductase reduces the activity of the enzyme that generates N^{5-10} methylene FH_4, the cofactor for TS, and thereby increases MTX toxicity (Pullarkat et al., 2001). The presence of this polymorphism in leukemic cells confers increased sensitivity to MTX and might also modulate the toxicity and therapeutic effect of *pemetrexed*, a TS inhibitor. Likewise, polymorphisms in the promoter region of TS affect its expression and, by altering the intracellular levels of TS, modulate the response and toxicity of both antifolates and fluoropyrimidines (Pui et al., 2004).

Therapeutic Uses

Methotrexate is a critical drug in the management of childhood ALL. High-dose MTX is of great value in remission induction and consolidation and in the maintenance of remissions in this highly curable disease. A 6- to 24-h infusion of relatively large doses of MTX may be employed every 2 to 4 weeks but only when *leucovorin* rescue follows within 24 h of the MTX infusion. For maintenance therapy, it is administered at a lower dose, orally every week. Outcome of treatment in children correlates inversely with the rate of drug clearance. During MTX infusion, high steady-state levels are associated with a lower leukemia relapse rate. MTX is of limited value in adults with AML, except for treatment and prevention of leukemic meningitis.

The intrathecal administration of MTX has been employed for treatment or prophylaxis of meningeal leukemia or lymphoma and for treatment of meningeal carcinomatosis. This route of administration achieves high concentrations of MTX in the CSF and also is effective in patients whose systemic disease has become resistant to MTX. The treatment is repeated every 4 days until malignant cells no longer are evident in the CSF. *Leucovorin* may be administered to counteract the potential toxicity of MTX that escapes into the systemic circulation, although this generally is not necessary. Because MTX administered into the lumbar space distributes poorly over the cerebral convexities, the drug may be given via an intraventricular catheter system in the treatment of active intrathecal disease. MTX is of established value in choriocarcinoma and related trophoblastic tumors of women; cure is achieved in about 75% of advanced cases treated sequentially with MTX and *dactinomycin* and in more than 90% when early diagnosis is made. For choriocarcinoma, MTX is administered intramuscularly every other day for four doses, alternating with *leucovorin*. Courses are repeated at 3-week intervals, toxicity permitting, and urinary β-human chorionic gonadotropin titers are used as a guide for persistence of disease.

Beneficial effects also are observed in the combination therapy of Burkitt and other non-Hodgkin lymphomas. MTX is a component of regimens for carcinomas of the breast, head and neck, ovary, and bladder. High-dose MTX with *leucovorin* rescue (HDM-L) is a standard approach for adjuvant therapy of osteosarcoma and produces a high complete response rate in CNS lymphomas. The administration of HDM-L has the potential for renal toxicity, probably related to the precipitation of the drug, a weak acid, in the acidic tubular fluid. Thus, vigorous hydration and alkalinization of urine pH are required prior to drug administration. If MTX values measured 48 h after drug administration are 1 μM or higher, higher doses (100 mg/m^2) of *leucovorin* must be given until the plasma concentration of MTX falls to less than 50 nM. With appropriate hydration and urine alkalinization, and in patients with normal renal function, the incidence of nephrotoxicity following HDM-L is less than 2%. In patients who become oliguric, intermittent hemodialysis is ineffective in reducing MTX levels. Continuous-flow hemodialysis can eliminate MTX at about 50% of the clearance rate in patients with intact renal function. Alternatively, an MTX-cleaving enzyme, *glucarpidase*, is FDA approved for the treatment of MTX toxicity. *Glucarpidase* is a recombinant bacterial carboxypeptidase G2 that converts MTX into glutamate and 2,4-diamino-N(10)-methylpteroic acid. These metabolites are less toxic and are excreted by the liver. MTX concentrations in plasma fall by 99% or more within 5 to 15 min following enzyme administration. However, systemically administered carboxypeptidase G2 has little effect on MTX levels in the CSF.

Methotrexate is used in the treatment of severe, disabling psoriasis (see Chapter 75), given orally for 5 days, followed by a rest period of at least 2 days, or intravenously weekly. It also is used at low dosage to induce remission in refractory rheumatoid arthritis. MTX inhibits cell-mediated immune reactions and is employed to suppress graft-versus-host disease in allogeneic bone marrow and organ transplantation and for the treatment of dermatomyositis, Wegener granulomatosis, and Crohn's disease (see Chapters 39 and 55). MTX is also used as an abortifacient, generally in combination with a *prostaglandin* (see Chapter 48).

Adverse Effects

The primary toxicities of antifolates are on the bone marrow and the intestinal epithelium. Patients may be at risk for spontaneous hemorrhage or life-threatening infection and may require prophylactic transfusion of platelets and broad-spectrum antibiotics if febrile. Side effects usually reverse completely within 2 weeks, but prolonged myelosuppression may occur in patients with compromised renal function who have delayed drug excretion. The dosage of MTX (and likely *pemetrexed*) must be reduced in proportion to any reduction in CL_{Cr}. Additional toxicities of MTX include alopecia, dermatitis, an allergic interstitial pneumonitis, nephrotoxicity (after high-dose therapy), defective oogenesis or spermatogenesis, abortion, and teratogenesis. Low-dose MTX may lead to cirrhosis after long-term continuous treatment, as in patients with psoriasis. Intrathecal administration of MTX often causes meningismus and an inflammatory response in the CSF. Seizures, coma, and death may occur rarely. Note that *leucovorin* does not reverse neurotoxicity.

Pemetrexed toxicity mirrors that of MTX, with the additional feature of a prominent erythematous and pruritic rash in 40% of patients. *Dexamethasone*, 4 mg twice daily on days –1, 0, and +1, markedly diminishes this toxicity. Unpredictably severe myelosuppression with *pemetrexed*, seen especially in patients with preexisting homocystinemia, largely is eliminated by concurrent administration of low dosages of folic acid, 350 to 1000 mg/day, beginning 1 to 2 weeks prior to *pemetrexed* and continuing while the drug is administered. Patients should receive intramuscular vitamin B_{12} (1 mg) with the first dose of *pemetrexed* to correct possible B_{12} deficiency. These small doses of folate and B_{12} do not compromise the therapeutic effect.

Pyrimidine Analogues

The pyrimidine antimetabolites encompass a diverse group of drugs that inhibit RNA and DNA function. The fluoropyrimidines and certain purine analogues (*6-mercaptopurine* [6MP] and *6-thioguanine* [6TG]) inhibit the synthesis of essential precursors of DNA. Others, such as the cytidine and adenosine nucleoside analogues, become incorporated into DNA and block its further elongation and function (Wilson et al., 2014). Other inhibitory effects of these analogues may contribute to their cytotoxicity and their capacity to induce differentiation.

Cellular Actions of Pyrimidine Antimetabolites

Four bases (Figure 70–7) form DNA: two pyrimidines (thymine and cytosine) and two purines (guanine and adenine). RNA differs in that it incorporates uracil instead of thymine as one of its bases. Strategies for inhibiting DNA synthesis are based on the ability to create analogues of these precursors that readily enter tumor cells and become activated intracellularly. As an example, the pyrimidine analogue 5FU is converted to a fluorodeoxyuridine monophosphate (FdUMP), which in turn blocks TS, an enzyme required for the physiological conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP),

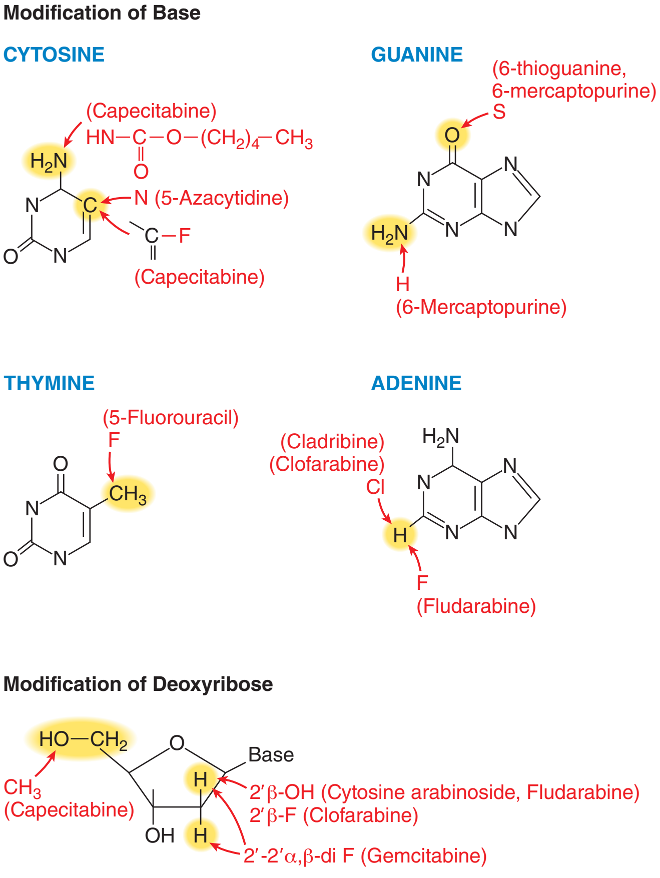
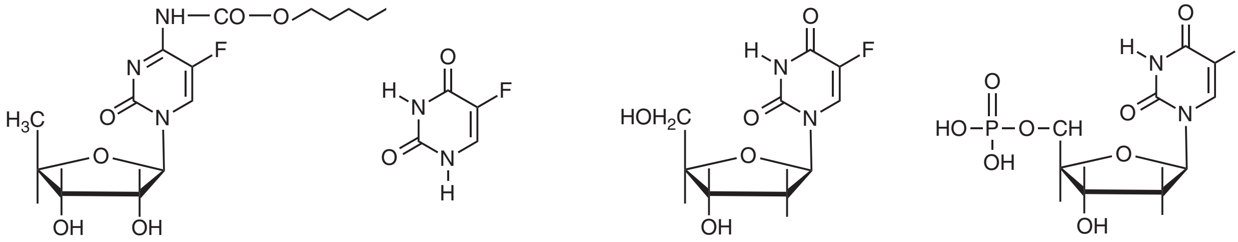


Figure 70–7 *Structural modification of base and deoxyribonucleoside analogues. Yellow ellipses* indicate sites modified to create antimetabolites. Specific substitutions are noted in red for each drug. Modifications occur in the base ring systems, in their amino or hydroxyl side groups, and in the deoxyribose sugar found in deoxyribonucleosides. See structures in Figure 70–8.

Fluoropyrimidine Analogue



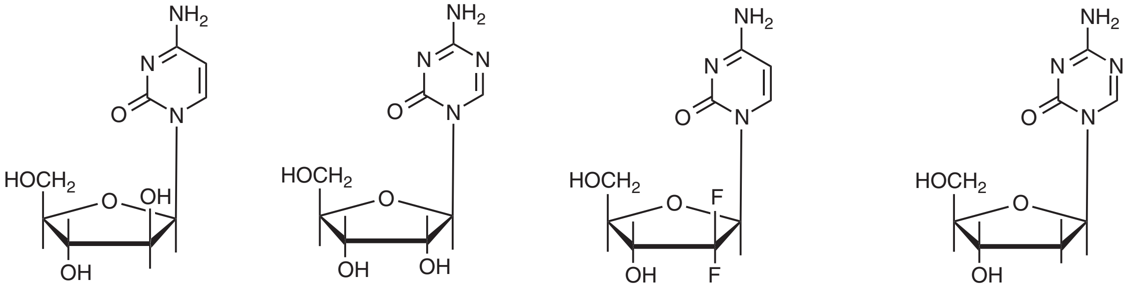
CAPECITABINE

5-FLUOROURACIL (5FU)

5-FLUORODEOXYURIDINE (FLOXURIDINE)

5-FLUORODEOXYURIDINE MONOPHOSPHATE

Cytidine Analogue



CYTOSINE ARABINOSIDE (CYTARABINE; Ara-C)

5-AZACYTIDINE

2′,2′-Difluorodeoxycytidine (gemcitabine)

Decitabine

Figure 70–8 *Pyrimidine analogues.*

a component of DNA (see Figure 70–6). Other analogues incorporate into DNA itself and thereby block its function (Wilson et al., 2014).

Cells can make the purine and pyrimidine bases *de novo* and convert them to their active triphosphates (deoxynucleotide triphosphates [dNTPs]), providing substrates for DNA polymerase. Alternatively, cells can salvage free bases or their deoxynucleosides from the bloodstream. Thus, cells can take up uracil, guanine, and their analogues and convert them to (deoxy)nucleotides by the addition of deoxyribose and phosphate groups. Antitumor analogues of these bases (5FU, 6TG) can be formulated as simple substituted bases. Other bases, including cytosine, thymine, and adenine and their analogues can only be used as deoxynucleosides, which are readily transported into cells and activated to deoxynucleotides by intracellular kinase. Thus, *cytarabine* (cytosine arabinoside [Ara-C]), *gemcitabine*, *5-azacytidine*, and adenosine analogues (*cladribine*) (Figures 70–7 and 70–8) are nucleosides readily taken up by cells, converted to nucleotides, and incorporated into DNA.

Fludarabine phosphate, a nucleotide, is dephosphorylated rapidly in plasma, releasing the nucleoside that is readily taken up by cells. Analogues may differ from the physiological bases in a variety of ways: by altering the purine or pyrimidine ring; by altering the sugar attached to the base, as in the arabinoside, Ara-C; or by altering both the base and sugar, as in *fludarabine phosphate* (see Figure 70–7). These alterations produce inhibitory effects on vital enzymatic pathways and prevent DNA synthesis.

Fluorouracil, Floxuridine, Capecitabine

Fluorouracil is available as 5FU, as the derivative fluorodeoxyuridine (FUdR) (not often used in clinical practice), and as a prodrug, *capecitabine*, which is ultimately converted to 5FU.

Mechanisms of Action

5-Fluorouracil requires enzymatic conversion (ribosylation and phosphorylation) to the nucleotide form to exert its cytotoxic activity. As the triphosphate 5-fluorouridine triphosphate (FUTP), the drug is incorporated into RNA. Alternative reactions can produce the deoxy derivative FdUMP; FdUMP inhibits TS and blocks the synthesis of deoxythymidine triphosphate (dTTP), a necessary constituent of DNA (see Figure 70–6). The folate cofactor, 5,10-methylene FH$_4$, and FdUMP form a covalently bound ternary complex with TS. The physiological complex of TS-folate-dUMP progresses to the synthesis of thymidylate by transfer of the methylene group and two hydrogen atoms from folate to dUMP, but this reaction is blocked in the inhibited complex of TS-FdUMP-folate by the stability of the fluorine carbon bond on FdUMP; sustained inhibition of the enzyme results.

5-Fluorouracil is incorporated into both RNA and DNA. In 5FU-treated cells, both fluorodeoxyuridine triphosphate (FdUTP) and deoxyuridine triphosphate (dUTP; which accumulates behind the blocked TS reaction) incorporate into DNA in place of the depleted physiological TTP. Presumably, such incorporation into DNA calls into action the excision-repair process, which can lead to DNA strand breakage because DNA repair requires TTP, which is lacking as a result of TS inhibition. 5FU incorporation into RNA also causes toxicity as the result of major effects on both the processing and the functions of RNA.

Mechanisms of Resistance

Resistance to the cytotoxic effects of 5FU or FUdR has been ascribed to loss or decreased activity of the enzymes necessary for activation of 5FU, amplification of TS, mutation of TS to a form that is not inhibited by FdUMP, and high levels of the degradative enzymes dihydrouracil dehydrogenase and thymidine phosphorylase. TS levels are finely controlled by an autoregulatory feedback mechanism wherein the unbound enzyme interacts with and inhibits the translational efficiency of its own mRNA, which provides for the rapid TS modulation needed for cellular division. When TS is bound to FdUMP, inhibition of translation is relieved and levels of free TS rise, restoring thymidylate synthesis. Thus, TS autoregulation may be an important mechanism by which malignant cells become insensitive to the effects of 5FU.

Combination with Leucovorin

Some malignant cells appear to have insufficient concentrations of 5,10-methylene FH$_4$ and thus cannot form maximal levels of the inhibited ternary complex with TS. Addition of exogenous folate in the form of *leucovorin* increases formation of the complex and enhances responses to 5FU. A number of other agents have been combined with 5FU in attempts to enhance the cytotoxic activity through biochemical modulation. MTX, by inhibiting purine synthesis and increasing cellular pools of 5-phosphoribosyl-1-pyrophosphate (PRPP), enhances the activation of 5FU and increases antitumor activity of 5FU when given prior to but not following 5FU. The combination of *cisplatin* and 5FU has yielded impressive responses in tumors of the upper aerodigestive tract, but the molecular basis of their interaction is unclear. A combination with *oxaliplatin*, which downregulates TS expression, is commonly used with 5FU and *leucovorin* for treating patients with metastatic colorectal cancer; the combination is abbreviated as FOLFOX. Addition of *irinotecan* (see discussion that follows) is abbreviated as FOLFIRINOX and used in the treatment of patients with colorectal or pancreatic cancer. A most important interaction is the enhancement of radiation therapy by fluoropyrimidines, the basis for which is unclear. 5FU with simultaneous radiation is effective in patients with anal cancer and enhances local tumor control in patients with head, neck, cervical, rectal, gastroesophageal, and pancreatic cancer.

ADME

5-Fluorouracil is administered parenterally because absorption after oral ingestion of the drug is unpredictable and incomplete. 5FU is inactivated by reduction of the pyrimidine ring in a reaction carried out by dihydropyrimidine dehydrogenase (DPD), which is found in liver, intestinal mucosa, tumor cells, and other tissues. Inherited deficiency of this enzyme leads to greatly increased sensitivity to the drug (Milano et al., 1999). DPD deficiency can be detected by either enzymatic or molecular assays using peripheral white blood cells or by determining the plasma ratio of 5FU to its metabolite, 5-fluoro-5,6-dihydrouracil.

Plasma clearance is rapid ($t_{1/2}$ about 10–20 min). Only 5% to 10% of a single intravenous dose of 5FU is excreted intact in the urine. The dose does not have to be modified in patients with hepatic dysfunction, presumably because of sufficient degradation of the drug at extrahepatic sites. 5FU enters the CSF in minimal amounts.

Therapeutic Uses

5-Fluorouracil. *5-Fluorouracil* produces partial responses in 10% to 20% of patients with metastatic colon carcinomas, upper GI tract carcinomas, and breast carcinomas but rarely is used as a single agent. 5FU in combination with *leucovorin* and *oxaliplatin* or *irinotecan* (FOLFOX or FOLFIRINOX) in adjuvant therapy is associated with a survival advantage for patients with colorectal cancers. For average-risk patients in good nutritional status with adequate hematopoietic function, the dosage regimen employs *leucovorin* once each week for 6 of 8 weeks. Other regimens use daily doses for 5 days, repeated in monthly cycles. When used with *leucovorin*, doses of daily 5FU for 5 days must be reduced because of mucositis and diarrhea. 5FU increasingly is used as a biweekly infusion, a schedule that has less overall toxicity as well as superior response rates and progression-free survival for patients with metastatic colon cancer. Also, topical application of 5FU is effective in the treatment of premalignant keratoses of the skin and multiple superficial basal cell carcinomas.

Floxuridine. Fluorodeoxyuridine is converted directly to FdUMP by thymidine kinase. The drug is administered primarily by continuous infusion into the hepatic artery for treatment of patients with metastatic carcinoma of the colon or following resection of hepatic metastases; the response rate of intrahepatic infusion (40%–50%) is twice that of intravenous administration. Intrahepatic arterial infusion for 14 to 21 days causes minimal systemic toxicity; however, there is a significant risk of biliary sclerosis if this route is used for multiple cycles of therapy. Treatment should be discontinued at the earliest manifestation of toxicity (usually stomatitis or diarrhea) because the maximal effects of bone marrow suppression and gut toxicity will not be evident until days 7 to 14.

Capecitabine. *Capecitabine*, an orally administered prodrug of 5FU, is approved for the treatment of patients with (1) metastatic breast cancer who have not responded to a regimen of *paclitaxel* and an anthracycline; (2) metastatic breast cancer when used in combination with *docetaxel* in patients who have had a prior anthracycline-containing regimen; and (3) metastatic colorectal cancer.

The recommended dosage is given in two divided doses with food, for 2 weeks, followed by a rest period of 1 week. *Capecitabine* is well absorbed orally. It is rapidly deesterified and deaminated, yielding high plasma concentrations of an inactive prodrug 5′-deoxyfluorodeoxyuridine (5′-dFdU), which disappears with a $t_{1/2}$ of about 1 h. The conversion of 5′-dFdU to 5FU by thymidine phosphorylase occurs in liver tissues, peripheral tissues, and tumors. 5FU levels are less than 10% of those of 5′-dFdU, reaching a maximum of 0.3 mg/L or 1 μM at 2 h. Liver dysfunction delays the conversion of the parent compound to 5′-dFdU and 5FU, but there is no consistent effect on toxicity.

Combination Therapy. Higher response rates are seen when 5FU or *capecitabine* is used in combination with other agents (e.g., with *cisplatin* in head and neck cancer, with *oxaliplatin* or *irinotecan* in colon cancer). The combination of 5FU and *oxaliplatin* or *irinotecan* has become the standard first-line treatment of patients with metastatic colorectal cancer (FOLFOX and FOLFIRINOX). The use of 5FU in combination regimens has improved survival in the adjuvant treatment of breast cancer and, with *oxaliplatin* and *leucovorin*, of colorectal cancer. 5FU also is a potent radiation sensitizer. Beneficial effects have been reported when combined with irradiation as primary treatment of patients with locally advanced cancers of the esophagus, stomach, pancreas, cervix, anus, and head and neck.

Adverse Effects

The clinical manifestations of toxicity caused by 5FU and *floxuridine* are similar. The earliest untoward symptoms during a course of therapy are anorexia and nausea, followed by stomatitis and diarrhea, reliable warning signs that a sufficient dose has been administered. Mucosal ulcerations occur throughout the GI tract and may lead to fulminant diarrhea, shock, and death, particularly in patients who are DPD deficient. The major toxic effects of bolus-dose regimens result from the myelosuppressive action of 5FU. The nadir of leukopenia usually occurs 9 to 14 days after the first injection of drug. Thrombocytopenia and anemia also may occur, as may loss of hair (occasionally progressing to total alopecia), nail changes, dermatitis, and increased pigmentation and atrophy of the skin. Hand-foot syndrome, a particularly prominent adverse effect of *capecitabine*, consists of erythema, desquamation, pain, and sensitivity to touch of the palms and soles. Acute chest pain with evidence of ischemia in the electrocardiogram may result from coronary artery vasospasms during or shortly after 5FU infusion. In general, myelosuppression, mucositis, and diarrhea occur less often with infusional than with bolus regimens, while hand-foot syndrome occurs more often with infusional than with bolus regimens. The significant risk of toxicity with fluoropyrimidines requires close supervision by physicians familiar with the drug's effects and possible hazards.

Capecitabine causes a similar spectrum of toxicities as 5FU (diarrhea, myelosuppression), but the hand-foot syndrome occurs more frequently and may require dose reduction or cessation of therapy.

Trifluridine

Trifluridine is the 5-trifluoromethyl pyrimidine nucleoside analogue of 5-FUdR (*floxuridine*; Figure 70–8). It is used in eye drops for the treatment of herpes simplex virus (see Table 74–5)) and in a fixed combination with *tipiracil*, a thymidine phosphorylase inhibitor, for the treatment of patients with metastatic colorectal cancers who have previously received other standard combination treatments that included FOLFIRINOX. *Trifluridine* and *tipiracil* are formulated together in a single tablet with *tipiracil* added to prevent rapid breakdown of *trifluridine*. The *trifluridine* mechanism of action mimics that of 5FU (Davidson et al., 2016).

Cytidine Analogues

Cytarabine (Cytosine Arabinoside; Ara-C)

Cytarabine is the most important antimetabolite used in the therapy of AML; it is the single most effective agent for induction of remission in this disease.

Mechanisms of Action

Cytarabine is an analogue of 2′-deoxycytidine; the 2′-hydroxyl in a position *trans* to the 3′-hydroxyl of the sugar (see Figures 70–7 and 70–8) hinders rotation of the pyrimidine base around the nucleoside bond and interferes with base pairing. The drug enters cells via ENT1 (SLC29A1). It is then converted to its active form, the 5′-monophosphate ribonucleotide, by deoxycytidine kinase (dCK), an enzyme that shows polymorphic expression among patients (see discussion that follows). Ara-CMP then reacts with deoxynucleotide kinases to form diphosphate and triphosphates (Ara-CDP and Ara-CTP). Ara-CTP competes with deoxycytidine triphosphate (dCTP) for incorporation into DNA by DNA polymerases. The incorporated Ara-CMP residue is a potent inhibitor of DNA polymerase, in both replication and repair synthesis, and blocks the further elongation of the nascent DNA molecule. If DNA breaks are not repaired, apoptosis ensues. Ara-C cytotoxicity correlates with the total Ara-C incorporated into DNA; incorporation of about 5 molecules of Ara-C per 10^4 bases of DNA decreases cellular clonogenicity by about 50% (Kufe et al., 1984).

In infants and adults with ALL and t(4;11) MLL translocation, usefulness of high-dose Ara-C is mediated by the highly expressed nucleoside transporter ENT1, and its expression correlates with sensitivity to Ara-C (Pui et al., 2004). At extracellular drug concentrations greater than 10 μM (levels achievable with high-dose Ara-C), the nucleoside transporter no longer limits drug accumulation, and intracellular metabolism to a triphosphate becomes rate limiting. Patients with particular subtypes of AML derive benefit from high-dose Ara-C treatment; these types include t(8;21), inv(16), t(9;16), and del(16).

Mechanisms of Resistance

Response to Ara-C is strongly influenced by the relative activities of anabolic and catabolic enzymes that determine the proportion of drug converted to Ara-CTP. The rate-limiting activating enzyme dCK produces Ara-CMP. It is opposed by the degradative enzyme, cytidine deaminase, which converts Ara-C to a nontoxic metabolite, ara-uridine (Ara-U). Cytidine deaminase activity is high in many normal tissues, including intestinal mucosa, liver, and neutrophils, but lower in AML cells and other human tumors. A second degradative enzyme, dCMP deaminase, converts Ara-CMP to the inactive metabolite Ara-UMP. Increased synthesis and retention of Ara-CTP in leukemic cells lead to a longer duration of complete remission in patients with AML. The capacity of cells to transport Ara-C also may affect response. Clinical studies have implicated a loss of dCK as the primary mechanism of resistance to Ara-C in AML.

ADME

Due to the presence of high concentrations of cytidine deaminase in the GI mucosa and liver, only about 20% of the drug reaches the circulation after *oral* Ara-C administration; thus, the drug must be given intravenously. Peak concentrations of 2 to 50 μM are measurable in plasma after intravenous injection of 30 to 300 mg/m^2 but fall rapidly ($t_{1/2} \approx 10$ min). Less than 10% of the injected dose is excreted unchanged in the urine within 12 to 24 h; most appears as the inactive deaminated product, Ara-U. Higher concentrations of Ara-C are found in CSF after continuous infusion than after rapid intravenous injection but are 10% or less of concentrations in plasma. After *intrathecal* administration of the drug at a dose of 50 mg/m^2, deamination proceeds slowly, with a $t_{1/2}$ of 3 to 4 h, and peak concentrations of 1 to 2 μM are achieved. CSF concentrations remain above the threshold for cytotoxicity (0.4 μM) for 24 h or longer. A depot liposomal formulation of Ara-C provides sustained release into the CSF. After a standard dose, liposomal Ara-C remains above cytotoxic levels for an average of 12 days, thus avoiding the need for frequent lumbar punctures.

Therapeutic Uses

Continuous inhibition of DNA synthesis for a duration equivalent to at least one cell cycle or 24 h is necessary to expose most tumor cells during the S phase of the cell cycle. The optimal interval between bolus doses of Ara-C is about 8 to 12 h, a schedule that maintains intracellular concentrations of Ara-CTP at inhibitory levels during a multiday cycle of treatment. In general, children tolerate higher doses than adults. The intrathecal administration of liposomal Ara-C every 2 weeks seems equally effective as the regimen every 4 days with the standard drug. Ara-C is indicated for induction and maintenance of remission in AML and is useful in the treatment of patients with other leukemias, such as acute lymphoblastic leukemia (ALL), CML in the blast phase, acute promyelocytic leukemia (APL), and high-grade lymphomas. Because drug concentration in plasma rapidly falls below the level needed to saturate transport and intracellular activation, clinicians have employed high-dose regimens every 12 h for 3 to 4 days to achieve 20 to 50 times higher serum levels, with improved results in remission induction and consolidation for AML. Injection of the liposomal formulation is indicated for the intrathecal treatment of lymphomatous meningitis.

Adverse Effects

Cytarabine is myelosuppressive and can produce acute, severe leukopenia, thrombocytopenia, and anemia with striking megaloblastic changes. Other toxic manifestations include GI disturbances, stomatitis, conjunctivitis, reversible hepatic enzyme elevations, noncardiogenic pulmonary edema, and dermatitis. The onset of dyspnea, fever, and pulmonary infiltrates on chest computed tomographic scans may follow 1 to 2 weeks after high-dose Ara-C and may be fatal in 10% to 20% of patients, especially in patients being treated for relapsed leukemia. No specific therapy, other than Ara-C discontinuation, is indicated. Intrathecal Ara-C, either the free drug or the liposomal preparation, may cause arachnoiditis, seizures, delirium, myelopathy, or coma, especially if given concomitantly with systemic high-dose MTX or systemic Ara-C. Cerebellar toxicity, manifesting as ataxia and slurred speech, and cerebral toxicity (seizures, dementia, and coma) may follow intrathecal administration or high-dose systemic administration, especially in patients older than 50 years or patients with poor renal function.

Azacitidine (5-Azacytidine); Decitabine

5-Azacytidine and *decitabine* (2′-deoxy-5-azacytidine; see Figure 70–8) have antileukemic activity as cytotoxic agents and particularly at lower doses induce leukemic cell differentiation by inhibiting DNA cytosine methyltransferase activity. Both drugs are approved for treatment of myelodysplasia, for which they induce normalization of bone marrow in 15% to 20% of patients and reduce the transfusion requirement in one-third of patients. *5-Azacytidine* improves survival.

Mechanism of Action

The aza-nucleosides enter cells by ENT1 (SLC29A1). The drugs incorporate into DNA, where they become covalently bound to the DNA methyltransferase, depleting intracellular enzyme and leading to global demethylation of DNA that results in tumor cell differentiation and apoptosis. *Decitabine* also induces double-strand DNA breaks, perhaps as a consequence of the effort to repair the protein-DNA adduct.

ADME

After subcutaneous administration, *5-azacytidine* undergoes rapid deamination by cytidine deaminase (plasma $t_{1/2}$ ~20–40 min). Due to the formation of intracellular nucleotides that become incorporated into DNA, the effects of the aza-nucleosides persist for many hours.

Therapeutic Use

The usual treatment regimen for *5-azacytidine* in patients with myelodysplastic syndrome is daily for 7 days every 28 days, while *decitabine* is given intravenously every day for 5 days every 4 weeks. Best responses may become apparent only after two to five courses of treatment.

Adverse Effects

The major toxicities of the aza-nucleosides include myelosuppression and mild GI symptoms. *5-Azacytidine* produces severe nausea and vomiting when given intravenously in large doses (150–200 mg/m^2 per day for 5 days).

Gemcitabine

Gemcitabine, a difluoro analogue of *deoxycytidine* (dFdC; see Figure 70–8), is used in the treatment of patients with pancreatic; nonsquamous, non–small cell lung; ovarian; breast; and bladder cancer.

Mechanism of Action

Gemcitabine enters cells via three distinct nucleoside transporters: ENT1 (SLC29A1; the major route), CNT1 (SLC28A1), and a nucleobase transporter found in malignant mesothelioma cells. Intracellularly, dCK phosphorylates *gemcitabine* to the monophosphate (dFdCMP), which is converted to di- and triphosphates (dFdCDP and dFdCTP, respectively). Although *gemcitabine*'s anabolism and effects on DNA in general mimic those of *cytarabine*, there are distinct differences in kinetics of inhibition, additional enzymatic sites of action, different effects of incorporation into DNA, and a distinct spectrum of clinical activity. Unlike that of *cytarabine*, the cytotoxicity of *gemcitabine* is not confined to the S phase of the cell cycle. The cytotoxic activity may reflect several actions on DNA synthesis. dFdCTP competes with dCTP as a weak inhibitor of DNA polymerase. dFdCDP is a stoichiometric inhibitor of ribonucleoside diphosphate reductase (RNR), resulting in depletion of deoxyribonucleotide pools necessary for DNA synthesis. Incorporation of dFdCTP into DNA causes DNA strand termination (Heinemann et al., 1988) and appears resistant to repair. The capacity of cells to incorporate dFdCTP into DNA is critical for *gemcitabine*-induced apoptosis. *Gemcitabine* is inactivated by cytidine deaminase, which is found both in tumor cells and throughout the body.

ADME

Gemcitabine is administered as an intravenous infusion. The pharmacokinetics of the parent compound are largely determined by deamination in liver, plasma, and other organs, and the predominant urinary elimination product is 2′,2′-difluorodeoxycytidine (dFdU). In patients with significant renal dysfunction, dFdU and its triphosphate accumulate to high and potentially toxic levels. *Gemcitabine* has a short plasma $t_{1/2}$ (~15 min); women and elderly patients clear the drug more slowly.

Therapeutic Uses

The standard dosing schedule for *gemcitabine* is an intravenous infusion on days 1, 8, and 15 of each 21- to 28-day cycle, depending on the indication. Conversion of *gemcitabine* to dFdCMP by dCK is saturated at infusion rates of about 10 mg/m^2/min. To increase dFdCTP formation, the duration of infusion at this maximum concentration has been extended to 100 to 150 min at a fixed rate of 10 mg/min. The 150-min infusion produces a higher level of dFdCTP within peripheral blood mononuclear cells and increases the degree of myelosuppression. The inhibition of DNA repair by *gemcitabine* may increase cytotoxicity of other agents, particularly platinum compounds, and with radiation therapy.

Adverse Effects

The principal toxicity is myelosuppression. Longer duration infusions lead to greater myelosuppression and hepatic toxicity. Nonhematological toxicities include a flu-like syndrome, asthenia, and rarely a posterior leukoencephalopathy syndrome. Mild, reversible elevation in liver transaminases may occur in 40% or more of patients. Interstitial pneumonitis, at times progressing to acute respiratory distress syndrome, may occur within the first two cycles of treatment and usually responds to corticosteroids. Rarely, patients treated for many months may develop a slowly progressive hemolytic uremic syndrome, necessitating drug discontinuation. *Gemcitabine* is a very potent radiosensitizer and should not be used with radiotherapy.

Purine Analogues

The pioneering studies of Hitchings and Elion identified analogues of naturally occurring purine bases with antileukemic and immunosuppressant properties. Figure 70–9 shows structural formulas of several purine analogues, with *adenosine* for comparison. Other purine analogues that have valuable roles in leukemia and lymphoid malignancies include *cladribine* (standard therapy of hairy cell leukemia), *fludarabine phosphate* (standard treatment of CLL), *nelarabine* (pediatric ALL), and *clofarabine* (T-cell leukemia/lymphoma). The apparent selectivity of these agents may relate to their effective uptake, activation, and apoptotic effects in lymphoid tissue.

6-Thiopurine Analogues

6-Mercaptopurine and 6TG are approved agents for human leukemias and function as analogues of the natural purines hypoxanthine and guanine. The substitution of sulfur for oxygen on C6 of the purine ring creates compounds that are readily transported into cells, including activated malignant cells. Nucleotides formed from 6MP and 6TG inhibit *de novo* purine synthesis and also become incorporated into nucleic acids (see Figure 55–5).

Mechanism of Action

Hypoxanthine guanine phosphoribosyl transferase converts 6TG and 6MP to the ribonucleotides 6-thioGMP and 6-thioIMP (T-IMP), respectively. Because T-IMP is a poor substrate for guanylyl kinase (the enzyme that converts GMP to GDP), T-IMP accumulates intracellularly. T-IMP inhibits the new formation of ribosyl-5-phosphate, as well as conversion of IMP to adenine and guanine nucleotides. The most important point of attack seems to be the reaction of glutamine and PRPP to form ribosyl-5-phosphate, the first committed step in the *de novo* pathway. 6TG nucleotide is incorporated into DNA, where it induces strand breaks and base mispairing.

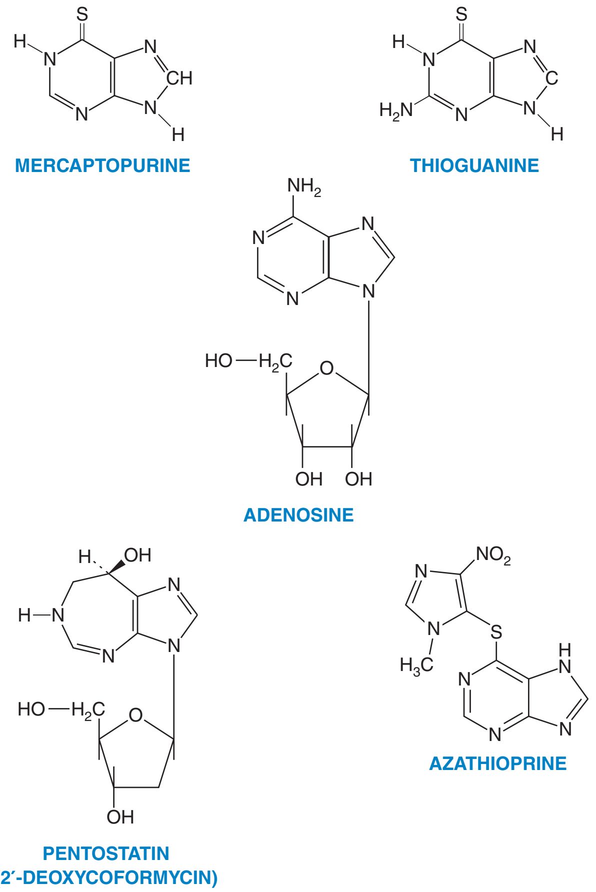


Figure 70–9 *Adenosine and various purine analogues.*

Mechanisms of Resistance

The most common mechanism of 6MP resistance observed *in vitro* is deficiency or complete lack of the activating enzyme HGPRT or increased alkaline phosphatase activity. Other mechanisms for resistance include:

- Decreased drug uptake or increased efflux due to active transporters
- Alteration in allosteric inhibition of ribosylamine 5-phosphate synthase
- Impaired recognition of DNA breaks and mismatches due to loss of a component (MSH6, mutator S homologue 6) of the MMR system (Karran and Attard, 2008)

ADME and Toxicity

Absorption of oral *mercaptopurine* is incomplete (10%–50%); the drug is subject to first-pass metabolism by xanthine oxidase in the liver. Food or oral antibiotics decrease absorption. Oral bioavailability is increased when *mercaptopurine* is combined with high-dose MTX. After an intravenous dose, the $t_{1/2}$ of the drug is about 50 min in adults due to rapid metabolic degradation by xanthine oxidase and by thiopurine methyltransferase (TPMT). Restricted brain distribution of *mercaptopurine* results from an efficient efflux transport system in the blood-brain barrier. In addition to the HGPRT-catalyzed anabolism of *mercaptopurine*, there are two other pathways for its metabolism. The first involves methylation of the sulfhydryl group and subsequent oxidation of the methylated derivatives. Activity of the enzyme TPMT reflects the inheritance of polymorphic alleles; up to 15% of the Caucasian population has decreased enzyme activity. Low levels of erythrocyte TPMT activity are associated with increased drug toxicity in individual patients and a lower risk of relapse. In patients with autoimmune disease treated with *mercaptopurine*, those with polymorphic alleles may experience bone marrow aplasia and life-threatening toxicity. Testing for these polymorphisms prior to treatment is recommended in this patient population.

A relatively large percentage of the administered sulfur appears in the urine as inorganic sulfate. The second major pathway for 6MP metabolism involves its oxidation by xanthine oxidase to 6-thiourate, an inactive metabolite. Oral doses of 6MP should be reduced by 75% in patients receiving the xanthine oxidase inhibitor *allopurinol*; no dose adjustment is required for intravenous dosing.

Therapeutic Uses

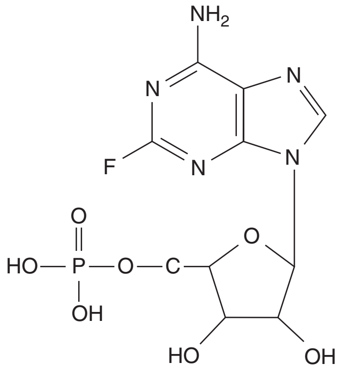
In the maintenance therapy of ALL, an initial daily oral dose of 6MP is adjusted according to white blood cell and platelet counts. The combination of MTX and 6MP appears to be synergistic. By inhibiting the earliest steps in purine synthesis, MTX elevates the intracellular concentration of PRPP, a cofactor required for 6MP activation.

Adverse Effects

The principal toxicity of 6MP is myelosuppression. Thrombocytopenia, granulocytopenia, or anemia may not become apparent for several weeks. Dose reduction usually results in prompt recovery, although myelosuppression may be severe and prolonged in patients with a polymorphism affecting TPMT. Anorexia, nausea, or vomiting is seen in about 25% of adults, but stomatitis and diarrhea are rare; manifestations of GI effects are less frequent in children than in adults. Jaundice and hepatic enzyme elevations occur in up to one-third of adult patients treated with 6MP and usually resolve on discontinuation of therapy. 6MP and its derivative *azathioprine* predispose to opportunistic infection (e.g., reactivation of hepatitis B, fungal infection, and *Pneumocystis* pneumonia) and an increased incidence of squamous cell malignancies of the skin. 6MP is teratogenic during the first trimester of pregnancy, and AML has been reported after prolonged 6MP therapy for Crohn's disease.

Fludarabine Phosphate

Fludarabine phosphate is a fluorinated, deamination-resistant, phosphorylated analogue of the antiviral agent *vidarabine* (9-β-D-arabinofuranosyl-adenine). It is active in CLL and low-grade lymphomas and is also effective as a potent immunosuppressant.



FLUDARABINE PHOSPHATE

Mechanisms of Action and Resistance

The drug is dephosphorylated extracellularly to the nucleoside *fludarabine*, which enters the cell and is rephosphorylated by dCK to the active triphosphate. This antimetabolite inhibits DNA polymerase, DNA primase, DNA ligase, and RNR and becomes incorporated into DNA and RNA. The nucleotide is an effective chain terminator when incorporated into DNA (Kamiya et al., 1996). Incorporation of *fludarabine* into RNA inhibits RNA function, RNA processing, and mRNA translation.

In experimental tumors, resistance to *fludarabine* is associated with decreased activity of dCK (the enzyme that phosphorylates the drug), increased drug efflux, and increased RNR activity. Its mechanism of immunosuppression and paradoxical stimulation of autoimmunity stems from the particular susceptibility of lymphoid cells to purine analogues and the specific effects on the $CD4^+$ subset of T cells, as well as its inhibition of regulatory T-cell responses.

ADME

Fludarabine phosphate is administered both intravenously and orally and is rapidly converted to *fludarabine* in the plasma. The median time to reach maximal concentrations of drug in plasma after oral administration is 1.5 h, and oral bioavailability averages 55% to 60%. The $t_{1/2}$ of *fludarabine* in plasma is about 10 h. The compound is eliminated primarily by renal excretion.

Therapeutic Uses

Fludarabine phosphate is approved for intravenous and oral use and is equally active by both routes. The recommended dose interval is daily for 5 days and may be repeated every 4 weeks. Gradual improvement in CLL usually occurs within two to three cycles. Dosage should be reduced in patients with renal impairment in proportion to the reduction in CL_{Cr}. *Fludarabine phosphate* is highly active alone or with *rituximab* and *cyclophosphamide* for the treatment of patients with CLL; overall response rates in previously untreated patients approximate 80%, and the duration of response averages 22 months. The synergy of *fludarabine* with alkylators may stem from the observation that it blocks the repair of double-strand DNA breaks and interstrand cross-links induced by alkylating agents. It also is effective in follicular B-cell lymphomas refractory to standard therapy. It is increasingly used as a potent immunosuppressive agent in nonmyeloablative allogeneic bone marrow transplantation.

Adverse Effects

Oral and intravenous therapy cause myelosuppression in about 50% of patients, nausea and vomiting in a minor fraction, and, uncommonly, chills and fever, malaise, anorexia, peripheral neuropathy, and weakness. Lymphopenia and thrombocytopenia and cumulative side effects are expected. Depletion of $CD4^+$ T cells with therapy predisposes to opportunistic infections. Tumor lysis syndrome, a rare complication, occurs primarily in previously untreated patients with CLL. Altered mental status, seizures, optic neuritis, and coma have been observed at higher doses and in older patients. Autoimmune events may occur after *fludarabine* treatment. Patients with CLL may develop an acute hemolytic anemia or pure red cell aplasia during or following *fludarabine* treatment. Prolonged cytopenias, probably mediated by autoimmunity, also complicate *fludarabine* treatment. Myelodysplasia and acute leukemias may arise as late complications. Pneumonitis is an occasional side effect and responds to corticosteroids. In patients with compromised renal function, the initial doses should be reduced in proportion to the reduction in CL_{Cr}.

Cladribine

Cladribine is an adenosine deaminase (ADA)-resistant purine analogue that has potent and curative activity in hairy cell leukemia, CLL, and low-grade lymphomas.

Mechanisms of Action and Resistance

Cladribine enters cells via active nucleoside transport. After phosphorylation by dCK and conversion to cladribine triphosphate, it is incorporated into DNA. It produces DNA strand breaks and depletion of NAD and ATP, leading to apoptosis. It is a potent inhibitor of RNR. The drug does not require cell division to be cytotoxic. Resistance is associated with loss of the activating enzyme dCK; increased expression of RNR; or increased active efflux by ABCG2 or other members of the ATP binding cassette (ABC) family of transporters.

ADME

Cladribine is absorbed orally (55%) but is routinely administered intravenously. It is excreted by the kidneys, with a terminal $t_{1/2}$ in plasma of 6.7 h. *Cladribine* crosses the blood-brain barrier and reaches CSF concentrations of about 25% of those seen in plasma. Doses should be adjusted for renal dysfunction.

Therapeutic Uses

Cladribine is administered daily for 7 days by continuous intravenous infusion. It is the drug of choice in hairy cell leukemia. Eighty percent of patients achieve a complete response after a single course of therapy. The drug also is active in CLL; low-grade lymphomas; Langerhans cell histiocytosis; CTCLs, including mycosis fungoides and the Sézary syndrome; and Waldenström macroglobulinemia.

Adverse Effects

The major dose-limiting toxicity of *cladribine* is myelosuppression. Cumulative thrombocytopenia may occur with repeated courses. Opportunistic infections are common and correlate with decreased $CD4^+$ cell counts. Other toxic effects include nausea, infections, high fever, headache, fatigue, skin rashes, and tumor lysis syndrome.

Clofarabine (2-Chloro-2′-fluoro-arabinosyladenine)

The analogue *clofarabine* (2-chloro-2′-fluoro-arabinosyladenine) incorporates the 2-chloro, glycosylase-resistant substituent of *cladribine* and a 2′-fluoro-arabinosyl substitution, which adds stability and enhances uptake and phosphorylation. The resulting compound is approved for the treatment of pediatric ALL after failure of two prior therapies. *Clofarabine* produces complete remissions in 20% to 30% of these patients. It has activity in pediatric and adult AML and in myelodysplasia. The uptake and metabolic activation of *clofarabine* in tumor cells follow the same path as *cladribine* and the other purine nucleosides, although *clofarabine* is more readily phosphorylated by dCK. *Clofarabine triphosphate* has a long intracellular $t_{1/2}$ (24 h). It incorporates into DNA, where it terminates DNA synthesis and leads to apoptosis; *clofarabine* also inhibits RNR.

Therapeutic Uses and Adverse Effects

In children, *clofarabine* is administered as a 2-h infusion daily for 5 days. The primary elimination $t_{1/2}$ in plasma is 6.5 h. Most of the drug is excreted unchanged in the urine. Doses should be adjusted according to reductions in CL_{Cr}. The primary toxicities are myelosuppression; a clinical syndrome of hypotension, tachyphemia, pulmonary edema, organ dysfunction, and fever, all suggestive of capillary leak syndrome and cytokine release that necessitate immediate discontinuation of the drug; elevated hepatic enzymes and increased bilirubin; nausea, vomiting, and diarrhea; and hypokalemia and hypophosphatemia.

Nelarabine (6-Methoxy-arabinosyl-guanine)

Nelarabine (6-methoxy-arabinosyl-guanine) is the only guanine nucleoside in clinical use. It has selective activity against acute T-cell leukemia (20% complete responses) and the closely related T-cell lymphoblastic lymphoma and is approved for use in patients with relapsed/refractory disease. Its basic mechanism of action closely resembles that of the other purine nucleosides, in that it is incorporated into DNA and terminates DNA synthesis.

ADME

Following infusion, the parent methoxy compound is rapidly activated in blood and tissues by ADA-mediated cleavage of the methyl group, yielding the phosphorylase-resistant Ara-G, which has a plasma $t_{1/2}$ of 3 h. The active metabolite is transported into tumor cells, where it is activated by dCK to Ara-GTP, which incorporates into DNA and terminates DNA synthesis. The drug and its metabolite Ara-G are primarily eliminated by metabolism to guanine, and a smaller fraction is eliminated by renal excretion of Ara-G. The drug should be used with close clinical monitoring in patients with renal impairment (CL_{Cr} <50 mL/min). Adults are given a 2-h infusion on days 1, 3, and 5 of a 21-day cycle, and children are given a lower dose for 5 days, repeated every 21 days.

Adverse Effects

The adverse effects include myelosuppression and liver function test abnormalities, as well as infrequent serious neurological sequelae, such as seizures, delirium, somnolence, peripheral neuropathy, or Guillain-Barré syndrome. Neurological adverse effects may not be reversible.

Pentostatin (2′-Deoxycoformycin)

Pentostatin (2′-deoxycoformycin; see Figure 70–9), a transition-state analogue of the intermediate in the ADA reaction, potently inhibits ADA. Its effects mimic the phenotype of genetic ADA deficiency (severe immunodeficiency affecting T-cell and B-cell functions).

Mechanism of Action

Inhibition of ADA by *pentostatin* leads to accumulation of intracellular adenosine and deoxyadenosine nucleotides, which can block DNA synthesis by inhibiting RNR. Deoxyadenosine also inactivates *S*-adenosyl homocysteine hydrolase. The resulting accumulation of *S*-adenosyl homocysteine is particularly toxic to lymphocytes. *Pentostatin* also can inhibit RNA synthesis, and its triphosphate derivative is incorporated into DNA, resulting in strand breakage. Although the precise mechanism of cytotoxicity is not known, it is probable that the imbalance in purine nucleotide pools accounts for its antineoplastic effect in hairy cell leukemia and T-cell lymphomas.

ADME

Pentostatin is administered intravenously every other week and has a mean terminal $t_{1/2}$ of 5.7 h. After hydration with 500 to 1000 mL of 5% dextrose in half-normal (0.45%) saline, the drug is administered by rapid intravenous injection or by infusion over a period of 30 min or less, followed by an additional 500 mL of fluids. The drug is eliminated almost entirely by renal excretion. Proportional reduction of dosage is recommended in patients with renal impairment as measured by reduced CL_{Cr}.

Therapeutic Use

Pentostatin is effective in producing complete remissions (58%) and partial responses (28%) in patients with hairy cell leukemia. It largely has been superseded by *cladribine* (see previous discussion). Toxic manifestations include myelosuppression, GI symptoms, skin rashes, and abnormal liver function studies. Depletion of normal T cells occurs, and neutropenic fever and opportunistic infections may result. Immunosuppression may persist for several years after discontinuation. At high doses, major renal and neurological complications are encountered. *Pentostatin* in combination with *fludarabine phosphate* may result in severe or even fatal pulmonary toxicity.

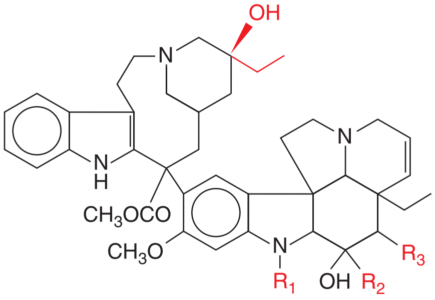
III. Natural Products

Microtubule-Damaging Agents

Structural biology analyses have revealed multiple binding sites of microtubule targeting drugs with different mechanisms of action; the major distinct binding sites are for vinca, taxane, and colchicine (Steinmetz and Prota, 2018).

Vinca Alkaloids

Purified alkaloids from the periwinkle plant, including *vinblastine* and *vincristine*, were among the earliest clinical agents for the treatment of patients with leukemias, lymphomas, and testicular cancer. A closely related derivative, *vinorelbine*, has activity against lung and breast cancer.



Basic structure of vinca alkaloids

Mechanism of Action

The vinca alkaloids are cell-cycle–specific agents and, in common with other drugs, such as *colchicine*, *podophyllotoxin*, the taxanes, and the epothilones, block cells in mitosis. The biological activities of the vincas can be explained by their ability to bind to a specific site on β-tubulin and to block its polymerization with α-tubulin into microtubules (Figure 70–10) (Akhmanova and Steinmetz, 2015). The mitotic spindle cannot form, duplicated chromosomes cannot align along the division plate, and cell division arrests in metaphase. Cells blocked in mitosis undergo changes characteristic of apoptosis. Microtubules are found in high concentration in the brain and contribute to other cellular functions, such as movement, phagocytosis, and axonal transport. Adverse effects of the vinca alkaloids, such as their neurotoxicity, may relate to disruption of these functions.

Resistance

Despite their structural similarity, the individual vinca alkaloids have unique patterns of clinical efficacy (see discussion that follows). However, in most experimental systems, they share cross-resistance. Their antitumor effects are blocked by multidrug resistance mediated by P-glycoprotein (Pgp), which confers resistance to a broad range of agents (vinca alkaloids, epipodophyllotoxins, anthracyclines, and taxanes). Pgp, also known as MDR1, ABCB1, and CD243, is an ATP-dependent efflux pump with broad substrate specificity that provides a cellular defense mechanism against potentially harmful substances. Chromosomal abnormalities consistent with gene amplification and markedly increased levels of Pgp have been observed in resistant cells in culture. Other membrane transporters, such as the multidrug resistance-associated protein (MRP) and the closely related breast cancer resistance protein (BCRP), may contribute to resistance. Other forms of resistance to vinca alkaloids stem from mutations in β-tubulin or in the relative expression of isoforms of β-tubulin that prevent the inhibitors from effectively binding to their target.

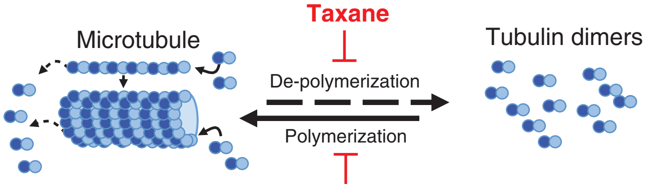


Figure 70–10 *Inhibitors of microtubular function.*

Adverse Effects

The limited myelosuppressive activity of *vincristine* makes it a valuable component of several combination therapy regimens for leukemia and lymphoma, while the lack of severe neurotoxicity of *vinblastine* is a decided advantage in lymphomas and in combination with *cisplatin* against testicular cancer. *Vinorelbine*, which causes mild neurotoxicity and myelosuppression, has an intermediate toxicity profile.

ADME

Hepatic CYPs extensively metabolize all three drugs, and the metabolites are excreted in the bile. Only a small fraction of a dose (<15%) is found unchanged in the urine. In patients with hepatic dysfunction (bilirubin >3 mg/dL), a 50% to 75% reduction in dose of any of the vinca alkaloids is advisable. The elimination $t_{1/2}$ is 20 h for *vincristine*, 23 h for *vinblastine*, and 24 h for *vinorelbine*.

Vinblastine

Therapeutic Uses. *Vinblastine sulfate* is given intravenously; special precautions must be taken against subcutaneous extravasation, which may cause painful irritation and ulceration. The drug should not be injected into an extremity with impaired circulation. After a single dose, myelosuppression reaches its maximum in 7 to 10 days. If a moderate level of leukopenia (~3000 cells/mm^3) is not attained, the weekly dose may be increased gradually. For testicular cancer, *vinblastine* is administered every 3 weeks. Doses should be reduced by 50% for patients with plasma bilirubin greater than 1.5 mg/dL. *Vinblastine* is used with *bleomycin* and *cisplatin* in the curative therapy of patients with metastatic testicular cancer, although it has been mainly supplanted by *etoposide* (see further discussion) or *ifosfamide* (see previous material). It is a component of the standard curative ABVD (***a***driamycin [doxorubicin]), *bleomycin*, *vinblastine*, *dacarbazine*) regimen for Hodgkin lymphoma. *Vinblastine* also is active against Kaposi sarcoma, neuroblastoma, Langerhans cell histiocytosis, bladder cancer, carcinoma of the breast, and choriocarcinoma.

Adverse Effects. Maximal leukopenia occurs within 7 to 10 days, after which recovery ensues within 7 days. Other toxic effects of *vinblastine* include mild neurological manifestations. GI disturbances, including nausea, vomiting, anorexia, and diarrhea, may be encountered. The syndrome of inappropriate secretion of antidiuretic hormone has been reported. Loss of hair, stomatitis, and dermatitis occur infrequently. Extravasation during injection may lead to cellulitis and phlebitis.

Vincristine

Therapeutic Uses. *Vincristine* is a standard component of regimens for treating pediatric patients with leukemias, lymphomas, and solid tumors, such as Wilms tumor, neuroblastoma, and rhabdomyosarcoma. In large-cell non-Hodgkin lymphomas, *vincristine* remains an important agent, particularly when used in the CHOP (*cyclophosphamide*, *doxorubicin*, *vincristine*, and *prednisone*) regimen. *Vincristine* with glucocorticoids is the treatment of choice to induce remissions in patients with childhood leukemia and in combination with alkylating agents and *anthracycline* for those with pediatric sarcomas. The common adult intravenous dosage for *vincristine* is 1.4 mg/m^2 of body surface area at weekly or longer intervals, with a maximum dose of 2 mg at each periodic administration. *Vincristine* is tolerated better by children than by adults, who may experience severe, progressive neurological toxicity and require lowering of the dose. Administration of the drug more frequently than every 7 days or at higher doses increases the toxic manifestations without proportional improvement in the response rate. Precautions also should be used to avoid extravasation during intravenous administration. Doses should be reduced for patients with elevated plasma bilirubin.

Adverse Effects. The clinical toxicity of *vincristine* is mostly neurological. Severe neurological manifestations may be reversed by suspending therapy or reducing the dosage at the first sign of motor dysfunction. Severe constipation, sometimes resulting in colicky abdominal pain and obstruction, may be prevented by laxatives and hydrophilic (bulk-forming) agents. Reversible alopecia occurs in about 20% of patients. Modest leukopenia may occur. Thrombocytopenia, anemia, and the syndrome of inappropriate secretion of antidiuretic hormone are less common. Inadvertent injection of *vincristine* into the CSF causes a devastating and often-fatal irreversible coma and seizures.

Vinorelbine

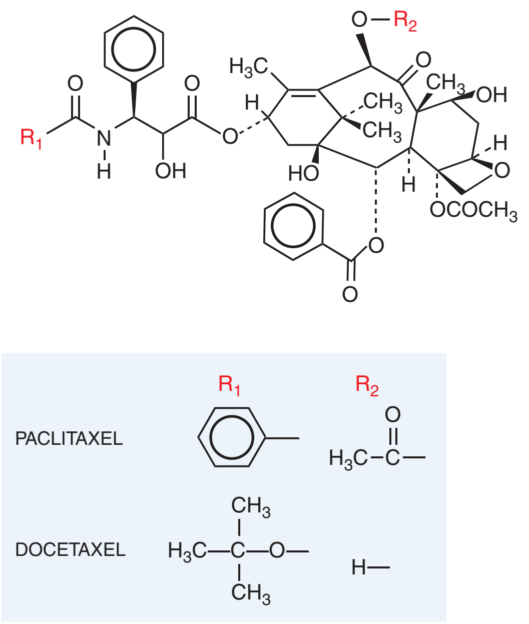
Vinorelbine has activity against non–small cell lung cancer and breast cancer. *Vinorelbine* is administered in normal saline as an intravenous infusion over 6 to 10 min or as an oral capsule formulation. When used alone, it is given intravenously at doses of 30 mg/m^2 either weekly or for 2 of every 3 weeks. When used with *cisplatin* for the treatment of non–small cell lung cancer, it is given at doses of 25 mg/m^2 either weekly or for 3 of every 4 weeks. A lower dose (20–25 mg/m^2) may be required for patients who have received prior chemotherapy and for hematological toxicity. The primary adverse effect of *vinorelbine* is granulocytopenia, with only modest thrombocytopenia and less neurotoxicity than other vinca alkaloids. *Vinorelbine* may cause allergic reactions and mild, reversible changes in liver enzymes. Doses should be reduced in patients with elevated plasma bilirubin.

Eribulin

Eribulin is a synthetic analogue of halichondrin, a poly-ether macrolide, originally isolated from the Pacific marine sponge *Halichondria okadai*. The drug binds to the vinca site on β-tubulin and inhibits microtubule assembly. *Eribulin* is a poorer substrate than other microtubule disruptors for the Pgp efflux pump and is effective in drug-resistant tumors that overexpress Pgp. *Eribulin* is approved for the treatment of patients with drug-resistant metastatic breast cancer and liposarcoma. Adverse effects overlap with those of the vinca alkaloids and include neutropenia, neuropathies, and GI toxicities. *Tirbanibulin*, a mitotic inhibitor that binds to the colchicine binding site on β-tubulin, was FDA-approved in 2020 for local treatment of actinic keratosis, a precursor lesion of squamous cell carcinoma.

Taxanes

Paclitaxel was first isolated from the bark of the Western yew tree, *Taxus brevifolia*. *Paclitaxel* and its semisynthetic congeners *docetaxel* and *cabazitaxel* exhibit unique pharmacological properties as inhibitors of mitosis. These taxanes differ from the vinca alkaloids and colchicine derivatives in that they bind to a different site on β-tubulin and promote rather than inhibit microtubule formation (see Figure 70–10), stabilizing tubulin-GDP (Akhmanova and Steinmetz, 2015). The taxanes have a central role in the therapy of patients with ovarian, breast, lung, GI, genitourinary, prostate, and head and neck cancers.



Core structure of taxanes

Mechanism of Action

Paclitaxel binds to the β-tubulin subunit on the inner surface of microtubules and antagonizes their disassembly (see Figure 70–10), with the result that bundles of microtubules and aberrant structures derived from microtubules appear in the mitotic phase of the cell cycle (Schiff et al., 1979). Arrest in mitosis follows. Cell death occurs by apoptosis and depends on both drug concentration and duration of drug exposure. Drugs that block cell-cycle progression prior to mitosis antagonize the toxic effects of taxanes.

Drug interactions have been noted. The sequence of *cisplatin* preceding *paclitaxel* decreases *paclitaxel* clearance and produces greater toxicity than the opposite schedule. *Paclitaxel* decreases *doxorubicin* clearance and enhances its cardiotoxicity; *docetaxel* has no apparent effect on anthracycline pharmacokinetics.

Resistance to taxanes can be a result of decreased cellular drug accumulation due to increased expression of membrane-bound efflux proteins, including MRP1 and Pgp. *Cabazitaxel* is a poor substrate for Pgp and may therefore be useful for treating multidrug-resistant tumors. Other mechanisms of resistance may include an increase in *survivin*, an antiapoptotic factor; an increase in *α-aurora kinase,* which promotes completion of mitosis; an upregulation of the βIII-isoform of tubulin that lacks taxane-binding capacity; or a direct alteration of the drug target by mutation.

ADME

Paclitaxel has limited water solubility and is administered in a vehicle of 50% ethanol and 50% polyethoxylated castor oil. Hepatic CYPs (primarily CYP2C8, secondarily CYP3A4) extensively metabolize the drug. The primary metabolite is 6-OH paclitaxel, which is inactive; multiple additional hydroxylation products are found in plasma; less than 10% of a dose is excreted in the urine intact. Dose reductions in patients with abnormal hepatic function have been suggested, and 50% to 75% of doses of taxanes should be used in the presence of hepatic metastases larger than 2 cm in size or in patients with abnormal serum bilirubin. Drugs that induce CYP2C8 or CYP3A4, such as *phenytoin* and *phenobarbital*, or those that inhibit these CYPs, such as antifungal imidazoles, significantly alter drug clearance and toxicity.

Paclitaxel clearance is nonlinear and decreases with increasing dose or dose rate; the plasma $t_{1/2}$ is 10 to 14 h. The critical plasma concentration for myelosuppression depends on the duration of exposure but likely is 50 to 100 nM. *Paclitaxel* clearance is markedly delayed by *cyclosporine A* and other drugs that inhibit Pgp.

A nanoparticle albumin-bound *paclitaxel* (*nab-paclitaxel*) is soluble and used for infusion. This form of *paclitaxel* has increased cellular uptake via an albumin-specific mechanism. *Nab-paclitaxel* achieves a higher serum concentration than *paclitaxel*, but the increased clearance of *nab-paclitaxel* results in a similar systemic drug exposure. It is somewhat less neurotoxic than *paclitaxel* and is used in combination treatment of metastatic breast, pancreas, and non–small cell lung cancer. Like the other taxanes, *nab*-paclitaxel should not be given to patients with an absolute neutrophil count below 1500 cells/mm^3.

Docetaxel, somewhat more soluble than *paclitaxel*, is administered intravenously in an emulsifier (polysorbate 80). *Docetaxel* pharmacokinetics are similar to those of *paclitaxel*, with an elimination $t_{1/2}$ of about 12 h. Clearance is primarily through CYP3A4- and CYP3A5-mediated hydroxylation, leading to inactive metabolites.

Therapeutic Uses

The taxanes have become central components of regimens for treating patients with metastatic ovarian, breast, lung, GI, genitourinary, and head and neck cancers. These drugs are administered once weekly or once every 3 weeks. *Cabazitaxel* is a poor substrate for Pgp and is approved for hormone-refractory metastatic prostate cancer previously treated with a *docetaxel*-containing regimen.

Adverse Effects

Paclitaxel exerts its primary toxic effects on the bone marrow. Neutropenia usually occurs 8 to 11 days after a dose and reverses rapidly by days 15 to 21. Used with granulocyte colony-stimulating factor (G-CSF), high doses over 24 h are well tolerated, and peripheral neuropathy becomes dose limiting. Many patients experience myalgias after receiving *paclitaxel*. In high-dose schedules or with prolonged use, a stocking-glove sensory neuropathy can be disabling, particularly in patients with underlying diabetic neuropathy or concurrent *cisplatin* therapy. Mucositis is prominent in 72- or 96-h infusions and in the weekly schedule. Hypersensitivity reactions can occur in patients receiving *paclitaxel* infusions of short duration (1–6 h) but are largely averted by pretreatment with *dexamethasone*, *diphenhydramine*, and histamine H_2 receptor antagonists. Premedication is not necessary with 96-h infusions. Many patients experience asymptomatic bradycardia; occasional episodes of silent ventricular tachycardia also occur and resolve spontaneously during 3- or 24-h infusions. *Nab-paclitaxel* produces increased rates of peripheral neuropathy compared to the original *cremophor*-delivered *paclitaxel* but rarely causes hypersensitivity reactions.

Docetaxel causes greater degrees of neutropenia than *paclitaxel* but less peripheral neuropathy and asthenia and less frequent hypersensitivity. Fluid retention is a progressive problem with multiple cycles of *docetaxel* therapy, leading to peripheral edema, pleural and peritoneal fluid, and pulmonary edema in extreme cases. Oral *dexamethasone*, begun 1 day prior to drug infusion and continuing for 3 days, greatly ameliorates fluid retention. In rare cases, *docetaxel* may cause progressive interstitial pneumonitis and respiratory failure if the drug is not discontinued.

Estramustine

Estramustine combines estradiol and normustine (nornitrogen mustard) through a carbamate link. Although the intent of the combination was to enhance the uptake of the alkylating agent into estradiol-sensitive prostate cancer cells, *estramustine* does not function *in vivo* as an alkylating agent; rather, it binds to β-tubulin and microtubule-associated proteins, causing microtubule disassembly and antimitotic actions (Tew and Stearns, 1987).

Therapeutic Use

Estramustine is approved for the palliative treatment of patients with progressive or metastatic prostate cancer.

ADME

Following oral administration, at least 75% of a dose of *estramustine phosphate* is absorbed from the GI tract and rapidly dephosphorylated. The drug undergoes extensive first-pass metabolism by hepatic CYPs to an active 17-keto derivative, estromustine, and to multiple inactive products; the active drug forms accumulate in the prostate. Some hydrolysis of the carbamate linkage occurs in the liver, releasing estradiol, estrone, and the normustine group. *Estramustine* and estromustine have plasma half-lives of 10 and 14 h, respectively, and are excreted as inactive metabolites, mainly in the feces.

Adverse Effects, Drug Interactions

In addition to myelosuppression, *estramustine* also possesses estrogenic side effects (gynecomastia, impotence, elevated risk of thrombosis, and fluid retention); hypercalcemia; acute attacks of porphyria; impaired glucose tolerance; and hypersensitivity reactions, including angioedema. *Estramustine* inhibits the clearance of taxanes.

Epothilones

The epothilones are polyketides discovered as cytotoxic metabolites from a strain of *Sorangium cellulosum*, a myxobacterium isolated from soil on the bank of the Zambezi River in southern Africa (Gerth et al., 1996). One of these, *ixabepilone*, is approved for breast cancer treatment; others are under development (Lee and Swain, 2008).

Ixabepilone

Mechanism of Action; Resistance. Epothilones bind to a β-tubulin pocket located on the luminal side of microtubules, the taxane site (Steinmetz and Prota, 2018), and can trigger microtubule nucleation at multiple sites away from the centriole. This dysfunctional microtubule stabilization triggers cell-cycle arrest at the G_2-M interface and apoptosis. *In vitro* studies suggested that *ixabepilone* is less susceptible to Pgp-mediated export and multidrug resistance than taxanes. Mechanisms implicated in epothilone resistance include mutation of the β-tubulin binding site and upregulation of isoforms of β-tubulin.

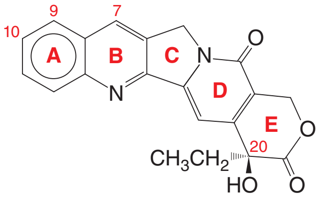
ADME. *Ixabepilone* is administered intravenously. Because of its minimal aqueous solubility, it is delivered in the solubilizing agent *cremophor* (polyoxyethylated castor oil/ethanol), which has been implicated as the cause of infusion reactions; such reactions are infrequent when administration is preceded by premedication with H_1 and H_2 antagonists. The drug is cleared by hepatic CYPs and has a plasma $t_{1/2}$ of 52 h.

Therapeutic Uses. In patients with metastatic breast cancer resistant to or pretreated with anthracyclines and resistant to taxanes, *ixabepilone* plus *capecitabine* provides an improved progression-free survival of 1.6 months compared to *capecitabine* alone. *Ixabepilone* also is indicated as monotherapy for metastatic breast cancer in patients who have previously progressed through treatment with anthracyclines, taxanes, and *capecitabine*. *Ixabepilone* (~40 mg/m^2) is administered as monotherapy or in combination with *capecitabine* over 3 h every 3 weeks. Patients should be premedicated with both an H_1 and an H_2 antagonist before receiving *ixabepilone* to minimize hypersensitivity reactions. In patients with mild-to-moderate hepatic dysfunction who receive *ixabepilone* monotherapy, lower starting doses are recommended due to delayed drug clearance.

Adverse Effects. Epothilones have toxicities similar to those of the taxanes: neutropenia, peripheral sensory neuropathy, fatigue, diarrhea, and asthenia.

Camptothecin Analogues

The camptothecins are potent, cytotoxic antineoplastic agents that target the nuclear enzyme *topoisomerase I*. The lead compound in this class, camptothecin, was isolated from the tree *Camptotheca acuminata*. *Irinotecan* and *topotecan*, currently the only camptothecin analogues approved for clinical use, have activity in colorectal, ovarian, and small cell lung cancer.



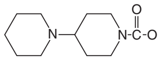
	C-10	C-9	C-7
Camptothecin	H	H	H
Topotecan	OH	$(CH_3)_2NHCH_2$	H
Irinotecan		H	CH_2CH_3
SN-38	OH	H	CH_2CH_3

Figure 70–11 *Camptothecin and its analogues.*

Chemistry

All camptothecins have a fused five-ring backbone that includes a labile lactone ring (Figure 70–11). The hydroxyl group and S conformation of the chiral center at C20 in the lactone ring are required for biological activity. Appropriate substitutions on the A and B rings of the quinoline subunit enhance water solubility and increase potency for inhibiting topoisomerase I. *Topotecan* is a semisynthetic molecule with a basic dimethylamino group that increases its water solubility. *Irinotecan* (CPT-11) differs from *topotecan* in that it is a prodrug. The carbamate bond between the camptothecin moiety and the dibasic bis-piperidine side chain at position C10 (which makes the molecule water soluble) is cleaved by a carboxylesterase to form the active metabolite SN-38 (see Figure 6–6).

Mechanism of Action

The DNA topoisomerases are nuclear enzymes that reduce torsional stress in supercoiled DNA, allowing selected regions of DNA to become sufficiently untangled to permit replication, repair, and transcription (Pommier, 2013). Two classes of topoisomerase (I and II) mediate DNA strand breakage and resealing. Camptothecin analogues inhibit the function of topoisomerase I; other drugs, such as anthracyclines, epipodophyllotoxins, and acridines, inhibit topoisomerase II. The camptothecins bind to and stabilize the normally transient DNA–topoisomerase I cleavable complex. Although the initial cleavage action of topoisomerase I is not affected, the religation step is inhibited, leading to the accumulation of single-stranded breaks in DNA. These lesions are reversible and not by themselves toxic to the cell. However, the collision of a DNA replication fork with this cleaved strand of DNA causes an irreversible double-strand DNA break, ultimately leading to cell death.

Camptothecins are *S phase–specific drugs* because ongoing DNA synthesis is necessary for cytotoxicity. This has important clinical implications. S phase–specific cytotoxic agents generally require prolonged exposures of tumor cells to drug concentrations above a minimum threshold for optimal therapeutic efficacy. In fact, low-dose, protracted administration of camptothecin analogues results in less toxicity and equal or greater antitumor activity than shorter, more intense courses.

Mechanisms of Resistance

Decreased intracellular drug accumulation may underlie resistance. The BCRP/xenobiotic exporter ABCG2/BCRP is overexpressed in cultured cells that have become resistant to *irinotecan* after exposure to camptothecins. Cell lines that lack carboxylesterase activity demonstrate resistance to *irinotecan*, but the liver and red blood cells may have sufficient carboxylesterase activity to convert *irinotecan* to the active metabolite SN-38. Camptothecin resistance also may result from decreased expression or mutation of topoisomerase I. A transient downregulation of topoisomerase I has been demonstrated following prolonged exposure to camptothecins *in vitro* and *in vivo*. Mutations leading to reduced topoisomerase I enzyme catalytic activity or DNA-binding affinity have been associated with experimental camptothecin resistance. Finally, exposure of cells to topoisomerase I–targeted agents upregulates topoisomerase II, an alternative enzyme for DNA strand passage.

Topotecan

ADME

Topotecan is approved for intravenous administration. An oral dosage form in development has a bioavailability of 30% to 40% in patients with cancer. *Topotecan* exhibits linear pharmacokinetics, and it is rapidly eliminated from systemic circulation with a $t_{1/2}$ of about 3.5 to 4.1 h. Only 20% to 35% of the total drug in plasma is found to be in the active lactone form. Within 24 h, 30% to 40% of the administered dose appears in the urine. Doses should be reduced in proportion to reductions in CL_{Cr}. Hepatic metabolism appears to be a relatively minor route of drug elimination. Plasma protein binding of topotecan is low (7%–35%), which may explain its relatively greater CNS penetration.

Therapeutic Uses

Topotecan is indicated for previously treated patients with ovarian and small cell lung cancer. Significant hematological toxicity limits its use in combination with other active agents in these diseases (e.g., *cisplatin*). The recommended dosing regimen of *topotecan* for ovarian cancer and small cell lung cancer is a 30-min infusion for 5 consecutive days every 3 weeks. For the treatment of patients with cervical cancer in conjunction with *cisplatin*, *topotecan* is administered on days 1, 2, and 3, repeated every 21 days. The dose of *topotecan* should be reduced in patients with moderate renal dysfunction (CL_{Cr} of 20–40 mL/min); *topotecan* should not be administered to patients with severe renal impairment (CL_{Cr} <20 mL/min). Hepatic dysfunction does not alter *topotecan* clearance and toxicity. A baseline neutrophil count greater than 1500 cells/mm^3 and a platelet count above 100,000 is necessary prior to *topotecan* administration.

Adverse Effects

The dose-limiting toxicity with all dosing schedules is neutropenia, with or without thrombocytopenia. The incidence of severe neutropenia at 1.5 mg/m^2 daily for 5 days every 3 weeks may be as high as 81%, with a 26% incidence of febrile neutropenia. In patients with hematological malignancies, GI side effects such as mucositis and diarrhea become dose limiting. Other less common and generally mild *topotecan*-related toxicities include nausea and vomiting, elevated liver transaminases, fever, fatigue, and rash.

Irinotecan

ADME

The conversion of *irinotecan* to its active metabolite SN-38 is mediated predominantly by carboxylesterases in the liver (see Figures 5–6 and 5–8). Although SN-38 can be measured in plasma shortly after beginning an intravenous infusion of *irinotecan*, the AUC of SN-38 is only about 4% of the AUC of *irinotecan*, suggesting that only a relatively small fraction of the dose is ultimately converted to the active form of the drug. *Irinotecan* exhibits linear pharmacokinetics. In comparison to *topotecan*, a relatively large fraction of both *irinotecan* and SN-38 are present in plasma as the biologically active intact lactone form. The $t_{1/2}$ of SN-38 is 11.5 h, three times that of *topotecan*. CSF penetration of SN-38 in humans has not been characterized.

In contrast to *topotecan*, hepatic metabolism of *irinotecan* and SN-38 represents an important route of elimination for both. Oxidative metabolites have been identified in plasma, all of which result from CYP3A-mediated reactions directed at the bis-piperidine side chain. These metabolites are not significantly converted to SN-38. The total body clearance of *irinotecan* was found to be two times greater in patients with brain cancer taking antiseizure drugs that induce hepatic CYPs.

UGT1A1 glucuronidates the hydroxyl group at position C10 (resulting from cleavage of the bispiperidine promoiety), producing the inactive glucuronide metabolite SN-38G (see Figures 5–6, 5–8, and 5–9). Biliary excretion appears to be the primary elimination route of *irinotecan*, SN-38, and metabolites, although urinary excretion also contributes (14%–37%). The extent of SN-38 glucuronidation inversely correlates with the risk of severe diarrhea after *irinotecan* therapy. UGT1A1 polymorphisms associated with familial hyperbilirubinemia syndromes may have a major impact on the clinical use of *irinotecan*. In patients treated with *irinotecan*, there is a positive correlation between baseline serum unconjugated bilirubin concentration and both severity of neutropenia and the AUC of *irinotecan* and SN-38. Severe *irinotecan* toxicity has been observed in patients with cancer with Gilbert syndrome, presumably due to decreased glucuronidation of SN-38. In patients with the syndrome, elevated levels of unconjugated bilirubin in the bloodstream are due to reduced activity of glucuronyltransferase (Figure 5–7). The presence of bacterial glucuronidase in the intestinal lumen potentially can contribute to *irinotecan*'s GI toxicity by releasing unconjugated SN-38 from the inactive glucuronide metabolite.

Therapeutic Uses

Single-agent dosage of *irinotecan* is by weekly infusion for 4 of 6 weeks, with a higher dose given every 3 weeks. In patients with advanced colorectal cancer, *irinotecan* is used as first-line therapy in combination with fluoropyrimidines or as a single agent or in combination with *cetuximab* following failure of a 5FU/*oxaliplatin* regimen. It also has activity in small cell lung cancer. An *irinotecan* liposome injection in combination with 5FU and *leucovorin* is FDA-approved for treatment of metastatic pancreatic cancer after disease progression following *gemcitabine* therapy.

Adverse Effects

The dose-limiting toxicity with all dosing schedules is delayed diarrhea (35%), with or without neutropenia. An intensive regimen of *loperamide* (see Chapter 54) reduces this incidence by more than half. However, once severe diarrhea occurs, standard doses of antidiarrheal agents tend to be ineffective. Diarrhea generally resolves within a week and, unless associated with fever and neutropenia, rarely is fatal.

The second most common *irinotecan*-associated toxicity is myelosuppression. Severe neutropenia occurs in 14% to 47% of the patients treated with a schedule of administration every 3 weeks and is less frequently encountered among patients treated with the weekly schedule. Febrile neutropenia is observed in 3% of patients and may be fatal, particularly when associated with concomitant diarrhea. A cholinergic syndrome resulting from the inhibition of acetylcholinesterase activity by *irinotecan* may occur within the first 24 h after *irinotecan* administration. Symptoms include acute diarrhea, diaphoresis, hypersalivation, abdominal cramps, visual accommodation disturbances, lacrimation, rhinorrhea, and, less often, asymptomatic bradycardia. These effects are short lasting and respond within minutes to *atropine*. Other common toxicities include nausea and vomiting, fatigue, vasodilation or skin flushing, mucositis, elevation in liver transaminases, and alopecia. There have been case reports of dyspnea and interstitial pneumonitis associated with *irinotecan* therapy.

Antibiotics

Dactinomycin (Actinomycin D)

Actinomycins are chromopeptides isolated from *Streptomyces* soil bacteria. Most contain the same chromophore, the planar phenoxazone actinosin, which is responsible for their yellow-red color. The differences among naturally occurring actinomycins are confined to variations in the structure of the amino acids of the peptide side chains. *Actinomycin D* has beneficial effects in the treatment of solid tumors in children and choriocarcinoma in adult women.

Mechanism of Action

The capacity of actinomycins to bind to double-helical DNA is responsible for their biological activity and cytotoxicity (Reich, 1963). The planar phenoxazone ring intercalates between adjacent guanine-cytosine base pairs of DNA, while the polypeptide chains extend along the minor groove of the helix, resulting in a *dactinomycin*-DNA complex with stability sufficient to block the transcription of DNA by RNA polymerase. The DNA-dependent RNA polymerases are much more sensitive to the effects of *dactinomycin* than are the DNA polymerases. In addition, *dactinomycin* causes single-strand breaks in DNA, possibly through a free-radical intermediate or as a result of the action of topoisomerase II. *Dactinomycin* inhibits rapidly proliferating cells of normal and neoplastic origin and is among the most potent antitumor agents known.

ADME

Dactinomycin is administered by intravenous injection. Metabolism of the drug is minimal. The drug is excreted in both bile and urine and disappears from plasma with a terminal $t_{1/2}$ of 36 h. *Dactinomycin* does not cross the blood-brain barrier.

Therapeutic Uses

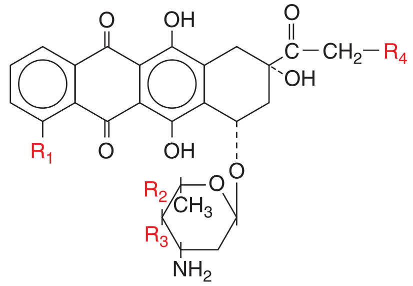
Dactinomycin is given intravenously for 5 days, usually in the range of 10 to 15 μg/kg. If no manifestations of toxicity are encountered, additional courses may be given at intervals of 2 to 4 weeks, although weekly maintenance doses have been used. The main clinical use of *dactinomycin* is in the treatment of rhabdomyosarcoma and Wilms tumor in children, where it is curative in combination with primary surgery, radiotherapy, and other drugs, particularly *vincristine* and *cyclophosphamide*. Ewing, Kaposi, and soft-tissue sarcomas also respond. *Dactinomycin* and MTX form a curative therapy for choriocarcinoma.

Adverse Effects

Toxic manifestations include anorexia, nausea, and vomiting, usually beginning a few hours after administration. Hematopoietic suppression with pancytopenia may occur in the first week after completion of therapy. Proctitis, diarrhea, glossitis, cheilitis, and ulcerations of the oral mucosa are common; dermatological manifestations include alopecia, as well as erythema, desquamation, and increased inflammation and pigmentation in areas previously or concomitantly subjected to X-ray radiation. Severe injury may occur as a result of local drug extravasation; the drug is extremely corrosive to soft tissues.

Anthracyclines and Anthracenediones

Anthracyclines are derived from *Streptomyces peucetius* var. *caesius*. *Idarubicin* and *epirubicin* are analogues of the naturally produced anthracyclines *doxorubicin* and *daunorubicin*, differing only slightly in chemical structure, but having somewhat distinct patterns of clinical activity. *Daunorubicin* and *idarubicin* primarily have been used in acute leukemias, whereas *doxorubicin* and *epirubicin* display broader activity against solid tumors. These agents, which possess the potential for generating free radicals, cause an unusual and often-irreversible cardiomyopathy, the occurrence of which is related to the total dose of the drug. The structurally similar agent *mitoxantrone* has less cardiotoxicity and is useful against prostate cancer and AML and in high-dose chemotherapy.



	DOXORUBICIN	DAUNORUBICIN	EPIRUBICIN	IDARUBICIN
R_1 =	OCH_3	OCH_3	OCH_3	H
R_2 =	H	H	OH	H
R_3 =	OH	OH	H	OH
R_4 =	OH	H	OH	H

Mechanisms of Action and Resistance

Anthracyclines and anthracenediones can intercalate with DNA, directly affecting transcription and replication. More important is their capacity to form a heterotrimeric complex with topoisomerase II and DNA (Pommier, 2013). Topoisomerase II produces double-strand breaks at the 3′-phosphate backbone, allowing strand passage and uncoiling of supercoiled DNA. Following strand passage, topoisomerase II religates the DNA strands; this enzymatic function is essential for DNA replication and repair. Formation of the ternary complex with anthracyclines or with *etoposide* inhibits the religation of the broken DNA strands, leading to apoptosis. Defects in DNA double-strand break repair sensitize cells to damage by these drugs, while altered DNA damage-sensing and DNA repair capacity may contribute to resistance.

The quinone moieties of anthracyclines can form radical intermediates that react with O_2 to produce superoxide anion radicals, which can generate H_2O_2 and •OH that attack DNA and oxidize DNA bases, leading to apoptosis (Serrano et al., 1999). The production of free radicals is significantly stimulated by the interaction of *doxorubicin* with iron. Enzymatic defenses such as superoxide dismutase and catalase protect cells against the toxicity of the anthracyclines, and these defenses can be augmented by exogenous antioxidants such as α-tocopherol or by the iron chelator *dexrazoxane*, which protects against cardiac toxicity. Multidrug resistance is observed in tumor cell populations exposed to anthracyclines. Anthracyclines also are exported from tumor cells by members of the MRP transporter family and by ABCG2 (BCRP). Other biochemical changes in resistant cells include increased glutathione peroxidase activity, decreased activity or mutation of topoisomerase II, and enhanced ability to repair DNA strand breaks.

ADME

Daunorubicin, *doxorubicin*, *epirubicin*, and *idarubicin* are administered intravenously and are cleared by a complex pattern of hepatic metabolism and biliary excretion. Each anthracycline is converted to an active alcohol intermediate that plays a variable role in the therapeutic activity. The plasma disappearance curves for *doxorubicin* and *daunorubicin* are multiphasic, with a terminal $t_{1/2}$ of 30 h. *Idarubicin* has a $t_{1/2}$ of 15 h, and its active metabolite, idarubicinol, has a $t_{1/2}$ of 40 h. The drugs rapidly enter the heart, kidneys, lungs, liver, and spleen; they do not cross the blood-brain barrier. Clearance is delayed in the presence of hepatic dysfunction; at least a 50% initial reduction in dose should be considered in patients with elevated serum bilirubin levels.

Idarubicin and Daunorubicin

Therapeutic Use. *Idarubicin* (~12 mg/m^2 per day for 3 days) is administered by slow intravenous injection (10–15 min) to avoid extravasation. *Idarubicin* has less cardiotoxicity than the other anthracyclines. *Daunorubicin* (also named daunomycin or rubidomycin) is administered (at variable doses according to the regimen, 45–90 mg/m^2 per day) intravenously for 3 days, with care to prevent extravasation. Total doses greater than 550 mg/m^2 are associated with a high risk of cardiotoxicity. Radiation therapy to the mediastinum may enhance the risk of anthracycline toxicity. *Daunorubicin* may impart a red color to the urine. *Daunorubicin* and *idarubicin* are used in the treatment of patients with AML in combination with Ara-C.

Adverse Effects. Toxic effects of *daunorubicin* and *idarubicin* include bone marrow depression, stomatitis, alopecia, GI disturbances, rash, and cardiac toxicity. Cardiac toxicity of anthracyclines can be acute or chronic and is described in detail next for *doxorubicin*.

Doxorubicin

Therapeutic Uses. The dose (60–75 mg/m^2) is administered as a single rapid intravenous infusion that is repeated after 21 days. A *doxorubicin* liposomal product is available for treatment of AIDS-related Kaposi sarcoma and is given intravenously in a dose of 20 mg/m^2 over 60 min and repeated every 3 weeks. The liposomal formulation also is approved for ovarian cancer for treatment at a dose of 50 mg/m^2 every 4 weeks and as a treatment of multiple myeloma, where it is given as a 30-mg/m^2 dose on day 4 of each 21-day cycle. Patients should be advised that the drug may impart a red color to the urine. *Doxorubicin* is effective in malignant lymphomas. In combination with *cyclophosphamide*, vinca alkaloids, and other agents, it is an important ingredient for the successful treatment of lymphomas. It is a valuable component of various regimens adjunct of chemotherapy for and metastatic carcinoma of the breast. The drug also is beneficial in pediatric and adult sarcomas, including osteogenic, Ewing, and soft-tissue sarcomas.

Adverse Effects. Toxicities of *doxorubicin* are similar to those of *daunorubicin*. Myelosuppression is a major dose-limiting complication, with maximal leukopenia usually occurring during the second week of therapy and recovering by the fourth week; thrombocytopenia and anemia follow a similar pattern but usually are less pronounced. Stomatitis, mucositis, diarrhea, and alopecia are common but reversible. Erythematous streaking near the site of infusion ("flare") is a benign local allergic reaction and should not be confused with extravasation. Facial flushing, conjunctivitis, and lacrimation may occur rarely. The drug may produce severe local toxicity in irradiated tissues (e.g., the skin, heart, lung, esophagus, and GI mucosa) even when the two therapies are not administered concomitantly.

Cardiomyopathy is the most important long-term toxicity (Rochette et al., 2015) and may take two forms:

- **An acute form, characterized by abnormal electrocardiographic changes, including ST- and T-wave alterations and arrhythmias.** This is brief and rarely a serious problem. An acute reversible reduction in ejection fraction is observed in some patients in the 24 h after a single dose, and plasma troponin T may increase in a minority of patients in the first few days following drug administration (Lipshultz et al., 2004). Acute myocardial damage, the "pericarditis-myocarditis syndrome," may begin in the days following drug infusion and is characterized by severe disturbances in impulse conduction and frank congestive heart failure, often associated with pericardial effusion.
- **Chronic, cumulative, dose-related toxicity (usually total doses of ≥550 mg/m^2) progressing to congestive heart failure.** The mortality rate in patients with congestive heart failure approaches 50%. The risk of cardiomyopathy increases markedly with dose; estimates run as high as 20% at total doses of 550 mg/m^2 (a total dose limit of 300 mg/m^2 is advised for pediatric cases). These total dosages should be exceeded only under exceptional circumstances or with the concomitant use of *dexrazoxane*, a cardioprotective iron-chelating agent (Swain et al., 1997), as the mechanism of cardiotoxicity is thought to derive in part from cardiac lipid peroxidation (Myers et al., 1977) promoted by Fe^{2+} (Zweier, 1984). More recent studies have also proposed that *dexrazoxane* can prevent anthracycline-derived DNA damage by interaction with topoisomerase II in cardiac cells (Lyu et al., 2007). Cardiac irradiation, administration of high doses of *cyclophosphamide* or another anthracycline, or concomitant *trastuzumab* increases the risk of cardiotoxicity (Slamon et al., 2001). Late-onset cardiac toxicity, with congestive heart failure years after treatment, may occur in both pediatric and adult populations. In children treated with anthracyclines, there is a 3- to 10-fold elevated risk of arrhythmias, congestive heart failure, and sudden death in adult life. Concomitant administration of *dexrazoxane* may reduce troponin T elevations and avert later cardiotoxicity (Lipshultz et al., 2004).

Epirubicin

The anthracycline *epirubicin* is indicated as a component of adjunctive therapy for treatment of breast cancer. It is administered intravenously in doses of 100 to 120 mg/m^2 every 3 to 4 weeks. Total doses greater than 900 mg/m^2 sharply increase the risk of cardiotoxicity. Its toxicity profile is the same as that of *doxorubicin*.

Valrubicin

Valrubicin is a semisynthetic analogue of *doxorubicin* used exclusively for intravesicular treatment of bladder cancer. Once a week for 6 weeks, 800 mg are instilled into the bladder. Less than 10% of instilled drug is absorbed systemically. Side effects relate to bladder irritation.

Mitoxantrone

Mitoxantrone is a synthetic anthracenedione derivative topoisomerase II inhibitor that is approved for use in AML, prostate cancer, and late-stage, secondary progressive multiple sclerosis. *Mitoxantrone* has limited ability to produce quinone-type free radicals and causes less cardiac toxicity than does *doxorubicin*. It produces acute myelosuppression, cardiac toxicity, and mucositis as its major toxicities; the drug also causes nausea, vomiting, and alopecia, although less than *doxorubicin*. *Mitoxantrone* is administered by intravenous infusion. To induce remission in acute nonlymphocytic leukemia in adults, the drug is given in a daily dose of 12 mg/m^2 for 3 days with *cytarabine*. It also is used in advanced hormone-resistant prostate cancer in a dose of 12 to 14 mg/m^2 every 21 days.

Epipodophyllotoxins

Podophyllotoxin Derivatives

Two synthetic derivatives of podophyllotoxins have significant therapeutic activity in pediatric leukemia, small cell carcinomas of the lung, testicular tumors, Hodgkin disease, and large cell lymphomas. These derivatives are *etoposide* (VP-16-213) and *teniposide* (VM-26). Although podophyllotoxin binds to tubulin, *etoposide* and *teniposide* have no effect on microtubular structure or function at usual concentrations.

Mechanisms of Action and Resistance

Etoposide and *teniposide* form ternary complexes with topoisomerase II and DNA and prevent resealing of the break that normally follows topoisomerase binding to DNA. The enzyme remains bound to the free end of the broken DNA strand, leading to an accumulation of DNA breaks and cell death. Cells in the S and G_2 phases of the cell cycle are most sensitive to *etoposide* and *teniposide*. Resistant cells demonstrate (1) amplification of the *MDR1* gene that encodes the Pgp drug efflux transporter, (2) mutation or decreased expression of topoisomerase II, or (3) mutations of the p53 tumor suppressor gene, a required component of the apoptotic pathway (Lowe et al., 1993).

Etoposide

ADME

Oral administration of *etoposide* results in variable absorption that averages about 50%. After intravenous injection, there is a biphasic pattern of clearance with a terminal $t_{1/2}$ of about 6 to 8 h in patients with normal renal function. Approximately 40% of an administered dose is excreted intact in the urine. In patients with compromised renal function, dosage should be reduced in proportion to the reduction in CL_{Cr}. In patients with advanced liver disease, increased toxicity may result from a low serum albumin (decreased drug binding) and elevated bilirubin (which displaces *etoposide* from albumin); guidelines for dose reduction in this circumstance have not been defined. Drug concentrations in the CSF average 1% to 10% of those in plasma.

Therapeutic Uses

The intravenous dose of *etoposide* for testicular cancer in combination therapy (with *bleomycin* and *cisplatin*) is 50 to 100 mg/m^2 for 5 days or 100 mg/m^2 on alternate days for three doses. For small cell carcinoma of the lung, the dosage in combination therapy (with *cisplatin*) is 100 to 200 mg/m^2/d intravenously for 3 days. Cycles of therapy usually are repeated every 3 to 4 weeks. After relapse, oral administration of 50 mg/m^2 per day for 21 days is one treatment option. When given intravenously, the drug should be administered slowly over a 30- to 60-min period to avoid hypotension and bronchospasm, which likely result from the additives used to dissolve *etoposide*.

Etoposide is also active against non-Hodgkin lymphomas, acute nonlymphocytic leukemia, and Kaposi sarcoma associated with AIDS. *Etoposide* has a favorable toxicity profile for dose escalation in that its primary acute toxicity is myelosuppression. In combination with *ifosfamide* and *carboplatin*, it frequently is used for high-dose chemotherapy in total doses of 1500 to 2000 mg/m^2. *Trilaciclib*, a cyclin-dependent kinase (CDK) 4/6 kinase inhibitor, was approved in 2021 for administration prior to cytotoxic chemotherapy to decrease the incidence of myelosuppression in patients receiving a platinum/*etoposide*-containing regimen or *topotecan*-containing regimen for extensive-stage small cell lung cancer (see Chapter 71).

Adverse Effects

The dose-limiting toxicity of *etoposide* is leukopenia (nadir at 10–14 days, recovery by 3 weeks). Thrombocytopenia occurs less often and usually is not severe. Nausea, vomiting, stomatitis, and diarrhea complicate treatment in about 15% of patients. Alopecia is common but reversible. Hepatic toxicity is particularly evident after high-dose treatment. For both *etoposide* and *teniposide*, toxicity increases in patients with decreased serum albumin, an effect related to decreased protein binding of the drug.

A disturbing complication of *etoposide* therapy is the development of an unusual form of acute nonlymphocytic leukemia with a translocation in chromosome 11q23. At this locus is a gene (the *MLL* gene) that regulates the proliferation of pluripotent stem cells. The leukemic cells have the cytological appearance of acute monocytic or monomyelocytic leukemia. Another distinguishing feature of *etoposide*-related leukemia is the short time interval between the end of treatment and the onset of leukemia (1–3 years), compared to the 4- to 5-year interval for secondary leukemias related to alkylating agents, and the absence of a myelodysplastic period preceding leukemia (Pui et al., 1995). Patients receiving weekly or twice-weekly doses of *etoposide*, with cumulative doses greater than 2000 mg/m^2, seem to be at higher risk of leukemia.

Teniposide

Teniposide is administered intravenously. It has a multiphasic pattern of clearance from plasma: After distribution, a $t_{1/2}$ of 4 h and another $t_{1/2}$ of 10 to 40 h are observed. Approximately 45% of the drug is excreted in the urine; in contrast to *etoposide*, as much as 80% is recovered as metabolites. Anticonvulsants such as *phenytoin* increase the hepatic metabolism of *teniposide* and reduce systemic exposure. Dosage need not be reduced for patients with impaired renal function. Less than 1% of the drug crosses the blood-brain barrier. *Teniposide* is available for treatment of refractory ALL in children and is synergistic with *cytarabine*. *Teniposide* is administered by intravenous infusion for 5 days or twice weekly. The drug has limited utility and is given primarily for acute leukemia in children and monocytic leukemia in infants, as well as glioblastoma, neuroblastoma, and brain metastases from small cell carcinomas of the lung. Myelosuppression, nausea, and vomiting are its primary toxic effects.

Trabectedin Analogues

Trabectedin

Trabectedin is derived from the marine invertebrate tunicate *Ecteinascidin turbinate*. *Trabectedin* is an alkylating drug that binds to the minor groove of DNA, allowing the alkylation of the N2 position of guanine and bending the helix toward the major groove. The bulky DNA adduct is recognized by the transcription-coupled NER complex, and these proteins initiate attempts to repair the damaged strand, converting the adduct to a double-stranded break. *Trabectedin* has particular cytotoxic effects on cells that lack components of the Fanconi anemia complex or those that lack the ability to repair double-strand DNA breaks through homologous recombination (Soares et al., 2011). Unlike *cisplatin* and other DNA adduct–forming drugs, the activity of *trabectedin* requires the presence of intact components of NER, including xeroderma pigmentosum endonuclease G, which may be important for initiation of single breaks and attempts at adduct removal.

ADME

Trabectedin is administered as a 24-h infusion of 1.3 mg/m^2 every 3 weeks. It is administered with *dexamethasone*, 4 mg twice daily, starting 24 h before drug infusion to diminish hepatic toxicity. The drug is slowly cleared by CYP3A4, with a plasma $t_{1/2}$ of about 24 to 40 h.

Therapeutic Uses

Trabectedin is approved for the treatment of patients with unresectable or metastatic liposarcoma or leiomyosarcoma after an anthracycline-containing regimen and is under study for the treatment of patients with ovarian and pancreatic cancer.

Adverse Effects

Without *dexamethasone* pretreatment, *trabectedin* causes significant hepatic enzyme elevations and fatigue in at least one-third of patients. With the steroid, the increases in transaminase are less pronounced and rapidly reversible. Other toxicities include mild myelosuppression and, rarely, rhabdomyolysis.

Lurbinectedin

Lurbinectedin is structurally similar to *trabectedin*. It covalently binds to residues in the minor groove of DNA and delays progression through S phase, causing cell cycle arrest in the G2/M phase and cell death. The FDA approved *lurbinectedin* in 2020 for metastatic small cell lung cancer disease progression on or after platinum-based chemotherapy. It has similar adverse effects as *trabectedin*. *Lurbinectedin* can be administered as a 60-min infusion, also every 3 weeks.

Drugs of Diverse Mechanisms of Action

Bleomycin

The bleomycins, an unusual group of DNA-cleaving antibiotics, are fermentation products of *Streptomyces verticillus,* comprising a family of peptide polyketides. The drug currently employed clinically is a mixture of two copper-chelating peptides, bleomycins A_2 and B_2, that differ only in their terminal amino acid. Because their toxicities do not overlap with those of other cytotoxic drugs and because of their unique mechanism of action, *bleomycin* maintains an important role in treating Hodgkin disease and testicular cancer.

Mechanisms of Action and Resistance

Bleomycin's cytotoxicity results from its capacity to cause oxidative damage to DNA. *Bleomycin* is a glycopeptide that has a DNA-binding portion (Chien et al., 1977) and, through a distinct functionality, binds to metal ions. In the presence of O_2, the Fe^{2+}-drug complex becomes activated and transfers electrons from Fe^{2+} to molecular oxygen to produce oxygen radicals, leading to single- and double-stranded breaks with release of free bases from DNA (Sausville et al., 1978). *Bleomycin* causes accumulation of cells in the G_2 phase of the cell cycle, and many of these cells display chromosomal aberrations, including chromatid breaks, gaps, fragments, and translocations.

Bleomycin is degraded by a specific hydrolase found in various normal tissues, including liver. Hydrolase activity is low in skin and lung, perhaps contributing to the serious toxicity. Some *bleomycin*-resistant cells contain high levels of hydrolase activity. In other cell lines, resistance has been attributed to decreased uptake, repair of strand breaks, or drug inactivation by thiols or thiol-rich proteins.

ADME

Bleomycin is administered intravenously, intramuscularly, or subcutaneously or instilled into the bladder for local treatment of bladder cancer. Having a high molecular mass, *bleomycin* crosses the blood-brain barrier poorly. The elimination $t_{1/2}$ is about 3 h. About two-thirds of the drug is excreted intact in the urine. Concentrations in plasma are greatly elevated in patients with renal impairment, and doses of *bleomycin* should be reduced in the presence of a CL_{Cr} less than 60 mL/min.

Therapeutic Uses

Bleomycin is given weekly or twice weekly by the intravenous, intramuscular, or subcutaneous route. A test dose of 2 units or less is recommended for patients with lymphoma. Myriad regimens are employed clinically, with *bleomycin* doses expressed in units. Total courses exceeding 250 mg should be given with caution, and usually only in high-risk testicular cancer treatment, because of a marked increase in the risk of pulmonary toxicity. *Bleomycin* also may be instilled into the pleural cavity in doses of 5 to 60 mg to ablate the pleural space in patients with malignant effusions. *Bleomycin* is highly effective against germ cell tumors of the testis and ovary. In testicular cancer, it is curative when used with *cisplatin* and *vinblastine* or *cisplatin* and *etoposide*. It is a component of the standard curative ABVD regimen for Hodgkin disease.

Adverse Effects

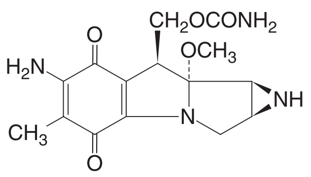
Because *bleomycin* causes little myelosuppression, it has significant advantages in combination with other cytotoxic drugs. However, it does cause a constellation of cutaneous toxicities, including hyperpigmentation, hyperkeratosis, erythema, even ulceration, and rarely, Raynaud phenomenon. Skin lesions may recur when patients are treated with other antineoplastic drugs. Rarely, *bleomycin* causes a flagellate dermatitis consisting of bands of pruritic erythema on the arms, back, scalp, and hands; this rash responds readily to topical corticosteroids.

The most serious adverse reaction to *bleomycin* is pulmonary toxicity, which begins with a dry cough, fine rales, and diffuse basilar infiltrates on X-ray and may progress to life-threatening pulmonary fibrosis. Approximately 5% to 10% of patients receiving *bleomycin* develop clinically apparent pulmonary toxicity, and about 1% die of this complication. Most who recover experience a significant improvement in pulmonary function, but fibrosis may be irreversible. Pulmonary function tests are not of predictive value for detecting early onset of this complication. The risk of pulmonary toxicity is related to total dose, with a significant increase in risk in total doses greater than 250 mg and in patients more than 40 years of age, in those with a CL_{Cr} less than 80 mL/min, and in those with underlying pulmonary disease; single doses of 30 mg/m^2 or more also are associated with an increased risk of pulmonary toxicity. Administration of high O_2 concentrations during anesthesia or respiratory therapy may aggravate or precipitate pulmonary toxicity in patients previously treated with the drug, in keeping with the clear ability of metallobleomycin complexes to generate O_2-derived radicals. There is no known specific therapy for *bleomycin* lung injury except for symptomatic management and standard pulmonary care. Steroids are of variable benefit, with greatest effectiveness in the earliest inflammatory stages of the lesion.

Other toxic reactions to *bleomycin* include hyperthermia, headache, nausea and vomiting, and a peculiar acute fulminant reaction observed in patients with lymphomas. This reaction is characterized by profound hyperthermia, hypotension, and sustained cardiorespiratory collapse; it does not appear to be a classical anaphylactic reaction and may be related to release of an endogenous pyrogen. This reaction has occurred in about 1% of patients with lymphomas or testicular cancer.

Mitomycin

Mitomycin has limited clinical utility, having been replaced by less toxic and more effective drugs, with the exception of the use in patients with anal cancers, for which it is potentially curative.



MITOMYCIN

Mechanisms of Action and Resistance

After intracellular enzymatic or spontaneous chemical alteration, *mitomycin* becomes a bifunctional or trifunctional alkylating agent. The drug inhibits DNA synthesis and cross-links DNA at the N6 position of adenine and at the O6 and N7 positions of guanine. Attempts to repair DNA lead to strand breaks. *Mitomycin* is a potent radiosensitizer, teratogen, and carcinogen in rodents. Resistance has been ascribed to deficient activation, intracellular inactivation of the reduced Q form, and Pgp-mediated drug efflux.

ADME

Mitomycin is administered intravenously. It has a $t_{1/2}$ of 25 to 90 min. The drug distributes widely throughout the body but is not detected in the CNS. Inactivation occurs by hepatic metabolism or chemical conjugation with sulfhydryls. Less than 10% of the active drug is excreted in the urine or the bile.

Therapeutic Uses

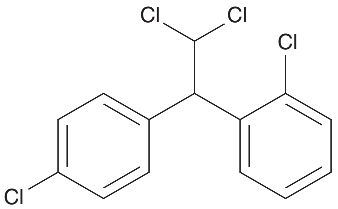
Mitomycin is administered as a single bolus (6–20 mg/m^2) every 6 to 8 weeks. Dosage should be modified based on hematological recovery. *Mitomycin* also may be used by direct instillation into the bladder to treat superficial transitional cell carcinomas. *Mitomycin* is used in combination with 5FU and *cisplatin* for the treatment of anal cancer. *Mitomycin* is also approved for local use as an adjunct to glaucoma surgery to reduce scarring.

Adverse Effects

The major toxic effect is myelosuppression, characterized by marked leukopenia and thrombocytopenia; after higher doses, maximal suppression may be delayed and cumulative, with recovery only after 6 to 8 weeks of pancytopenia. Nausea, vomiting, diarrhea, stomatitis, rash, fever, and malaise also are observed. Patients who have received more than 50 mg/m^2 total dose may acutely develop hemolysis, neurological abnormalities, interstitial pneumonia, and glomerular damage resulting in renal failure. The incidence of renal failure increases to 28% in patients who receive total doses of 70 mg/m^2 or more. There is no effective treatment of the disorder. It must be recognized early, and *mitomycin* must be discontinued immediately. *Mitomycin* causes interstitial pulmonary fibrosis; total doses greater than 30 mg/m^2 have infrequently led to congestive heart failure. *Mitomycin* may potentiate the cardiotoxicity of *doxorubicin*.

Mitotane

Mitotane (*o,p*′-DDD, dichlorodiphenyldichloroethane), a compound chemically similar to the insecticides DDT and DDD, is used in the treatment of adrenal cortex carcinoma. The mechanism of action of *mitotane* has not been elucidated, but its relatively selective destruction of adrenocortical cells, normal or neoplastic, is well established. Administration of the drug causes a rapid reduction in the levels of adrenocorticosteroids and their metabolites in blood and urine, a response that is useful in both guiding dosage and following the course of hyperadrenocorticism (Cushing syndrome) resulting from an adrenal tumor or adrenal hyperplasia. It does not damage other organs.



MITOTANE (*o*,*p*′-DDD)

ADME

Approximately 40% of *mitotane* is absorbed after oral administration. Plasma concentrations of mitotane are still measurable for 6 to 9 weeks following discontinuation of therapy. Although the drug is found in all tissues, fat is the primary site of storage. A water-soluble metabolite of *mitotane* found in the urine constitutes 25% of an oral or parenteral dose. About 60% of an oral dose is excreted unchanged in the stool.

Therapeutic Uses

Mitotane initial daily oral doses are 2 to 6 g, usually in three or four divided portions. The maximal tolerated dose may vary from 2 to 16 g/day. Treatment should continue for at least 3 months; if beneficial effects are observed, therapy should be maintained indefinitely. *Spironolactone* should not be administered concomitantly because it interferes with the adrenal suppression produced by *mitotane*. Treatment with *mitotane* is indicated for the palliation of inoperable adrenocortical carcinoma, producing symptomatic benefit in 30% to 50% of such patients.

Adverse Effects

Although the administration of *mitotane* produces anorexia and nausea in most patients, somnolence and lethargy in about 34%, and dermatitis in 15% to 20%, these effects do not contraindicate the use of the drug at lower doses. Because this drug damages the adrenal cortex, administration of replacement doses of adrenocorticosteroids is necessary.

L-Asparaginase

Malignant lymphoid cells depend on exogenous sources of L-asparagine. Thus, L-*asparaginase* (L-ASP) has become a standard agent for treating ALL.

Mechanism of Action

Most normal tissues synthesize L-asparagine in amounts sufficient for protein synthesis, but lymphocytic leukemias lack adequate amounts of asparagine synthase and derive the required amino acid from plasma. L-ASP, by catalyzing the hydrolysis of circulating asparagine to aspartic acid and ammonia, deprives these malignant cells of asparagine, leading to cell death. L-ASP is used in combination with other agents, including MTX, *doxorubicin*, *vincristine*, and *prednisone*, for the treatment of ALL and high-grade lymphomas. Resistance arises through induction of asparagine synthetase in tumor cells.

ADME and Therapeutic Use

Asparaginase is given intramuscularly or intravenously. After intravenous administration, *Escherichia coli*–derived L-ASP has a clearance rate from plasma of 0.035 mL/min/kg, a volume of distribution that approximates the volume of plasma in humans, and a $t_{1/2}$ of 1 day. The enzyme is given in doses of 6000 to 10,000 IU every third day for 3 to 4 weeks. *Pegaspargase* is a preparation in which the enzyme is conjugated to 5000-Da units of monomethoxy polyethylene glycol, resulting in a much longer plasma $t_{1/2}$ (6–7 days); it is administered intramuscularly every 14 days, producing rapid and complete depletion of plasma and tumor cell asparagine for 21 days in most patients. *Pegaspargase* has much reduced immunogenicity (<20% of patients develop antibodies) and has been approved for first-line ALL therapy. The conjugated preparation *calaspargase pegol* is administered intravenously every 21 days.

Intermittent dosage regimens and longer durations of treatment increase the risk of inducing hypersensitivity. In hypersensitive patients, neutralizing antibodies inactivate L-ASP. Not all patients with neutralizing antibodies experience clinical hypersensitivity, although the enzyme may be inactivated and therapy may be ineffective. In previously untreated ALL, *pegaspargase* produces more rapid clearance of lymphoblasts from bone marrow than does the *E. coli* preparation and circumvents the rapid antibody-mediated clearance seen with *E. coli* enzyme in relapsed patients. Asparaginase preparations only partially deplete CSF asparagine.

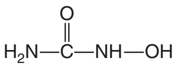
Adverse Effects

The L-ASP toxicities result from its antigenicity as a foreign protein and its inhibition of protein synthesis. Hypersensitivity reactions, including urticaria and full-blown anaphylaxis, occur in 5% to 20% of patients and may be fatal. In these patients, *pegaspargase* is a safe and effective alternative. So-called silent enzyme inactivation by antibodies occurs in a higher percentage of patients than overt hypersensitivity and may be associated with a negative clinical outcome, especially in high-risk patients with ALL.

Other toxicities result from inhibition of protein synthesis in normal tissues (e.g., hyperglycemia due to insulin deficiency, clotting abnormalities due to deficient clotting factors, hypertriglyceridemia due to effects on lipoprotein production, hypoalbuminemia). Pancreatitis also has been observed. The clotting problems may take the form of spontaneous thrombosis or, less frequently, hemorrhagic episodes. Brain magnetic resonance imaging studies should be considered in patients treated with L-ASP who present with seizures, headache, or altered mental status. Intracranial hemorrhage in the first week of L-ASP treatment is an infrequent but devastating complication. L-ASP also suppresses immune function. L-ASP terminates the antitumor activity of MTX when given shortly after the antimetabolite. By lowering serum albumin concentrations, L-ASP may decrease protein binding and accelerate plasma clearance of other drugs.

Hydroxyurea

Hydroxyurea (HU) inhibits the enzyme ribonuleotide reductase (RNR) and has unique and diverse biological effects as an antileukemic drug, radiation sensitizer, and an inducer of fetal hemoglobin (HbF) in patients with sickle cell disease. It is orally administered, and its toxicity is modest and limited to myelosuppression.



$$H_2N{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}NH{-}OH$$

HYDROXYUREA

Mechanisms of Action and Resistance

Hydroxyurea inhibits RNR, which catalyzes the reductive conversion of ribonucleotides to deoxyribonucleotides, a rate-limiting step in the biosynthesis of DNA. HU binds non-heme iron atoms that are essential for activation of a tyrosyl radical in the catalytic subunit of RNR. The drug is specific for the S phase of the cell cycle, during which RNR concentrations are maximal. HU produces arrest at or near the G_1-S interface through both p53-dependent and-independent mechanisms. Because cells are highly sensitive to irradiation at the G_1-S boundary, HU and irradiation cause synergistic antitumor effects. Through depletion of deoxynucleotides, HU potentiates the antiproliferative effects of DNA-damaging agents such as *cisplatin*, alkylating agents, or topoisomerase II inhibitors and facilitates the incorporation of antimetabolites such as Ara-C, *gemcitabine*, and *fludarabine* into DNA. It also promotes degradation of the p21 cell-cycle checkpoint and thereby enhances the effects of histone deacetylase (HDAC) inhibitors *in vitro* (Kramer et al., 2008).

Hydroxyurea is the primary drug for improving control of sickle cell (HbS) disease in adults and for inducing HbF in patients with thalassemia HbC and HbC/S. It reduces vaso-occlusive events, painful crises, hospitalizations, and the need for blood transfusions in patients with sickle cell disease. The mechanism of stimulated HbF production is uncertain. HU stimulates nitric oxide production, causing nitrosylation of low-molecular-weight GTPases, a process that stimulates γ-globin production in erythroid precursors. Another property of HU that may be therapeutically relevant is its capacity to reduce L-selectin expression and thereby to reduce adhesion of red cells and neutrophils to vascular endothelium. Also, by suppressing the production of neutrophils, it decreases their contribution to vascular occlusion. Tumor cells become resistant to HU through increased synthesis of the catalytic subunit of RNR, thereby restoring enzyme activity.

ADME

The oral bioavailability of HU is 80% to 100%; comparable plasma concentrations are seen after oral or intravenous dosing. HU disappears from plasma with a $t_{1/2}$ of 3.5 to 4.5 h. The drug readily crosses the blood-brain barrier; significant quantities appear in human breast milk. From 40% to 80% of the drug is recovered in the urine within 12 h after administration. It is advisable to modify initial doses for patients with renal dysfunction.

Therapeutic Uses

In cancer treatment, HU is used alone or in combination with other drugs with two dosage schedules: (1) intermittent therapy administered orally as a single dose (~80 mg/kg) every third day or (2) continuous therapy administered as a single daily dose (20–30 mg/kg). In patients with essential thrombocythemia and in sickle cell disease, HU is given in a daily dose of 15 mg/kg, adjusting that dose upward or downward according to blood counts. The neutrophil count responds within 1 to 2 weeks to discontinuation of the drug. In treating subjects with sickle cell and related diseases, a neutrophil count of at least 2500 cells/mL should be maintained. Treatment typically is continued for 6 weeks to determine effectiveness; if satisfactory results are obtained, therapy can be continued indefinitely, although leukocyte counts at weekly intervals are advisable.

The principal use of HU has been as a myelosuppressive agent in various myeloproliferative syndromes, particularly CML, polycythemia vera, myeloid metaplasia, and essential thrombocytosis, for controlling high platelet or white cell counts. Many of the myeloproliferative syndromes harbor activating mutations of Janus kinase 2 (*JAK2*), a gene that is down-regulated by HU. In essential thrombocythemia, it is the drug of choice for patients with a platelet count greater than 1.5 million cells/mm^3 or with a history of arterial or venous thrombosis. In CML, HU has been largely replaced by *imatinib* (see Chapter 71). HU is a potent radiosensitizer as a consequence of its inhibition of RNR and has been incorporated into several treatment regimens with concurrent irradiation (i.e., cervical carcinoma, primary brain tumors, head and neck cancer, non–small cell lung cancer).

Adverse Effects

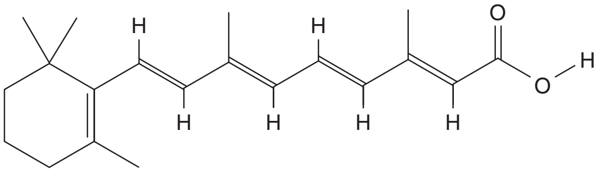
Leukopenia, anemia, and occasionally thrombocytopenia are the major toxic effects; recovery of the bone marrow is prompt if the drug is discontinued for a few days. Other adverse reactions include a desquamative interstitial pneumonitis, GI disturbances, mild dermatological reactions, and, more rarely, stomatitis, alopecia, and neurological manifestations. Increased skin and fingernail pigmentation may occur, as well as painful leg ulcers, especially in elderly patients or in those with renal dysfunction. HU does not increase the risk of secondary leukemia in patients with myeloproliferative disorders or sickle cell disease. It is a potent teratogen in animals and should not be used in women with childbearing potential.

Retinoids

One of the hallmarks of malignant transformation is a block in differentiation. A number of chemical entities (vitamin D and its analogues, retinoids, benzamides and other inhibitors of HDAC [see Chapter 71], various cytotoxic and biological agents, and inhibitors of DNA methylation) can induce differentiation in tumor cell lines. The most important of these for cancer treatment are retinoids, in particular *tretinoin* (ATRA), which induces a high rate of complete remission in patients with APL as a single agent and, in combination with anthracyclines, cures most patients with this disease. The biology and pharmacology of retinoids are discussed in detail in Chapter 75.

Tretinoin (ATRA)

Mechanism of Action. Under physiological conditions, the retinoic acid receptor (RAR) A dimerizes with the retinoid X receptor to form a complex that binds ATRA tightly. ATRA binding displaces a repressor from the transcriptional complex and alters the expression of genes that control differentiation of cells of multiple lineages. Most APLs are characterized by a chromosomal translocation of the *RARA* gene on chromosome 17 and fusion with the *PML* gene on chromosome 15 that is denoted as t(15;17)(q22;q12). In APL cells, physiological concentrations of retinoid are inadequate to displace the repressor, but pharmacological concentrations are effective in activating the differentiation program and in promoting degradation of the PML-RARA fusion protein (Collins, 2008). The *PML* gene encodes a transcription factor important in inhibiting proliferation and promoting myeloid differentiation. The oncogenic *PML-RARA* gene produces a protein that binds retinoids with much decreased affinity, lacks PML regulatory function, and fails to upregulate transcription factors (C/EBP and PU.1) that promote myeloid differentiation. ATRA also binds and activates RAR-γ and thereby promotes stem cell renewal, and this action may help restore normal bone marrow renewal. Resistance to ATRA arises by further mutation of the fusion gene, abolishing ATRA binding; by induction of the CYP26A1; or by loss of expression of the *PML-RARA* fusion gene.



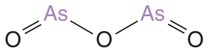
Tretinoin

Therapeutic Use, ADME and Adverse Effects. Oral administration of ATRA is 45 mg/m^2 per day until 30 days after remission is achieved (maximum course of therapy is 90 days). ATRA as a single agent reverses the hemorrhagic diathesis associated with APL and induces a high rate of temporary remission. ATRA in combination with an anthracycline induces remission with 80% or greater relapse-free long-term survival.

ATRA is cleared by a CYP3A4-mediated elimination with a $t_{1/2}$ of less than 1 h. Treatment with inducers of CYP3A4 leads to more rapid drug disappearance and resistance to ATRA. Inhibitors of CYPs, such as antifungal imidazoles, block ATRA degradation and may lead to hypercalcemia and renal failure, which respond to diuresis, bisphosphonates, and ATRA discontinuation. When used as a single agent for remission induction, especially in patients with more than 5000 leukemic cells/mm^3 in the peripheral blood, ATRA induces an outpouring of cytokines and mature-appearing neutrophils of leukemic origin. These cells express high concentrations of integrins and other adhesion molecules on their surface and clog small vessels in the pulmonary circulation, leading to significant morbidity in 15% to 20% of patients. Corticosteroids (e.g., *dexamethasone* 5–10 mg twice daily) and chemotherapy (e.g., hydroxyurea) sharply decrease the occurrence of "retinoic acid differentiation syndrome," which is characterized by fever, dyspnea, weight gain, pulmonary infiltrates, and pleural or pericardial effusions. The syndrome results from the differentiation of leukemia cells and their migration into peripheral tissues, most notably the lungs. Retinoids also cause dry skin, cheilitis, reversible hepatic enzyme abnormalities, bone tenderness, pseudotumor cerebri, hypercalcemia, and hyperlipidemia.

Arsenic Trioxide

Arsenic trioxide (As_2O_3), known as ATO, is a highly effective treatment of relapsed APL, producing complete responses in more than 85% of such patients. The chemistry and toxicity of *arsenic* are considered in Chapter 76.



ARSENIC TRIOXIDE

Mechanism of Action

The basis for ATO's antitumor activity remains uncertain. APL cells have high levels of reactive oxygen species (ROS) and are quite sensitive to further ROS induction. ATO inhibits thioredoxin reductase and thereby generates ROS. It inactivates glutathione and other sulfhydryls that scavenge ROS and thereby aggravates ROS damage. Cells exposed to ATO also upregulate p53, Jun kinase, and caspases associated with the intrinsic pathway of apoptosis and downregulate antiapoptotic proteins such as bcl-2 (see Figure 3–25). ATO's cytotoxic effects are antagonized by cell survival signals emanating from activation of components of the PI3 kinase cell survival pathway, including PKB, S6 kinase, and mTOR (see Figure 71–4). ATO also induces differentiation of leukemic cell lines in experimental and human leukemias, with evidence of enhanced degradation of the PML-RARα fusion protein that acts to inhibit normal differentiation of APL cells (Zhang et al., 2010). Recent studies indicate that ATO binding to p53 DNA binding domain mutants restores wild-type transcriptional function to the mutant p53 proteins (Chen et al., 2021).

ADME

Arsenic trioxide is well absorbed orally but in cancer treatment is administered as a 2-h daily intravenous infusion in dosages of 0.15 mg/kg per day for up to 60 days, until remission is documented. The drug enters cells via one of several glucose transporters. The primary mechanism of elimination is through enzymatic methylation. Multiple methylated metabolites (see Figure 71–9) form rapidly and are excreted in urine. Less than 20% of administered drug is excreted unchanged in the urine. No dose reductions are indicated for hepatic or renal dysfunction.

Adverse Effects

Pharmacological doses of ATO are well tolerated. Patients may experience reversible side effects, including hyperglycemia, hepatic enzyme elevations, fatigue, dysesthesias, and light-headedness. Fewer than 10% of patients experience a leukocyte maturation syndrome similar to that seen with ATRA, including pulmonary distress, effusions, and mental status changes. Oxygen, corticosteroids, and temporary discontinuation of ATO lead to full reversal of this syndrome. Lengthening of the QT interval on the electrocardiogram occurs in 40% of patients, but rarely do patients develop torsade de pointes. Simultaneous treatment with other QT-prolonging drugs should be avoided. Monitoring of serum electrolytes and repletion of serum K$^+$ in patients with hypokalemia are precautionary measures in patients receiving ATO therapy. In patients exhibiting a significantly prolonged QT (>470 msec), treatment should be suspended, K$^+$ supplemented, and therapy resumed only if the QT returns to normal.

Drug Facts for Your Personal Formulary: *Cytotoxic Drugs*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Section I: Alkylating Agents and Platinum Coordination Complexes		
Mechanism of action: covalent modification of DNA. Adverse effects of all alkylating drugs: myelosuppression and immunosuppression; toxicity to dividing mucosal cells and hair follicles (e.g., oral mucosal ulceration, intestinal denudation, alopecia); delayed pulmonary fibrosis; reproductive system toxicity (premature menopause, sterility); and leukemogenesis (up to 5%, highest for melphalan, procarbazine, nitrosoureas).		
Nitrogen Mustards: DNA alkylation		
Mechlorethamine	• Hodgkin lymphoma • Topical: cutaneous T-cell lymphoma	• Vascular damage during injection due to vesicant properties
Cyclophosphamide	• Acute and chronic lymphocytic leukemia; Hodgkin lymphoma; non-Hodgkin lymphoma; multiple myeloma; neuroblastoma; breast, ovary, Wilms tumor; soft-tissue sarcoma • Autoimmune disease (Wegener granulomatosis, rheumatoid arthritis, nephrotic syndrome)	• Oral or intravenous administration • Active alkylating moieties generated through hepatic metabolism • Nephrotoxic and urotoxic metabolite, acrolein; severe hemorrhagic cystitis in high-dose regimens; prevented by MESNA • Provide vigorous hydration during high-dose treatment • Elimination not affected by renal dysfunction; reduce dose in patients with hepatic dysfunction
Ifosfamide	• Germ cell testicular cancer • Pediatric and adult sarcoma • High-dose chemotherapy with bone marrow rescue	• See cyclophosphamide • Can cause neurotoxicity (including seizures) • Methylene blue treatment of CNS toxicity possibly useful
Melphalan	• Multiple myeloma	• Oral and intravenous administration
Chlorambucil	• Chronic lymphocytic leukemia	• Oral administration
Bendamustine	• Non-Hodgkin lymphoma • Chronic lymphocytic leukemia	• Lacks cross-resistance with other classical alkylators
Alkyl Sulfonate: DNA alkylation		
Busulfan	• Chronic myelogenous leukemia • High-dose chemotherapy regimen with bone marrow transplantation	• Oral administration • Adverse effects: prolonged (up to years) pancytopenia; suppression of stem cells; seizures; lung fibrosis • Clearance of phenytoin; hepatic VOD
Nitrosoureas: DNA alkylation		
Carmustine (BCNU)	• Malignant gliomas • Hodgkin lymphoma; non-Hodgkin lymphoma	• Vascular damage during injection due to vesicant properties • Profound and delayed myelosuppression
Streptozocin (or streptozotocin)	• Malignant pancreatic insulinoma • Carcinoid	• Frequent renal toxicity, sometimes renal failure
Methylhydrazine Derivatives: Monofunctional DNA alkylation		
Procarbazine (*N*-methylhydrazine, MIH)	• Hodgkin lymphoma • Gliomas	• Greater capacity for mutagenesis and carcinogenesis than bifunctional alkylators (e.g., cyclophosphamide)
Triazenes: Methyl transfer to DNA		
Dacarbazine (DTIC)	• Hodgkin lymphoma; soft-tissue sarcomas • Melanoma	• Intravenous administration • Activation by hepatic CYPs • Adverse effects: nausea, vomiting • Rare hepatotoxicity and neurotoxicity
Temozolomide	• Malignant gliomas	• Oral administration • Combined with radiation therapy • Greater capacity for mutagenesis and carcinogenesis than bifunctional alkylators; more active in MGMT-deficient tumors
Platinum Coordination Complexes: Form covalent metal adducts with DNA		

Cisplatin	• Testicular, ovarian, bladder, esophageal, gastric, lung, head and neck, anal, and breast cancer	• Intravenous administration
		• Adverse effects:
		  • Nephrotoxicity (reduce by forced pretreatment hydration, diuresis, and use of *amifostine*)
		  • Ototoxicity (tinnitus, high-frequency hearing loss)
		  • Nausea and vomiting (antidote, *aprepitant*)
		  • Peripheral sensory and motor neuropathy (may worsen after discontinuation; may be aggravated by taxane treatment)
		  • Drug resistance due to loss of mismatch repair proteins

Drug Facts for Your Personal Formulary: *Cytotoxic Drugs* (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Carboplatin	• Same as above	• Less nausea, neuro-, oto-, and nephrotoxicity than cisplatin • Dose-limiting toxicity: myelosuppression • May cause hypersensitivity reaction
Oxaliplatin	• Colorectal, gastric, and pancreatic cancer	• Peripheral neuropathy is dose limiting • Some nausea • Efficacy not dependent on intact mismatch repair
Section II: Antimetabolites		
Folic Acid Analogues: Inhibit dihydrofolate reductase		
Methotrexate (amethopterin)	• Acute lymphocytic leukemia; choriocarcinoma; breast, head and neck, ovary, bladder, and lung cancers; osteogenic sarcoma • Noncancer use: psoriasis, rheumatoid arthritis	• Oral, intravenous, or intramuscular administration • Adverse effects: myelosuppression, GI toxicity • Leucovorin can reverse toxic effects; used as "rescue" in high-dose therapy • *Glucarpidase*, a methotrexate-cleaving enzyme, is approved to treat toxicity • ↓ Dose in renal insufficiency
Pemetrexed	• Mesothelioma, lung cancer	• Similar effects and side effects as methotrexate • Attenuate toxicity with folate and vitamin B_{12} supplementation
Pyrimidine Analogues		
5-Fluorouracil (5FU) *Thymidylate synthase inhibitor*	• Breast, colon, esophageal, stomach, anal cancer • In FOLFOX or FOLFIRINOX combination to treat pancreatic or colorectal cancer • Combined with cisplatin in head and neck cancer • Premalignant skin lesion (topical)	• Intravenous administration • Nausea, mucositis, diarrhea, myelosuppression, hand and foot syndrome • Combined with leucovorin to enhance efficacy • Enhanced toxicity with DPD deficiency; may rescue with uridine triacetate
Capecitabine *Thymidylate synthase inhibitor*	• Metastatic breast, colorectal cancer	• Orally administered prodrug of 5FU • Similar adverse effects as 5FU; hand and foot syndrome more frequent than with 5FU
Cytarabine (cytosine arabinoside) *Interferes with base pairing in DNA; inhibits DNA polymerase*	• Acute myelogenous and acute lymphocytic leukemia; non-Hodgkin lymphoma	• Intravenous administration • Myelosuppressive; can cause acute, severe leukopenia, thrombocytopenia, anemia • GI disturbances • Noncardiogenic pulmonary edema • Dermatitis
Gemcitabine (difluoro analogue of deoxycytidine) *Inhibits DNA polymerase; causes strand termination*	• Pancreatic, ovarian, lung, bladder cancer	• Intravenous administration • Female and elderly patients clear the drug more slowly • Myelosuppression, hepatic toxicity • Rare posterior leukoencephalopathy syndrome; sometimes interstitial pneumonitis • Radiosensitizer; should be used with caution in radiotherapy
5-Azacytidine (azacitidine) *Inhibits DNA cytosine methyltransferase*	• Myelodysplasia	• Subcutaneous or intravenous administration • Myelosuppression and mild GI symptoms • After intravenous administration, severe nausea possible
Purine Analogues and Related Inhibitors		
6-Mercaptopurine *Inhibits purine nucleotide synthesis and metabolism*	• Acute lymphocytic and myelogenous leukemia; small cell non-Hodgkin lymphoma • Noncancer: Crohn's disease, ulcerative colitis	• Oral absorption incomplete, thus intravenous administration • Reduce oral dose by 75% in patients receiving allopurinol; no adjustment needed for intravenous administration • Myelosuppression; anorexia, nausea, vomiting; GI side effects less frequent in children than adults • Secondary malignancy: squamous cell skin cancer; AML
Fludarabine *A chain terminator when incorporated into DNA; inhibits RNA function and processing*	• Chronic lymphocytic leukemia • Follicular B-cell lymphoma • Allogeneic bone marrow transplant	• Oral or intravenous administration • Frequently myelosuppression • Less frequent: nausea, vomiting; altered mental status; seizures • Secondary myelodysplasia and acute leukemias • Adjust dose for renal dysfunction
Cladribine *Incorporated into DNA, produces strand breaks; inhibits conversion of ribo- to deoxyribonucleotides*	• Hairy cell leukemia • Chronic lymphocytic leukemia • Low-grade lymphoma • CTCL, Waldenström macroglobulinemia	• Intravenous administration • Adjust dose for renal dysfunction • Myelosuppression, opportunistic infections, nausea, high fever, tumor lysis syndrome

Drug Facts for Your Personal Formulary: *Cytotoxic Drugs* (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Clofarabine (mechanism as above)	• Acute myelogenous or lymphocytic leukemia	• Intravenous administration • Adjust dose to creatinine clearance • Myelosuppression • Capillary leak syndrome: discontinue drug • Nausea, vomiting, diarrhea
Nelarabine *Incorporated into DNA, terminates DNA synthesis*	• T-cell leukemia, lymphoma	• Intravenous administration • Myelosuppression; liver function abnormalities; infrequent neurologic sequelae
Pentostatin (2′-deoxycoformycin) *Inhibits adenosine deaminase; causes immunodeficiency (T and B cells)*	• Hairy cell leukemia; chronic lymphocytic leukemia; small cell non-Hodgkin lymphoma	• Intravenous administration • Adjust dose for renal dysfunction • Myelosuppression, GI symptoms, skin rashes, opportunistic infections • Renal, neurologic, pulmonary toxicity
Section III: Natural Products		
Vinca Alkaloids: Inhibit tubulin polymerization and microtubule formation		
Vinblastine	• Hodgkin and non-Hodgkin lymphoma • Breast, bladder, lung, testicular cancer • Kaposi sarcoma, neuroblastoma • Part of ABVD combination with doxorubicin (Adriamycin, bleomycin, dacarbazine) for Hodgkin lymphoma	• Administered IV; extravasation causes irritation and ulceration • Reduce dose in patients with impaired liver function • Least neurotoxic vinca alkaloid • Myelosuppressive • GI side effects nausea, vomiting, diarrhea • Vinca alkaloids are substrates of the Pgp efflux pump
Vinorelbine	• Breast cancer • Non–small cell lung cancer	• Intravenous administration • Reduce dose in patients with impaired liver function • Intermediate neurotoxicity among the vinca alkaloids • Myelosuppressive (granulocytopenia)
Vincristine	• Acute lymphocytic leukemia; neuroblastoma; Wilms tumor; rhabdomyosarcoma; Hodgkin and non-Hodgkin lymphoma • Part of CHOP regimen: cyclophosphamide, doxorubicin (H), vincristine (O), prednisone	• Administered IV; extravasation causes irritation and ulceration • Reduce dose in patients with impaired liver function • Least myelosuppressive vinca alkaloid • Dose-limiting neurotoxicity • Better tolerated by children than adults
Eribulin	• Breast cancer, liposarcoma	• Side effects overlap with those of vinca but less sensitive to extrusion by Pgp
Taxanes: Stabilize microtubules, inhibit depolymerization		
Paclitaxel	• Ovarian, breast, lung, prostate, bladder, head and neck cancer	• Intravenous administration • Metabolized by hepatic CYPs, ↓ dose in patients with hepatic dysfunction • Substrate of Pgp efflux pump • Myelosuppressive, alleviated by G-CSF • Peripheral neuropathy is dose limiting • Mucositis
Docetaxel	• Same as above	• No effect on doxorubicin clearance • Pharmacokinetics similar to paclitaxel's • ↓ Neutropenia, ↓ neuropathy than paclitaxel
Camptothecins: Inhibit topoisomerase I; DNA religation is inhibited: accumulation of single-strand breaks		
Topotecan	• Ovarian cancer; small cell lung cancer	• Intravenous or oral administration • Reduce dose in patients with renal dysfunction • Neutropenia, GI side effects, nausea, vomiting • Substrate for Pgp
Irinotecan	• Colorectal cancer, small cell lung cancer • Part of FOLFIRI (leucovorin, 5FU, and irinotecan) or FOLFIRINOX combination for GI tumors	• Intravenous administration • Prodrug activated in the liver; CYP substrate • Diarrhea and neutropenia • Acetylcholinesterase inhibition results in cholinergic syndrome: treat with atropine

Drug Facts for Your Personal Formulary: *Cytotoxic Drugs* (*continued*)

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Antibiotics		
Dactinomycin (actinomycin D) *Intercalates between GC base pairs of DNA*	• Wilms tumor; rhabdomyosarcoma; Ewing, Kaposi, and other sarcomas; choriocarcinoma	• Intravenous administration; severe injury on extravasation • Nausea, vomiting; myelosuppression; GI side effects; erythema, inflammation of the skin
Anthracyclines and Anthracenediones: Inhibit topoisomerase II and intercalate DNA		
Daunorubicin (daunomycin, rubidomycin)	• Acute myelogenous and acute lymphocytic leukemia	• Intravenous administration • Imparts a red color to the urine • Myelosuppression, GI side effects • Most important long-term side effect is cardiotoxicity, including tachycardia, arrhythmias, congestive heart failure • Alopecia
Doxorubicin	• Soft-tissue, osteogenic, and other sarcomas; Hodgkin and non-Hodgkin lymphoma; acute leukemia; breast, genitourinary, thyroid, and stomach cancer; neuroblastoma	• See Daunorubicin above
Mitoxantrone (an anthracenedione)	• Acute myelocytic leukemia; breast and prostate cancer	• Similar side effects as above • Less cardiotoxic
Epipodophyllotoxins: Inhibit topoisomerase II and religation of cleaved DNA strand		
Etoposide	• Testicular and lung cancer; Hodgkin lymphoma; non-Hodgkin lymphomas; acute myelogenous leukemia; Kaposi sarcoma	• Oral and intravenous administration • Reduce dose in patients with renal dysfunction • Leukopenia, GI side effects; hepatic toxicity after high doses • Secondary leukemia
Teniposide	• Acute lymphoblastic leukemia in children; glioblastoma, neuroblastoma	• Intravenous administration • Myelosuppression, nausea, vomiting
Drugs With Diverse Mechanisms of Action		
Bleomycin *Binds to DNA, generates free radicals, and induces DNA cleavage via deoxyribose ring damage*	• Testicular cancer; Hodgkin and non-Hodgkin lymphoma; local treatment of bladder cancer • Part of the ABVD regimen (doxorubicin [Adriamycin], bleomycin, vinblastine, and dacarbazine)	• IV, IM, or SC administration; instilled into bladder • Reduce dose in patients with renal dysfunction • Most serious: pulmonary toxicity • Cutaneous toxicity (erythema, ulcerations) • Less myelosuppression than other cytotoxics
L-Asparaginase *Hydrolyzes asparagine; deprives leukemia cells that lack asparagine synthase*	• Acute lymphocytic leukemia	• IV and IM administration • Hypersensitivity reactions, anaphylaxis • Hyperglycemia, clotting abnormalities
Hydroxyurea *Inhibits RNR (conversion of ribo- to deoxyribonucleotides)*	• Chronic myelogenous leukemia; polycythemia vera; essential thrombocytosis; sickle cell disease in adults	• Oral administration • Reduce dose in patients with renal dysfunction • Myelosuppression; some GI side effects
Tretinoin (all-*trans* retinoic acid) *Promotes degradation of PML-RARA fusion protein*	• Acute promyelocytic leukemia	• Oral administration • CYP substrate • Leukocyte maturation syndrome, pulmonary distress, effusions, fever, dyspnea • Dry skin, cheilitis • Hypercalcemia and renal failure
Arsenic trioxide *Inhibits thioredoxin and generates reactive oxygen species*	• Acute promyelocytic leukemia	• Oral or intravenous administration • Leukocyte maturation syndrome as above with ATRA • QT prolongation; rare torsade de pointes • See Chapter 76 (section on arsenic)

Note: For drugs that are subject to hepatic metabolism by CYP enzymes, drug exposure of a patient can be affected by coadministration of inhibitors or inducers of CYP3A4 and can then reduce efficacy or increase side effects.

Embryo-fetal toxicity: Consider that all of these drugs can cause fetal harm. Advise women of the potential risk to a fetus and to avoid pregnancy while taking the drug and for 1 month after cessation of therapy. Advise men to avoid fathering a child during the same time period. Avoid lactation during therapies.

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Chapter 71

Protein Kinase Inhibitors and Pathway-Targeted Small Molecules

Anton Wellstein and Giuseppe Giaccone

I. INHIBITORS OF RECEPTOR SIGNALING IN CANCER CELLS

TYROSINE KINASE RECEPTORS
- Inhibitors of Human Epidermal Growth Factor Receptor (EGFR/HER1)
- Inhibitors of Human Epidermal Growth Factor Receptor 2 (HER2/Neu/ErbB2)
- ALK and ROS1
- NRTK/TRK Fusions
- Inhibitors of Platelet-Derived Growth Factor Receptor and KIT
- FGFR Inhibitors
- MET/HGFR Inhibitors
- RET Inhibitors

HEDGEHOG PATHWAY INHIBITORS
- SMO Inhibitors
- GLI Inhibitor

II. INHIBITORS OF INTRACELLULAR PROTEIN KINASE SIGNALING IN CANCER CELLS

INHIBITORS OF RAS
- Sotorasib
- Adagrasib

INHIBITORS OF RAF KINASE: VEMURAFENIB, DABRAFENIB, AND ENCORAFENIB
- Combination of BRAF and MEK Inhibitors
- Vemurafenib
- Dabrafenib
- Encorafenib

INHIBITORS OF MEK: TRAMETINIB, COBIMETINIB, BINIMETINIB, AND SELUMETINIB
- Trametinib
- Cobimetinib
- Binimetinib
- Selumetinib

INHIBITORS OF JAK1 AND JAK2
- Ruxolitinib
- Fedratinib

CYCLIN-DEPENDENT KINASE (CDK) 4/6 INHIBITORS
- Palbociclib
- Abemaciclib
- Ribociclib
- Trilaciclib

BRUTON TYROSINE KINASE (BTK) INHIBITORS
- Ibrutinib
- Acalabrutinib
- Zanubrutinib

INHIBITORS OF THE BCR-ABL KINASE
- Imatinib, Dasatinib, and Nilotinib
- Bosutinib
- Ponatinib

INHIBITORS OF THE PI3K/AKT/mTOR PATHWAY
- Background
- Impact of PI3K Inhibitors on the Tumor Microenvironment
- Idelalisib
- Copanlisib
- Duvelisib
- Umbralisib
- Alpelisib
- mTOR Inhibitors: Rapamycin Analogues

MULTIKINASE INHIBITORS: CABOZANTINIB, VANDETANIB, MIDOSTAURIN, AND GILTERITINIB
- Vandetanib
- Midostaurin
- Gilteritinib

III. INHIBITORS OF TUMOR ANGIOGENESIS

INHIBITION VEGF AND THE VEGFR PATHWAY

ANTIANGIOGENIC SMALL-MOLECULE KINASE INHIBITORS
- Sunitinib
- Sorafenib
- Pazopanib
- Axitinib
- Tivozanib
- Lenvatinib
- Regorafenib
- Belzutifan

IV. INHIBITORS OF POLY(ADP-RIBOSE) POLYMERASE (PARP)
- Olaparib
- Rucaparib
- Niraparib
- Talazoparib

V. MODULATORS OF PROTEIN DEGRADATION

TARGETING PROTEIN DEGRADATION

THALIDOMIDE AND LENALIDOMIDE
- Thalidomide
- Lenalidomide
- Adverse Effects of Thalidomide and Lenalidomide
- Pomalidomide

PROTEASOME INHIBITORS
- First Generation
- Second Generation

VI. EPIGENETIC MODULATORS: INHIBITORS OF HDAC, HMT, AND IDH1/2

INHIBITORS OF HISTONE DEACETYLASE

- Panobinostat
- Romidepsin
- Vorinostat
- Belinostat

INHIBITORS OF HISTONE METHYLTRANSFERASE

- Tazemetostat

IDH1/2 INHIBITORS

VII. OTHER INHIBITORS (BCL2, NUCLEAR EXPORT, TRANSLATION, CXCR4)

BCL2 INHIBITORS

- Venetoclax

NUCLEAR EXPORT INHIBITOR

- Selinexor

PROTEIN TRANSLATION INHIBITOR

- Omacetaxine

CXCR4 INHIBITOR

- Plerixafor

Abbreviations

ABC: ATP-binding cassette
ABL: Abelson murine leukemia viral oncogene homolog
ALK: anaplastic lymphoma kinase
ALL: acute lymphoblastic leukemia
AML: acute myelocytic leukemia
APML: acute promyelocytic leukemia
ARNT: aryl hydrocarbon receptor nuclear translocator (also known as HIF-1β)
ATO: arsenic trioxide
AUC: area under the curve
BCC: basal cell carcinoma
BCL: B-cell lymphoma
BCR: breakpoint cluster region (chr22)
BCRP: breast cancer resistance protein
BH: BCL2 homology domain
BIM: BCL2-like protein 11
BRCA: breast cancer susceptibility gene
BRK: breast tumor kinase, PTK6
BTK: Bruton tyrosine kinase
CDK: cyclin-dependent kinase
CLL: chronic lymphocytic leukemia
CML: chronic myelocytic leukemia
CRL4-CRBN: cullin-RING ubiquitin ligase cereblon complex
cuSCC: cutaneous squamous cell carcinoma
CXCR4: C-X-C chemokine receptor 4
DNMT: DNA methyltransferase
ECG: electrocardiogram
EGF(R): epidermal growth factor (receptor) = HER1
EML4: echinoderm microtubule-associated protein-like 4
ER: estrogen receptor
ERK: extracellular signal-related kinase = MAPK
FGF(R): fibroblast growth factor (receptor)
FIP1L1: factor interacting with poly (A) polymerase
FKBP12: immunophilin-binding protein for tacrolimus (FK506)
FL: follicular B-cell non-Hodgkin lymphoma
FLT(1 or 4): fms-related tyrosine kinase (1 or 4) (= VEGFR1 or 3)
FMO3: flavin containing monooxygenase 3
GI: gastrointestinal
GIST: gastrointestinal stromal tumor
HDAC: histone deacetylase
HER1/2: human epidermal growth factor receptor; 1 = EGFR; 2 = erbB2
2-HG: 2-hydroxyglutarate
HGF(R): hepatocyte growth factor (receptor) = cMET
HIF-1 or -2: hypoxia-inducible factor 1 or 2
HMT: histone methyltransferase
HSC: hematopoietic stem cell
IFN: interferon
IGF1R: insulin-like growth factor 1 receptor
IκB: inhibitor of nuclear factor kappa B
IL: interleukin
IMiDs: immunomodulatory imide drugs (e.g., thalidomide)
ITK: inducible T-cell kinase
KDR: kinase insert domain receptor = VEGFR2
KIT: feline sarcoma virus oncogene homolog
LCK: lymphocyte-specific kinase
MAPK: mitogen-activated extracellular signal regulated protein kinase = ERK
MCL: mantle cell lymphoma
MCyR: major cytogenetic response
MDS: myelodysplastic syndrome
MEK: mitogen-activated extracellular signal regulated protein kinase kinase = MKK
MET: mesenchymal-epithelial transition factor (= HGFR)
MM: multiple myeloma
mTOR: mammalian or mechanistic target of rapamycin
NCCN: National Comprehensive Cancer Network
NET: neuroendocrine tumor
NF-κB: nuclear factor-κB
NK: natural killer
NPM: nucleophosmin (gene)
NRAS: neuroblastoma RAS virus homolog
NSCLC: non–small cell lung cancer
OATP: organic anion-transporting polypeptide
PARP: poly(ADP-ribose) polymerase
PDGF(R): platelet-derived growth factor (receptor)
Pgp: P-glycoprotein
PI3K: phosphatidylinositol 3-kinase
PIK3CA: PI3K catalytic subunit α gene; codes for the p110α enzyme
PIP2: phosphatidylinositol 4,5-bisphosphate
PIP3: phosphatidylinositol (3,4,5)-trisphosphate
PNET: peripheral neuroendocrine tumor
PPES: palmar-plantar erythrodysesthesia syndrome

PPI: proton pump inhibitor
PROTAC: proteolysis-targeting chimera
PTEN: phosphatase and tensin homolog
Rb: retinoblastoma (protein)
RCC: renal cell carcinoma
RET: "rearranged during transfection" receptor tyrosine kinase
ROS1: orphan receptor tyrosine kinase
SDF: stromal cell-derived factor
SLL: small lymphocytic lymphoma
TGF: transforming growth factor
TIE: tyrosine kinase with Ig and EGF domains
TKI: tyrosine kinase inhibitor
TSC: tuberous sclerosis complex
VEGF(R): vascular endothelial growth factor (receptor)
VHL: von Hippel-Lindau (pVHL = VHL protein)

A Note on Treatment Regimens

Cancer treatment regimens change to reflect continuous advances in basic and clinical science. As a consequence, this chapter presents relatively few detailed treatment regimens; rather, we refer the reader to the web-based resources of the U.S. FDA and the National Comprehensive Cancer Network (NCCN). Table 71–1 provides two examples of therapeutic regimens that illustrate the complexity of current cancer drug therapy.

Development of targeted therapies for treating cancer relies on the discovery of molecular changes that drive malignant progression of human cancers. A rapidly growing number of drugs are being developed to block oncogenic pathways that lead to dysregulated cancer cell growth and survival and may be combined with cytotoxic cancer drugs described in Chapter 70 to improve efficacy. Growth factor receptors and downstream signaling molecules have been productively targeted by cancer chemotherapeutic agents. Indeed, the use of pathway-targeted therapies has likely contributed to reduced mortality from lung cancer (Howlader et al., 2020). The drivers of cancer growth are oncogenic pathways in malignant cells (e.g., mutant receptors and kinases), the reaction of the tumor microenvironment (e.g., angiogenesis), and the escape of malignant cells from the host's immune surveillance (Hanahan and Weinberg, 2011). The primary tools for targeting oncogenic pathways are *small molecules* (discussed in this chapter) that enter cells and engage intracellular targets (e.g., kinases) and *monoclonal antibodies* (see Chapter 72) that recognize cell surface or shed antigens (e.g., growth factor receptors or receptor ligands). These two classes of drugs have different pharmacological properties (see box titled Two Classes of Pathway-Targeted Drugs Used in Cancer Treatment). The sections of this chapter focus on inhibitory drugs that target these drivers of cancer cell growth and survival:

I. Altered growth factor receptor signaling
II. Activation of intracellular kinases
III. Cancer cell-host interactions and aberrant angiogenesis
IV. Defects in DNA repair
V. Altered protein degradation
VI. Altered epigenetic regulation
VII. Targets that control cancer cell behavior

TABLE 71–1 ■ CANCER TREATMENT REGIMENS: COMPLEXITIES AND RESOURCES

In treating cancers, the selection of the most appropriate treatment(s), dose, and dose intervals and the management of adverse effects require specialized knowledge and a team effort. In addition, cancer treatment regimens are undergoing frequent updates due to findings in ongoing clinical trials. The availability of drugs with new mechanisms of action (e.g., immune checkpoint inhibitors), good target selectivity (e.g., kinase inhibitors), efficacy in specific cancers, and different adverse effect profiles permits the use of new drug combinations and regimens. The chapters on the pharmacotherapy of cancer focus on mechanism of action, ADME, and adverse effects, with less emphasis on treatment regimens. There are several excellent sources of up-to-date treatment information. The U.S. FDA provides authoritative information on approved drugs online (http://www.accessdata.fda.gov/scripts/cder/daf/). Detailed treatment guidelines for most cancers are available from the National Comprehensive Cancer Network (NCCN), a nonprofit alliance of 31 cancer centers that establishes evidence-based guidelines for cancer treatment. The NCCN guidelines are continuously updated to reflect new information that may alter clinical practice. The guidelines can be accessed online (https://www.nccn.org/professionals/physician_gls/f_guidelines.asp).

This table provides examples that illustrate the complexities of current treatment for two cancers: HER2-positive breast cancer and advanced colorectal cancer.

SAMPLE TREATMENT REGIMENS

SUMMARY	DRUGS, SCHEDULE, AND DOSING
HER2-Positive Breast Cancer	
Doxorubicin and cyclophosphamide (AC) followed by paclitaxel, trastuzumab, and pertuzumab	**Day 1:** Doxorubicin 60 mg/m^2 IV **Day 1:** Cyclophosphamide 600 mg/m^2 IV Repeat cycle every 21 days for 4 cycles **Followed by** **Day 1:** Pertuzumab 840 mg IV followed by 420 mg IV **Day 1:** Trastuzumab 8 mg/kg IV followed by 6 mg/kg IV **Days 1, 8, and 15:** Paclitaxel 80 mg/m^2 IV Repeat cycle every 21 days for 4 cycles **Day 1:** Trastuzumab 6 mg/kg IV Repeat cycle every 21 days to complete 1 year of trastuzumab therapy Cardiac monitoring at baseline and 3-month intervals
Advanced or Metastatic Colorectal Cancer RAS Wild-Type	
Oxaliplatin, leucovorin, and 5FU (FOLFOX) plus cetuximab	**Day 1:** Oxaliplatin 85 mg/m^2 IV over 2 h **Day 1:** Leucovorin 400 mg/m^2 IV over 2 h **Days 1–3:** 5FU 400 mg/m^2 IV bolus on day 1, then 1200 mg/m^2 per day × 2 days (total 2400 mg/m^2 over 46–48 h) IV continuous infusion **Repeat cycle every 2 weeks, plus** Cetuximab 400 mg/m^2 IV over 2 h for the first infusion, then 250 mg/m^2 IV over 60 min weekly **OR** **Day 1:** Cetuximab 500 mg/m^2 IV over 2 h every 2 weeks

Source: Data from NCCN.

Two Classes of Pathway-Targeted Drugs Used in Cancer Treatment

Small-molecule inhibitors described in this chapter and **large-molecule drugs** (e.g., monoclonal antibodies) described in Chapter 72 constitute pathway-targeted anticancer drugs. Small molecules (molecular mass <1 kDa) may attack the same targets as monoclonal antibodies but mainly exert their effects by entering cells. A monoclonal antibody (molecular mass of immunoglobulin [Ig] G is ~150 kDa) is generally specific for a single antigen; in contrast, small molecules often inhibit multiple targets (e.g., kinases) with different selectivities and thus are likely to have a broader spectrum of activity and produce a broader spectrum of desired effects, off-target effects, and adverse effects than monoclonal antibodies. Many small-molecule drugs have elimination half-lives of 12 to 24 h and typically require at least daily oral administration, whereas antibodies are typically eliminated with half-lives of many days to weeks and require less frequent administration. For small-molecule drugs that are metabolized by hepatic CYPs, the concomitant use of strong CYP inhibitors (e.g., *ketoconazole*, *nefazodone*, *clarithromycin*, *gemfibrozil*, *cobicistat*) or inducers (e.g., *rifampin*, *phenytoin*, *carbamazepine*, *phenobarbital*, St John's wort) can lead to increased adverse effects due to reduced drug elimination or to reduced efficacy due to accelerated clearance. Syllables contained in the generic names of small-molecule drugs indicate their targeted pathways and are listed in Table 71–2.

I. Inhibitors of Receptor Signaling in Cancer Cells

Tyrosine Kinase Receptors

Inhibitors of Human Epidermal Growth Factor Receptor (EGFR/HER1)

Background

EGFR is essential for the growth and differentiation of epithelial cells. Ligand binding to the extracellular domain of EGFR family members causes receptor dimerization and stimulates the protein tyrosine kinase activity of the intracellular domain, resulting in autophosphorylation of several Tyr residues located in the C-terminal tail of the receptor monomers. These phosphotyrosines provide interaction sites for a variety of adapter proteins, resulting in stimulation of signaling pathways, including the mitogen-activated extracellular signal regulated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt pathways (Figure 71–1).

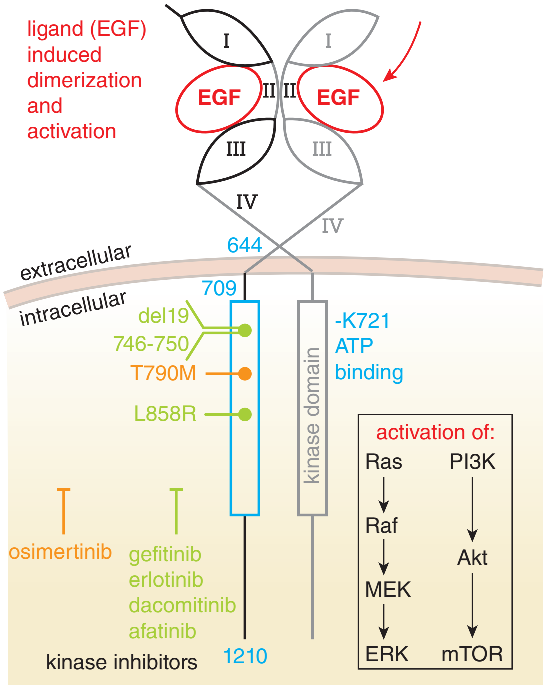


Figure 71–1 *Targeting the EGFR in cancer.* The extracellular portion of the EGFR contains the binding domains for growth factors that include EGF, TGF-α, and amphiregulin. Ligand binding to extracellular domains I and III induces conformational change and receptor dimerization through domains II and IV and activates intracellular signaling by cross-phosphorylation. The intracellular portion contains the tyrosine kinase activity and a C-terminal tail that recruits intracellular substrates after phosphorylation (see Chapter 3). EGFR mutations enhance downstream signaling, which provides a growth and survival advantage for cancer cells and confers sensitivity to treatment with the TKIs *afatinib*, *dacomitinib*, *erlotinib*, *gefitinib*, and *osimertinib*. The most common activating mutations are in-frame deletions in exon 19 (del 19) between E746 and A750 (~45%) and a missense mutation in exon 21 that leads to an L858R substitution (~40%). Lung cancers with these mutations are initially sensitive to first- and second-generation TKIs shown in green font. Resistance develops, however, most commonly via the T790M substitution in exon 20 found in about 50% of resistant tumors. *Osimertinib*, a third-generation inhibitor shown in orange font, is the only TKI that is active against cancers with the T790M mutation. Other insertional mutations in exon 20 are relatively rare and discussed in the text.

Two separate classes of drugs that target the EGFR pathway are important in the therapy of solid tumors: protein tyrosine kinase inhibitors (TKIs) and inhibitors of extracellular ligand binding. EGFR TKIs (*erlotinib*, *gefitinib*, *afatinib*, *osimertinib, dacomitinib*) bind to the intracellular kinase domain and inhibit the enzymatic function of EGFR. Monoclonal antibodies (*cetuximab*, *panitumumab*, *necitumumab*) that recognize the extracellular domain of the EGFR, inhibit ligand-induced signaling, and can elicit an immune response are discussed in Chapter 72. In epithelial cancers, overexpression of the EGFR is a common finding, and mutational activation of the EGFR (e.g., in 10%–40% of non–small cell lung cancers [NSCLCs]) creates a dependence on EGFR signaling. The most common EGFR mutations are in-frame deletions in exon 19 or leucine-to-arginine point mutations (L858R) in exon 21 that cause conformational changes and constitutive activation of the kinase. First-generation (*erlotinib*, *gefitinib*) and second-generation (*afatinib, dacomitinib*) drugs are available that inhibit EGFR kinase activity driven by these mutations. Resistant cancer cell subpopulations that bear the T790M mutation that emerges during treatment with first- and second-generation inhibitors in approximately 50% to 60% of patients are sensitive to the third-generation inhibitor *osimertinib* (see Figure 71–1).

TABLE 71–2 ■ THE NAMING OF SMALL-MOLECULE INHIBITORS

ENDING WITH	INHIBITOR CATEGORY	EXAMPLE
-anib	Angiogenesis	Pazopanib
-ciclib	Cyclin-dependent kinase 4 and 6	Palbociclib
-degib	Hedgehog signaling	Sonidegib
-denib	IDH1 and IDH2	Enasidenib
-lisib	PI3K	Idelalisib
-parib	PARP	Olaparib
-rafenib	BRAF	Vemurafenib
-tinib	Tyrosine kinase	Erlotinib
-zomib	Proteasome	Bortezomib

Erlotinib

Mechanism of Action. *Erlotinib* is a reversible inhibitor of the EGFR tyrosine kinase, competitively inhibiting ATP binding at the active site of the kinase. Erlotinib has an IC_{50} of 2 nM for EGFR kinase activity. By comparison, for many protein kinases, the K_d for ATP at the substrate site is in the range of 20 μM.

ADME. *Erlotinib* is about 60% absorbed after oral administration. Peak plasma levels occur after 4 h. *Erlotinib* has a $t_{1/2}$ of 36 h and is metabolized by CYP3A4 and to a lesser extent by CYPs 1A2 and 1A1.

Therapeutic Uses. *Erlotinib* is approved for treatment of patients with advanced or metastatic NSCLC after failure of platinum-based chemotherapy treatment. In newly diagnosed NSCLC, *erlotinib* is approved in patients with EGFR mutations only (see Figure 71–1). *Erlotinib* in combination with *gemcitabine* is also approved for the treatment of patients with locally advanced, unresectable, or metastatic pancreatic cancer.

Adverse Effects and Drug Interactions. The most common adverse reactions (≥20%) are rash, diarrhea, anorexia, fatigue, dyspnea, nail disorders, nausea, and vomiting. Serious adverse reactions include severe rash (>10%). Fatal interstitial lung disease occurs with a frequency of 0.7% to 2.5%, and fatal hepatic failure has been reported, particularly in patients with baseline hepatic dysfunction. Other rare but serious toxicities include GI perforation, renal failure, arterial thrombosis, microangiopathic hemolytic anemia, hand-foot skin reaction, and corneal perforation or ulceration. *Erlotinib* therapy may cause rare cases of Stevens-Johnson syndrome/toxic epidermal necrolysis.

Concurrent use of proton pump inhibitors decreases the bioavailability of *erlotinib* by 50%. Plasma levels can vary due to drug interactions with inducers or inhibitors of CYP3A4. Patients using *warfarin* may experience poorer extrinsic coagulation while taking *erlotinib*. Smoking accelerates metabolic clearance of *erlotinib* and may decrease its antitumor effects.

Gefitinib

Mechanism of Action. *Gefitinib* inhibits the EGFR tyrosine kinase by competitive blockade of ATP binding with an IC_{50} of 15 to 57 nM for the kinase activity.

ADME. Oral bioavailability is about 60%; peak plasma concentrations are achieved within 3 to 7 h. Absorption of *gefitinib* is not significantly altered by food but is reduced by drugs that cause elevations in gastric pH. Metabolism of *gefitinib* is predominantly via CYP3A4, with a terminal $t_{1/2}$ of 41 h. Inducers of CYP3A4 activity decrease *gefitinib* plasma concentrations and efficacy; conversely, CYP3A4 inhibitors increase plasma concentrations.

Therapeutic Uses. *Gefitinib* is approved for treatment of patients with metastatic NSCLC with EGFR mutations (see Figure 71–1).

Adverse Effects and Drug Interactions. Diarrhea and skin reactions occur in more than 20% of patients. In addition to pustular/papular rash, other side effects include dry skin, nail changes, nausea, vomiting, pruritus, anorexia, and fatigue. Most adverse effects occur within the first month of therapy and are manageable with medications. Asymptomatic increases in liver transaminases may necessitate discontinuation of therapy. Respiratory compromise and interstitial lung disease, especially in patients with prior radiation, occurs in fewer than 2% of patients but may have a fatal outcome. Inducers and inhibitors of CYP3A4 will alter plasma concentrations. Patients using *warfarin* should be monitored for poorer extrinsic coagulation while taking *gefitinib*.

Afatinib

Mechanism of Action. *Afatinib* is a second-generation, orally bioavailable, *irreversible* inhibitor of EGFR (HER1) and HER2 receptor kinases with IC_{50} values of 0.5 and 14 nM, respectively.

ADME. The elimination $t_{1/2}$ of *afatinib* is 37 h after repeat dosing in patients with cancer. In patients with severe renal impairment, a dose reduction is recommended. *Afatinib* is a substrate of P-glycoprotein (Pgp), and coadministration of Pgp-modulating drugs can alter drug concentrations of *afatinib*.

Therapeutic Uses. *Afatinib* is approved for the first-line treatment of patients with metastatic NSCLC with EGFR mutations (see Figure 71–1) and patients with metastatic squamous NSCLC that has progressed subsequent to platinum-based chemotherapy.

Adverse Effects and Drug Interactions. Most common adverse effects are diarrhea and skin rash/acneiform dermatitis, stomatitis, and hand-foot skin reactions and nail changes. Also observed are interstitial lung disease, liver function abnormalities, left ventricular dysfunction, and rarely, gastrointestinal perforation (0.2% of patients).

Dacomitinib

Dacomitinib, approved in 2018, is an orally available, second-generation irreversible inhibitor of EGFR indicated for first-line treatment of patients with metastatic NSCLC with EGFR exon 19 deletion or exon 21 L858R substitution mutations. Plasma $t_{1/2}$ of *dacomitinib* is 70 h. Hepatic metabolism is the main route of clearance of *dacomitinib*. There is no impact of hepatic or renal impairment on drug elimination. Adverse effects are similar to *afatinib*, with diarrhea and skin rashes being the most common.

Mobocertinib

Mobocertinib is an irreversible kinase inhibitor that was approved in 2021 for the treatment of patients with advanced NSCLC with EGFR exon 20 insertional mutations progressing after chemotherapy. Exon 20 insertional mutation in EGFR are relatively infrequent (approximately 2%) and are not sensitive to first-, second-, and third-generation EGFR inhibitors. The response rate to mobocertinib is lower (28%) than that of EGFR inhibitors targeting NSCLC with the more common mutations (50%–80%). Adverse effects are similar to the second-generation EGFR inhibitors, in particular skin rash and diarrhea.

Resistance to Gefitinib, Erlotinib, Afatinib, and Dacomitinib

The majority of tumors with activating mutations in the EGFR initially respond to the kinase inhibitors of the first (*gefitinib*, *erlotinib*) and second generation (*afatinib, dacomitinib*). Eventually, however, the tumors progress. Approximately 60% of NSCLCs acquire a second EGFR mutation in the so-called EGFR gatekeeper residue in exon 20, T790M (see Figure 71–1), that reduces binding of these inhibitors to the kinase domain. The second-generation irreversible inhibitors that bind covalently to the kinase can overcome resistance in preclinical models, but their activity against wild-type EGFR exacerbates adverse effects such as skin rash and diarrhea and thus has limited their clinical use. *Osimertinib*, a third-generation inhibitor designed to recognize the T790M-mutant EGFR, is described below. Other mechanisms of resistance include mutational activation of downstream signaling molecules such as *KRAS* or activation of parallel signaling pathways, for example, through *MET* mutation or amplification, *EML4-ALK* translocation (Figure 71–2), and transformation to small cell lung cancer. Anaplastic lymphoma kinase (ALK) and mesenchymal-epithelial transition factor (MET) kinase inhibitors are described further below (Smit and Baas, 2015).

Osimertinib

Mechanism of Action. *Osimertinib* is a third-generation, orally bioavailable, *irreversible* inhibitor of T790M-mutant EGFR, with an IC_{50} value of 10 nM. In addition, *osimertinib* is also a very potent inhibitor of the EGFR mutants that are sensitive to first- and second-generation EGFR inhibitors. The T790M gatekeeper mutation in Exon 20 is often acquired after prior treatment with other EGFR kinase inhibitors (see previous discussion). Very sensitive detection methods can identify the presence of T790M in up to 30% of untreated patients, suggesting preexisting resistant subpopulations (see Chapter 69, Figure 69–3).

ADME. The mean $t_{1/2}$ of *osimertinib* is 48 h. *Osimertinib* is a substrate of CYP3A. The drug is primarily eliminated in the feces and to a lesser extent in the urine. Concurrent administration of inducers of CYP3A4 may necessitate an increase in the dose.

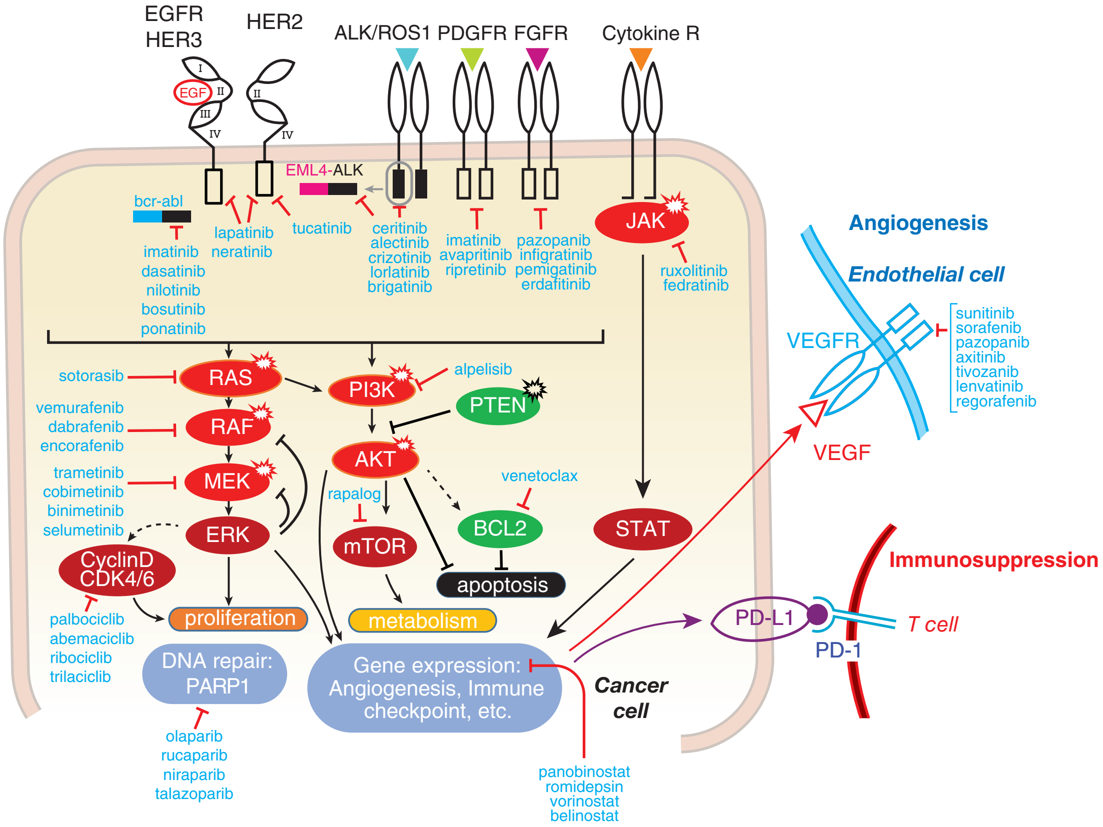

EGFR
HER3
HER2
ALK/ROS1
PDGFR
FGFR
Cytokine R
EGF
I
II
III
IV
EML4-ALK
bcr-abl
imatinib
dasatinib
nilotinib
bosutinib
ponatinib
lapatinib
neratinib
tucatinib
ceritinib
alectinib
crizotinib
lorlatinib
brigatinib
imatinib
avapritinib
ripretinib
pazopanib
infigratinib
pemigatinib
erdafitinib
JAK
ruxolitinib
fedratinib
Angiogenesis
Endothelial cell
VEGFR
sunitinib
sorafenib
pazopanib
axitinib
tivozanib
lenvatinib
regorafenib
VEGF
sotorasib
RAS
PI3K
alpelisib
PTEN
vemurafenib
dabrafenib
encorafenib
RAF
AKT
venetoclax
trametinib
cobimetinib
binimetinib
selumetinib
MEK
rapalog
BCL2
STAT
ERK
mTOR
apoptosis
CyclinD
CDK4/6
Immunosuppression
palbociclib
abemaciclib
ribociclib
trilaciclib
proliferation
metabolism
PD-L1
T cell
PD-1
DNA repair:
PARP1
Gene expression:
Angiogenesis, Immune
checkpoint, etc.
Cancer
cell
olaparib
rucaparib
niraparib
talazoparib
panobinostat
romidepsin
vorinostat
belinostat

Figure 71–2 *Cancer cell signaling pathways and drug targets.* Extracellular binding of agonist ligands to transmembrane growth factor receptors causes receptor dimerization and activation of a C-terminal protein kinase that initiates intracellular signaling cascades that regulate gene expression and control cancer cell proliferation, apoptosis, metabolism, and metastasis. Crosstalk between cancer cells and the host stroma (including the vasculature and immune cells) is modulated through the release of angiogenic factors and the expression of immune checkpoint proteins. Cancer cell signaling can be altered through increased expression of receptors (e.g., HER2) or ligands, activating mutations in receptors (e.g., ALK, EGFR, FGFR) or in intracellular kinases (e.g., RAS, RAF, MEK, PI3K, AKT, JAK), gene translocations resulting in activated fusion proteins (e.g., bcr-abl, EML4-ALK), and the loss of function of an inhibitor of a pathway (e.g., the PTEN phosphatase) through mutation, gene deletion, or promoter methylation. Inhibitors are shown acting on a cancer cell and an endothelial cell. A T cell is included to represent the crosstalk with the immune system and effects of combination therapies. Inhibitors (in blue) are depicted adjacent to their intracellular target proteins. Proteins within red ellipses provide activating signals, those in green ellipses, inhibitory signals. Star bursts (✸) indicate signaling components in which mutations are common. More details of mechanisms of action are shown below for CDK4/6 inhibitors (Figure 71–3), BTK and PI3Kδ inhibitors in B cells (Figure 71–4), the mTOR pathway (Figure 71–5), HIF-2α inhibition (Figure 71–6), PARP inhibition (Figure 71–7), *thalidomide* congeners (Figures 71–8 and 71–9), and BH3 mimics (Figure 71–10). Immune suppression by mTOR inhibition during organ transplants is discussed in Chapter 39.

Therapeutic Uses. *Osimertinib* is approved for first-line treatment of patients with metastatic NSCLC and adjuvant therapy after resection of NSCLC with EGFR exon 19 deletions or exon 21 L858R mutations; it is also approved in patients with metastatic NSCLC that progressed after prior EGFR TKI treatment and is positive for the EGFR T790M mutation. Genotyping of tumors for the T790M mutation is required after resistance develops and, besides a tumor biopsy, can be done noninvasively from blood samples analyzing DNA shed from tumor cells into the bloodstream (Oxnard et al., 2016). Response rates are greater than 60% in patients with metastatic NSCLC. *Osimertinib* improved survival in patients with NSCLC EGFR T790M mutations and in first-line treatment compared to first-generation inhibitors and thus has become the standard of care irrespective of the presence of the EGFR T790M mutation (Cohen et al., 2021). Acquired resistance after *osimertinib* treatment is manifested by the appearance of a third mutation, C797S, in less than 20% of tumors. The mutation of Cys^{797} to Ser^{797} prevents formation of the potency-conferring covalent bond of the inhibitor (Thress et al., 2015); however, MET amplification is a more common resistance mechanism along with several other mechanisms that have been described. Inhibitors of C797S-mutant EGFR are under development for combination treatment with an EGFR-targeted antibody (Jia et al., 2016).

Adverse Effects and Drug Interactions. The most common adverse effects after *osimertinib* treatment are diarrhea and skin rash; however, these are much less common and severe than for first- and second-generation inhibitors, because the activity on wild-type EGFR is reduced with the third-generation inhibitor. Also observed are interstitial lung disease, QTc prolongation, and left ventricular dysfunction.

Inhibitors of Human Epidermal Growth Factor Receptor 2 (HER2/Neu/ErbB2)

Human epidermal growth factor receptor 2 (also named Neu or ErbB2; see History box) is a member of the HER family, which also includes EGFR (HER1), HER3, and HER4 (see Figure 71–2). The fixed conformation of the extracellular domain of HER2 resembles the ligand-activated state of the other HER family members and explains its unique function as a coreceptor that does not require ligand activation. In addition, due to this conformation, the overexpression of wild-type HER2 is sufficient to activate the intracellular tyrosine kinase and oncogenic signaling even in the absence of activating mutations, coreceptors, or ligands. Overexpression of HER2 is found in 20% to 30% of human breast cancers due to gene amplification on chromosome 17 and results in more aggressive tumors, lower response rates to hormonal therapies, and higher risk of disease recurrence after treatment.

HISTORY EGFR/HER PATHWAY-TARGETED CANCER THERAPY

The discovery and analysis of the EGFR (HER1) in 1984 (Ullrich et al., 1984) revealed a remarkable homology of its protein kinase domain to an oncogenic protein in the avian erythroblastosis retrovirus, v-erb (Downward et al., 1984). The first oncogenic protein isolated from the erb virus is a homologue of the thyroid hormone receptor and was named erbA; the second oncogenic protein isolated from v-erb was named erbB, an alternate name for members of the EGFR/HER gene family members. The *HER2* gene was discovered based on its homology to v-erbB in human breast cancer and its oncogenic function in rat ***neu***ral tumors; hence the different names HER2/ErbB2/Neu (King et al., 1985; Schechter et al., 1985). The association of HER2 overexpression with poor prognosis in breast cancer (Slamon et al., 1987) provided the impetus for the development of HER2-targeted therapies and a new concept in the treatment of HER2-positive breast cancers. The most recent addition in 2020 to drugs that target HER2 is *tucatinib*, an HER2-selective, small-molecule kinase inhibitor approved for use in HER2-positive breast cancer.

Lapatinib

Mechanism of Action; ADME. *Lapatinib* is an orally bioavailable, small-molecule inhibitor of the EGFR and HER2 tyrosine kinases with an IC_{50} of about 10 nM for both kinases. *Lapatinib* is metabolized by CYP3A4 with a plasma $t_{1/2}$ of 14 h. Concurrent administration of inducers and inhibitors of CYP3A4 may necessitate adjustment of the dose.

Therapeutic Uses; Adverse Effects. *Lapatinib* in combination with *capecitabine* is approved for the treatment of patients with metastatic HER2-positive *trastuzumab*-refractory breast cancer. *Lapatinib* is also used in combination with the aromatase inhibitor *letrozole* (see Chapter 73) to treat postmenopausal women with hormone receptor–positive, metastatic breast cancer that overexpresses HER2. Frequent adverse effects include acneiform rash, diarrhea, cramping, and exacerbation of gastroesophageal reflux. When *lapatinib* is combined with *capecitabine*, diarrhea is worsened. Cardiac toxicity appears less pronounced than with *trastuzumab*, although *lapatinib* should be used with caution in combination with cardiotoxic drugs. A dose-dependent prolongation of the QT interval has been reported; thus, careful monitoring of patients with heart disease is recommended. Hepatotoxicity that may be severe has been observed.

Neratinib

Neratinib is an orally bioavailable, *irreversible* inhibitor of the HER2 and EGFR protein tyrosine kinase with IC_{50} values of 59 nM and 92 nM, respectively. It is approved as a single agent in early-stage HER2-positive breast cancer following *trastuzumab*–based therapy and in advanced or metastatic HER2-positive breast cancer after two or more anti-HER2–based regimens in combination with *capecitabine*. Diarrhea is the most common and frequent adverse effect, with grade 3 or 4 diarrhea occurring in over one-third of patients (Park et al., 2016); prophylaxis with *loperamide* is recommended. The metabolism is via CYP3A in the liver with an elimination $t_{1/2}$ of 7 to 17 h; concomitant use of *ketoconazole*, a strong inhibitor of CYP3A4 and Pgp, increased maximal plasma concentration by over 2-fold.

Tucatinib

Tucatinib is an orally bioavailable, reversible, highly selective TKI of HER2 with minimal inhibition of EGFR (HER1). *Tucatinib* demonstrated efficacy compared with placebo in progression-free and overall survival of heavily pretreated patients with HER2-positive metastatic breast cancer, including patients with brain metastases (Murthy et al., 2020). In this study, addition of *tucatinib* to the anti-HER2 antibody *trastuzumab* improved outcomes, demonstrating that targeting of the intracellular kinase with a small-molecule inhibitor in addition to targeting the extracellular domain with a monoclonal antibody can be beneficial. The FDA approved its use in 2020 for the treatment of patients with unresectable or metastatic HER2-positive breast cancers, including patients with brain metastases and pretreated patients.

Tucatinib is metabolized mostly by CYP2C8 and less by CYP3A and has an elimination $t_{1/2}$ of 8.5 h. The most common adverse effects are diarrhea and hepatotoxicity. Skin toxicity that is frequently dose limiting and associated with EGFR inhibition is significantly less common with *tucatinib*.

ALK and ROS1

Background

ALK is a membrane monospan with an extracellular domain and an intracellular protein tyrosine kinase domain (see Figure 71–2). The importance of this protein kinase in human cancers is the capacity of the *ALK* gene to form fusion genes that become oncogenic drivers. A 2;5 chromosomal translocation yields an oncogenic fusion gene in which a 3′ portion of *ALK* (chromosome 2) contributes coding for the catalytic domain and is joined to a 5′ region of the *NPM* gene. The extracellular and transmembrane sequences of ALK are absent in the *ALK-NPM* fusion gene, which is the oncogenic driver of malignant progression in anaplastic large cell lymphoma. A similar translocation of the ALK kinase and fusion with EML4 creates the oncogenic driver in a subset of NSCLC (Martelli et al., 2010), as shown in Figure 71–2. *ALK* also fuses with numerous additional 5′ partners (Roskoski, 2017). *Alectinib*, *brigatinib*, *crizotinib*, *ceritinib*, and *lorlatinib* are small-molecule inhibitors of ALK kinase that are used in the treatment of ALK translocation–positive cancers.

The overall organization of ROS1 (orphan receptor tyrosine kinase) is similar to ALK, and the intracellular kinase portion of ROS1 also can become part of chimeric oncoproteins due to different chromosomal translocation partners most frequently found in glioblastoma and NSCLC but also in other cancers. Several of the kinase inhibitors discussed below also target the ROS1 kinase due to protein sequence homologies in the kinase domains of approximately 70% (reviewed by Drilon et al., 2021). ROS1 inhibition correlates with increased efficacy and improved survival in patients with ROS1-positive NSCLCs (Nokin et al., 2020).

Crizotinib, originally developed as a MET inhibitor, was found to also target ALK and ROS1 and became the first inhibitor to be approved for ALK-positive cancers. The next-generation ALK inhibitors have mostly replaced *crizotinib* due to their improved clinical efficacy in ALK-positive NSCLC.

Crizotinib

Mechanism of Action. *Crizotinib* is an orally bioavailable inhibitor of receptor tyrosine kinases that include ROS1, HGFR/cMET, ALK, and ROS1. IC_{50} values are 0.6, 11, and 24 nM for ROS1, hepatocyte growth factor receptor (HGFR)/cMet, and ALK, respectively.

ADME. *Crizotinib* is predominantly metabolized by CYP3A4/5 and shows a terminal $t_{1/2}$ of 42 h. The typical twice daily dosing is reduced to once daily in patients with severe renal impairment and a creatinine clearance below 30 mL/min.

Therapeutic Uses. *Crizotinib* was initially developed and approved for the treatment of patients with locally advanced or metastatic NSCLC harboring *ROS1* or *ALK* gene rearrangements described above (see Figure 71–2) (Thomas et al., 2015).

Adverse Effects. The most common adverse reactions are GI toxicities including nausea, diarrhea, and vomiting as well as hepatic, respiratory, and ocular toxicity and neuropathy. Also observed are QT prolongation and bradycardia.

Alectinib

Mechanism of Action. *Alectinib* is an orally bioavailable inhibitor of ALK kinase activity with an IC_{50} of 1.9 nM. *Alectinib* shows a higher selectivity for ALK than *crizotinib* and is active against mutant forms of ALK that are found in tumors resistant to *crizotinib*.

ADME. *Alectinib* has an elimination half-life of 33 h and is metabolized by CYP3A4. Coadministration of *alectinib* with CYP3A inhibitors or inducers will alter plasma concentrations.

Therapeutic Uses. *Alectinib* is approved for the treatment of patients with advanced or recurrent NSCLC harboring the ALK fusion gene (ALK$^+$). Patients with ALK$^+$ NSCLC who have progressed on treatment with *crizotinib* or are intolerant to *crizotinib* also qualify. *Alectinib* is also approved for first-line treatment of ALK-positive advanced NSCLC.

Adverse Effects. The most common adverse effects are fatigue, constipation, edema, and myalgia. Pneumonitis, GI toxicity, hepatotoxicity, bradycardia, and prolonged QT intervals are also observed (Katayama et al., 2015).

Ceritinib

Ceritinib is an orally bioavailable, competitive inhibitor of the ALK kinase with an IC_{50} of 0.2 nM and is 40-fold selective relative to the homologous insulin-like growth factor 1 receptor (IGF1R) kinase. *Ceritinib* is approved for the treatment of ALK-positive metastatic NSCLC unresponsive to *crizotinib*. *Ceritinib* is metabolized by CYP3A and eliminated with a $t_{1/2}$ of 41 h. Common adverse effects are GI toxicity and hepatotoxicity. Bradycardia and prolonged QT intervals also occur.

Brigatinib

Brigatinib is an inhibitor of the ALK and ROS1 (IC_{50} 0.9 nM) and of IGF1R and FLT3 tyrosine kinases at lower potency. *Brigatinib* is approved for the treatment of ALK-positive metastatic NSCLC, where treatment resulted in a longer progression-free survival and higher response rate (78%) against brain metastases than *crizotinib* (29%) (Camidge et al., 2018). *Brigatinib* is approved for first-line treatment. *Brigatinib* is metabolized by CYP3A and excreted through the urine. Reduced dosing is recommended with hepatic or renal impairment. The elimination $t_{1/2}$ is 25 h. Adverse effects can include the induction of interstitial lung disease/pneumonitis, hypertension, and bradycardia. Blood pressure and heart rate monitoring are recommended.

Lorlatinib

Lorlatinib (originally named PF-06463922) is an orally active, third-generation inhibitor of the ALK and ROS1 kinase with an IC_{50} of less than 1 nM. It was approved in 2018 for the treatment of metastatic, ALK-positive NSCLC who failed 1 or 2 prior ALK inhibitors. In 2021 it was also approved for first-line treatment.

Lorlatinib is metabolized by CYP3A4 and eliminated with a $t_{1/2}$ of 24 h. CNS adverse effects including seizures, cognitive dysfunction, and psychoses or mood changes are a risk during therapy. Potential induction of hypertension requires monitoring of blood pressure. Hepatotoxicity is a further risk.

NRTK/TRK Fusions

Chromosomal rearrangements resulting in in-frame fusions of the kinases with different partners can result in constitutively active chimeric TRK that act as oncogenic drivers, promoting proliferation and survival in tumor cells. Fusions involving one of three TRKs (A, B, or C) occur in diverse cancers (e.g., salivary gland, soft-tissue sarcoma, fibrosarcoma) and define a unique molecular subgroup of advanced tumors. TRK refers to tropomyosin receptor kinase, one of a family of receptor tyrosine kinases encoded by neurotrophic tyrosine receptor kinase (NTRK) gene fusions. Assays to detect TRK fusions are used to identify cancers that contain such a drug-sensitive driver (Nokin et al., 2020).

Larotrectinib

Larotrectinib is an orally available inhibitor of the TRKA, TRKB, and TRKC kinases with IC_{50} values of 5 to 11 nM that showed antitumor activity against constitutively active TRK fusion proteins or TRK overexpression. *Larotrectinib* was not active against mutant TRKA or TRKC. It was approved in 2018 for the treatment of any cancers with TRK gene fusions without resistance mutation (Drilon et al., 2018). Adverse effects include neurotoxicity and hepatotoxicity. It is metabolized by CYP3A and eliminated with a $t_{1/2}$ of 2.9 h.

Entrectinib

Entrectinib is an orally active inhibitor of the TRKA, TRKB, TRKC, ROS1, and ALK kinases with IC_{50} values of 0.1 to 2 nM. It was approved in 2019 for the treatment of any cancers with TRK gene fusions without resistance mutation and for metastatic NSCLC positive for ROS1. Adverse effects include an increased risk for congestive heart failure, cognitive impairment, hepatotoxicity, and prolongation of QT intervals requiring periodic monitoring. It is metabolized by CYP3A with an elimination $t_{1/2}$ of approximately 20 h.

Inhibitors of Platelet-Derived Growth Factor Receptor and KIT

Signaling by the platelet-derived growth factor receptor (PDGFR) plays a significant part in mesenchymal biology, including stem cell growth, and is involved in oncogenesis through aberrant cancer cell signaling, modulation of the tumor microenvironment, and facilitation of angiogenesis and metastasis. Small-molecule protein kinase inhibitors, including *imatinib* (see discussion below), have been used in the treatment of patients with gastrointestinal stromal tumors (GISTs) that carry mutations in *KIT* (feline sarcoma virus oncogene homolog) or *PDGFRA* (see Figure 71–2).

Ripretinib

Ripretinib is an orally bioavailable kinase inhibitor of the PDGFR, wild-type and mutant KIT, and other kinases including VEGFR2 and tyrosine kinase with Ig and EGF domains (TIE) 2 with IC_{50} values of 4 to 18 nM. It was approved in 2020 for the treatment of patients with advanced GIST who have received prior treatment with three or more kinase inhibitors, including *imatinib*. Adverse effects include palmar-plantar erythrodysesthesia syndrome (PPES), nausea, vomiting, constipation, diarrhea, and alopecia. Hypertension and cardiac dysfunction have also been reported. It is metabolized by CYP3A and eliminated with a $t_{1/2}$ of 14.8 h.

Avapritinib

Avapritinib is an orally bioavailable inhibitor of KIT and PDGFRA mutant kinases with IC_{50}s of less than 25 nM. It was approved in 2020 for the treatment of metastatic GIST harboring *PDGFRA* mutations and for advanced systemic mastocytosis and mast cell leukemia. Adverse events include risk of intracranial hemorrhage and cognitive impairment. *Avapritinib* is primarily metabolized by CYP3A. The elimination $t_{1/2}$ ranges from 20 to 57 h.

FGFR Inhibitors

Physiological fibroblast growth factor (FGF) receptor (FGFR) signaling is modulated by 22 human protein ligands that bind to four transmembrane tyrosine kinase receptors expressed as seven distinct splice variant receptors. In approximately 7% of cancers, FGFR genes are mutated or amplified or become part of translocations that lead to constitutively active gene fusions and thus contribute to cancer initiation and malignant progression. *FGFR1* is amplified in NSCLC and breast cancer and mutated in gliomas. FGFR2 is activated by gene fusions in approximately 20% of cholangiocarcinomas, leading to recent approval of kinase inhibitors. The first approval of FGFR kinase inhibitors was based on FGFR3 mutations in metastatic urothelial carcinomas but FGFR3 fusions are also found in brain tumors and bladder cancers. Unusual for tyrosine kinase receptors, the most common FGFR3 mutations occur in the ligand-binding domain, mimicking a constitutive extracellular signaling. A more detailed overview is provided by Facchinetti et al. (2020).

Erdafitinib

Erdafitinib is a selective pan-FGFR kinase inhibitor that also inhibits other kinases including RET, CSF1R, PDGFR, KIT, and VEGFR2. It was approved in 2019 for the treatment of patients with locally advanced or metastatic urothelial carcinoma with FGFR2 or FGFR3 genetic alterations that has progressed after chemotherapy. Adverse effects include ocular disorders that require monthly ophthalmological monitoring. Hyperphosphatemia as a consequence of FGFR inhibition was observed in 76% of patients with a median onset time of 20 days. About one-third of patients received phosphate binders during treatment. *Erdafitinib* is metabolized by CYP2C9 and CYP3A4 and eliminated with a $t_{1/2}$ of 59 h.

Pemigatinib

Pemigatinib is an orally bioavailable selective FGFR1–3 kinase inhibitor (IC$_{50}$ <1.2 nM; FGFR4 IC$_{50}$ is 30 nM). It was approved in 2020 for the treatment of pretreated, locally advanced, or metastatic cholangiocarcinoma with an FGFR2 fusion or rearrangement. Adverse events include diarrhea, ocular toxicity, and hyperphosphatemia as a consequence of FGFR inhibition, resulting in soft-tissue mineralization and vascular and myocardial calcification. It is metabolized by CYP3A and eliminated with a $t_{1/2}$ of 15.4 h.

Infigratinib

Infigratinib (previously named BGJ398) is an orally bioavailable selective FGFR1–3 kinase inhibitor (IC$_{50}$ <2 nM) approved in 2021 for the treatment of pretreated, locally advanced, or metastatic cholangiocarcinoma with an FGFR2 fusion or rearrangement. Adverse events include diarrhea, ocular toxicity, and hyperphosphatemia as a consequence of FGFR inhibition, resulting in soft-tissue mineralization and vascular and myocardial calcification. It is metabolized by CYP3A and eliminated with a $t_{1/2}$ of 33.5 h.

MET/HGFR Inhibitors

Under physiological conditions, the MET/HGFR transmembrane receptor is activated by HGF ligand binding. Malignant transformation observed in NSCLC and other cancers can lead to alternate MET activation through gene rearrangements that code for constitutively active cytosolic fusion proteins (Nokin et al., 2020). Also, somatic mutations in MET are known to result in aberrant mRNA splicing resulting in increased MET activity: Exon 14 codes for a binding site for the E3 ubiquitin ligase CBL that is responsible for ubiquitination of the MET protein and for its degradation. Skipping of exon 14 thus leads to decreased ubiquitination and results in increased protein levels and activity of MET (Guo et al., 2020) that can be targeted therapeutically (Paik et al., 2015).

Capmatinib

Capmatinib is an orally bioavailable selective inhibitor of the MET kinase (IC$_{50}$ of 0.13 nM) that was approved in 2020 for the treatment of patients with metastatic NSCLC with a mutation that leads to MET exon 14 skipping (see above). Adverse effects include peripheral edema, dyspepsia, and fatigue. *Capmatinib* is predominantly metabolized by CYP3A4 and eliminated with a $t_{1/2}$ of 6.5 h.

Tepotinib

Tepotinib is an orally bioavailable inhibitor of the MET kinase (IC$_{50}$ of 4 nM) approved in 2021 for the treatment of patients with metastatic NSCLC with a mutation that leads to MET exon 14 skipping (see above). Adverse effects include peripheral edema, dyspepsia, fatigue, and musculoskeletal pain. *Tepotinib* is predominantly metabolized by CYP3A4 and eliminated with a $t_{1/2}$ of 32 h.

RET Inhibitors

Physiologically, the RET (rearranged during transfection) transmembrane tyrosine kinase receptor is activated through extracellular interactions with co-receptors and ligands from the glial cell–derived neurotrophic factor family and is involved in development of different organ systems including hematopoiesis, the CNS and the gastrointestinal (GI) tract. Activating RET mutations or gene fusions can become oncogenic drivers in NSCLC (1%–2%), thyroid cancer (10%–20%), and other cancers (Thein et al., 2021).

Selpercatinib

Selpercatinib is an orally bioavailable inhibitor of wild-type and mutated RET isoforms (IC$_{50}$ of 1–4 nM) as well as VEGFRs and FGFRs at higher concentrations. It was approved in 2020 for the treatment of patients with metastatic RET fusion–positive NSCLC and advanced or metastatic RET-mutant medullary thyroid cancer. Adverse effects include the risk of hepatotoxicity that requires close monitoring every 2 weeks; hypertension requiring monitoring; QTc prolongation; impaired wound healing risk after surgery; and tumor lysis syndrome. It is metabolized predominantly by CYP3A4 and eliminated with a $t_{1/2}$ of 32 h.

Pralsetinib

Pralsetinib is an orally bioavailable inhibitor of wild-type and oncogenic RET fusions and mutations (IC$_{50}$ <0.5 nM) that is over 10-fold selective relative to other kinases. It was approved in 2020 for the treatment of patients with metastatic RET fusion–positive NSCLC and advanced or metastatic RET-mutant medullary thyroid cancer. Adverse effects include the risk of severe interstitial lung disease/pneumonitis (10% of patients; 0.5% with fatal reactions) and hypertension (29% of patients; 14% with grade 3) that needs medications. It is metabolized predominantly by CYP3A; elimination $t_{1/2}$ is 15.7 h.

Hedgehog Pathway Inhibitors

The hedgehog signaling pathway was discovered by genetic studies in fruit flies: Mutations resulting in stubby and hairy *Drosophila melanogaster* larvae inspired the naming of the signaling pathway as "hedgehog" or Hh, which is the name of the pathway's polypeptide ligand. The hedgehog pathway controls embryonic cell differentiation in tissues of different species through distinct gradients of hedgehog signaling proteins that are key regulators during development. In adults, hedgehog signaling plays roles in stem cell regulation and tissue regeneration. In mammals, binding of one of three Hh ligands, named Sonic, Indian, and Desert Hh, to Patched-1 (PTCH1), a 12-transmembrane domain cell surface receptor protein, releases the PTCH1-mediated suppression of the seven-transmembrane receptor protein SMO (smoothens hedgehog), resulting in its activation of the glioma-associated (GLI) family of transcription factors that control the expression of Hh target genes. Mutations in PTCH1 are found in patients with Gorlin's syndrome predisposed to basal cell carcinoma (BCC), medulloblastoma, and rhabdomyosarcoma due to increased Hh pathway signaling. Overexpression of Hh ligands occurs in glioblastoma and GI and breast cancers, and abnormal activation of the pathway is seen in BCC and in some hematological malignancies, resulting in increased self-renewal capacity of leukemic cells (Jamieson et al., 2020). Hh pathway inhibitors induce differentiation of stem cells and can thus be used in combination with chemotherapy to target both dormant stem cells and growing cancer cells in the bulk of the tumor (Amakye et al., 2013). Clinical trials in solid tumors and hematological malignancies are ongoing to test the efficacy of such combinations (Jamieson et al., 2020). Three inhibitors of SMO and arsenic trioxide, an inhibitor of GLI transcription factors, are currently FDA-approved for cancer treatment and discussed next.

SMO Inhibitors

Vismodegib

Vismodegib, a 32% orally bioavailable first-in-class hedgehog signaling pathway inhibitor, is approved for the treatment of adult patients with BCC. It acts as a competitive antagonist of SMO and is indicated for patients with metastatic or relapsed BCC. Common adverse effects include GI disorders (nausea, vomiting, diarrhea, constipation), muscle spasms, fatigue, hair loss, and dysgeusia (distortion of the sense of taste). These adverse effects are mostly mild to moderate. *Vismodegib* can cause embryo-fetal death and severe birth defects and thus must not be used during pregnancy. Also, patients are advised not to donate blood for 24 months after the final dose and males not to donate semen during and for 3 months after therapy. The estimated elimination $t_{1/2}$ is 4 days after continuous dosing.

Sonidegib

Sonidegib is a less than 10% orally bioavailable hedgehog pathway inhibitor that binds to and inhibits SMO signal transduction similar to *vismodegib*. *Sonidegib* is approved for the treatment of patients with locally advanced BCC that has recurred following surgery or radiation therapy. Warnings and adverse effects are similar to those of *vismodegib*. It is predominantly metabolized by CYP3A and has an elimination $t_{1/2}$ of approximately 28 days.

Glasdegib

Glasdegib is a 77% orally active hedgehog pathway inhibitor that was approved in 2018 for the treatment of patients 75 years of age or older with newly diagnosed acute myelocytic leukemia (AML) in combination with low-dose *cytarabine* if comorbidities preclude use of intensive induction chemotherapy. In addition to the warnings and adverse effects in common with *vismodegib*, *glasdegib* can cause QTc prolongation and thus requires electrocardiogram (ECG) monitoring. *Glasdegib* is metabolized by CYP3A4 and has a mean elimination $t_{1/2}$ of 17.4 h.

GLI Inhibitor

Arsenic Trioxide

Arsenic trioxide (ATO) is administered IV and binds to GLI1 and GLI2, resulting in inhibition of hedgehog target gene expression. ATO is approved in combination with *tretinoin* for the treatment of acute promyelocytic leukemia (APML) with a t(15;17) translocation that fuses PML to the retinoic acid receptor RAR-α. Patients with newly diagnosed, low-risk APML or patients after relapse from retinoid or chemotherapy are eligible. Adverse effects and warnings associated with the treatment include the occurrence of differentiation syndrome, which may be life-threatening and requires high-dose corticosteroids; risk of QTc interval prolongation; complete atrioventricular block; and torsades de pointes that can be fatal and requires ECG and electrolyte monitoring. Patients with ventricular arrhythmias or prolonged QTc should be excluded from treatment. Encephalopathy, including Wernicke's, can occur and requires parenteral thiamine treatment. Hepatic function should be monitored due to potential hepatotoxicity, and patients should be surveyed for new primary malignancies due to the carcinogenic effect of ATO. Patients should also be advised on the potential risk to a fetus and use effective contraception. Other symptoms indicative of adverse effects are nausea, abdominal pain, dyspnea, and pruritus. The elimination $t_{1/2}$ of ATO is 10 to 14 h. Chapter 76 presents aspects of the toxicology of ATO.

II. Inhibitors of Intracellular Protein Kinase Signaling in Cancer Cells

Inhibitors of RAS

The RAS GTPase family is a major switch that transduces extracellular signals from transmembrane receptors to multiple intracellular effectors, including the RAF family of kinases and PI3 kinase. This initiates a cascade of coordinated cellular events required for cell growth and maintenance, including regulation of gene expression and metabolic steady state. The cascade is visualized in Figure 71–2. GTP-bound RAS activates downstream signaling by binding to the RAS-binding domain of effector proteins with the GTPase activity limiting the signal strength and duration. Mutations in codons for glycines G12 or G13 or for glutamine Q61, impact GTP binding and disrupt GTPase activity of RAS proteins. Mutations in codons 12 and 61 decrease the rate of GTP hydrolysis, and mutations in codon 61 also accelerate the rate of GDP-GTP exchange. This results in elevated RAS^{GTP} and dysregulated activation of the downstream effectors characteristic of malignant transformation. Notably, almost all of the components of the downstream pathway can be found mutated in different cancers, and inhibitors are described in this section. RAS mutations occur in a tumor-specific manner for the K-, N-, and HRAS isoforms (reviewed in Moore et al., 2020). *KRAS* is one of the most highly mutated oncogenes in human cancer, occurring in 95% of pancreatic, 50% of colorectal, and 32% of lung adenocarcinomas (Rosen, 2021). Ostrem et al. (2013) developed drugs that bind covalently to the cysteine in the G12C mutant *KRAS* and disrupt downstream signaling of mutant and not wild-type protein. The recently approved KRAS G12C inhibitor *sotorasib* inhibits activation of mutant KRAS by preventing its regeneration from the inactive RAS-GDP state. The promise of mutant RAS inhibition in cancer treatment is supported by the results from the initial *sotorasib* trial. In previously treated patients with NSCLC harboring G12C KRAS mutations, a complete or partial response was observed in 37.1% of patients and disease control was observed in 80.6% of patients (Skoulidis et al., 2021).

HISTORY **THE RAS-RAF-MEK-ERK PATHWAY**

HRAS and *KRAS*, named after ***H***arvey and ***K***irsten ***ra***t ***s***arcoma viruses and discovered in the early 1980s, were the first oncogenes discovered in human tumors. *NRAS* was identified in human ***n***euroblastoma. These oncogenes were found to be mutated and constitutively active in approximately 20% of all cancers, up to 95% in some cancers (pancreatic adenocarcinoma). RAS proteins are GTPases that toggle between the active, GTP-bound and inactive, GDP-bound state. The first *RAF* gene was identified from a murine retrovirus named "***r***apidly ***a***ccelerated ***f***ibrosarcoma" due to its oncogenic effects. Following the discovery of the first human *RAF* gene (*RAF1*, also named *cRAF* = normal cellular *RAF*), the *RAF* genes *ARAF* and *BRAF* were identified; their gene products are ser/thre protein kinases. The signaling cascade of RAS-RAF-MEK-ERK was established by the early 1990s. This pathway plays a central role in cell proliferation and differentiation, is activated in many human cancers, and controls the crosstalk of cancer cells with the tumor microenvironment (see Figure 71–2). Mutations in *RAF* genes can lead to one (BRAF) or two (cRAF, BRAF) changes in amino acids, rendering these gene products oncogenic and resulting in continuous cell proliferation and the development of cancer. Activating mutations of *BRAF* were detected in melanoma and other tumors in 2002. A small-molecule inhibitor targeting mutated BRAF, *vemurafenib*, was approved by the FDA in 2011 for treatment of advanced melanoma; the first inhibitor of MEK, *trametinib*, was approved in 2013; the first inhibitor of mutant $\text{KRAS}^{\text{G12C}}$, *sotorasib*, was approved in 2021. The role of this pathway for immune crosstalk led to combination trials with the intent of triggering cancer cell death by pathway-targeted inhibitors and promoting cytotoxic T-cell efficacy. Ongoing combination trials have been reviewed a workshop of the National Academy of Sciences (Balogh and Nass, 2019), Hughes et al. (2016), and Petroni et al. (2021).

Sotorasib

Sotorasib (initially named AMG-510) is a first-in-class covalent inhibitor of the RAS GTPase family that targets G12C-mutant oncogenic *KRAS* and was approved in 2021 for the treatment of patients with locally advanced or metastatic NSCLC who have received at least one prior systemic therapy. Approximately 13% of NSCLCs carry mutant KRAS G12C, along with 3% of colorectal cancers and other cancers at lower frequencies. *Sotorasib* forms an irreversible, covalent bond with the unique cysteine in mutant KRAS G12C, locking the protein in an inactive state that prevents downstream signaling. *Sotorasib* showed minimal off-target activity and was associated with antitumor immunity in KRAS G12C preclinical models. Adverse effects include diarrhea, nausea, low-grade hepatotoxicity, and rarely, pneumonitis and dyspnea. The main metabolic pathways of *sotorasib* are nonenzymatic conjugation and metabolism by CYP3A. The mean elimination $t_{1/2}$ is 5 h.

Adagrasib

Adagrasib is a covalent inhibitor of KRAS G12C currently in clinical trials in NSCLC and colorectal cancer (Awad et al., 2021).

Emerging resistance mechanisms to KRAS G12C inhibition by *adagrasib*, an investigational drug still in clinical trials, showed a multitude of resistance pathways in patients with NSCLC and colorectal cancer (Awad et al., 2021). Treatment-resistant cancers contained secondary mutations or amplifications in the targeted KRAS, activation of the signaling pathway without alterations of KRAS itself, and histologic transformation from lung adenocarcinoma to squamous cell carcinoma. Trials exploring various combinations are ongoing to take advantage of this novel pathway inhibitor.

Inhibitors of RAF Kinase: Vemurafenib, Dabrafenib, and Encorafenib

BRAF belongs to the RAF/Mil family of serine/threonine protein kinases and plays a role in regulating signaling via MAPK (also named extracellular signal-related kinase [ERK]) (see Figure 71–2). *RAF* is the most frequently altered gene (7%–10% of all cancers) in the MAPK pathway downstream of RAS.

In melanoma, MAPK signaling can be constitutively activated through alterations in membrane receptors or through mutations of RAS or BRAF. BRAF mutations confer constitutive activation of the kinase pathway independent of RAS activation. BRAF is mutated in about 55% of melanomas, and 90% of these mutations display a substitution of valine to glutamic acid (V600E) or to lysine (V600K), which results in constitutive protein kinase activation (Vultur and Herlyn, 2013). Approximately 8% of other cancers, colon cancer and NSCLC among them, also carry an activating mutation in *BRAF*. The BRAF inhibitors *vemurafenib* and *dabrafenib* are superior to traditional chemotherapy in improving survival in patients with mutant BRAF melanoma and are discussed in the material that follows.

Combination of BRAF and MEK Inhibitors

Since the introduction of BRAF inhibitors in 2011 and MEK inhibitors in 2013 (see below), their combination has shown improved efficacy and is now standard of care in melanoma and NSCLC including *dabrafenib* plus *trametinib*, *vemurafenib* plus *cobimetinib*, and *encorafenib* plus *binimetinib* (Curti and Faries, 2021). Notably, in patients with BRAF-mutant colon cancers, no therapeutic benefit was observed, indicating that colon cancers do not depend on the activated mutant BRAF as a rate-limiting oncogenic driver.

Vemurafenib

Mechanism of Action

Vemurafenib is an orally bioavailable inhibitor of mutated BRAF (V600E). The name *vemurafenib* is derived from the target, that is, ***V****600****E*** ***mu****tated* B***RAF***. *Vemurafenib* also is effective against the less common BRAF V600K mutation. Melanoma cells lacking these mutations are not inhibited by *vemurafenib*.

ADME

Following oral administration, the elimination $t_{1/2}$ for *vemurafenib* is 57 h. *Vemurafenib* is a substrate of CYP3A4 and a substrate and an inhibitor of the Pgp exporter. *Vemurafenib* can increase concentrations of CYP1A2 substrates. Thus, concomitant use with the centrally acting α_2 adrenergic receptor agonist *tizanidine*, which has a narrow therapeutic window and is used as a muscle relaxant, should be avoided.

Therapeutic Uses

Vemurafenib is approved for the treatment of patients with metastatic melanoma harboring activating BRAF (V600E/K) mutations. It is not indicated for the treatment of patients with melanoma carrying wild-type BRAF. It is also approved for treatment of patients with Erdheim-Chester disease, a rare blood cancer (600–700 patients worldwide). Half of this slow-growing, histiocyte malignancy carries mutant BRAF as a driver.

Adverse Effects

The most common adverse effects (30%–60% of patients) are cutaneous events. Arthralgia, fatigue, and nausea are also observed but at a lower frequency. Cutaneous squamous cell carcinomas (cuSCCs) and keratoacanthomas that may need surgical removal appear in more than 20% of patients. The median time to the first appearance of these skin lesions is 7 to 8 weeks, and they are caused by a paradoxical stimulation of wild-type BRAF. Increased photosensitivity reactions can be prevented with sunblock. QT prolongation has been observed, necessitating controlling for risk factors and monitoring ECG and electrolytes.

Drug Resistance

Melanoma is one of the most aggressive malignancies, with a high mutation rate that renders these tumors highly heterogeneous and thus prone to the quick development of resistance to drug treatments. After the initial response of melanoma lesions to treatment with *vemurafenib*, resistant cancer cell subpopulations are selected, typically in less than 6 months. Constitutively active mutant *NRAS*; downstream mutations in MEK; overexpression of the growth receptor PDGFR-B; activation of parallel pathways such as PI3K/Akt, mammalian or mechanistic target of rapamycin (mTOR), and STAT3 signaling; or stromal cell secretion of HGF can drive this drug resistance (Chapman et al., 2011). Combination treatment with a MEK inhibitor (see below) that acts downstream of BRAF (see Figure 71–2) can delay development of resistance to single-agent BRAF inhibitors (Robert et al., 2015).

Dabrafenib

Mechanism of Action

Dabrafenib is an orally bioavailable, small-molecule inhibitor of mutated forms of BRAF kinases. *Dabrafenib* can inhibit the proliferation of tumor cells that contain a constitutively active mutated BRAF with *in vitro* IC_{50} values of 0.65, 0.5, and 1.84 nM for the V600E, V600K, and V600D mutations, respectively. At about 5-fold higher concentrations, *dabrafenib* also inhibits wild-type BRAF and cRAF (IC_{50} values of 3.2 and 5.0 nM, respectively). *Dabrafenib*, in combination with *trametinib* (see discussion that follows), is approved for the treatment of patients with unresectable or metastatic melanoma and NSCLC with BRAF mutations. The drug is not indicated for the treatment of patients with wild-type BRAF melanoma.

ADME

The mean terminal $t_{1/2}$ of *dabrafenib* is 8 h after oral administration. *Dabrafenib* is metabolized primarily by CYP2C8 and CYP3A4. Patients should be monitored closely for adverse reactions and for loss of efficacy if treated with drugs that induce CYP2C8 and CYP3A4 activity.

Therapeutic Uses

Dabrafenib was FDA approved as a single-agent treatment of patients with BRAF V600E mutation–positive advanced melanoma in 2013. Resistance to *dabrafenib* and other BRAF inhibitors occurs within about 6 months, but a combination with the MEK inhibitor *trametinib* can delay the development of resistance (see discussion that follows). Thus, the FDA has approved the combination of *dabrafenib* and *trametinib* for the treatment of patients with BRAF V600E/K–mutant metastatic melanoma and BRAF V600E–mutant NSCLC.

Adverse Effects and Drug Interactions

Adverse effects, in order of decreasing frequency, are hyperkeratosis, headache, pyrexia, arthralgia, papilloma, alopecia, and PPES. An increased incidence of cuSCC is expected in about 10% of patients, with a median time of 2 months to the first diagnosis. About one-third of those patients develop more than one lesion with continued administration of *dabrafenib*. With the *dabrafenib-trametinib* combination, the appearance of cuSCC is delayed to a median time of 7 months and occurs in only 3% of patients. Most common adverse reactions (≥20%) for *dabrafenib* in combination with *trametinib* are pyrexia, rash, chills, headache, arthralgia, and cough. Cardiomyopathy has also been reported.

Encorafenib

Encorafenib is an orally active protein kinase inhibitor that targets BRAF V600E, as well as wild-type BRAF and CRAF. It was approved in 2018 in combination with *binimetinib* (a MEK inhibitor) for the treatment of patients with unresectable metastatic melanoma with BRAF mutations. A combination of *encorafenib* and *cetuximab* (anti-EGFR monoclonal antibody; see Chapter 72) is approved for the treatment of patients with metastatic colorectal cancer with a BRAF V600E mutation in whom treatment increased progression-free and overall survival of patients. Adverse events include fatigue, nausea, diarrhea, acneiform dermatitis, abdominal pain, the risk of cuSCC, and dose-dependent QTc interval prolongation. *Encorafenib* is mostly metabolized by CYP3A4, and dosage adjustment is recommended in patients with mild to moderate renal or hepatic impairment. The elimination $t_{1/2}$ of *encorafenib* is 3.5 h.

Inhibitors of MEK: Trametinib, Cobimetinib, Binimetinib, and Selumetinib

The MKKs or MEKs (mitogen-activated extracellular signal regulated protein kinase kinases) are serine/threonine kinases that act downstream of RAF. They provide additional targets for the inhibition of the RAS-RAF-MEK-ERK pathway that is frequently activated in cancer and controls cell survival, differentiation, and growth (see Figure 71–2). Most MEK inhibitors are allosteric kinase inhibitors and their use as a single drug is susceptible to adaptive feedback reactivation of MAPK signaling that can reduce their efficacy (Yaeger and Corcoran, 2019).

Trametinib

Trametinib was the first inhibitor of the MEK family to be FDA-approved.

Mechanism of Action

Trametinib is an orally bioavailable, reversible, allosteric inhibitor of ATP binding to the MEK1/2 kinases. *Trametinib* inhibits MEK1/2 kinase activities with an IC_{50} of about 2 nM.

ADME

Bioavailability of oral *trametinib* is 72% and is reduced if taken with a high-calorie meal. Peak plasma concentrations are achieved 1.5 h postdose. The drug has a relatively long $t_{1/2}$ of 4 to 5 days. *Trametinib* is not a substrate of CYP enzymes, and mild renal or hepatic impairment does not affect exposure to the drug significantly.

Therapeutic Uses

The FDA approved *trametinib* as a monotherapy for patients with mutant BRAF V600E/K melanoma due to improved survival relative to standard therapy. *Trametinib* in combination with the BRAF inhibitor *dabrafenib* is also approved for mutant V600E/K metastatic melanoma and mutant V600E metastatic NSCLC as well as metastatic anaplastic thyroid cancer. *Trametinib* treatment lacks efficacy in patients who received prior treatment with BRAF inhibitors. This suggests that resistance to BRAF and MEK inhibitors involves similar mechanisms (Kim et al., 2013).

Adverse Effects

The most frequent adverse effects are cutaneous rash, acneiform dermatitis, and diarrhea. Fatigue, nausea, and lymphedema also occur. Serious grade 3 to 4 skin toxicity is found in 6% of patients. Other serious adverse effects are cardiomyopathy, hypertension, hemorrhage, interstitial lung disease, and ocular toxic effects. In contrast to the BRAF inhibitors, *trametinib* does not cause cuSCC. Because *trametinib* can cause fetal harm when administered to a pregnant woman, this risk must be weighed against the potential benefit.

Drug Resistance

Activation of alternative signaling pathways that include PI3K/Akt, mTOR, and STAT3 signaling can bypass MEK inhibition and result in drug-resistant tumors. Also, mutations in the allosteric binding pocket and activation loop of MEK1 can inhibit the binding of the inhibitor and cause resistance to *trametinib*. Resistance to single-agent *trametinib* occurs within 6 to 7 months of the initiation of treatment at a rate approaching 50%. As a strategy to overcome resistance, *trametinib* has been combined with the BRAF inhibitor *dabrafenib* for the treatment of patients with BRAF V600E/K–mutant metastatic melanoma (Flaherty et al., 2012). This combination is FDA-approved.

Cobimetinib

Mechanism of Action; ADME

Cobimetinib is an orally bioavailable, reversible inhibitor of MEK1/2 protein kinase activity with an IC_{50} of about 4 nM. Its elimination $t_{1/2}$ ranges from 1 to 3 days. *Cobimetinib* is a substrate for CYP3A, and concomitant treatment with inducers or inhibitors of CYP3A will reduce or increase the systemic exposure of patients.

Therapeutic Uses

A combination of *cobimetinib* and *vemurafenib* is approved for the treatment of patients with unresectable or metastatic melanoma with a BRAF V600E/K mutation.

Adverse Effects

The most common adverse effects are diarrhea, photosensitivity reaction, nausea, pyrexia, and vomiting. Major hemorrhagic events can occur with *cobimetinib*; patients should be monitored for signs and symptoms of bleeding. The drug also increases the risk of cardiomyopathy. The safety in patients with decreased left ventricular ejection fraction has not been established.

Binimetinib

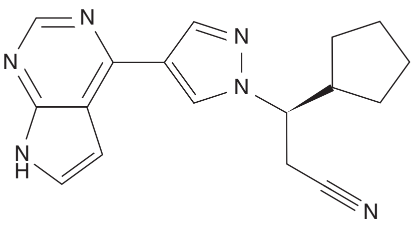
Binimetinib is an orally bioavailable MEK1/2 kinase inhibitor with an IC_{50} of 12 nM that was approved in 2018, in combination with *encorafenib* (see above), for the treatment of patients with unresectable or metastatic melanoma with a BRAF V600E or V600K mutation. After moderate to severe hepatic impairment, the area under the curve (AUC) is increased 2-fold. Impaired renal function does not impact drug elimination. The elimination $t_{1/2}$ is 3.5 h.

Selumetinib

Selumetinib is an orally bioavailable inhibitor of MEK1 (IC_{50}, 14 nM) and MEK2 with a lower affinity (IC_{50}, 530 nM). *Selumetinib* was approved in 2020 for the treatment of pediatric patients with neurofibromatosis type 1 and inoperable, plexiform neurofibromas. Warnings include the risk of cardiomyopathy requiring assessment of ejection fraction prior to treatment, ocular and skin toxicity, and possible GI toxicity requiring antidiarrheal treatment and increased fluid intake. Additional adverse reactions (≥40%) are vomiting, rash, and abdominal pain. *Selumetinib* is primarily metabolized by CYP3A4 and eliminated with a $t_{1/2}$ of 6.2 h.

Inhibitors of JAK1 and JAK2

Janus ***a***ssociated ***k***inases (JAKs) mediate the signaling of cytokines and growth factors in hematopoiesis and immune function (see, for instance, Figure 39–2). Intracellular JAK signaling recruits STATs to cytokine membrane receptors (see Figure 71–2). After STAT activation and subsequent localization to the nucleus, STATs modulate gene expression. JAK signaling is dysregulated in myelofibrosis and polycythemia vera, which prompted the development of JAK inhibitors (JAKinibs). *Ruxolitinib* is the first drug in this class.



Ruxolitinib

Ruxolitinib

Mechanism of Action; ADME

Ruxolitinib is an orally bioavailable (95%) analogue of ATP that inhibits the protein kinase activities of JAK1 and JAK2 (see Figure 72–4) with an IC_{50} of 3 nM and more than 100-fold selectivity over JAK3. The elimination $t_{1/2}$ of *ruxolitinib* is about 3 h; its metabolism is mainly via hepatic CYP3A4, with products excreted largely in the urine. In patients with renal or hepatic impairment, a dose reduction is recommended.

Therapeutic Uses

Ruxolitinib is indicated for the treatment of patients with polycythemia vera who have had an inadequate response to *hydroxyurea* and for the treatment of myelofibrosis and steroid-refractory, acute graft-versus-host disease (>12 years of age).

Adverse Effects and Drug Interactions

The most common adverse reactions, thrombocytopenia and anemia, may require a dose reduction or discontinuation of treatment. Infections should be resolved before treatment with *ruxolitinib*. In a few patients, BCC or squamous cell skin carcinoma may occur, warranting periodic skin examinations during treatment. Strong CYP3A4 inhibitors (e.g., *ketoconazole*, *fluconazole*) increase exposure to *ruxolitinib* and prolong its $t_{1/2}$ to 6 h. Due to the adverse hematopoietic effects, postpartum patients should discontinue nursing during drug treatment.

Fedratinib

Fedratinib is an orally bioavailable JAK2-selective inhibitor (IC_{50} is 3 nM; >35-fold selective vs. JAK1 and JAK3) with activity against wild-type and mutationally activated JAK2 as well as FLT3 (IC_{50} is 15 nM) and RET (IC_{50} is 48 nM). Abnormal activation of JAK2 is associated with myeloproliferative disease and polycythemia vera. *Fedratinib* was approved in 2019 for the treatment of patients with myelofibrosis. Warnings include the risk of serious and fatal encephalopathy requiring monitoring and possibly replenishment of thiamine levels, anemia and thrombocytopenia, as well as GI and hepatic toxicity. Adverse reactions include diarrhea and nausea. The metabolism is through CYP3A with a terminal elimination $t_{1/2}$ of 114 h.

Cyclin-Dependent Kinase (CDK) 4/6 Inhibitors

The CDKs are a family of over 20 serine/threonine protein kinases that modulate intracellular signaling during cell cycle progression (see Figure 71–2). Given their pivotal role in cellular proliferation, CDKs are prime targets for development of inhibitors. However, different tissue selectivity and distinct cell cycle–specific activity periods of different CDKs provide a challenge.

CDK4/6 are attractive targets because they control cell cycle progression from the G_0/G_1 to S phase. Interaction of cyclin D with CDK4/6 enhances phosphorylation of the Rb (retinoblastoma) tumor suppressor protein, inactivating Rb and permitting the transcription of factors that control transition into the S phase. Inhibition of CDK4/6 will inhibit the phosphorylation and inactivation of Rb and cause a G_1 arrest in susceptible cells that utilize this pathway, thereby providing the rationale for the use of inhibitors of CDK4/6 (Figure 71–3). This cell cycle checkpoint is frequently compromised in cancers due to cyclin D amplification, loss of Rb function, or loss of negative regulators of CDK4/6, such as $p16^{INK4A}$, a tumor suppressor named for its molecular mass (it is a small protein of 16 kDa) and its capacity to inhibit CDK4. The class of CDK inhibitory drugs is identified by the syllable *ciclib*. CDK4/6 inhibitors with inhibitory profiles similar to that of *palbociclib* include *abemaciclib*, *ribociclib*, and *trilaciclib*, which are now approved for the treatment mostly of patients with breast cancer and are in clinical trials for other cancers. The efficacy and adverse effects of these inhibitors overlap, although differences in the selectivity for the targeted kinases result in different adverse effect profiles and approved uses of these inhibitors (O'Leary et al., 2016).

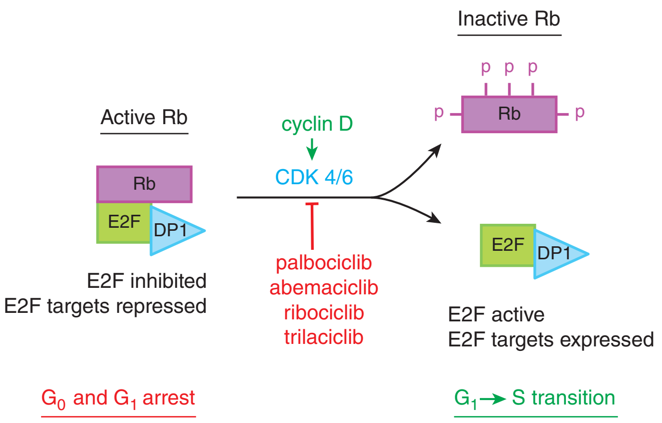


Figure 71–3 *CDK4/6 inhibitors: retinoblastoma protein, cyclin-dependent protein kinases, and the regulation of cell cycle progression.* In quiescent and differentiated cells (in G_0 or in cells arrested in G_1), the Rb protein is active and interacts with a heterodimer of the transcription factor E2F and its dimerization partner DP1, repressing transcription of promoters regulated by E2F. As a cell begins a cycle of division, cyclins activate CDKs that phosphorylate Rb, disrupting the Rb-E2F/DP complexes and permitting accumulation of active E2F complexes that drive transcription. This cell cycle checkpoint is frequently compromised in cancers due to cyclin D amplification, loss of Rb function, or loss of negative regulators of CDK4/6. Hyperphosphorylation of Rb can occur via mutations in Rb or expression of viral oncoproteins targeting Rb, placing the cell in a state of extensive proliferation, with reduced capacity to exit the cell cycle. Inhibition of CDK4/6 can cause G_1 arrest in susceptible cells (Dyson, 2016). CDK inhibitors (in red lettering) are identified by the disyllabic suffix "ciclib."

Palbociclib

Mechanism of Action

Palbociclib is an orally bioavailable, small-molecule inhibitor of CDK4 and CDK6, with IC_{50} values of 11 and 16 nM, respectively, and without inhibitory activity against CDK1, 2, or 5.

ADME

The mean plasma elimination $t_{1/2}$ is 29 h. *Palbociclib* is a substrate and an inhibitor of CYP3A; thus, doses of *palbociclib* or of substrates of CYP3A4 may need to be reduced when they are given concurrently, with special attention to coadministered agents with narrow therapeutic indexes (see the Adverse Effects and Drug Interactions section). The sulfotransferase SULT2A1 also participates in *palbociclib* metabolism.

Therapeutic Uses

Palbociclib is the first CDK inhibitor approved by the FDA (in 2015) for the treatment of advanced or metastatic breast cancer that is estrogen receptor (ER) positive and HER2 negative. *Palbociclib* is used in combination with *letrozole*, an aromatase inhibitor, as initial endocrine-based therapy in postmenopausal women or with the antiestrogen *fulvestrant* in women with disease progression after endocrine-based therapy. *Letrozole* and *fulvestrant* are described in Chapter 73. The inclusion of *palbociclib* with different endocrine-based treatments almost doubles the progression-free survival of patients (Cristofanilli et al., 2016; Finn et al., 2016).

Adverse Effects and Drug Interactions

The most common adverse effects of *palbociclib* are neutropenia, leukopenia, infections, stomatitis, fatigue, nausea, anemia, headache, diarrhea, and thrombocytopenia. The most common grade 3 and 4 adverse effects are neutropenia (>60%), leukopenia (~25%), and anemia (~5%). Patients should avoid breastfeeding while taking *palbociclib*. *Letrozole* and *fulvestrant* do not alter the pharmacokinetics of *palbociclib*. No effect on the QTc interval was observed.

Patients should avoid concomitant use of strong CYP3A inhibitors (e.g., *clarithromycin, indinavir, itraconazole, ketoconazole, lopinavir/ritonavir, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin*, and *voriconazole*). Coadministration of a strong

CYP3A inhibitor (e.g., *itraconazole*) can markedly increase exposure to *palbociclib* (by 87% in healthy subjects). Patients should also avoid grapefruit or grapefruit juice. If *palbociclib* coadministration with a strong CYP3A inhibitor cannot be avoided, the dose of *palbociclib* should be reduced.

Coadministration of a strong CYP3A inducer will decrease exposure to *palbociclib*. Thus, concomitant use of *phenytoin*, *rifampin*, *carbamazepine*, *enzalutamide*, and St. John's wort should be avoided.

Palbociclib may increase exposure to coadministered CYP3A substrates with narrow therapeutic indexes (e.g., *midazolam, alfentanil, cyclosporine, dihydroergotamine, ergotamine, everolimus, fentanyl, pimozide, quinidine, sirolimus*, and *tacrolimus*), requiring reduction of *palbociclib* dose.

Abemaciclib

Abemaciclib is an orally bioavailable inhibitor of CDK4/6 approved in 2017 in the treatment of patients with ER-positive and HER2-negative, advanced or metastatic breast cancer. One indication is in postmenopausal women in combination with an aromatase inhibitor (see Chapter 73); a second indication is in patients whose disease progressed following endocrine therapy in combination with the antiestrogen *fulvestrant* (see Chapter 73); a third indication is in patients with disease progression following endocrine therapy and prior chemotherapy in the metastatic setting. Most common adverse reactions (incidence ≥20%) are diarrhea, neutropenia, nausea, abdominal pain, infections, fatigue, anemia, decreased appetite, vomiting, headache, alopecia, and thrombocytopenia.

Abemaciclib is metabolized by CYP3A, and it is recommended to avoid concomitant use of inhibitors (see discussion above on *palbociclib* metabolism). The elimination $t_{1/2}$ of *abemaciclib* is 18.3 h.

Ribociclib

Ribociclib, an orally bioavailable inhibitor of CDK4/6, was approved in 2017 for the treatment of patients with ER-positive and HER2-negative advanced or metastatic breast cancer. One indication is in combination with an aromatase inhibitor (see Chapter 73) for the treatment of pre-, peri-, or postmenopausal patients as initial endocrine-based therapy; a second indication is for the treatment of postmenopausal patients in combination with the antiestrogen *fulvestrant* (see Chapter 73) as initial endocrine-based therapy or following disease progression on endocrine therapy. Recent warnings indicate an increased risk of interstitial lung disease/pneumonitis during treatment. Monitoring of patients for pulmonary symptoms is thus recommended. Other risks are concentration-dependent increases in the QTc interval, with pretreatment and continuous ECG and electrolyte monitoring recommended; severe cutaneous adverse reactions, including Stevens-Johnson syndrome and toxic epidermal necrolysis; and neutropenia, with pretreatment and every-2-week monitoring of CBC counts recommended. Common adverse effects (incidence ≥20%) are nausea, fatigue, diarrhea, leukopenia, vomiting, alopecia, headache, rash, and cough. *Ribociclib* is metabolized through CYP3A, and concomitant use of interacting drugs should be avoided (see above discussion for *palbociclib*). The elimination $t_{1/2}$ of *ribociclib* ranges from 29.7 to 54.7 h.

Trilaciclib

Trilaciclib is a CDK4/6 kinase inhibitor that was approved in 2021 to decrease the incidence of chemotherapy-induced myelosuppression in patients receiving a platinum/*etoposide*-containing regimen or *topotecan*-containing regimen (see Chapter 70) for extensive-stage small cell lung cancer. *Trilaciclib* is administered as a single IV 30-min infusion within 4 h prior to chemotherapy on each day of chemotherapy. *Trilaciclib* transiently maintains immune cells and hemopoietic stem and progenitor cells in G_1 cell cycle arrest to reduce bone marrow toxicity of chemotherapy. The elimination $t_{1/2}$ is approximately 14 h, and bone marrow cell cycle arrest lasts for up to 32 h after a single dose. After that time, bone marrow progenitor subsets resume proliferation.

An intriguing possibility is that the intermittent administration of *trilaciclib* may preserve anticancer immune reactivity and contribute to increased efficacy of chemotherapy. This is suggested from a study using *gemcitabine* and *carboplatin* chemotherapy in patients with hormone receptor–negative and HER2-negative ("triple negative") metastatic breast cancer that is not considered responsive to CDK4/6 inhibitors (Tan et al., 2019). An editorial in *Lancet Oncology* (Goel and Tolaney, 2019) speculates that *trilaciclib* before chemotherapy may preserve immune cell function.

Bruton Tyrosine Kinase (BTK) Inhibitors

The BTK protein tyr kinase plays important roles in the signal transduction and normal function of B cells as well as their malignant transformation. B-cell receptor signaling recruits SYK followed by the activation of BTK and PI3Kδ, leading to further downstream responses and multiple cellular effects (Figure 71–4). Integrin signaling and chemokine receptor signaling can modulate the activity of the pathway. A SYK kinase inhibitor, *fostamatinib*, was approved for the treatment of patients with chronic immune thrombocytopenia. BTK inhibitors are discussed next, and inhibitors of the PI3Kδ isoform that is frequently activated in B-cell malignancies are discussed further below. BTK and PI3K inhibitors can disrupt oncogenic B-cell receptor signaling and accelerate tumor cell death (reviewed by Burger and O'Brien, 2018).

Ibrutinib

Mechanism of Action

Ibrutinib is an orally bioavailable, small-molecule inhibitor that inactivates BTK through covalent binding to cys^{481} near the ATP-binding domain.

ADME

Administration with food (vs. on an empty stomach) roughly doubles absorption. The elimination $t_{1/2}$ of *ibrutinib* is 4 to 6 h. It is a substrate of CYP3A; CYP2D6 participates to a minor degree. Metabolites are excreted mainly via the feces; renal excretion is minor. Hepatic impairment is likely to increase *ibrutinib* exposure. *Ibrutinib* is not a substrate for Pgp.

Therapeutic Uses

Ibrutinib inhibits malignant B-cell proliferation and is indicated for the treatment of patients with mantle cell lymphoma (MCL) and marginal zone lymphoma who have received at least one prior therapy and patients with chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), Waldenström's macroglobulinemia, and chronic graft-versus-host disease after failure of systemic therapy.

Adverse Effects and Drug Interactions

The most common adverse effects (≥20%) in patients with B-cell malignancies are neutropenia, pyrexia, thrombocytopenia, hemorrhage, anemia, diarrhea, nausea, musculoskeletal pain, rash, and fatigue. The onset of hypertension has been observed within less than a month and up

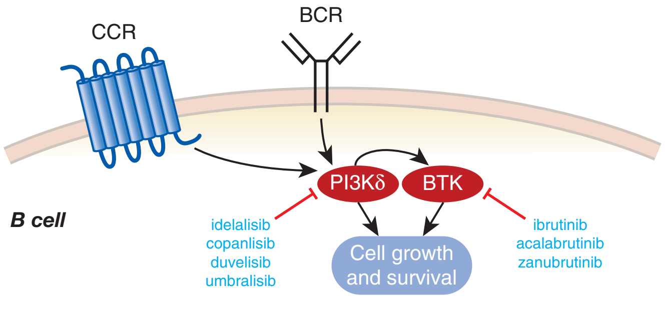


Figure 71–4 *Inhibitors of signaling in B cells.* The signaling by B-cell receptors (BCRs) and chemokine receptors (CCRs) enables the recruitment of intracellular signal transducers, which is followed by the activation of BTK and PI3Kδ. The BTK and PI3K inhibitors indicated can disrupt oncogenic BCR signaling and thus lead to cell death.

to 2 years after the start of *ibrutinib* treatment. Thus, blood pressure should be monitored and antihypertensive treatment initiated or modified. Atrial fibrillation is observed in up to 7% of patients; this requires monitoring and treatment. Second primary malignancies occur in up to 16% of patients, most of them nonmelanoma skin cancers.

Strong (e.g., azole antifungals) and moderate (e.g., *diltiazem, erythromycin*) CYP inhibitors will increase *ibrutinib*'s AUC; thus, dose reduction is recommended when using CYP3A inhibitors concomitantly. The reverse is true for strong and moderate CYP inducers (e.g., *rifampin*, *efavirenz*), which will decrease *ibrutinib* exposure.

Ibrutinib may cause fetal harm. Pregnant patients and those at risk for pregnancy should be apprised of the drug's hazardous potential.

Acalabrutinib

Acalabrutinib is a second-generation, orally bioavailable, irreversible BTK inhibitor that shows higher selectivity for BTK versus other kinases (e.g., EGFR, ITK [inducible T-cell kinase]) than *ibrutinib*. *Acalabrutinib* was first approved in 2017 and is indicated for the treatment of patients with MCL who have received at least one prior therapy and patients with CLL and SLL. Adverse effects include the risk of infections, hemorrhage, cytopenias, second primary malignancies including nonmelanoma skin cancers, and cardiac arrhythmias. The elimination $t_{1/2}$ is 1 h for *acalabrutinib* and 3.5 h for an active metabolite. Still, dosing every 12 h generates BTK occupancy of 95% or greater in peripheral blood and inactivation of BTK likely due to the covalent binding to a cysteine residue in the active site of the kinase.

Zanubrutinib

Zanubrutinib is an orally bioavailable inhibitor of BTK that forms a covalent bond with a cysteine residue in the BTK active site and shows a higher selectivity for BTK versus other kinases (e.g., ITK, EGFR), resulting in less off-target effects. *Zanubrutinib* was approved in 2019 for the treatment of patients with MCL who have received at least one prior therapy. Adverse events and warnings overlap with those of *acalabrutinib* (above) and include skin cancers as second primary malignancies, hemorrhage, and arrhythmias. The elimination $t_{1/2}$ is 2 to 4 h. Still, BTK occupancy in peripheral mononuclear cells was maintained at 100% over 24 h and at 94% to 100% in lymph nodes.

Inhibitors of the BCR-ABL Kinase

A single molecular event, the Philadelphia chromosome translocation t(9;22), leads to expression of ABL (Abelson murine leukemia viral oncogene homolog) and BCR. This fusion generates a constitutively active protein kinase, BCR-ABL, resulting in continuous and uncontrollable cell division. BCR-ABL drives the malignant phenotype of chronic myelocytic leukemia (CML) (see Figure 71–2).

HISTORY BCR-ABL KINASE INHIBITORS

Imatinib mesylate, originally named "sti 571" (signal transduction inhibitor 571), was the first protein kinase inhibitor designed to target a driver mutation in a cancer and to receive FDA approval. It was approved in 2001 under the name *Gleevec* and is now designated as an essential drug by the World Health Organization. *Imatinib* targets the BCR-ABL tyrosine kinase fusion protein that drives CML. Imatinib-resistant ABL mutations were uncovered in 2002 and led to the development of next-generation inhibitors *dasatinib* and *nilotinib*, which overcome the resistance.

Imatinib and the second-generation inhibitors *dasatinib* and *nilotinib* induce clinical and molecular remissions in more than 90% of patients with CML in the chronic phase of disease. Due to its inhibition of other kinases, *imatinib* is also effective in some other tumors, including GISTs (driven by a c-KIT mutation), hypereosinophilia syndrome, and dermatofibrosarcoma protuberans (all driven by activating mutations in the PDGFR; see Figure 71–2).

Imatinib, Dasatinib, and Nilotinib

Mechanisms of Action

These are orally bioavailable, small-molecule kinase inhibitors. *Imatinib* was identified through high-throughput screening against the BCR-ABL kinase. *Dasatinib*, a second-generation BCR-ABL inhibitor, also inhibits the Src kinase, and unlike *imatinib*, it binds both the open (active) and closed (inactive) configurations of the BCR-ABL kinase. *Nilotinib* was designed to have increased potency and specificity compared to *imatinib*. Its structure overcomes mutations that cause *imatinib* resistance. *Imatinib* and *nilotinib* bind to a segment of the kinase domain that fixes the enzyme in a closed or nonfunctional state, in which the protein is unable to bind its substrate/phosphate donor, ATP. These three BCR-ABL kinase inhibitors differ in their inhibitory potencies, binding specificities, and susceptibility to resistance mutations in the target enzyme (reviewed by Braun et al., 2020). *Dasatinib* (IC_{50} ~1 nM) and *nilotinib* (IC_{50} ~20 nM) inhibit BCR-ABL kinase more potently than *imatinib* (IC_{50} ~100 nM).

Mechanisms of Resistance

Resistance to these TKIs arises from point mutations in three separate segments of the kinase domain. The contact points between *imatinib* and the enzyme become sites of mutations in drug-resistant leukemic cells; these mutations prevent tight binding of the drug and lock the enzyme in its open configuration, in which it has access to substrate and is enzymatically active. *Nilotinib* retains inhibitory activity in the presence of most point mutations that confer resistance to *imatinib*. Other mutations affect the phosphate-binding region and the "activation loop" of the domain with varying degrees of associated resistance. Some mutations, such as those at amino acids 351 and 355, confer low levels of resistance to *imatinib*, possibly explaining the clinical response of some resistant tumors to dose escalation of *imatinib*.

Molecular studies have detected cells with resistance-mediating kinase mutations *prior* to initiation of therapy, particularly in patients with Ph^+ acute lymphoblastic leukemia (ALL) or CML in blast crisis. This finding indicates that drug-resistant cells arise through spontaneous mutation and expand under the selective pressure of drug exposure. Mechanisms other than BCR-ABL kinase mutations play a minor role in resistance to *imatinib*. Amplification of the wild-type kinase gene, leading to overexpression of the enzyme, has been identified in tumor samples from patients resistant to treatment. The *MDR* gene confers resistance experimentally but has not been implicated in clinical resistance. Ph^- clones lacking the BCR-ABL translocation and displaying the karyotype of myelodysplastic cells may emerge in patients receiving imatinib for CML; these may progress to myelodysplastic syndrome (MDS) and AML. Their origin is unclear.

A comparison among secondary mutations in the EGFR, ABL, and KIT kinases following treatment with BCR-ABL kinase inhibitors shows striking differences: The T790M gatekeeper mutation in EGFR (see Figure 71–1) accounts for about 90% of secondary mutations. In contrast, secondary resistance mutations in ABL or KIT after treatment of CML or GIST with *imatinib* are found across the kinase domain and rarely at the analogous gatekeeper residue in ABL (T^{315}) or in KIT (T^{670}).

ADME

Imatinib. *Imatinib* is well absorbed after oral administration and reaches maximal plasma concentrations within 2 to 4 h. The elimination $t_{1/2}$ of *imatinib* and its major active metabolite, the *N*-desmethyl derivative, are about 18 and 40 h, respectively. Food does not influence the pharmacokinetic profile. Doses of more than 300 mg/day achieve trough levels of 1 μM, which correspond to *in vitro* levels required to kill BCR-ABL–expressing cells. In the treatment of GISTs, higher doses may improve response rates. CYP3A4 is the major metabolizer of *imatinib*; thus, drugs that induce or interact with CYP3A4 can alter the pharmacokinetics of *imatinib*. Coadministration of *imatinib* and *rifampin*, an inducer of

CYP3A4, lowers the plasma *imatinib* AUC by 70%. *Imatinib*, as a competitive CYP3A4 substrate, inhibits the metabolism of *simvastatin* and increases its plasma AUC by 3.5-fold.

Dasatinib. Oral *dasatinib* is well absorbed; its bioavailability is significantly reduced at neutral gastric pH (i.e., after antacids and H_2 blockers) but is unaffected by food. The plasma $t_{1/2}$ of *dasatinib* is 3 to 5 h. *Dasatinib* exhibits dose-proportional increases in AUC, and its clearance is constant over the dose range of 15 to 240 mg/day. *Dasatinib* is metabolized primarily by CYP3A4, with minor contributions by FMO3 (flavin containing monooxygenase 3) and UGT. The major metabolite is equipotent to the parent drug but represents only 5% of the AUC. Plasma concentrations of *dasatinib* are affected by inducers and inhibitors of CYP3A4 in a similar fashion to *imatinib*.

Nilotinib. Approximately 30% of an oral dose of *nilotinib* is absorbed after administration, with peak concentrations in plasma 3 h after dosing. Unlike the other BCR-ABL inhibitors, *nilotinib*'s bioavailability increases significantly in the presence of food. The drug has a plasma $t_{1/2}$ about 17 h, and plasma concentrations reach a steady state only after 8 days of daily dosing. *Nilotinib* is metabolized by CYP3A4, with predictable alteration by inducers, inhibitors, and competitors of CYP3A4. *Nilotinib* is a substrate and an inhibitor of Pgp.

Therapeutic Uses

These protein TKIs have efficacy in diseases in which ABL, *KIT*, or PDGFR has dominant roles in driving tumor growth, reflecting the presence of a mutation that results in constitutive activation of the kinase. *Imatinib* shows therapeutic benefits in patients with chronic-phase CML (BCR-ABL) or GIST and a subset of patients with mucosal or acral lentiginous melanoma (*KIT* mutation positive), chronic myelomonocytic leukemia (EVT6-PDGFR translocation), hypereosinophilia syndrome (FIP1L1-PDGFR), and dermatofibrosarcoma protuberans (constitutive production of the ligand for PDGFR). It is the agent of choice for patients with metastatic GIST and is used as adjuvant therapy of *c-KIT*–positive GIST. *Dasatinib* is approved for patients with newly diagnosed CML with resistance to or intolerance of *imatinib* in both chronic and advanced phases of disease and for use combined with cytotoxic chemotherapy in patients with Ph$^+$ ALL who are resistant or intolerant to prior therapies.

Adverse Effects

Imatinib, *dasatinib*, and *nilotinib* cause GI symptoms (diarrhea, nausea, and vomiting) that are usually readily controlled. All three drugs promote fluid retention, edema, and periorbital swelling. Myelosuppression occurs infrequently but may require transfusion support and dose reduction or discontinuation of the drug. These drugs can be associated with hepatotoxicity. *Dasatinib* may cause pleural effusions and pulmonary hypertension in a small subset of patients. *Nilotinib* and *dasatinib* may prolong the QT interval; *nilotinib* has been associated with cardiac and vascular events, including ischemia. Most nonhematological adverse reactions are self-limited and respond to dose adjustments. After the adverse reactions have resolved, the drug may be reinitiated and titrated back to effective doses.

Bosutinib

Bosutinib is another second-generation, orally bioavailable BCR-ABL kinase inhibitor (IC_{50} = 1.2 nM). *Bosutinib* also inhibits activity of Src (IC_{50} = 1 nM) and other members of the Src family. It is FDA approved for the treatment of patients with chronic, accelerated, or blast-phase Ph$^+$ CML with resistance or intolerance to prior therapy. The most common adverse reactions (incidence >20%) are diarrhea, nausea, thrombocytopenia, vomiting, abdominal pain, rash, anemia, pyrexia, and fatigue.

Ponatinib

Ponatinib is a third-generation BCR-ABL kinase inhibitor. *Imatinib* (first-generation) lacks efficacy for the more advanced disease phases, and cells with mutations in the BCR-ABL tyrosine kinase domain are resistant. Second-generation inhibitors (*nilotinib*, *dasatinib*, *bosutinib*) were developed to address these weaknesses, although they do not inhibit the T315I mutant of BCR-ABL that is found in 15% to 20% of patients with CML.

Mechanism of Action

Ponatinib is a third-generation, orally bioavailable kinase inhibitor that is active against the T315I mutant BCR-ABL and is also effective toward all other known BCR-ABL1 kinase mutants. IC_{50} values (nM) for different kinases are as follows: ABL, 0.37; PDGFR, 1.1; VEGFR2, 1.5; FGFR1, 2.2; and Src, 5.4.

ADME

Peak *ponatinib* concentrations are observed within 6 h and are unaffected by food or fasting. Drug solubility is pH dependent, with higher pH resulting in decreased solubility. Accordingly, drugs that affect gastric pH (e.g., H_2 antagonists, antacids, and proton pump inhibitors [PPIs]) may significantly alter *ponatinib* bioavailability. *Ponatinib* is highly (99%) bound to plasma proteins and is a weak substrate for Pgp and ABCG2 transporters. Metabolism is primarily via CYP3A4 and to a lesser extent by CYPs 2C8, 2D6, and 3A511; esterases; and amidases. Coadministration of CYP3A inhibitors significantly increases *ponatinib* concentrations. The $t_{1/2}$ of *ponatinib* is about 24 h. Unmetabolized *ponatinib* is primarily excreted in the feces (87%), with a small portion (5%) in the urine.

Therapeutic Uses

Ponatinib is approved for resistant CML and Ph$^+$ ALL. Clinical cytogenetics measurements are a key tool for monitoring the response to therapy, reported as the percentage of Ph$^+$ karyotypes in 20 bone marrow metaphases (major cytogenetic response [MCyR]). *Ponatinib* produces an overall 54% MCyR in patients with early-phase CML and a 70% MCyR in patients with early-phase CML with the T315I mutation. Because of *ponatinib*'s potency and efficacy against all known BCR-ABL single mutants, trials are under way that may advance this therapy to a first-line treatment.

Adverse Effects

Arterial thrombosis and hepatotoxicity are major adverse effects; thus, appropriate precautions, dose reduction, monitoring, or discontinuation is recommended. Dose-limiting toxicities include elevated lipase and amylase levels and pancreatitis.

Inhibitors of the PI3K/Akt/mTOR Pathway

Background

Activation of PI3K and the consequent activation of signaling events through protein kinase B (Akt) and the mTOR pathway are important in cell growth and survival and in the regulation of cell metabolism (Figures 71–2 and 71–5). Excessive signaling through the PI3K pathway is a frequent aberration in many human cancers; thus, this pathway is an attractive target in cancer therapy. There are three classes of PI3Ks, with the class I being the most important in cancer. The class I PI3K enzymes are heterodimers of a p85 regulatory subunit and a p110 catalytic subunit. The p110 catalytic subunit exists in four identified isoforms: α, β, γ, and δ. The α and β isoforms are ubiquitously expressed, whereas expression of the γ and δ isoforms is restricted to cells of immune lineage. The p110 subunit of the enzyme is a lipid phosphokinase that catalyzes synthesis of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) from the phosphatidylinositol 4,5-bisphosphate (PIP2) substrate, an abundant lipid at the plasma membrane. The different catalytic subunits have distinct roles in normal and malignant cells. The gene coding for PI3Kα (*PIK3CA*) is the most frequently mutated PI3K in human cancer (Fruman and Rommel, 2014). In B-cell malignancies, the PI3Kδ isoform is frequently activated. A PI3Kδ inhibitor, *idelalisib*, is described below followed by the discussion of additional inhibitors of PI3Kδ, *copanlisib*, *duvelisib*, and *umbralisib*, that are approved for the treatment of B-cell lymphoma. An inhibitor of PI3Kα, *alpelisib*, shows a distinct efficacy profile, was approved in 2019 for the treatment of patients with advanced breast cancer, and is described at the end of this section.

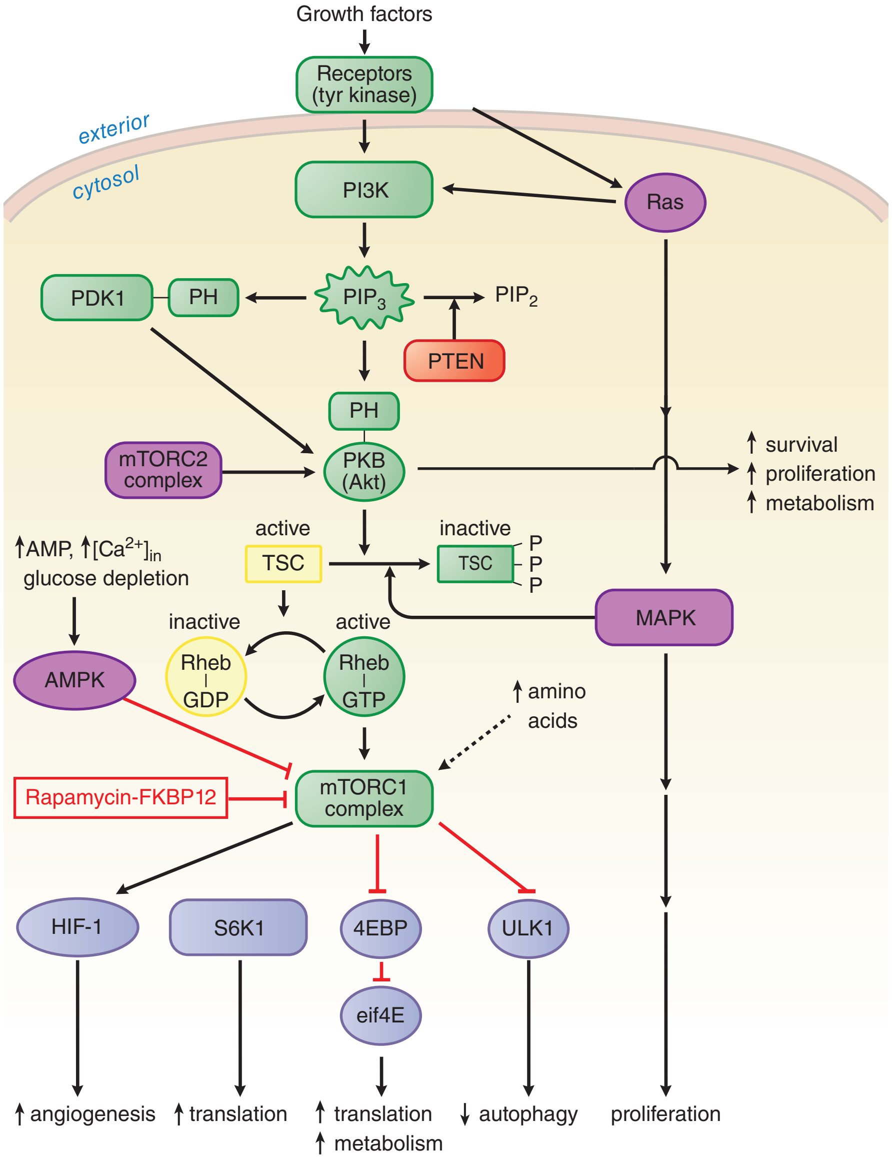

Growth factors
Receptors (tyr kinase)
exterior
cytosol
PI3K
Ras
PDK1
PH
PIP3
PIP2
PTEN
PH
PKB (Akt)
mTORC2 complex
↑ survival
↑ proliferation
↑ metabolism
↑AMP, ↑[Ca2+]in
glucose depletion
active
TSC
inactive
TSC
P
P
P
MAPK
inactive
Rheb GDP
active
Rheb GTP
AMPK
↑ amino acids
Rapamycin-FKBP12
mTORC1 complex
HIF-1
S6K1
4EBP
ULK1
eif4E
↑ angiogenesis
↑ translation
↑ translation
↑ metabolism
↓ autophagy
proliferation

Figure 71–5 *Caveat mTOR: effect of rapamycin on growth factor signaling.* The PI3K-mTOR pathway responds to extracellular signals, the metabolic and nutrient status, and the energy charge of the cell, transmitting signals downstream that drive maintenance and proliferation (Hall, 2016). mTOR activity appears to contribute to resistance to many targeted cancer therapies (Guri and Hall, 2016), and dysregulation of the pathway is implicated in diabetes, cancer, and other pathologies (Dibble and Cantley, 2015). In this simplified version of the PI3K-mTOR pathway, the green shapes show the action pathway from membrane receptor to the mTOR multiprotein complex, mTORC1. Substrates of the mTOR ser/thr protein kinase activity and integrated responses are shown in blue across the bottom of the figure. Red T bars represent inhibitory influence. Signaling through the pathway is initiated by agonist activation of membrane receptors such as IGF1R and stimulation of their tyrosine kinase activities, leading to activation of PI3K, which phosphorylates the inositol ring of the PIP2 in the membrane to produce PIP3. PIP3 recruits to the membrane several proteins containing PH domains, including PKD1 and Akt. Akt, also known as PKB, phosphorylates TSC-2 (a subunit of TSC) at multiple sites. TSC is a GTPase-activating protein (GAP) for Rheb. Phosphorylation of TSC inhibits its GAP activity, thereby reducing the GTPase activity of Rheb and increasing the fraction of Rheb in the GTP-liganded state. Rheb, in its GTP-liganded configuration, activates mTORC1. TSC phosphorylation and PIP3 phosphorylation can be reversed by the phosphatase PTEN. mTORC1 phosphorylates substrates that mediate downstream effects; among them are the following:

- HIF-1, leading to increased angiogenesis. HIF-1 and -2 are overexpressed or upregulated in many cancers.
- S6K1 (ribosomal protein S6 kinase β1), enhancing protein synthesis. Dephospho S6K1 exists in a complex with eIF3; phosphorylation by mTOR dissociates the complex, freeing S6K1 to phosphorylate its targets that promote translation.
- 4EBP, enhancing transcription. The phosphorylation of 4EBP by mTOR reduces the capacity of 4EBP to inhibit eIF4E and slow transcription.
- ULK1, whose phosphorylation reduces autophagic activity. mTOR activity prevents ULK1 activation by disrupting its interaction with AMPK, a key promoter of autophagy. Conversely, inhibition of mTOR will stimulate autophagy.

Rapamycin (*sirolimus*) and the "rapalogs" *everolimus* and *temsirolimus* inhibit mTOR by associating with the intracellular protein FKBP12. The FKBP12-rapalog complex binds directly to mTOR and inhibits its functions in the multiprotein complex mTORC1. mTORC2 remains relatively unaffected by mTORC1 inhibitors and still can respond by upregulating Akt activity.

Impact of PI3K Inhibitors on the Tumor Microenvironment

Tumor angiogenesis is dependent on PI3Kα signaling and its inhibition may contribute to the treatment effects. Also, immune recognition of tumor cells may be enhanced by inhibition of myeloid suppressive cells that are sensitive to PI3Kδ/γ inhibition and macrophages that require PI3Kγ signaling for their suppression of T-cell activation. For further details, see the review by Castel et al. (2021).

Idelalisib

Mechanisms of Action

Idelalisib is an orally bioavailable inhibitor of the PI3Kδ isoform with an IC_{50} of about 19 nM. It is 6-fold selective versus the PI3Kγ and over 200-fold more selective versus the PI3Kα and β isoforms. PI3Kδ is expressed in normal and malignant B cells. *Idelalisib* induces apoptosis and inhibits proliferation in cell lines derived from malignant B cells and in primary tumor cells. *Idelalisib* inhibits several cell signaling pathways, including BCR, C-X-C chemokine receptor (CXCR) 4, and CXCR5 signaling, which are involved in trafficking and homing of B cells to the lymph nodes and bone marrow. Treatment of lymphoma cells with *idelalisib* resulted in inhibition of chemotaxis and adhesion and reduced cell viability.

ADME

Food does not affect absorption of *idelalisib*. The drug is metabolized by aldehyde oxidase, CYP3A, and UGT1A4, yielding an elimination $t_{1/2}$ of 8.2 h. *Idelalisib* inhibits CYP3A and is a substrate of Pgp and of the efflux transporter breast cancer resistance protein (BCRP)/ATP-binding cassette (ABC) G2 *in vitro*. *Idelalisib* increases plasma levels of *midazolam* (a CYP3A substrate) but does not affect plasma levels of *digoxin* (a Pgp substrate) or *rosuvastatin* (an OATP1B1/B3 substrate). Patients receiving strong CYP inhibitors may be at increased risk for *idelalisib* toxicity. Hepatic impairment increases systemic exposure, putting patients at risk for toxicity.

Clinical Use

Idelalisib is approved for the treatment of relapsed or refractory B-cell malignancies in patients who have received at least two prior systemic therapies. This includes CLL in combination with rituximab, follicular B-cell non-Hodgkin lymphoma (FL), and SLL. *Idelalisib* is not indicated and is not recommended for first-line treatment.

Adverse Effects

Idelalisib may cause serious and sometimes fatal adverse effects, including liver toxicity in 16% to 18%, fatal diarrhea or colitis in 14% to 20%, pneumonitis in 4%, or serious infections in 21% to 48% of patients. Patients taking *idelalisib* should avoid other hepatotoxic drugs. Other common side effects include fever, chills, cough, pneumonia, fatigue, nausea, rash, hyperglycemia, and elevated levels of triglycerides and liver enzymes. *Idelalisib* may cause embryofetal toxicity. Breastfeeding during therapy is contraindicated because of the potential for adverse reactions in nursing infants.

Copanlisib

Copanlisib is an inhibitor of PI3Kα (IC_{50} of 0.5 nM) and PI3Kδ (IC_{50} of 0.7 nM) isoforms that are expressed in malignant B cells. Parenterally administered, *copanlisib* was approved in 2017 for the treatment of patients with relapsed FL who have received at least two prior systemic therapies. The efficacy of *copanlisib* has been linked to inhibition of nuclear factor-κB (NF-κB) signaling and reduced release of interleukin (IL)-6 and IL-10. Adverse effects include hyperglycemia, hypertension that may require treatment, pneumonitis, diarrhea, and decreased general strength and energy. *Copanlisib* is a CYP3A substrate and eliminated with a $t_{1/2}$ of 39.1 h.

Duvelisib

Duvelisib is an orally bioavailable inhibitor of PI3Kδ (IC_{50} of 2.5 nM) and PI3Kγ (IC_{50} of 27 nM), which are expressed in normal and malignant B cells. The potency of *duvelisib* against the β (IC_{50} of 85 nM) and α isoforms (IC_{50} of 1602 nM) is lower. *Duvelisib* was approved in 2018 for the treatment of patients with relapsed or refractory CLL, SLL, or FL after at least two prior therapies. Adverse effects include the risk of infections in 31% of patients, diarrhea and colitis in 18%, and pneumonitis in 5%. Metabolism of *duvelisib* is through CYP3A4. The elimination $t_{1/2}$ is 4.7 h.

Umbralisib

Umbralisib is an orally bioavailable inhibitor of PI3Kδ (IC_{50} of 6.2 nM) with a greater than 100-fold selectivity versus PI3Kα, β, or γ. It also inhibits casein kinase ε. *Umbralisib* was approved in 2021 for the treatment of patients with marginal zone lymphoma after at least one prior therapy and for relapsed or refractory FL after three prior therapies. It is in trials for the treatment of other hematological malignancies. Adverse effects include neutropenia, increased risk of infections, diarrhea, noninfectious colitis, and hepatotoxicity. *Umbralisib* is metabolized by CYP2C9, CYP3A4, and CYP1A2. The elimination $t_{1/2}$ is 91 h.

Alpelisib

Alpelisib is an orally bioavailable inhibitor of PI3Kα (IC_{50} of 4.6 nM) with a greater than 50-fold selectivity relative to the other isoforms of PI3K. PIK3CA gene mutations are found in approximately 40% of patients with hormone receptor–positive, HER2-negative advanced breast cancer and indicate a worse prognosis and may mediate resistance to endocrine therapy. *Alpelisib* was approved in 2019 in combination with *fulvestrant* for the treatment of postmenopausal women, and men, with hormone receptor–positive, HER2-negative, PIK3CA-mutated, advanced breast cancer following progression on or after endocrine-based therapy (André et al., 2019). The most frequent adverse events of higher grade are hyperglycemia (36%), rash (10%), and diarrhea (7%). *Alpelisib* is metabolized by CYP3A and has an elimination $t_{1/2}$ of 8 to 9 h.

mTOR Inhibitors: Rapamycin Analogues

Rapamycin (*sirolimus*) is a product from a strain of the soil bacterium *Streptomyces* found on Rapa Nui (Easter Island). It inhibits a serine/threonine protein kinase in mammalian cells named mTOR. The PI3K/PKB(Akt)/mTOR pathway responds to a variety of signals from growth factors. The activation of the PI3K pathway is opposed by the phosphatase activity of the tumor suppressor PTEN (phosphatase and tensin homolog; see Figure 71–2). Activating mutations and amplification of genes in the receptor-PI3K pathway and loss-of-function alterations in PTEN occur frequently in cancer cells, with the result that PI3K signaling is exaggerated and cells exhibit enhanced survival. *Rapamycin* (*sirolimus*) and its congener rapalog *everolimus* are first-line drugs in posttransplant immunosuppression (see Chapter 39) and are used in coronary artery stents to prevent fibrotic growth. Rapalogs have also been approved for the treatment of patients with renal cancer; neuroendocrine tumors (NETs) of pancreatic, GI tract or lung origin; anti–hormone refractory, hormone receptor–positive breast cancer; and tuberous sclerosis complex (TSC)–related tumors.

Mechanism of Action

The rapalogs inhibit an enzyme complex, mTORC1, which occupies a downstream position in the PI3K pathway (see Figures 71–2 and 71–5). mTOR forms the mTORC1 complex with FKBP12 (immunophilin-binding protein for *tacrolimus* [FK506]), a member of the FK506-binding protein family. The antitumor actions of the rapalogs result from their binding to FKBP12 and preventing activation of mTOR, as detailed in Figure 71–5 and its legend.

ADME

For renal cell cancer, *temsirolimus* is given in weekly intravenous doses. It is metabolized by CYP3A4, with the parent drug elimination $t_{1/2}$ of 30 h and the primary active metabolite *sirolimus* (*rapamycin*) elimination $t_{1/2}$ of 53 h. Because *sirolimus* has equivalent activity as an inhibitor of mTORC1 and has a greater AUC, *sirolimus* is likely the more important contributor to antitumor action in patients. *Everolimus* is administered orally and is metabolized by CYP3A4. *Everolimus* has an elimination $t_{1/2}$

of 30 h and maintains inhibition of mTORC1 for 7 days in white blood cells. Both drugs are susceptible to interactions with other agents that affect CYP3A4 activity. For *everolimus*, the dose should be reduced for patients with moderate hepatic impairment; guidelines for dose reduction of *temsirolimus* in such patients have not been established. The drugs' pharmacokinetics do not depend on renal function, and hemodialysis does not hasten *temsirolimus* clearance.

Therapeutic Uses

Both *temsirolimus* and *everolimus* are approved for treatment of patients with advanced renal cell carcinoma. *Everolimus* prolongs survival in patients who had failed initial treatment with antiangiogenic drugs. *Everolimus* is also approved for the treatment of postmenopausal women with advanced hormone receptor–positive, HER2-negative breast cancer in combination with the aromatase inhibitor *exemestane* after failure of treatment with *letrozole* or *anastrozole*. Other indications of *everolimus* are peripheral neuroendocrine tumors (PNETs) and progressive, well-differentiated, nonfunctional NETs of GI or lung origin as well as TSC-related tumors.

Adverse Effects; Resistance

The rapamycin analogues cause similar patterns of adverse effects: a mild maculopapular rash, mucositis, anemia, and fatigue (30%–50%). A few patients develop leukopenia or thrombocytopenia, effects that are reversible if therapy is discontinued. Other, less common side effects include hyperglycemia, hypertriglyceridemia, and, rarely, pulmonary infiltrates and interstitial lung disease. Pulmonary infiltrates emerge in 8% of patients receiving *everolimus* and in a smaller percentage of those treated with *temsirolimus*. If symptoms such as cough or shortness of breath develop or radiological changes progress, the drug should be discontinued. *Prednisone* may hasten the resolution of radiological changes and symptoms.

Resistance to mTOR inhibitors is incompletely understood but may arise through the action of a second mTOR complex, mTORC2, which is unaffected by rapalogs and which can upregulate Akt activity (see Figure 71–5).

Multikinase Inhibitors: Cabozantinib, Vandetanib, Midostaurin, and Gilteritinib

Selectivity of small-molecule protein kinase inhibitors for their targets is dependent on the similarity of the targeted site with sites in other kinases and the chemical composition of the inhibitor. Most inhibitors interact with the ATP-binding site of their target that is relatively conserved within a kinase family; thus, such inhibitors have only relative specificity and will cross-react at higher concentrations with closely related kinases. Nonetheless, inhibition of distantly related kinases is possible. The selectivity of inhibitors for a range of targets in the kinome is determined experimentally using assays with recombinant kinase proteins and *in vivo* using intact cells that express the kinases (Elkins et al., 2015). Inhibitors that target multiple kinase families within the clinically used dose range can be therapeutically efficacious with tolerable adverse effects and are discussed in the material that follows.

Cabozantinib

Mechanism of Action

Cabozantinib is an orally bioavailable, small-molecule inhibitor of several tyrosine kinases; as ranked by the IC_{50} values (in nM) from in vitro assays, these protein kinases are VEGFR2, 0.035; MET, 1.3; RET, 4; KIT, 4.6; AXL, 7; FLT3, 11.3; VEGFR1, 12; and TIE2, 14.3. These receptor tyrosine kinases control normal cellular function and pathologic processes that include maintenance of the tumor microenvironment, tumor angiogenesis, and metastatic spread.

ADME

The elimination half-life of *cabozantinib* is about 99 h. *Cabozantinib* is a substrate of CYP3A4; thus, the dose of *cabozantinib* should be reduced in patients with mild-to-moderate hepatic impairment as well as for concurrent administration of CYP3A4 inhibitors. Conversely, strong inducers of CYP3A4 will reduce exposure to the drug and necessitate increased dosage of *cabozantinib* unless the CYP inducers can be avoided.

Therapeutic Uses

Cabozantinib is indicated for the treatment of patients with advanced RCC and as a first- line treatment in combination with nivolumab; for patients with HCC who have been previously treated with sorafenib and for patients with with progressive, metastatic medullary thyroid cancer.

Adverse Effects

The most commonly reported (≥25%) adverse reactions are diarrhea, fatigue, nausea, PPES, hypertension, vomiting, weight loss, and constipation. Cabozantinib should not be administered to patients with a recent history of bleeding and should be discontinued in patients with thrombotic events, GI perforations and fistulas. Blood pressure should be monitored regularly for the onset of hypertension. Due to the risk of fetal harm, women of childbearing age should use contraception during treatment and for 4 months thereafter.

Vandetanib

Mechanism of Action; ADME

Vandetanib is an orally bioavailable, small-molecule multikinase inhibitor of VEGFRs, the EGFR/HER family, RET, BRK (breast tumor kinase), TIE2, and members of the ephrin receptor and SRC kinase families. These receptor tyrosine kinases are involved in both normal cellular functions and pathological processes (see Figure 71–2). *Vandetanib* is a substrate of CYP3A4. Its median elimination $t_{1/2}$ is 19 days.

Therapeutic Uses

Vandetanib is indicated for the treatment of symptomatic or progressive medullary thyroid cancer that is unresectable, locally advanced, or metastatic. In patients with asymptomatic or slowly progressing disease, *vandetanib* is only indicated after careful consideration of the treatment-related risks.

Adverse Effects and Drug Interactions

The most common adverse effects (>20%) are diarrhea/colitis, rash, acneiform dermatitis, hypertension, nausea, headache, upper respiratory tract infections, decreased appetite, and abdominal pain. Less common are QT prolongation, torsades de pointes, and sudden death. *Vandetanib* should not be used in patients with hepatic impairment or congenital long QT syndrome.

Midostaurin

Midostaurin is an orally bioavailable multikinase inhibitor with activity against mutant FLT3, a driver in AML, as well as other kinases including KIT (wild-type and D816V mutant), PDGFR, SRC, and PKC. It was approved in 2017 for the treatment of patients with newly diagnosed FLT-mutant AML in combination with *cytarabine* and *daunorubicin*. Other indications include systemic mastocytosis with associated leukemia. Major adverse effects of treatment include a risk of prolonged severe leukopenia and thrombocytopenia, mucositis, upper respiratory tract infection, diarrhea, musculoskeletal pain, and QT prolongation. It is metabolized by CYP3A. The parent *midostaurin* has an elimination $t_{1/2}$ of 19 h. Two active metabolites that contribute 28% and 38% of circulating drug are eliminated much more slowly, with $t_{1/2}$ of 32 h and 482 h, respectively.

Gilteritinib

Gilteritinib is an orally bioavailable inhibitor of multiple receptor tyrosine kinases, including wild-type and mutant FLT3 (IC_{50} of 0.29 nM). *Gilteritinib* was approved in 2018 for the treatment of patients with relapsed or refractory AML with an FLT3 mutation. Resistance of leukemic cells to the treatment can be provided by the normal bone marrow microenvironment or by cancer cell intrinsic changes (Joshi et al., 2021) and may require combinatorial treatment. Serious adverse effects include the risk of posterior reversible encephalopathy syndrome (1% of patients) and differentiation syndrome (3% of patients) that is associated with rapid proliferation and differentiation of myeloid cells and may be life-threatening or fatal if not treated with corticosteroids. Symptoms may be fever, dyspnea, or pulmonary infiltrates. Differentiation syndrome (previously termed "retinoic acid syndrome") is also a risk of ATO treatment (see above). *Gilteritinib* is metabolized through CYP3A, and an increase in the AUC was observed when coadministered with an antifungal, *itraconazole*, that is also a strong CYP3A inhibitor. The elimination $t_{1/2}$ is 113 h.

III. Inhibitors of Tumor Angiogenesis

Cancer cells secrete angiogenic factors that induce the formation of new blood vessels and guarantee the flow of nutrients to the tumor cells to permit growth and metastasis. Many tumor types overexpress these angiogenic factors, turning on an "angiogenic switch" whereby the tumor cells adopt an invasive phenotype favoring proliferation of endothelial cells and neovascularization. In 1971, Judah Folkman hypothesized that the growth of solid tumors was dependent on angiogenesis and that blockade of the effects of putative angiogenic factors would be a good treatment modality for human cancers (Folkman, 1971). Folkman's hypothesis proved to be correct and led to the characterization of a number of secreted angiogenic factors, including VEGF, FGF, transforming growth factor (TGF)-β, and PDGF. Furthermore, inhibitors of these angiogenic factors have, indeed, become useful therapeutic agents against certain cancers. VEGF is a major driver of angiogenesis, and inhibitors of VEGF signaling comprise an important class of antitumor agents. Beyond nourishing an expanding cancerous lesion, the control of the vascular steady state by growth factors also impacts diseased tissue surveillance by circulating immune cells. Hence, combination therapies of immune checkpoint inhibitors and antiangiogenic drugs are investigated, and some have received approval as indicated below (e.g., *pembrolizumab* and *lenvatinib*) (Huinen et al., 2021). Recent research revealed the modulation of "angiogenic switch" genes by the activities of hypoxia-inducible transcription factors (HIF) that are controlled by oxygen tension in tissues. Indeed, cellular oxygen-sensing mechanisms are essential for the maintenance of complex multicellular life forms (Hammarlund et al., 2020). William G. Kaelin, Jr, Peter J. Ratcliffe, and Gregg L. Semenza shared the 2019 Nobel Prize in Medicine/Physiology "for their discoveries of how cells sense and adapt to oxygen availability." The HIF-2α inhibitor belzutifan, approved in 2021 for the treatment of renal cell carcinoma (RCC), is based on these discoveries (Choueiri and Kaelin, 2020).

Inhibition VEGF and the VEGFR Pathway

Vascular endothelial growth factor initiates endothelial cell proliferation and vascular permeability when it binds to a member of the VEGFR family, a group of highly homologous receptors with intracellular tyrosine kinase domains; these receptors include VEGFR1 (FLT1), VEGFR2 (KDR [kinase insert domain receptor]), and VEGFR3 (FLT4) (see Figure 71–2). The binding of VEGF to its receptors activates the intracellular VEGFR tyrosine kinase activity and initiates mitogenic and antiapoptotic signaling pathways (Nagy et al., 2007). Antibodies targeting VEGF, such as *bevacizumab*, sterically hinder the interaction of VEGF with its receptor (see Chapter 72). *Aflibercept* acts as a VEGF trap; it is a recombinant molecule that uses the VEGFR1-binding domain to sequester VEGF, basically acting as a "soluble decoy receptor" for VEGF. Several small-molecule drugs that inhibit the protein tyrosine kinase function of VEGFR (*pazopanib*, *sorafenib*, *sunitinib*, and *axitinib*) as well as monoclonal antibodies that target the receptor (*ramucirumab*) have been approved for clinical use. The inhibition of endothelial function by these different approaches results in a similar spectrum of adverse effects.

Antiangiogenic Small-Molecule Kinase Inhibitors

Sunitinib

Mechanism of Action

Sunitinib is a small-molecule, orally bioavailable inhibitor of multiple kinases, including KIT and VEGFR2.

ADME

The typical treatment cycle of *sunitinib* is 4 weeks on treatment followed by 2 weeks off. The dosage and schedule of *sunitinib* can be decreased in patients with adverse effects. More recently, a 2-week-on/1-week-off schedule has proven to be better tolerated and as effective as the original treatment schedule. *Sunitinib* is metabolized by CYP3A4 to produce an active metabolite, SU12662, the $t_{1/2}$ of which is 80 to 110 h; steady-state levels of the metabolite are reached after about 2 weeks of daily administration of the parent drug. Further metabolism results in the formation of inactive products. The pharmacokinetics of *sunitinib* are not affected by food intake.

Therapeutic Uses

Sunitinib is approved in metastatic renal cell cancer, producing a higher response rate and a longer progression-free survival than seen with *interferon* (IFN) or *bevacizumab*. *Sunitinib* is also approved for the treatment of pancreatic NETs and of GIST in patients who have developed resistance to *imatinib* as a consequence of *c-KIT* mutations. Specific *c-KIT* mutations correlate with the degree of response to *sunitinib* (e.g., patients with *c-KIT* exon 9 mutations have a response rate of 37%; patients with *c-KIT* exon 11 mutations have a 5% response rate).

Adverse Effects

The main adverse effects of *sunitinib* are shared by antiangiogenic inhibitors that target VEGF and VEGFR signaling and thus impact endothelial cell survival signaling: hypertension, proteinuria, and, uncommonly, bleeding, arterial thromboembolic events, and intestinal perforation. However, because *sunitinib* is a multitargeted TKI, it has a broader adverse effect profile than the monoclonal antibody *bevacizumab* that selectively targets VEGF (see Chapter 72). Fatigue affects 50% to 70% of patients and may be disabling. Hypothyroidism occurs in 40% to 60% of patients. Bone marrow suppression and diarrhea also are common side effects; severe neutropenia (neutrophils <1000/mL) develops in 10% of patients. Less common side effects include hepatotoxicity, congestive heart failure as a consequence of hypertension, and PPES. It is essential to check blood counts and thyroid function at regular intervals. Monitoring of blood pressure and periodic echocardiograms also are recommended.

Sorafenib

Mechanism of Action and Therapeutic Use

Sorafenib, like *sunitinib*, is an orally bioavailable inhibitor of multiple protein kinases. *Sorafenib* is approved for treatment of patients with thyroid and hepatocellular carcinoma as well as patients with metastatic renal cell cancer, but *sunitinib* and *pazopanib* are generally preferred first-line therapies (see previous discussion and below).

ADME

Sorafenib is given orally every day without treatment breaks. *Sorafenib* is metabolized to inactive products by CYP3A4 with a $t_{1/2}$ of 20 to 27 h; with repeated administration, steady-state concentrations are reached within 1 week.

Adverse Effects

Sorafenib-treated patients can experience the vascular toxicities seen with other antiangiogenic medications described under *sunitinib*. More common adverse effects include fatigue, nausea, diarrhea, anorexia, rash, and palmar-plantar erythrodysesthesias; uncommonly, bone marrow suppression, GI perforation, and cardiomyopathy occur.

Pazopanib

Pazopanib is an orally bioavailable kinase inhibitor of VEGFR-1, -2, and -3 as well as FGFRs, KIT, LCK (lymphocyte-specific kinase), PDGFR, and other kinases implicated in pathological angiogenesis and cancer progression. *Pazopanib* is approved for the treatment of patients with advanced RCC and advanced soft-tissue sarcoma after prior chemotherapy. It has less toxicity and equivalent efficacy as *sunitinib* in patients with treatment-naïve metastatic renal cancer and therefore has become the preferred first-line treatment. Major adverse effects include hypertension, thrombotic and hemorrhagic events, GI perforation, QT prolongation, and cardiomyopathy. *Pazopanib* carries a black-box warning indicating that it can produce severe and life-threatening hepatotoxicity; as a consequence, *pazopanib* should not be used in elderly patients or those with preexisting liver function test abnormalities. *Pazopanib* is metabolized mostly by CYP3A and eliminated with a $t_{1/2}$ of 31 h.

Axitinib

Axitinib is an orally bioavailable kinase inhibitor of VEGFR-1, -2, and -3, which are implicated in pathological angiogenesis, tumor growth, and cancer progression. *Axitinib* is approved for the treatment of patients with advanced RCC after failure of one prior systemic therapy. Major adverse effects include hypertension, thrombotic and hemorrhagic events, and GI perforation, similar to those of the other VEGFR pathway–targeting drugs (see above). *Axitinib* is metabolized mainly by CYP3A4/5 and eliminated with a variable $t_{1/2}$ of 2.5 to 6.1 h.

Tivozanib

Tivozanib is an orally bioavailable kinase inhibitor of VEGFR-1, -2, and -3 that also inhibits other kinases including c-KIT and PDGFR. *Tivozanib* was approved in 2021 for the treatment of patients with relapsed or refractory advanced RCC following two or more prior systemic therapies. Adverse effects are typical for inhibitors of the VEGFR pathway and include hypertension that requires continuous monitoring and potential treatment as well as monitoring of cardiac function, thromboembolic and hemorrhaging events, diarrhea, decreased appetite, nausea, and dysphonia. *Tivozanib* is mostly metabolized by CYP3A4; it showed an increased AUC with moderately impaired hepatic function and is eliminated with a $t_{1/2}$ of 111 h.

Lenvatinib

Lenvatinib is an orally bioavailable kinase inhibitor of VEGFRs, FGFRs, PDGFR, KIT, and RET and other kinases that are implicated in pathological angiogenesis and cancer progression. *Lenvatinib* is approved for the treatment of patients with recurrent or metastatic differentiated thyroid cancer, in combination with *everolimus* for advanced RCC following one prior antiangiogenic therapy, and for first-line treatment of unresectable hepatocellular carcinoma. In 2021, approval was granted for treatment of patients with endometrial carcinoma that progressed after systemic treatment, in combination with the immune checkpoint inhibitor *pembrolizumab*, even in absence of high levels of microsatellite instability or deficient mismatch repair, which are typical requirements for *pembrolizumab* treatment (see Chapter 72). Adverse effects and warnings are typical for inhibitors of the VEGFR signaling pathway including hypertension, cardiac dysfunction, thromboembolism, and impaired wound healing. Osteonecrosis of the jaw is a rarer adverse effect of treatment. *Lenvatinib* is metabolized by CYP3A and eliminated with a $t_{1/2}$ of 28 h.

Regorafenib

Regorafenib is an orally bioavailable kinase inhibitor of VEGFR1–3, PDGFR, KIT, RET, and RAF1 with IC_{50} values in the low nanomolar range and other kinases including FGFR1/2, TIE2, and ABL. Several of these kinases are implicated in pathological angiogenesis and malignant progression. *Regorafenib* is approved for the treatment of patients with metastatic colorectal cancer after previous chemotherapy, anti-VEGF therapy, or anti-EGFR therapy; for patients with hepatocellular carcinoma who have been previously treated with *sorafenib*; and for patients with locally advanced, unresectable, or metastatic GIST after *imatinib* or *sunitinib*. Major adverse effects include hypertension, thrombotic and hemorrhagic events, hepatotoxicity, GI perforation, QT prolongation, and wound-healing complications. *Regorafenib* is metabolized mostly by CYP3A and eliminated with a $t_{1/2}$ of about 28 h.

Belzutifan

Belzutifan is an orally bioavailable inhibitor of HIF-2α that was approved in 2021 for the treatment of patients with von Hippel-Lindau (VHL) disease and associated RCC, CNS hemangioblastomas, or pancreatic NET. HIF-2α is a transcription factor that plays a role in cellular responses to oxygen levels. Under hypoxic conditions, HIF-2α accumulates in the cytosol and translocates into the nucleus where it heterodimerizes with HIF-1β, resulting in the induction of genes associated with cellular growth and angiogenesis. *Belzutifan* binds to HIF-2α and blocks its heterodimerization with HIF-1β and activation of target genes. Under normoxic conditions, the VHL protein targets HIF-2α for proteasomal degradation, thus preventing its activity. VHL is a subunit of the E3 ubiquitin ligase complex, which mediates the proteasomal degradation of HIF-2α (Figure 71–6). The VHL syndrome is a hereditary condition associated mostly with hemangioblastomas that can arise in multiple organs. Loss of VHL is a crucial oncogenic event in approximately 90% of clear cell RCC, resulting in the accumulation of HIF-2α and induction of cell growth and angiogenesis. HIF-2α also plays a role in the function of immune cells, and combination therapy of *belzutifan* with other cancer therapies is being investigated (Choueiri and Kaelin, 2020) and has shown some promising efficacy in patients with RCC pretreated with immune checkpoint inhibitor and antiangiogenic therapy (Choueiri et al., 2021). Adverse effects of *belzutifan* treatment include anemia due to the reduction of erythropoietin, which occurs within 2 weeks of dosing and can return to baseline values within 3 months of treatment. Monitoring for anemia before initiation and throughout treatment to keep the hemoglobin level at 9 g/dL or greater is required. Blood transfusions may be considered. Other adverse effects include fatigue, headache, and dizziness. *Belzutifan* is primarily metabolized by UGT2B17 and CYP2C19 and to a lesser extent by CYP3A4 and is eliminated with a $t_{1/2}$ of 14 h.

IV. Inhibitors of Poly(ADP-Ribose) Polymerase (PARP)

DNA damage-repair genes are frequently inactivated in human cancer. PARP1 is the product of one such gene. PARP1 is a nuclear protein that transfers ADP-ribose from NAD^+ to target proteins, and this poly(ADP-ribosyl)ation (or PARylation) of nuclear proteins by PARP1 plays a significant role in the DNA damage response (Tallis et al., 2014). Inactive PARP1 associates with chromatin and helps to create a compact chromatin structure in the nucleosome. DNA damage (e.g., strand breaks) mobilizes the enzyme, promoting PARP-mediated PARylation, with the result that chromatin relaxes in the area of damage. The mechanisms of relaxation are the PARylation of PARP1 itself and of histones and other chromatin-associated proteins, resulting in their dissociation from DNA. PARylation produces branching chains up to 200 ADP-ribose units in length. These chains provide docking centers for the localized recruitment of PAR-binding factors, DNA repair enzymes (e.g., DNA polβ, DNA ligase III), and proteins that help to maintain an inactive environment in the open chromatin region while repairs are made to the damaged DNA. The synthesis and degradation of PAR chains *in vivo* are tightly regulated, with the chains having half-lives measured in minutes (Wei and Yu, 2016). Excessive activation of PARP1 leads to depletion of the cellular pool of NAD^+ and to cell death; teleologically, this could be viewed as a means of preserving genomic integrity by removing cells with heavily damaged DNA.

Deficient PARP1 activity leads to defective DNA repair. However, PARP-deficient cells are still able to carry out DNA repair through a different mechanism homologous recombination. The protein machinery

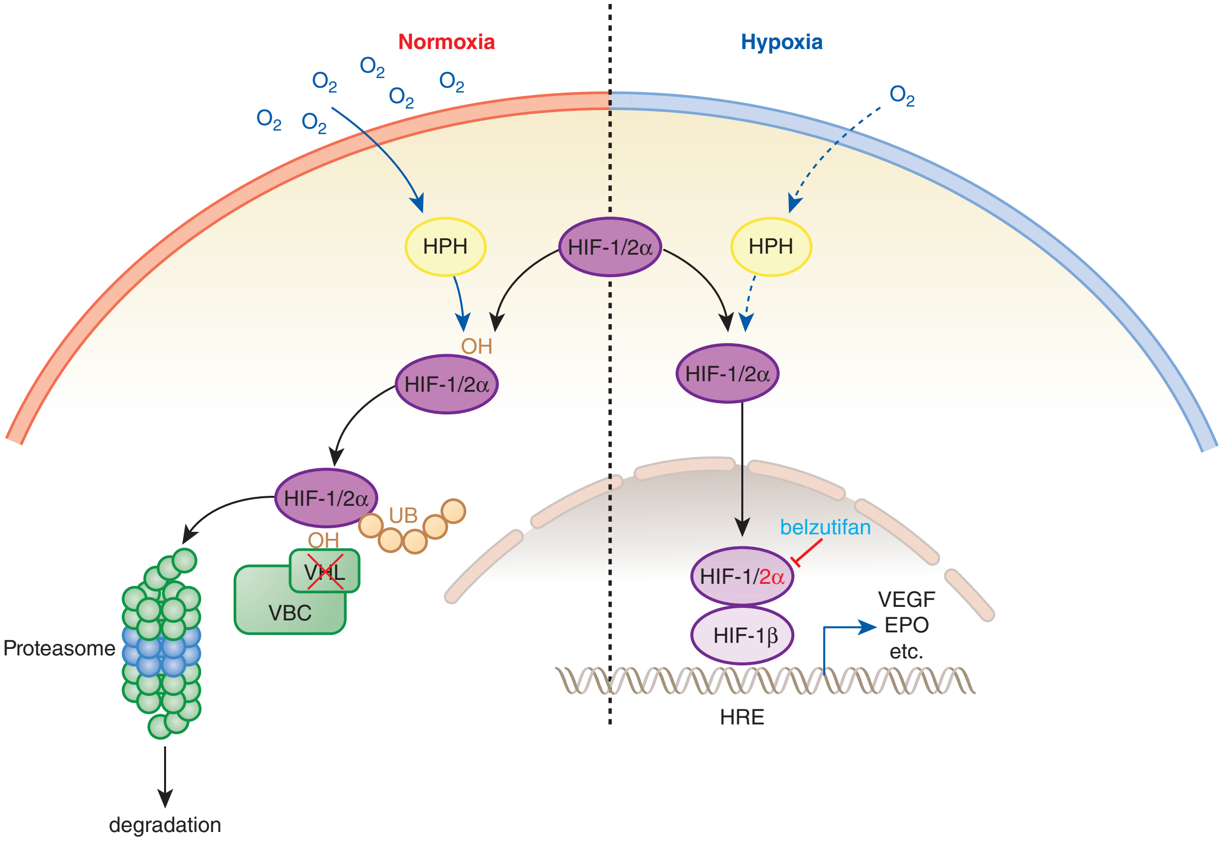


Figure 71–6 *Oxygen-sensing and hypoxia-inducible genes targeted.* Under normoxic conditions, HIF-1α and -2α (HIF-1/2α in short) are hydroxylated (symbolized by the "OH") on proline residues by HIF-prolyl hydroxylases (HPHs) in an oxygen-dependent manner. This allows the VHL protein to recognize and target HIF-1/2α for rapid degradation. VHL is part of an E3 ubiquitin ligase complex of VHL-Elongin B/C-CUL2 (VBC) that transfers ubiquitin (Ub). The ubiquitinated HIF is transported to the proteasome for degradation. In its canonical function depicted here, the VHL protein serves as the substrate recognition component of the VBC protein complex. Under hypoxic conditions, HIF-1/2α are not recognized by VHL and degraded but translocate into the nucleus. In the nucleus HIF-1/2α heterodimerize with HIF-1β (also named aryl hydrocarbon receptor nuclear translocator [ARNT]). The heterodimer binds to hypoxia response element (HRE) DNA sequences and recruits co-activators to drive transcription of hypoxia-inducible genes such as EPO (erythropoietin) and VEGF.

Loss of VHL, symbolized by the red X, is observed in the majority of clear cell RCC and is associated with a pseudohypoxic state that results in the accumulation of HIF-2α and upregulation of HRE target genes that include VEGF. Indeed, inhibition of VEGF and its pathway has shown efficacy and been approved for treatment of clear cell RCC as described in the text. Identification of a binding pocket in HIF-2α led to the development of small molecules that cause conformational changes of HIF-2α and disrupt its heterodimerization with HIF-1β. After successful clinical trials, the HIF-2α inhibitor *belzutifan* was approved in 2021 for the treatment of patients with VHL-deficient cancers (Choueiri et al., 2021).

for homologous recombination repair includes the breast cancer susceptibility genes *BRCA1* and *BRCA2*, suggesting that cells with diminished or absent BRCA1/2 function are unusually susceptible to the inhibition of PARP activity. BRCA1/2 mutations predispose carriers to breast, ovarian, and other cancers that can be resistant to chemotherapy. BRCA-deficient cells are more dependent on PARP1 and base excision repair to maintain genomic integrity. BRCA1/2-deficient cancer cells are less able to carry out repair and ultimately die; inhibition of PARP could accelerate this process (Curtin and Szabo, 2020) and is described in Figure 71–7. PARP inhibitors thus represent a successful example of a molecular medicine application to treatment that takes advantage of DNA repair defects in cancer cells and enhances cancer cell death. Inhibitors of PARP are designed to compete with NAD^+ at its binding site on the PARP enzyme. Catalytic activity is required for repulsion between auto-PARylated PARP1 and DNA, and thus, inhibition of PARP catalytic activity will trap PARP1 and PARP2 on DNA, contributing to the efficacy but also adverse effects and genotoxicity of these inhibitors (Slade, 2020). PARPs catalyze PARylation of a number of proteins that regulate cellular processes beyond DNA damage repair; these extra-chromatin actions may contribute to the clinical efficacy and adverse effects of PARP inhibitors. Different selectivities, subtle differences between the inhibitors, and future drug development in the field were reviewed by Slade (2020). *Olaparib*, *rucaparib*, *niraparib*, and *talazoparib* are currently approved for the treatment of different subsets of patients with ovarian, breast, prostate, and pancreatic cancer, discussed in the below sections.

Olaparib

Olaparib is an orally bioavailable inhibitor of PARP1, -2, and -3 enzymes and was initially approved in 2014 as a monotherapy in patients with BRCA-mutated ovarian cancer who have been treated with three or more prior lines of chemotherapy. The indications have been expanded in 2020 to patients with breast, pancreatic, and prostate cancers with mutant BRCA or DNA repair deficiency. Treatment of these ovarian cancer patients is now approved after first-line platinum-based chemotherapy. Other approvals are in patients with HER2-negative metastatic breast cancer after chemotherapy or after appropriate endocrine therapy for hormone receptor–positive tumors; metastatic pancreatic adenocarcinoma after platinum-based chemotherapy; and metastatic castration-resistant prostate cancer after endocrine therapy with *enzalutamide* or *abiraterone* (see Chapter 73). Adverse effects of treatment include the risk of MDS and AML with mostly fatal outcome in approximately 1.5% of patients after monotherapy. Monitoring of patients for hematological toxicity at baseline and during treatment is required. Pneumonitis with some risk of fatal outcome is another severe risk in 0.8% of patients and requires

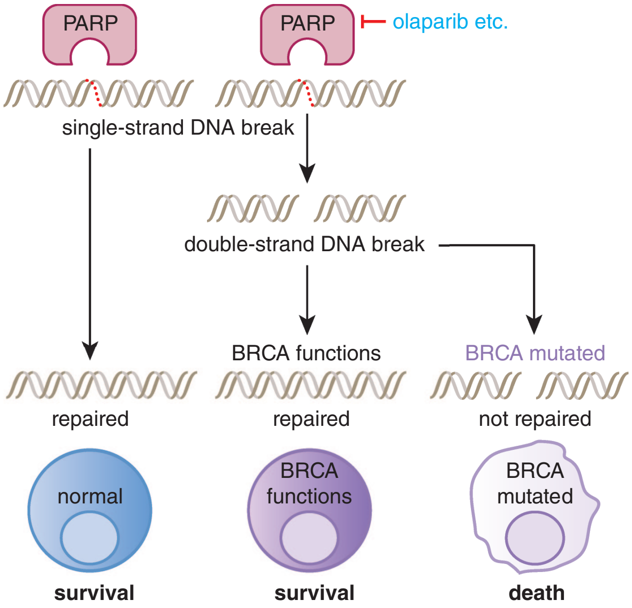


Figure 71–7 *Synthetic lethality from PARP inhibitors in cancer cells that lost BRCA function.* In normal cells, PARP functions in the repair of single-strand breaks in DNA. Without this function of PARP, double-strand DNA breaks accumulate during DNA replication. Cells with functional BRCA can repair these double-strand breaks in DNA through homology-directed repair and thus survive. In contrast, cancer cells with loss of function of BRCA cannot effectively repair double-strand DNA breaks and will become genomically unstable and undergo apoptosis. PARP inhibitors are approved for the treatment of patients with ovarian, breast, prostate, and pancreatic cancer.

monitoring. There is also risk to a fetus, and use of effective contraception is indicated while on treatment. Monitoring for potential venous thromboembolic events including pulmonary embolism is also required. Other adverse effects include nausea and vomiting, loss of appetite, fatigue, muscle and joint pain, low blood cell counts, and anemia. *Olaparib* is rapidly absorbed after oral administration and metabolized primarily by CYP3A4 and eliminated with a $t_{1/2}$ of 14.9 h. Drug exposure is increased or reduced when administered in combination with a CYP3A4 inhibitor or inducer, respectively.

Rucaparib

Rucaparib is an orally bioavailable inhibitor of PARP1–3. *Rucaparib* was approved in 2016 for the treatment of patients with advanced ovarian cancer with BRCA mutations and is used in the maintenance treatment of these patients after a partial response to platinum-based chemotherapy. Other uses include patients with BRCA-mutated, metastatic, castration-resistant prostate cancer after endocrine and taxane-based therapy. Warnings, adverse effects, and precautions overlap with those for *olaparib* (see previous paragraph) and include the risk for potentially fatal MDS and AML. *Rucaparib* is metabolized primarily by CYP2D6 and to a lesser extent by CYP1A2 and CYP3A4 and can impact metabolism of drugs that are substrates of CYP1A2, CYP3A, CYP2C9, or CYP2C19. If coadministered with *warfarin* (a CYP2C9 substrate), increased monitoring of coagulation parameters is required. The mean terminal $t_{1/2}$ of *rucaparib* is 25.9 h.

Niraparib

Niraparib is an orally bioavailable inhibitor of PARP1 and PARP2, was approved in 2017, and is used for the treatment of patients with BRCA-mutated or homologous recombination–deficient, advanced ovarian cancer after first-line platinum-based chemotherapy as maintenance treatment. Warnings and adverse effects include the risk of MDS, AML, and posterior reversible encephalopathy syndrome, which require discontinuation. Potential hypertension and cardiovascular effects due to the inhibition of the dopamine, norepinephrine, and serotonin transporters require monitoring of blood pressure and heart rate and appropriate treatment or dose adjustment. Nausea, thrombocytopenia, anemia, leukopenia, dyspnea, and diarrhea are common adverse events. *Niraparib* can cause fetal harm. *Niraparib* is metabolized and inactivated by carboxylesterases and eliminated with a $t_{1/2}$ of 36 h.

Talazoparib

Talazoparib is an orally bioavailable inhibitor of PARP1 and PARP2 that was approved in 2018 for the treatment of patients with BRCA-mutated, HER2-negative, locally advanced or metastatic breast cancer. *Talazoparib* is the strongest PARP trapper on DNA among the currently approved PARP inhibitors and has the highest occurrence rate of anemia.

Adverse effects are similar to the other PARP inhibitors and include the risk for MDS and AML (0.3% of patients), myelosuppression with anemia, neutropenia, thrombocytopenia, and potential risk to the fetus. *Talazoparib* is mostly excreted in the urine with minimal hepatic metabolism and eliminated with a $t_{1/2}$ of 90 h.

V. Modulators of Protein Degradation

Targeting Protein Degradation

The selective degradation of target proteins is an attractive approach to expand the druggable proteome. Targeted protein degradation uses proteolysis-targeting chimeras (PROTACs), which bind to a protein of interest and link that to an E3 ligase (Dale et al., 2021). An established example of this concept is the antiestrogen *fulvestrant*, which functions as a monovalent degrader of ERα by occupying the ligand-binding pocket of the receptor, exposing hydrophobic residues with its long aliphatic tail and thus tagging ERα for proteolysis (reviewed by Besten and Lipford, 2020). In 2013, the immunomodulatory imide drugs (IMiDs) *thalidomide* and *lenalidomide*, which contain a CO-NH-CO structure as their signature (see figure with structures and highlight), were discovered to exert their therapeutic effects in a similar manner (Bartlett et al., 2004; Jan et al., 2021). They act as a molecular glue that recruits disease-relevant proteins to an E3 ubiquitin ligase, resulting in proteasomal degradation. *Thalidomide* and its analogues bind to cereblon (CRBN), a component of a cullin-RING E3 ligase (CRL) complex, and their binding to CRBN mediates interaction with transcription factors of the Ikaros zinc finger family (IKZF), leading to their ubiquination and subsequent degradation. Figure 71–8 outlines the mechanism of action. Based on the protein targeted by this class of drugs, they may also be referred to as cereblon modulators (Kannt and Đikić, 2021). Proteasomal degradation of these transcription factors kills multiple myeloma cells. *Thalidomide* is associated with significant toxicity and was mostly replaced by *lenalidomide*, a more potent and less toxic IMiD used in the treatment of multiple myeloma (MM). *Pomalidomide*, a more potent, third-generation IMiD, shows efficacy even in patients with *lenalidomide*-refractory cancers. Also, in del(5q) MDS, *lenalidomide* induces the degradation of CK1α, which preferentially affects del(5q) cells because they express this gene at haploinsufficient levels.

Thalidomide and Lenalidomide

Thalidomide and *lenalidomide* have a most unusual history and a multiplicity of biological and immunological effects (see Chapters 39, 65, and 75). *Thalidomide* originally was used for the treatment of pregnancy-associated morning sickness but was withdrawn from the market due to teratogenicity and dysmelia (stunted limb growth). It reentered clinical practice for treatment of erythema nodosum leprosum (see Chapter 60). Further research revealed its antiangiogenic and immunomodulatory effects. At least four distinct mechanisms have been proposed to explain its antitumor activity, which are summarized in Figure 71–9 and enumerated in its legend.

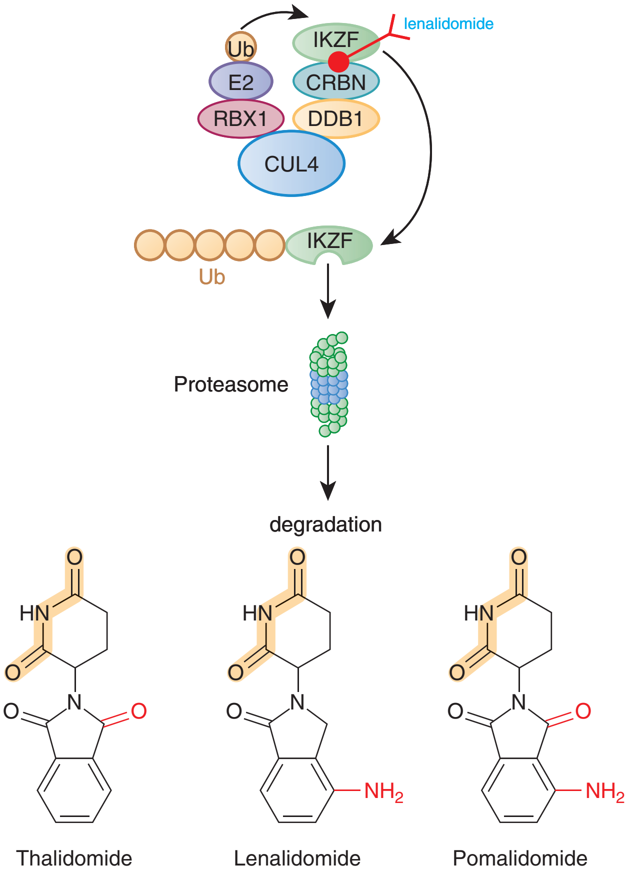


Figure 71–8 *Recruitment of target substrates by lenalidomide. Lenalidomide* functions as a molecular "glue" for the recruitment of proteins to the cereblon (CRBN) receptor component of the cullin–RING E3 ubiquitin ligase complex 4 (CRL4) comprising cullin 4 (CUL4), DNA damage-binding protein 1 (DDB1), and E3 ubiquitin–protein ligase RBX1. This recruitment results in protein ubiquitylation and degradation. Due to the structural differences, thalidomide, and its congeners, interact with and lead to the depletion of overlapping but distinct sets of proteins that include IKZF1/3 (Ikaros family zinc finger protein) and CK1α (casein kinase I isoform-α) (Jan et al., 2021).

Both *thalidomide* and *lenalidomide* possess potent activity in patients with newly diagnosed and heavily pretreated relapsed/refractory MM. *Lenalidomide* is also approved for its activity in the 5q− subset [or del(5q) subset] of MDS. A specific gene array profile identifies patients with MDS who lack the 5q− abnormality but respond to *lenalidomide*. A more recently added derivative of *thalidomide* is *pomalidomide*, approved for treatment of patients with MM resistant to *lenalidomide*.

Thalidomide

ADME

Thalidomide exists at physiological pH as a racemic mixture of cell-permeable and rapidly interconverting nonpolar S(−) and R(+) isomers. Of these mirror image molecules, the *S*-enantiomer is associated with the teratogenic, phocomelia-inducing, and growth control activities, while the *R*-enantiomer accounts for the sedative properties of *thalidomide*. It is noteworthy that the isomers interconvert under biological conditions. When treating MM, doses usually are escalated by 200 mg/day every 2 weeks until dose-limiting side effects (sedation, fatigue, constipation, or a sensory neuropathy) appear. With extended treatment, the neuropathy may necessitate dose reduction or discontinuation of treatment for a period of time. *Thalidomide* absorption from the GI tract is slow and highly variable. It distributes throughout most tissues and organs, without significant binding to plasma proteins. The enantiomers are eliminated with a $t_{1/2}$ of about 6 h, mainly due to spontaneous hydrolysis in all body fluids; the *S*-enantiomer is cleared more rapidly than the *R*-enantiomer. *Thalidomide* and its metabolites are excreted in the urine, while the nonabsorbed portion of the drug is excreted unchanged in feces. The inactive hydrolysis products undergo CYP-mediated metabolism. Longer plasma $t_{1/2}$ is reported at the highest doses (1.2 g daily). No dose adjustment is necessary in the presence of renal failure.

Lenalidomide

Lenalidomide constitutes the lead compound of immunomodulatory *thalidomide* derivatives. *Lenalidomide* induces the ubiquitination and degradation of target proteins by the E3 ubiquitin ligase CRL4-CRBN (Fink and Ebert, 2015). Target proteins in MM cells are IKZF1/3, which are crucial for cell survival (Lu et al., 2014) (Figure 71–9). In MDS, casein kinase 1A1 is the target protein (Krönke et al., 2015). Cellular effects of *lenalidomide* include direct suppression of tumor cell growth in culture, T-cell and natural killer (NK)-cell activation, suppression of tumor necrosis factor-α and other cytokines, antiangiogenesis, and promotion of HSC differentiation.

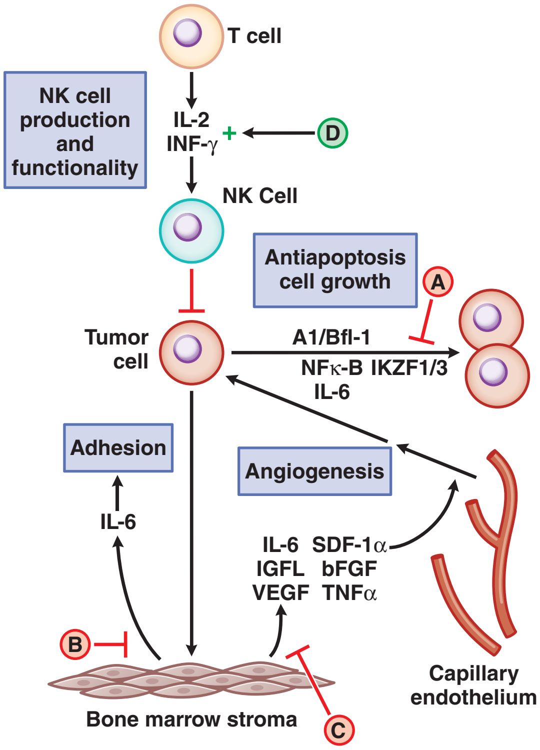


Figure 71–9 *Overview of proposed mechanisms of antimyeloma activity of thalidomide and its derivatives.* Some biological hallmarks of the malignant phenotype are indicated in blue *boxes*. The proposed sites of action for *thalidomide* (letters inside red and green circles) are hypothesized to also be operative for *thalidomide* derivatives. **A.** Direct anti-MM effect on tumor cells, including G_1 growth arrest or apoptosis, even against MM cells resistant to conventional therapy. This is due to the disruption of the antiapoptotic effect of BCL2 family members, blocking NF-κB signaling, and inhibition of the production of IL-6. Mechanistic studies show that *lenalidomide* can kill MM cells by inducing E3 ubiquitin ligase CRL4-CRBN–mediated ubiquitination and degradation of the crucial IKZF1 and -3 (zinc finger transcription factors). **B.** Inhibition of MM cell adhesion to bone marrow stromal cells partially due to the reduction of IL-6 release. **C.** Decreased angiogenesis due to the inhibition of cytokine and growth factor production and release. **D.** Enhanced T-cell production of cytokines, such as IL-2 and IFN-γ, that increase the number and cytotoxic functionality of NK cells.

ADME

The drug is rapidly absorbed following oral administration and eliminated with a $t_{1/2}$ of 9 h. Approximately 70% of the orally administered dose of *lenalidomide* is excreted intact by the kidney. Dose adjustments for reduced creatinine clearance are recommended.

Therapeutic Use

Lenalidomide exhibits antitumor activity in MM, MDS, and CLL; it causes fewer adverse effects and lacks the teratogenicity of *thalidomide*.

Adverse Effects of Thalidomide and Lenalidomide

Thalidomide is well tolerated at doses less than 200 mg daily. Common adverse effects are sedation and constipation. The most serious adverse effect is peripheral sensory neuropathy, which occurs in 10% to 30% of patients with MM or other malignancies in a dose- and time-dependent manner. *Thalidomide*-related neuropathy is an asymmetrical, painful, peripheral paresthesia with sensory loss, commonly presenting with numbness of toes and feet, muscle cramps, weakness, signs of pyramidal tract involvement, and carpal tunnel syndrome. Although symptoms improve upon drug discontinuation, long-standing sensory loss may not reverse. Particular caution should be applied in patients with preexisting neuropathy (e.g., related to diabetes) or prior exposure to drugs that can cause peripheral neuropathy (e.g., vinca alkaloids, *bortezomib*). *Thalidomide* enhances the sedative effects of barbiturates and alcohol and the catatonic effects of *chlorpromazine*. Conversely, CNS stimulants (such as *methamphetamine* and *methylphenidate*) counteract the depressant effects of *thalidomide*.

Adverse effects of *lenalidomide* are less severe; it causes little sedation, constipation, or neuropathy. *Lenalidomide* depresses bone marrow function and is associated with significant leukopenia (20% of patients). Hepatotoxicity and renal dysfunction are rare. In some patients with CLL, *lenalidomide* causes dramatic lymph node swelling and tumor lysis (tumor flare reaction). Patients with renal dysfunction are prone to this reaction; thus, patients with CLL should be started at lower doses of 10 mg/day, with escalation as tolerated. Patients with CLL should receive pretreatment hydration and *allopurinol* to avoid the consequences of tumor swelling and tumor lysis syndrome. A negative interaction with *rituximab*, an anti-CD20 antibody, may result from *lenalidomide*'s downregulation of CD20, an interaction that has clinical implications for their combined use in lymphoid malignancies.

Thalidomide and *lenalidomide* increase the risk for thromboembolic events, including deep vein thrombosis, stroke, and myocardial infarction, which occur with increased frequency in combination with glucocorticoids and with anthracyclines. Anticoagulation and antiplatelet medication reduce this risk and are indicated in patients with risk factors for clotting.

Pomalidomide

Pomalidomide, a third-generation *thalidomide* congener, was first approved in 2013 and is indicated in combination with *dexamethasone* for the treatment of patients with MM who have received at least two prior therapies including *lenalidomide*. It was also approved in 2020 for the treatment of AIDS-related Kaposi sarcoma. Adverse effects are similar to those of *thalidomide*. *Pomalidomide* is contraindicated during pregnancy and in females who may become pregnant. As a consequence, this drug is available only under a pregnancy control system to ensure that recipients are not pregnant and are using effective birth control.

Proteasome Inhibitors

First Generation

Bortezomib

Bortezomib is a first-generation inhibitor of proteasome-mediated protein degradation that has a central role in the treatment of MM.

Mechanism of Action

Bortezomib binds to the β5 subunit of the 20S core of the 26S proteasome and reversibly inhibits its chymotrypsin-like activity. This event disrupts multiple intracellular signaling cascades, leading to apoptosis. An important consequence of proteasome inhibition is its effect on NF-κB, a transcription factor that promotes cell damage response and cell survival. Most cellular NF-κB is cytosolic and bound to IκB (inhibitor of NF-κB); in this form, NF-κB is restricted to the cytosol and cannot enter the nucleus to regulate transcription. In response to stress signals resulting from hypoxia, chemotherapy, and DNA damage, IκB becomes ubiquitinated and then degraded via the proteasome. Its degradation releases NF-κB, which enters the nucleus and transcriptionally activates a host of genes involved in cell survival (e.g., cell adhesion proteins) and proliferation (e.g., cyclin D1) or antiapoptosis (e.g., cIAPs, BCL-2). NF-κB is highly expressed in many human tumors, including MM, and may be a key factor in tumor cell survival in a hypoxic environment and during chemotherapy. *Bortezomib* blocks proteasomal degradation of IκB, thereby preventing the transcriptional activity of NF-κB and downregulating survival responses.

Bortezomib also disrupts the ubiquitin-proteasomal degradation of p21, p27, p53, and other key regulators of the cell cycle and initiators of apoptosis. *Bortezomib* activates the cell's stereotypical "unfolded protein response," in which abnormal protein conformation activates adaptive signaling pathways in the cell. The composite effect leads to irreversible commitment of MM cells to apoptosis.

ADME

The recommended starting dose of *bortezomib* is 1.3 mg/m^2 given as an intravenous bolus on days 1, 4, 8, and 11 of every 21-day cycle (with a 10-day rest period per cycle). At least 72 h should elapse between doses. Drug administration should be withheld until resolution of any grade 3 nonhematological toxicity or grade 4 hematological toxicity, and subsequent doses should be reduced by 25%. The drug exhibits a terminal $t_{1/2}$ in plasma of 5.5 h. Peak proteasome inhibition reaches 60% within 1 h and declines thereafter, with a $t_{1/2}$ of about 24 h. *Bortezomib* clearance results from the deboronation of the parent compound (90%), followed by hydroxylation of the boron-free product by CYPs 3A4 and 2D6; administration of this drug with potent inducers or inhibitors/substrates of CYP3A4 requires caution. No dose adjustment is required for patients with renal dysfunction.

Therapeutic Uses

Bortezomib is used as initial therapy for patients with MM and as therapy for patients with MM after relapse from other drugs. It is also approved for treatment of patients with relapsed or refractory MCL. The drug is active in myeloma, including the induction of complete responses in up to 30% of patients when used in combination with other drugs (i.e., *thalidomide*, *lenalidomide*, liposomal *doxorubicin*, or *dexamethasone*).

Adverse Effects

Bortezomib toxicities include thrombocytopenia (28%), fatigue (12%), peripheral neuropathy (12%), neutropenia, anemia, vomiting, diarrhea, limb pain, dehydration, nausea, and weakness. Peripheral neuropathy, the most chronic of the toxicities, develops most frequently in patients with a prior history of neuropathy secondary to prior drug treatment (e.g., *thalidomide*) or diabetes or with prolonged use. Dose reductions or discontinuation of *bortezomib* ameliorates the neuropathic symptoms. Injection of *bortezomib* may precipitate hypotension, especially in volume-depleted patients, in those who have a history of syncope, or in patients taking antihypertensive medications. Cardiac toxicity is rare, but congestive heart failure and prolonged QT interval have been reported.

Second Generation

Carfilzomib is a second-generation selective proteasome inhibitor based on a tetrapeptide epoxyketone. It is FDA-approved as a single agent or in combination with *dexamethasone* or *lenalidomide* plus *dexamethasone* for the treatment of patients with relapsed or refractory MM who have received at least one prior therapy. Frequent adverse effects (>20% of patients) include anemia, thrombocytopenia, diarrhea, dyspnea, and peripheral edema. There is a risk for cardiac, renal, pulmonary, and hepatic toxicity as well as hypertension.

Ixazomib is an orally bioavailable second-generation peptide analogue proteasome inhibitor that interacts with subunit beta type 5 (PSMB5) of the 20S proteasome complex. *Ixazomib* has an elimination $t_{1/2}$ of 9.5 days and is approved for use in combination with *lenalidomide* and *dexamethasone* for the treatment of patients with MM after at least one prior therapy (Moreau et al., 2016). *Ixazomib* can cause diarrhea, peripheral neuropathy, and hepatotoxicity.

VI. Epigenetic Modulators: Inhibitors of HDAC, HMT, and IDH1/2

Inhibitors of Histone Deacetylase

Histone deacetylases (HDACs) are a class of enzymes that catalyze the removal of acetyl groups from acetylated lysine amino acids in histones, thereby altering the transcriptional activation of cellular genes. Thus, HDAC inhibitors produce a broad spectrum of epigenetic effects (Bates, 2020). HDACs can also deacetylate other proteins, including transcription factors. Overexpression of HDACs found in some cancers or aberrant recruitment of HDACs to oncogenic transcription factors can cause hypoacetylation of core nucleosomal histones. This hypoacetylation results in a condensed chromatin structure and repression of gene transcription. In contrast, inhibition of HDAC activity leads to the accumulation of acetyl groups on the histone lysine residues, an open chromatin structure, and activation of target genes that are selectively repressed in tumors. The result of HDAC inhibition can be differentiation with the emergence of a more normal cellular phenotype or cell cycle arrest with expression of endogenous regulators of cell cycle progression. Due to the diversity of the HDAC family of enzymes and their tissue distribution, HDAC inhibitors with different selectivity can induce a wide variety of cellular effects. Beyond the cellular effects, the impact of HDAC inhibitors on the crosstalk of cancer cells and the immune system is currently evaluated in trials using combination treatments with immune checkpoint inhibitors (Hogg et al., 2020). Four HDAC inhibitors, *panobinostat*, *romidepsin*, *vorinostat*, and *belinostat*, have gained FDA approval and are discussed below. An updated review of other epigenetic modulators is provided by Bates (2020).

Panobinostat

Panobinostat is an orally bioavailable, nonselective pan-HDAC inhibitor. The inhibitory activity leads to apoptosis of malignant cells via multiple pathways. *Panobinostat* is approved for the treatment of patients with MM who have received at least two previous treatments, including *bortezomib* and an immunomodulatory agent.

ADME

The oral bioavailability is about 21%. *Panobinostat* is metabolized through CYP3A. It is recommended to avoid concomitant use of strong CYP3A4 inducers or inhibitors; otherwise, dose adjustments will be necessary. The elimination $t_{1/2}$ is approximately 37 h.

Adverse Effects

The most common adverse effects are diarrhea (severe in 25% of patients), fatigue, nausea, peripheral edema, decreased appetite, pyrexia, and vomiting. The most common nonhematologic laboratory abnormalities (incidence ≥40%) are hypophosphatemia, hypokalemia, hyponatremia, and increased creatinine. Common hematological laboratory abnormalities (incidence ≥60%) are thrombocytopenia, lymphopenia, leukopenia, neutropenia, and anemia. Fatal cardiac ischemic events, severe arrhythmias, and ECG changes have occurred in patients receiving *panobinostat*. Arrhythmias may be exacerbated by electrolyte abnormalities. Avoid concomitant use of antiarrhythmic or QT-prolonging drugs. Close cardiac monitoring during treatment is recommended.

Romidepsin

Romidepsin is an HDAC inhibitor used in the treatment of cutaneous and peripheral T-cell lymphoma. Recently, its indication in peripheral T-cell lymphoma has been withdrawn. *Romidepsin* is a natural product obtained from the bacteria *Chromobacterium violaceum* and is also referred to as *depsipeptide*. *Romidepsin* functions as a prodrug. Within cells, a zinc-binding thiol of the drug is reduced and interacts with a zinc atom in the binding pocket of HDAC to block its activity.

ADME

After intravenous administration, the major metabolism is through CYP3A4. Thus, it is recommended to monitor for toxicities related to increased *romidepsin* exposure when coadministering strong CYP3A4 inhibitors. *Rifampin* and other strong CYP3A4 inducers should be avoided. The terminal $t_{1/2}$ is about 3 h.

Adverse Effects

The most commonly observed adverse effects are nausea and vomiting, anemia, thrombocytopenia, and leukopenia, as well as abnormal electrolyte levels and ECG changes.

Vorinostat

Vorinostat is a small-molecule, orally bioavailable HDAC inhibitor; it is also known as SAHA based on its chemical name ***s****uber****a****nilo****h****ydroxamic* ***a****cid*.

Mechanism of Action

Vorinostat binds to the active site of HDACs and chelates zinc ions in the active site. The resulting inhibition of HDACs causes the accumulation of acetylated histones and other acetylated proteins, among which are transcription factors crucial for cell differentiation. *Vorinostat* inhibits the enzymatic activities of HDAC1, -2, and -3 (class I) and HDAC6 (class II) at nanomolar concentrations (IC_{50} <100 nM). *In vitro*, *vorinostat* induces cell cycle arrest or apoptosis of some cancer cells.

ADME

The absorption of *vorinostat* is slightly improved when taken with a meal. Metabolism is mostly through glucuronidation and hydrolysis. The elimination $t_{1/2}$ is about 2 h.

Therapeutic Use; Adverse Effects

Vorinostat is approved for the treatment of patients with cutaneous T-cell lymphoma with persistent or recurrent disease after two systemic therapies. The most common adverse reactions are diarrhea, fatigue, nausea, thrombocytopenia, anorexia, and dysgeusia. Patients with severe hepatic impairment should be excluded from treatment. Women should be apprised of the potential harm to the fetus.

Belinostat

Belinostat is an HDAC inhibitor developed for the treatment of hematological malignancies and solid tumors. It is approved to treat peripheral T-cell lymphoma and is administered by intravenous infusion. Warnings and adverse effects include the risk of hematological toxicity, infections, and hepatotoxicity. Treatment can also cause fetal harm.

Inhibitors of Histone Methyltransferase

Tazemetostat

Tazemetostat is an orally available, S-adenosyl methionine (SAM) competitive inhibitor of histone methyltransferase (HMT) EZH2 (wild-type and mutant) that was approved in 2020 for the treatment of patients with metastatic or locally advanced epithelioid sarcoma not eligible for complete resection. Inhibition of EZH2 prevents the methylation of histone H3 lysine 27 (H3K27), alters gene expression patterns associated with cancer pathways, and inhibits growth of EZH2-mutated cancer cells. EZH2 is overexpressed or mutated in a variety of cancer cells and plays a key role in tumor cell proliferation. The most common adverse effects include fatigue, nausea, and decreased appetite. There is an increased risk of developing secondary malignancies and of fetal harm. *Tazemetostat* is metabolized by CYP3A, and coadministration of inhibitors can increase the AUC. The elimination $t_{1/2}$ is 3.1 h.

IDH1/2 Inhibitors

IDH1 and IDH2 mutations result in preferential production of 2-hydroxyglutarate (2-HG) over alpha-ketoglutarate, mimicking the loss-of-function of the ten-eleven translocation (TET) gene that controls DNA demethylation and resulting in DNA hypermethylation; simultaneously, inhibition of lysine demethylases by 2-HG leads to increased histone lysine methylation (Yang et al., 2012). Mutations in DNMT3A, TET2, IDH1, and IDH2 are found in 28%, 14%, 9%, and 10% of AMLs, respectively, with a similar mutation pattern in peripheral T-cell lymphoma. DNMT3A and TET2 mutations have been detected only at lower frequency in solid tumors, whereas IDH1 mutations were detected at higher frequency, including in 78% of low-grade gliomas, 14% of cholangiocarcinomas, and 4% of cutaneous melanomas. Two drugs targeting mutants IDH1 and IDH2 are currently approved and have shown some efficacy in the treatment of AML.

Enasidenib is an orally bioavailable IDH2 inhibitor that was approved in 2017 for the treatment of patients with relapsed or refractory AML with an IDH2 mutation. *Ivosidenib* is an orally bioavailable IDH1 inhibitor approved in 2018 for the treatment of AML with a susceptible IDH1 mutation (DiNardo et al., 2018; Issa and DiNardo, 2021) and recently for cholangiocarcinoma with IDH1 mutations. For both drugs, there is a risk of differentiation syndrome, which can be fatal and requires corticosteroid therapy and hemodynamic monitoring if suspected. For *ivosidenib*, risks include QTc prolongation and Guillain-Barré syndrome.

VII. Other Inhibitors (BCL2, Nuclear Export, Translation, CXCR4)

BCL2 Inhibitors

The B-cell lymphoma (BCL) 2 protein family comprises more than 20 proteins that govern mitochondrial outer membrane permeabilization and control programmed cell death (apoptosis). Proteins in this family can be either *pro-* or *anti*apoptotic depending on their content of BCL2 homology domains (BH1–4). *Proapoptotic* proteins contain a BH3 domain that is necessary for dimerization with other proteins of the BCL2 family. *Antiapoptotic* proteins contain BH1 and BH2 domains. The balance of these interacting proteins controls mitochondrial outer membrane permeabilization, cytochrome c release, and the activation of caspases that leads to apoptosis (Figure 71–10). BCL2 promotes cellular survival by inhibiting proapoptotic proteins like BIM (BCL2-like protein 11), BAX, and BAK and is overexpressed in CLL and some other tumors where it can support tumor cell survival and resistance to cancer treatments.

Venetoclax

Mechanism of Action

Venetoclax is a first-in-class, orally bioavailable, small-molecule inhibitor of BCL2. It was designed as a BH3 mimetic that inhibits BCL2 interaction with the proapoptotic BH3-only family members, such as BIM, BID, and BAD. *In vitro*, *venetoclax* inhibits BCL2 action with an IC_{50} of less than 0.01 nM and with a selectivity of two to three orders of magnitude over other BCL2 family members. In the presence of *venetoclax*, BH3-only proteins can translocate to mitochondria and initiate BAX/BAK-dependent apoptosis. Overexpression of BCL2 occurs in CLL cells, where it supports tumor cell survival and resistance to chemotherapeutic agents. Thus, *venetoclax* helps restore apoptosis in these cells.

Therapeutic Use; Adverse Effects; ADME

Venetoclax is approved for the treatment of patients with CLL or SLL and in combination with *azacitidine*, *decitabine*, or low-dose

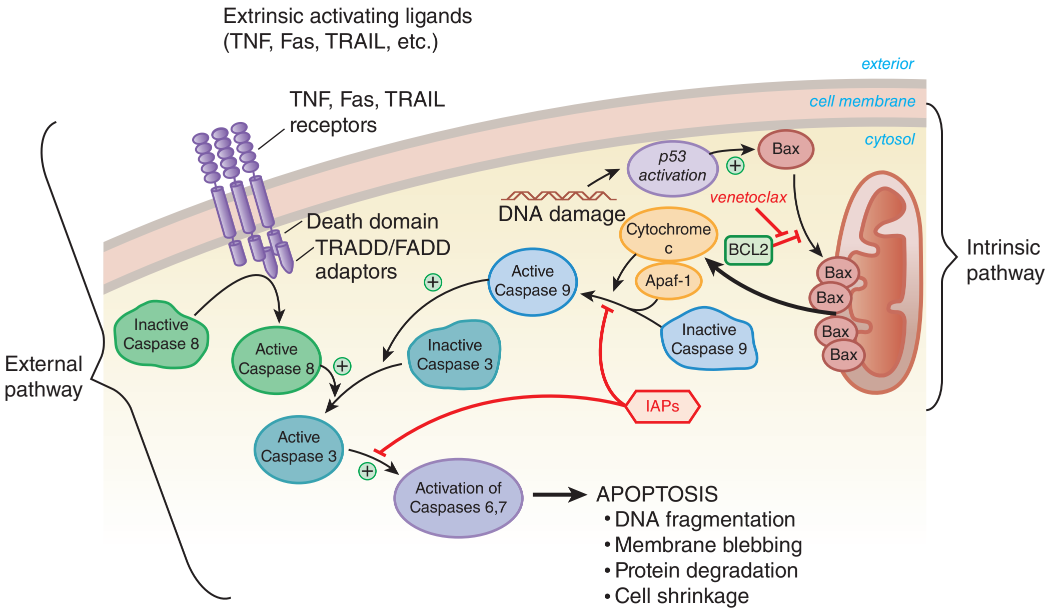


Figure 71–10 *BH3 mimetics enhance apoptosis.* Permeability of the outer mitochondrial membrane to cytochrome c is regulated by proteins required for forming pores, including proapoptotic BAX and BAK. BAK and BAX are rendered inactive by binding to the BH3 domains of antiapoptotic proteins such as BCL2. BH3 mimetic drugs (*venetoclax*) target BCL2 and its interaction with BAX/BAK, reducing the inhibitory effect of BCL2 on apoptosis progression, enhancing cytochrome c release, and sensitizing the cell to apoptosis. Cancer cells may be resistant to apoptosis by overexpression of BCL2; BH3 mimetics such as *venetoclax* release the resultant blockade and promote apoptosis.

cytarabine for the treatment of newly diagnosed AML in patients 75 years or older or with comorbidities that preclude the use of chemotherapy. The most common adverse effects are neutropenia, diarrhea, nausea, anemia, upper respiratory tract infection, thrombocytopenia, and fatigue. *Venetoclax* may cause fetal harm. Oral absorption is significantly improved 3- to 5-fold by administration with a meal. *Venetoclax* is metabolized by CYP3A4/5 and has an elimination $t_{1/2}$ of 18 to 26 h.

Nuclear Export Inhibitor

Selinexor

Selinexor is an orally bioavailable reversible inhibitor of exportin 1 (XPO1). It was approved in 2019 in combination with *bortezomib* and *dexamethasone* for the treatment of patients with MM who have received at least one prior therapy, and for the treatment of patients with relapsed or refractory diffuse large B-cell lymphoma after at least two lines of systemic therapy. Warnings and risks of treatment that require monitoring and management include thrombocytopenia, neutropenia, hyponatremia, and infection. Also, there is a risk of fetal harm. The most common adverse effects are fatigue, nausea, vomiting, decreased appetite, diarrhea, peripheral neuropathy, upper respiratory tract infection, decreased weight, and cataract. *Selinexor* is metabolized by CYP3A4 and eliminated with a $t_{1/2}$ of 6 to 8 h.

Protein Translation Inhibitor

Omacetaxine

Omacetaxine is administered by SC injection and is approved for the treatment of patients with CML with resistance or intolerance to two or more kinase inhibitors. The mechanisms of action include inhibition of protein translation by preventing the initial elongation step of protein synthesis, thereby depleting the cell of short-lived proteins. Warnings include the risk of myelosuppression, bleeding, and fetal toxicity. Adverse effects also include nausea, fatigue, and infections. *Omacetaxine* is hydrolyzed by plasma esterases and eliminated with a $t_{1/2}$ of 14.6 h.

CXCR4 Inhibitor

Plerixafor

Plerixafor is a small-molecule inhibitor of the CXCR4 chemokine receptor and is used in combination with granulocyte colony-stimulating factor to mobilize hematopoietic stem cells (HSC) to the peripheral blood for collection and subsequent autologous transplantation in patients with non-Hodgkin's lymphoma and MM. *Plerixafor* blocks binding of stromal cell-derived factor (SDF)-1α to CXCR4. CXCR4 activity supports homing of HSCs to the bone marrow, and *plerixafor* mobilizes the release and entry of HSCs into the circulation. The combination of granulocyte colony-stimulating factor with *plerixafor* increases the efficacy of HSC mobilization.

Drug Facts for Your Personal Formulary: *Pathway-Targeted Therapies*

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Section I: Inhibitors of Growth Factors and Receptors		
Epidermal Growth Factor Receptor Inhibitors		
Small-Molecule EGFR Kinase Inhibitors: Oral Administration		
Erlotinib	• Advanced NSCLC with mutant EGFR (del exon 19; L858R) • Advanced pancreatic cancer in combination with gemcitabine	• Skin rash, stomatitis, diarrhea, interstitial lung disease • CYP3A4 substrate • Anticoagulant effect of warfarin enhanced • Concurrent use of PPI ↓ bioavailability
Gefitinib	• Advanced NSCLC with mutant EGFR (del exon 19; L858R)	• Side effects similar to erlotinib, but bioavailability not affected by PPI
Afatinib	• Irreversible inhibitor EGFR > HER2 • Advanced NSCLC with mutant EGFR (del exon 19; L858R) • Squamous cell carcinoma of the lung progressing after systemic therapy	• Side effects stronger than gefitinib; can cause hepatotoxicity • Not affected by CYP3A4 modulation • ↓ Dose with renal impairment or Pgp inhibitors
Osimertinib	• Advanced NSCLC with EGFR mutations • Advanced NSCLC that is resistant to other EGFR kinase inhibitors and positive for exon 20 T790M-mutant EGFR	• Similar to gefitinib but less intense side effects; can ↑ QTc interval
Human Epidermal Growth Factor Receptor 2 Inhibitors		
Small-Molecule HER2 Kinase Inhibitors: Oral Administration, Also Inhibit EGFR		
Lapatinib	• HER2-positive breast cancer in combination with capecitabine • HER2-positive, hormone receptor–positive breast cancer in combination with letrozole (aromatase inhibitor)	• Skin rash, diarrhea • Cardiotoxicity (less than trastuzumab), QT interval prolongation • CYP3A4 substrate
Neratinib	• Irreversible inhibitor of EGFR and HER2 • HER2-positive breast cancer in addition to chemotherapy	• Diarrhea is major adverse effect, with one-third of patients with severe grade 3–4 diarrhea

Drug Facts for Your Personal Formulary: *Pathway-Targeted Therapies* (*continued*)

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Section II: Inhibitors of Intracellular Protein Kinases		
Mutant B-RAF Kinase Inhibitors		
Vemurafenib	• BRAF V600E/K mutant melanoma	• Cutaneous adverse effects up to 60%, with squamous cell carcinoma in >20% • Arthralgia, fatigue, nausea less frequently • Can cause QT prolongation • CYP3A4 substrate; CYP1A2 inhibitor
Dabrafenib	• BRAF V600E/K mutant melanoma also in combination with the MEK inhibitor trametinib • BRAF V600E mutant NSCLC with trametinib	• Cutaneous adverse effects include hyperkeratosis and papilloma • cuSCC in ~10% of patients • Combination with trametinib ↓ incidence of cuSCC to 3% and delays onset • CYP2C8 and CYP3A substrate
Mitogen-Activated Protein Kinase Kinase Inhibitors		
Cobimetinib	• BRAF V600E/K mutant melanoma	• Diarrhea, photosensitivity, nausea common • Risk of hemorrhage, cardiomyopathy • CYP3A4 substrate
Trametinib	• BRAF V600E/K mutant melanoma and BRAF V600E mutant NSCLC: with dabrafenib • Ineffective in patients who developed resistance to BRAF inhibitor treatment	• Cutaneous rash, acneiform dermatitis, diarrhea most frequent; serious skin toxicity in 6% • Risk of cardiomyopathy, hypertension, hemorrhage, interstitial lung disease • Reduced absorption after high-calorie meal
Janus-Associated Protein Kinase Inhibitors		
Ruxolitinib	• Polycythemia vera • Myelofibrosis	• Thrombocytopenia, anemia most frequent • Rare basal cell carcinoma or squamous cell carcinoma • ↓ Dose in renal or hepatic impairment • CYP3A4 substrate
Cyclin-Dependent Kinase 4/6 Inhibitors		
Palbociclib	• Advanced ER-positive, HER2-negative breast cancer • Combination with aromatase inhibitor or antiestrogen (fulvestrant)	• Common: neutropenia, leukopenia, infections, stomatitis, anemia, thrombocytopenia, nausea, diarrhea • Substrate and inhibitor of CYP3A4
Abemaciclib and ribociclib	• Same as for palbociclib	• Side effects similar to palbociclib
Bruton Tyrosine Kinase Inhibitors		
Ibrutinib	• Mantle cell lymphoma, CLL, SLL, Waldenström's macroglobulinemia	• Neutropenia, thrombocytopenia, diarrhea, anemia, musculoskeletal pain • Slow onset of hypertension possible: monitor blood pressure • Atrial fibrillation: monitor and treat • Secondary malignancies mostly skin, nonmelanoma • CYP3A4 substrate
BCR-ABL, PDGFR, KIT Kinase Inhibitors		
Imatinib	• Chronic-phase CML; mucosal and acral lentiginous melanoma (KIT mutation positive), GIST (KIT mutation positive), dermatofibrosarcoma protuberans, chronic myelomonocytic leukemia	• GI tract adverse effects: diarrhea, nausea, vomiting • Fluid retention, edema • Rare myelosuppression, hepatotoxicity • CYP3A4 substrate
Dasatinib	• CML resistant to imatinib after prior therapy	• Adverse effects: diarrhea, nausea, vomiting • Fluid retention, edema, pleural effusions • Rare myelosuppression, hepatotoxicity • Bioavailability ↓ after antacids or H_2 blockers • CYP3A4 substrate
Nilotinib	• CML resistant to imatinib after prior therapy	• Adverse effects: diarrhea, nausea, vomiting, fluid retention, edema • May ↑ QT interval; beware vascular events, including ischemia • Rare myelosuppression, hepatotoxicity • Bioavailability ↑ in the presence of food • CYP3A4 and Pgp substrate • Inhibitor of the Pgp
Bosutinib	• CML resistant to prior therapy	• Diarrhea, nausea, thrombocytopenia, vomiting, rash
Ponatinib	• Resistant CML and Ph^+ ALL	• Major adverse effects: thrombosis, hepatotoxicity, pancreatitis • Absorption ↓ by elevated gastric pH (H_2 antagonists, antacids, PPIs) • CYP3A4 substrate

Drug Facts for Your Personal Formulary: *Pathway-Targeted Therapies* (*continued*)

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Anaplastic Lymphoma Kinase Inhibitors		
Alectinib	• Advanced NSCLC with ALK kinase fusion gene • Advanced NSCLC with ALK kinase fusion gene and disease progression on crizotinib	• Fatigue, constipation, edema, and myalgia are common adverse effects • Pneumonitis, GI and hepatic toxicity; bradycardia and prolonged QT intervals observed
Ceritinib, crizotinib	• As above	• As above
PI3K (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase) Inhibitors		
Idelalisib	• Relapsed or refractory B-cell malignancies: CLL (with rituximab), FL, SLL • Not indicated for first-line treatment	• Serious and sometimes fatal adverse effects: hepatotoxicity, colitis, pneumonitis, intestinal perforations, skin toxicity • CYP3A substrate
mTOR (Mechanistic or Mammalian Target of Rapamycin) Inhibitors		
Temsirolimus	• Advanced renal cell carcinoma	• Adverse effects: frequent rash, mucositis, anemia, fatigue (30%–50%); rare leukopenia, thrombocytopenia, interstitial lung disease • Metabolized to a longer-lived active metabolite (sirolimus) by CYP3A4: avoid CYP3A4 inhibitors, including grapefruit juice
Everolimus	• Renal cell carcinoma • Breast cancer: advanced ER positive, HER2 negative in combination with aromatase inhibitor exemestane after failure of other aromatase inhibitors (letrozole, anastrozole) • PNETs • Progressive, well-differentiated, nonfunctional neuroendocrine GIST	• Adverse effects overlap with temsirolimus (see above) • CYP3A4 substrate
Multikinase Inhibitors		
Cabozantinib	• Advanced renal cell carcinoma	• Diarrhea, fatigue, nausea, abdominal pain • Hypertension: monitor blood pressure • Not indicated in patients with recent bleeding history or thromboembolic event • Discontinue in patients with GI perforation, fistulas • CYP3A4 substrate; ↓ dose with hepatic impairment
Vandetanib	• Progressing, locally advanced medullary thyroid cancer	• Diarrhea, colitis, rash • QT interval prolongation, torsades des pointes, sudden death • Do not use in patients with hepatic impairment, long QT syndrome • CYP3A4 substrate
Section III: Inhibitors of Tumor Angiogenesis		
Inhibitor of Hypoxia-Inducible Factor (HIF) Activity		
Belzutifan	• Inhibition of heterodimerization of HIF-2α with HIF-1β transcription factors • Clear cell RCC	Adverse effects: • Anemia due to reduction of erythropoietin • Hypertension
Inhibitors of Vascular Endothelial Growth Factor (VEGFR) and Intracellular Kinases Participating in Angiogenesis		

Sunitinib	• VEGFR2 and multiple other kinases inhibited
	• Metastatic RCC
	• GIST after imatinib resistance
	• Pancreatic neuroendocrine tumors
		• Adverse effects shared with anti-VEGF: bleeding, hypertension, proteinuria (frequent); thromboembolism, GI perforations (rare)
		• Adverse events distinct from anti-VEGF:
		  • Fatigue (50%–70%), hypothyroidism (40%–60%)
		  • Common: bone marrow suppression and diarrhea
		  • Less common: hepatotoxicity, congestive heart failure
		• Check blood pressure, blood counts, and thyroid functions at regular intervals
		• Elimination $t_{1/2}$ ~4 days: regimen in some cancers is 4 weeks on, 2 weeks off vs. continuous daily administration for other cancers

Sorafenib	• Hepatocellular carcinoma • Metastatic RCC (however, sunitinib is the first choice)	• Adverse vascular effects match with those of sunitinib • More common: fatigue, diarrhea, anorexia, rash • Less common: bone marrow suppression, GI perforation, cardiomyopathy
Axitinib	• Inhibition of VEGFR1–3 • Advanced renal cell cancer after failure of prior systemic therapy	• Adverse effects overlap with those of anti-VEGF: hypertension, thrombotic and hemorrhagic events, GI perforation • CYP3A4/5 substrate

Drug Facts for Your Personal Formulary: *Pathway-Targeted Therapies* (*continued*)

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Inhibitors of Vascular Endothelial Growth Factor (VEGFR) and Intracellular Kinases Participating in Angiogenesis (cont.)		
Lenvatinib	• Inhibition of VEGFR1–3, FGFRs, PDGFR • Recurrent or metastatic differentiated thyroid cancer • RCC in combination with everolimus • Endometrial cancer with pembrolizumab	• Adverse effects overlap with those of anti-VEGF: hypertension, thrombotic and hemorrhagic events, GI perforation • In addition: hepatotoxicity, QT prolongation • CYP3A substrate
Pazopanib	• Inhibition of VEGFR1–3, FGFRs, KIT, PDGFR • Advanced RCC and advanced soft-tissue sarcoma after prior chemotherapy	• Adverse effects overlap with those of anti-VEGF: hypertension, thrombotic and hemorrhagic events, GI perforation • In addition: hepatotoxicity, QT prolongation • CYP3A substrate
Regorafenib	• Inhibition of RET, VEGFR1, PDGFR, FGFR, TIE2, RAF1, BRAF, ABL • Metastatic colorectal cancer after previous chemotherapy and anti-VEGF or anti-EGFR • Advanced GIST after imatinib or sunitinib	• Major adverse effects: hepatotoxicity, hypertension, thrombotic and hemorrhagic events, GI perforation, and wound-healing complications • CYP3A substrate
Section IV: PARP Inhibitors		
Poly(ADP-Ribose) Polymerase Inhibitor		
Olaparib	• Ovarian cancer after three or more prior lines of treatment in patients with germline mutant BRCA	• Adverse effects: nausea, vomiting, loss of appetite, muscle and joint pain, anemia; leukemia (rare), potentially fatal MDS (rare), pneumonitis • CYP3A4 substrate
Section V: Modulators of Protein Degradation		
Thalidomide and Congeners		
Thalidomide	• Newly diagnosed multiple myeloma • Relapsed or refractory, pretreated multiple myeloma	• Most serious adverse effect: sensory neuropathy in 10%–30% of patients; may not be reversible after discontinuation of treatment; patients with preexisting neuropathy at higher risk • Teratogenic; do not use in pregnancy • Causes sedation (enhanced by CNS depressants), fatigue, constipation
Lenalidomide	• Multiple myeloma • MDS (5q– MDS) • Chronic lymphocytic leukemia (CLL)	• Marrow suppression and leukopenia (20% of patients), rare hepatic or renal toxicity • Tumor lysis in some patients with CLL ⟶ lymph node swelling and tumor flare: start at lower dose in patients with CLL • Downregulates CD20, a target for monoclonal antibody therapy • In contrast to thalidomide: little neuropathy, sedation, or constipation; lack of teratogenicity • Reduce dose in patients with reduced renal function
Pomalidomide	• AIDS-related Kaposi sarcoma	• Teratogenic; available only under a pregnancy control system
Proteasome Inhibitors		
Bortezomib	• Multiple myeloma: initial therapy and after relapse • Mantle cell lymphoma: relapsed or refractory	• Thrombocytopenia (28%), fatigue (12%), peripheral neuropathy (12%) • Neutropenia, anemia, vomiting, diarrhea, limb pain, weakness • Rare: congestive heart failure and prolonged QT intervals • Metabolized by CYP3A4; beware of drug interactions
Section VI: Epigenetic Modulators		
Panobinostat	• Patients with MM who have received at least two previous treatments, including bortezomib and an immunomodulatory agent	• Fatal cardiac ischemic events, severe arrhythmias, close cardiac monitoring during treatment • CYP3A substrate
Section VII: Other Inhibitors		
BCL2 (Antiapoptotic Protein): Orally Available Inhibitor		
Venetoclax	• CLL with 17p deletion (poor prognosis)	• Neutropenia, thrombocytopenia, diarrhea, nausea • Absorption ↑ 3- to 5-fold with a meal • CYP3A substrate

Note: CYP3A4 substrate: For drugs that are subject to hepatic metabolism by CYP enzymes, drug exposure of a patient can be affected by coadministration of inhibitors or inducers of CYP3A4 and can then reduce efficacy or increase side effects.

Embryo-fetal toxicity: Consider that all of these drugs can cause fetal harm. Advise women of the potential risk to a fetus and to avoid pregnancy while taking the drug and for 1 month after cessation of therapy. Advise men to avoid fathering a child during the same time period. Avoid lactation during therapies.

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Chapter 72

Antibodies, CAR T Cells, and Proteins to Treat Cancer

Anton Wellstein and Michael B. Atkins

I. INHIBITING GROWTH FACTOR RECEPTORS IN CANCER CELLS

INHIBITORS OF EPIDERMAL GROWTH FACTOR RECEPTOR

HER2/NEU INHIBITORS

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BISPECIFIC ANTIBODIES: CD19 AND CD3; EGFR AND MET

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ANTIBODY-DRUG CONJUGATES (ADCS)

CYTOKINE-CYTOTOXIN CONJUGATES

VI. CELLULAR THERAPY AND VACCINES

CAR T CELLS

CANCER VACCINES

VII. OTHER PROTEINS

COLONY-STIMULATING FACTORS

ASPARAGINASE

A Note on Treatment Regimens

Cancer treatment regimens change to reflect continuous advances in basic and clinical science: new drugs, both small molecules and biologicals; improved methods of targeting and timing of drug delivery; agents with altered pharmacokinetic properties and selectivities; the use of rational multidrug combinations; and greater knowledge of the basic cell biology of tumorigenesis, metastasis, and immune function, among other advances. As a consequence, this chapter presents only a few detailed treatment regimens; rather, we refer the reader to the web-based resources of the U.S. FDA (drugs@fda) and the NCCN (National Comprehensive Cancer Network). Table 71–1 provides two examples of therapeutic regimens that illustrate the complexity of current cancer drug therapy.

Development of targeted therapies for treating patients with cancer relies on the continuing process of discovery of molecular changes that drive malignant progression of human cancers. A growing number of drugs are being developed to block oncogenic pathways that lead to dysregulated cancer cell growth and survival. Targeted therapy may be combined with classical cytotoxic cancer drugs described in Chapter 70 for improved efficacy. Growth factor receptors and downstream signaling molecules are among the most actively explored targets for cancer drug discovery. The drivers of cancer growth are oncogenic pathways in malignant cells themselves (e.g., mutant or overexpressed receptors), the reaction of the tumor microenvironment (e.g., angiogenesis), and the escape of malignant cells from the host's immune surveillance (Hanahan and Weinberg, 2011). Chapter 71 describes small-molecule drugs used in targeting pathways altered in cancer. The discussion of drugs in this chapter focuses on large molecules and cell-based therapeutics of cancer and is organized into sections:

I. **Inhibiting growth factors receptors in cancer cells**
II. **Inhibiting tumor angiogenesis**
III. **Activating immune cells**
IV. **Targeting cancer cell surface molecules to engage immune cells**
V. **Cytotoxin conjugates with antibodies or cytokines**
VI. **Cellular therapy and vaccines**
VII. **Other proteins**

The proteins discussed here target oncogenic pathways that contribute to cancer growth and metastatic spread as well as immune evasion and for the most part consist of *monoclonal antibodies* or their derivatives that

Abbreviations

ADC: antibody-drug conjugate
ADCC: antibody-dependent cellular cytotoxicity
ADCP: antibody-dependent cellular phagocytosis
ALK: anaplastic lymphoma kinase
ALL: acute lymphoblastic leukemia
AML: acute myelocytic leukemia
APC: antigen-presenting cell
BCMA: B-cell maturation antigen, tumor necrosis factor receptor superfamily member 17
BiTE: bispecific T-cell–engaging antibody
CAR T: chimeric antigen receptor T cells
CCR: CC motif chemokine receptor
CDC: complement-dependent cytotoxicity
CDR: complementarity-determining regions
CLL: chronic lymphocytic leukemia
CTLA-4: cytotoxic T lymphocyte–associated protein 4
EGF(R): (human) epidermal growth factor (receptor) = HER1
FOLFIRI: folinic acid (leucovorin), 5-fluorouracil, irinotecan
FOLFOX: folinic acid (leucovorin), 5-fluorouracil, oxaliplatin
5FU: 5-fluorouracil
G(M)-CSF: granulocyte(-macrophage) colony-stimulating factor
HER1 or 2: *h*uman *E*GFR 1 or 2
HNSCC: head and neck squamous cell carcinoma
IFN: interferon
IL(-2R): interleukin (2 receptor)
JC: John Cunningham
KDR: kinase insert domain receptor = VEGFR2
KIT: feline sarcoma virus oncogene homolog
LAG3: lymphocyte activation gene 3
LVEF: left ventricular ejection fraction
mCRC: metastatic colorectal cancer
MET: mesenchymal-epithelial transition factor (= HGFR)
MHC: major histocompatibility complex protein
MM: multiple myeloma
MMAE/F: monomethyl auristatin E/F
MSI-H: microsatellite instability high
NHL: non-Hodgkin lymphoma
NK: natural killer
NSCLC: non-small cell lung cancer
PAP: prostate acidic phosphatase
PD-1: programmed cell death protein 1
PDGF(R): platelet-derived growth factor (receptor)
PD-L1: programmed cell death ligand 1
RCC: renal cell carcinoma
TCR: T-cell receptor
VEGF(R): vascular endothelial growth factor (receptor)

recognize cell surface proteins on cancer cells or on host cells or antigens shed from cancer cells (see box Antibodies as Cancer Drugs).

Over 40 different antibody-based therapeutics are currently FDA-approved for cancer treatment, many added in the past 5 years (see Figure 1–7 and Table 72–1). Notably, small-molecule inhibitors used to treat cancer impact the efficacy of antibody therapy and are used in treatment combinations that may target the same pathway or parallel and escape pathways. Indeed, triggering cell death in tumors by chemotherapy, pathway-targeted inhibitors, or radiotherapy can promote cytotoxic T-cell infiltration. The efficacy of such combinations is discussed below. Ongoing combination trials have been recently reviewed (Balogh and Nass, 2019; Petroni et al., 2021).

Antibodies as Cancer Drugs

The general concept of harnessing the immune system to treat cancer originated with William B. Coley, a professor of clinical surgery at Cornell University. He suggested that natural immunity becomes lowered during malignancy and thus thought to raise the immune reaction by injecting bacterial toxins into sarcoma (Coley, 1910) with some successes, although also with serious adverse events. The foundation for contemporary antibody-based drugs was laid by Köhler and Milstein who, in 1975, generated the first hybridoma cell lines capable of producing monoclonal antibodies. Georges Köhler, César Milstein, and Niels Jerne shared the 1984 Nobel Prize in Physiology/Medicine "for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies."

A monoclonal antibody is derived from a single B cell, recognizes a specific antigen, and can mediate cancer cell eradication by different mechanisms: (1) blocking ligands or function of cell surface receptors, (2) recruiting immune cells and complement to an antigen-antibody complex formed, (3) modulating immune cell function, or (4) carrying toxins or radionuclides as cytotoxic payloads to the cells of interest. A monoclonal antibody is generally specific for a single antigen, such as an epitope in a growth factor receptor; has a long plasma $t_{1/2}$ of days to weeks; and requires only intermittent parenteral administration. Immunoglobulin G1 (IgG1), the most frequently approved antibody isotype used in cancer treatment, can activate the complement pathway and interacts with Fcγ receptors on immune cells more potently than IgG2 or IgG4 (see Figure 72–1). Protein or carbohydrate engineering of the Fc region can alter the effector functions and mitigate complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent cellular phagocytosis (ADCP) (Goydel and Rader, 2021). Further background on monoclonal antibodies is provided in Chapters 38 and 39.

The naming of monoclonal antibodies (Table 72–1): *M*onoclonal *a*nti*b*ody names end with the syllable "mab" preceded by different morphemes that indicate the characteristics of a given antibody, mostly based on its molecular design: "xi"—fusion of human constant (Fc) and mouse variable (Fv) antibody domains to generate chimeric proteins; "zu"—insertion of antigen-binding regions (complementarity-determining regions [CDRs]) into a human IgG protein scaffold, known as humanization; and "u"—amino acid sequence fully matching the human IgG and generated in transgenic mice that carry human Ig gene loci or *in vitro* (e.g., by phage display of human antibody libraries and their affinity for the target, improved by randomization of amino acid sequences in the CDRs) (see Figure 72–1).

I. Inhibiting Growth Factor Receptors in Cancer Cells

Inhibitors of Epidermal Growth Factor Receptor

Background

The epidermal growth factor receptor (EGFR) belongs to the ErbB family of transmembrane receptor tyrosine kinases also known as ErbB1 or HER1 (*h*uman *EGFR 1*). The EGFR is expressed in epithelial tissues, including the skin, and essential for the growth and differentiation of epithelial cells. Ligand binding to the extracellular domain of EGFR family members causes receptor dimerization and stimulates the protein

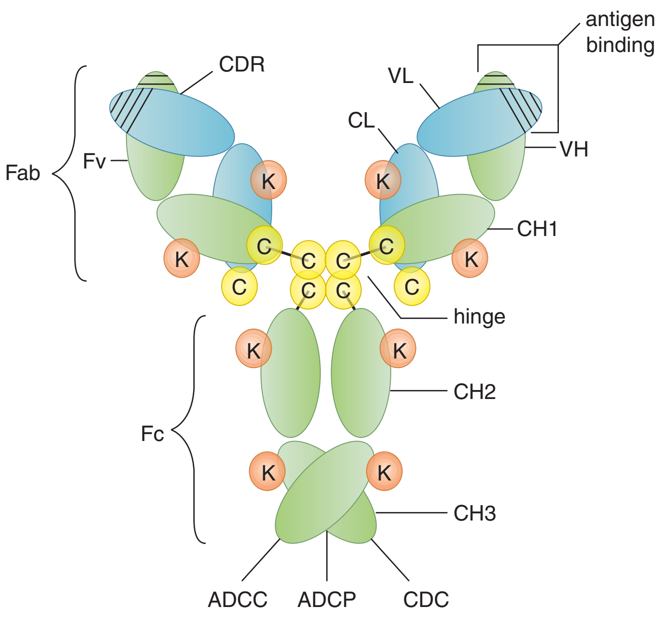

Figure 72–1 *IgG1 elements relevant for their use in cancer treatment.* Constant and variable heavy and light chains (CH, CL, VH, VL) are shown. The Fab domain contains the three complementarity-determining regions (CDR) in each of the variable chains that constitute the antigen binding site (paratope). The Fc domain modulates the effector functions, i.e., antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-dependent cellular phagocytosis (ADCP). Six disulfide bridges containing cysteine residues (C, yellow) connect the chains. The molecular mass of an IgG1 is approximately 150 kDa. Cytotoxic payloads can be linked to an IgG through the cysteine residues or through a multitude of lysine (K) residues in the different domains.

tyrosine kinase activity of the intracellular domain, resulting in autophosphorylation of several Tyr residues located in the C-terminal tail of the receptor monomers. These phosphotyrosines provide interaction sites for a variety of adapter proteins, resulting in stimulation of signaling pathways, including MAPK and PI3K/Akt pathways (Figure 72–2).

In epithelial cancers, overexpression of the EGFR is a common finding. Monoclonal antibodies (*cetuximab*, *panitumumab*, *necitumumab*) recognize the extracellular domain of the EGFR, inhibit ligand-induced signaling, and can elicit an immune response.

HISTORY EGFR/HER PATHWAY-TARGETED CANCER THERAPY

The discovery and analysis of the EGFR (= HER1) in 1984 revealed a remarkable homology of its protein kinase domain to an oncogenic protein in the avian erythroblastosis retrovirus, v-erb. This oncogenic protein was the second one isolated from v-erb, resulting in "erbB" as an alternate name for "HER" gene family members. The HER2 gene was discovered based on its homology to v-erbB in human breast cancer and its oncogenic function in rat **neu**ral tumors, hence the different names HER2/ErbB2/Neu (King et al., 1985; Schechter et al., 1985). The association of HER2 overexpression with poor prognosis in breast cancer (Slamon et al., 1987) provided the impetus for the development of HER2-targeted antibodies and a new concept in the treatment of HER2-positive breast cancers. *Trastuzumab* was the first anti-HER2 monoclonal antibody developed based on these discoveries; it was approved in the U.S. in 1998 and in Europe in 2000 for the treatment of patients with HER2-positive breast cancers. A monoclonal antibody targeting EGFR, *cetuximab*, was approved in Switzerland in 2003 and in the U.S. in 2004.

TABLE 72–1 ■ NAMING AND FORMAT OF ANTIBODY-BASED CANCER THERAPEUTICS

Please note that the nomenclature below applies to antibody drug names that were assigned by the WHO through the year 2021.

The nomenclature for future monoclonal antibodies was changed by the WHO in Oct 2021 to accommodate the expansion of this class of drugs that contains 880 "mab" entities. In newly named antibodies the stem "mab" will be replaced by four distinct stems to identify the composition of the antibody and will be indicated in future editions of this textbook and on *AccessMedicine.com* and *AccessPharmacy.com*.

ANTIBODY NOMENCLATURE	DEFINITION	EXAMPLE
"mab"	Names end with this syllable	Bevacizu*mab*
"xi"	Chimeric mAb consisting of human constant (Fc) and mouse variable (Fv) antibody domains	Cetu*xi*mab
"zu"	Humanization by insertion of antigen-binding regions (CDRs) into a human IgG protein scaffold	Trastu*zu*mab
"u"	Fully human amino acid sequence match with human IgG	Nivol*u*mab
"li"	Indicates immunomodulatory function	Cemip*li*mab

ANTIBODY FORMAT	EXAMPLES (TARGETS)	NUMBER BY 2021
IgG1	Bevacizumab (VEGF), cetuximab (EGFR), ipilimumab (CTLA-4), trastuzumab (HER2)	30
IgG2	Panitumumab (EGFR)	1
IgG4	Pembrolizumab, nivolumab (PD-1)	5
Bispecific, linked scFvs	Blinatumomab (CD3 and CD19)	2
CAR T cells	Tisagenlecleucel (CD19)	5

scFv, single-chain antibody fragment of the variable domain.

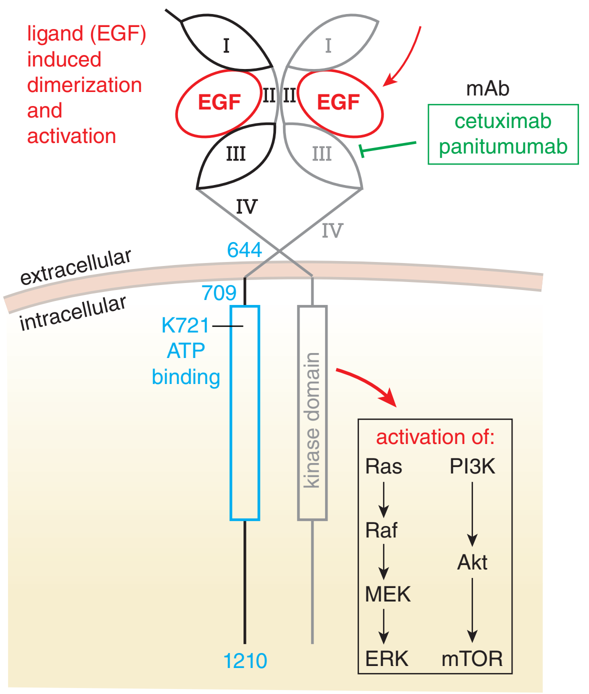

Figure 72–2 *Antibody targeting and domains of EGFR (HER1).* The extracellular portion of the EGFR contains the binding domains for growth factors that include EGF, transforming growth factor-α, and amphiregulin. Ligand binding to extracellular domains I and III induces conformational change and receptor dimerization through domains II and IV and activates intracellular signaling by cross-phosphorylation. The monoclonal antibodies *cetuximab*, *panitumumab*, and *necitumumab* block ligand binding. HER2, HER3, and HER4 share the extracellular domain organization of EGFR. mAb, monoclonal antibody. Amino acid residue numbers are in blue.

Antibody Inhibitors of EGFRs

Cetuximab

Mechanism of Action. *Cetuximab* is a chimeric human/mouse IgG1 antibody composed of the Fv regions of a murine anti-EGFR antibody with human IgG1 heavy and kappa light chain constant regions that bind to the extracellular domain III of EGFR (see Figure 72–2). Binding to EGFR prevents ligand-dependent signaling and receptor dimerization, thereby blocking growth and survival signals in normal and tumor cells. *Cetuximab* can also mediate ADCC against tumor cells that express high levels of EGFR.

ADME. *Cetuximab* exhibits nonlinear pharmacokinetics. A single loading dose of *cetuximab* intravenously is followed by weekly maintenance doses for the duration of treatment. Following intravenous administration, steady-state levels are achieved by the third weekly infusion. The volume of distribution approximates the intravascular space. The half-life of *cetuximab* is about 5 days.

Therapeutic Uses. Antibodies targeting EGFR show a different spectrum of antitumor activity than small-molecule EGFR kinase inhibitors (see Chapter 71), in part due to the additional ADCC of antibodies toward tumor cells. *Cetuximab* and related antibodies are used to treat patients with metastatic colon cancers and head and neck squamous cell carcinoma (HNSCC).

In colon cancer, *cetuximab* enhances the effectiveness of chemotherapy in patients with *KRAS* wild-type but not *KRAS*-mutant tumors (Van Cutsem et al., 2009). A combination of *cetuximab* with FOLFIRI (*folinic acid* [*leucovorin*], *5-fluorouracil* [5FU], and *irinotecan*; see Chapter 70) is typically used to treat patients with *KRAS* wild-type tumors that express EGFR. *Cetuximab* is also used as a single agent in patients who cannot tolerate *irinotecan*-based therapy and in patients with cancers that are resistant to *oxaliplatin*, *irinotecan*, and *5FU*.

In HNSCC, *cetuximab* is used in combination with radiation or with platinum complex–based chemotherapy (e.g., *cisplatin*; see Chapter 70) for locally or regionally advanced HNSCC. *Cetuximab* is also indicated as a monotherapy for patients with metastatic or recurrent HNSCC whose disease progresses on platinum-based chemotherapy.

Adverse Effects. Antibodies targeting EGFR have an adverse effect profile similar to that of first-generation EGFR protein tyrosine kinase inhibitors (see Chapter 71). Acneiform rash in the majority of patients, pruritus, nail changes, headache, and less frequently diarrhea are the most common adverse reactions. Rare but serious adverse effects include infusion reactions (~3% of patients), cardiopulmonary arrest in patients with HNSCC receiving radiation or chemotherapy (2%–3%), interstitial lung disease, and serum electrolyte imbalances, including hypomagnesemia. During pregnancy, *cetuximab* may be transmitted to the developing fetus and has the potential to cause fetal harm.

Panitumumab

Panitumumab is a human IgG2κ antibody that binds to the extracellular domain III of the EGFR and prevents ligand-dependent signaling. It is FDA approved for the treatment of EGFR-expressing metastatic colorectal cancer (mCRC) in combination with chemotherapy and as monotherapy after disease progression following chemotherapy regimens containing *fluoropyrimidine*, *oxaliplatin*, and *irinotecan* (Saltz et al., 2006). Although *panitumumab* effectively inhibits EGFR signaling similarly to *cetuximab*, it is less effective at triggering cellular immune mechanisms that may be crucial for effective therapy of HNSCC (Trivedi et al., 2016).

ADME; Adverse Effects. *Panitumumab* exhibits nonlinear pharmacokinetic characteristics. Following intravenous administration every 2 weeks, steady-state levels are achieved by the third infusion. The mean $t_{1/2}$ is 7.5 days. Adverse effects of *panitumumab* are similar to those of *cetuximab* and include rash and dermatological toxicity, infusion reactions, pulmonary fibrosis, and electrolyte abnormalities. Dermatological toxicities were reported in 90% of patients and were severe in 15% of patients receiving monotherapy.

Necitumumab

Necitumumab is a human IgG1 monoclonal antibody that binds to the extracellular domain of the EGFR. It is approved for first-line treatment of patients with metastatic, squamous NSCLC in combination with *gemcitabine* and *cisplatin*. *Necitumumab* is administered intravenously every 3 weeks prior to chemotherapy. Adverse effects are similar to those of *cetuximab*.

HER2/Neu Inhibitors

Human epidermal growth factor receptor 2 (HER2, also named Neu or ErbB2; see box on History) is a member of the HER1 to HER4 family, which shares a common extracellular domain organization that is shown in Figure 72–2 for EGFR (HER1). Of note, the fixed conformation of the extracellular domain of HER2 resembles the ligand-activated state of the other HER family members and explains the unique function of HER2 as a coreceptor that does not require ligand activation. In addition, due to this conformation, the overexpression of wild-type HER2 is sufficient to activate the intracellular tyrosine kinase and oncogenic signaling even in the absence of activating mutations, coreceptors, or ligands. Overexpression of HER2 is found in 20% to 30% of human breast cancers due to gene amplification on chromosome 17 and results in more aggressive tumors, lower response rates to hormonal therapies, and higher risk of disease recurrence after treatment. Antibodies targeting HER2 are discussed below; small-molecule kinase inhibitors of HER2 (*lapatinib*, *neratinib,* and *tucatinib*) are discussed in Chapter 71.

Trastuzumab

Mechanism of Action

Trastuzumab is a humanized IgG1 monoclonal antibody that binds to the extracellular domain IV of HER2 (see domain organization in

Figure 72–2), reduces HER2 signaling, and can induce antibody-dependent, immune cell–mediated cytotoxicity. HER2 protein overexpression or gene amplification is predictive of response to HER2-targeted therapies (Wolff et al., 2013).

ADME

Trastuzumab has dose-dependent pharmacokinetics with a mean $t_{1/2}$ of 5.8 days on a weekly maintenance dose with alternative dosing every 3 weeks. Steady-state levels are achieved between 16 and 32 weeks.

Therapeutic Uses

Trastuzumab is approved for HER2-overexpressing breast and gastric cancer. It is used in combination with cytotoxic chemotherapeutics such as taxanes as initial treatment or as a single agent following relapse of disease after cytotoxic chemotherapy (see Chapter 70).

Adverse Effects and Precautions

Acute adverse effects after infusion of *trastuzumab* are typical for monoclonal antibodies and can include fever, chills, nausea, dyspnea, and rashes. The most serious toxicity of *trastuzumab* is heart failure. Cardiotoxicity is caused by interruption of HER2/4 heterodimer signaling in cardiomyocytes, signaling that is essential for the maintenance of contractile function. The cardiotoxic potential was predicted from gene inactivation studies ("knockouts") in mice, which showed that mice lacking HER2, HER4, or HER ligands developed dilated cardiomyopathy and heart failure. Baseline electrocardiogram and cardiac ejection fraction measurement should be obtained before initiating treatment with *trastuzumab* to rule out underlying heart disease. Clinical monitoring for symptoms of congestive heart failure as well as periodic determination of left ventricular ejection fraction (LVEF) during and after the course of therapy are recommended. When *trastuzumab* is used as a single agent, fewer than 5% of patients will experience a decrease in LVEF, and about 1% will have clinical signs of congestive failure. However, left ventricular dysfunction occurs in up to 20% of patients who receive a combination of *doxorubicin* and *trastuzumab*, reflecting the added cardiotoxicity of *doxorubicin* (see Chapter 70). In contrast, the risk of cardiac toxicity is greatly reduced with the recommended combination of *trastuzumab* with taxanes.

Pertuzumab

Mechanism of Action

Pertuzumab is a humanized IgG1 monoclonal antibody directed against the extracellular receptor dimerization domain II of HER2 that is distinct from domain IV targeted by *trastuzumab* (see domain organization in Figure 72–2). *Pertuzumab*, in contrast to *trastuzumab*, prevents ligand-dependent heterodimerization of other HER family members with HER2 and thus inhibits ligand-induced signaling, cell growth, and survival. In addition to the inhibition of receptor heterodimerization, binding of *pertuzumab* to HER2-overexpressing cells can induce ADCC.

ADME

The median half-life of *pertuzumab* is 18 days; after an initial loading dose, maintenance doses are administered every 3 weeks.

Therapeutic Use

Preclinical studies with HER2-overexpressing human tumor cells grown as xenograft tumors in mice showed that the combination of *pertuzumab* and *trastuzumab* enhanced antitumor activity. In patients with HER2-positive metastatic breast cancer, the addition of *pertuzumab* to *trastuzumab* and *docetaxel* increases the median overall survival by over 1 year from 40.8 months to 56.5 months (Swain et al., 2015). Use of *pertuzumab* is approved for the treatment of patients with HER2-positive, locally advanced, inflammatory, or early-stage breast cancer in combination with *trastuzumab* and *docetaxel*.

Adverse Effects

Cardiotoxicity is similar to that of *trastuzumab*, and combinations with cardiotoxic anthracyclines are not indicated (Slamon et al., 2001). On the other hand, the combination of *pertuzumab* and *trastuzumab* does not cause any increase in cardiac toxicity (Swain et al., 2015). Based on its mechanism of action, *pertuzumab* can cause fetal harm when administered to a pregnant woman, and this risk should be weighed against the potential benefit.

Margetuximab

Margetuximab is a chimeric IgG1-targeting HER2 that was approved in 2020 for the treatment of patients with metastatic HER2-positive breast cancer who had received two or more prior anti-HER2 regimens. It is used in combination with chemotherapy and shares the risk of left ventricular dysfunction with *trastuzumab* and *pertuzumab*.

Inhibitors of Platelet-Derived Growth Factor Receptor

Signaling by the platelet-derived growth factor receptor (PDGFR) plays a significant part in mesenchymal biology, including stem cell growth, and is involved in oncogenesis through aberrant cancer cell signaling, modulation of the tumor microenvironment, and facilitation of angiogenesis and metastasis. Small-molecule protein kinase inhibitors, including *imatinib* (see discussion below), have been used in the treatment of patients with gastrointestinal stromal tumors that carry mutations in *KIT* or *PDGFRA* (see Figure 71–2).

Olaratumab

Mechanism of Action

Olaratumab is a human IgG1 monoclonal antibody that binds to PDGFRα and blocks ligand-mediated receptor activation (Tap et al., 2016).

Therapeutic Uses

Olaratumab is indicated, in combination with *doxorubicin*, for the treatment of adult patients with soft-tissue sarcoma with a histological subtype for which an anthracycline-containing regimen is appropriate and which is not amenable to curative treatment with radiotherapy or surgery. Recent studies showed that addition of *olaratumab* to *doxorubicin* doubled the median survival of patients with metastatic soft-tissue sarcoma to 26 months (Judson and van der Graaf, 2016).

Adverse Effects

The most common (≥20%) adverse effects of treatment with *olaratumab* are nausea, fatigue, neutropenia, musculoskeletal pain, inflammation of the mucous membranes (mucositis), alopecia, vomiting, diarrhea, decreased appetite, abdominal pain, neuropathy, and headache. Laboratory abnormalities are lymphopenia, neutropenia, thrombocytopenia, hyperglycemia, elevated activated partial thromboplastin time, hypokalemia, and hypophosphatemia. Other adverse effects include infusion-related reactions (low blood pressure, fever, chills, rashes) and embryofetal harm.

II. Inhibiting Tumor Angiogenesis

HISTORY

Cancer cells secrete angiogenic factors that induce the formation of new blood vessels and guarantee the flow of nutrients to the tumor cells to permit growth and metastasis. Many tumor types overexpress these angiogenic factors, turning on an "angiogenic switch" whereby the tumor cells adopt an invasive phenotype favoring proliferation of endothelial cells and neovascularization. In 1971, Judah Folkman hypothesized that the growth of solid tumors was dependent on angiogenesis and that blockade of the effects of putative angiogenic factors would be a good treatment modality for human cancers (Folkman, 1971). Folkman's hypothesis proved to be correct and led to the characterization of a number of secreted angiogenic factors, including vascular endothelial growth factor (VEGF), fibroblast growth factor, transforming growth factor-β, and PDGF. Furthermore, inhibitors of these angiogenic factors have, indeed, become useful therapeutic agents against certain cancers. VEGF is a major driver of angiogenesis, and inhibitors of VEGF signaling comprise an important class of antitumor agents. Most recently, a better understanding of the immunosuppressive features of angiogenesis has prompted the combination of antiangiogenic therapy with immune checkpoint inhibitors to enhance their efficacy, as discussed below (Huinen et al., 2021).

Inhibition of VEGF and the VEGF Receptor Pathway

Vascular endothelial growth factor initiates endothelial cell proliferation and vascular permeability when it binds to a member of the VEGF receptor (VEGFR) family, a group of highly homologous receptors with intracellular tyrosine kinase domains; these receptors include VEGFR1 (FLT1), VEGFR2 (KDR), and VEGFR3 (FLT4). The binding of VEGF to its receptors activates the intracellular VEGFR tyrosine kinase activity and initiates mitogenic and antiapoptotic signaling pathways (Nagy et al., 2007). Antibodies targeting VEGF, such as *bevacizumab*, sterically hinder the interaction of VEGF with its receptor. *Aflibercept* acts as a VEGF trap; it is a recombinant molecule that uses the VEGFR1-binding domain to sequester VEGF, basically acting as a "soluble decoy receptor" for VEGF. Several small-molecule drugs that inhibit the protein tyrosine kinase of VEGFR (*axitinib, cabozantinib, lenvatinib, pazopanib*, *sorafenib*, *sunitinib*, and *tivozanib*; see Chapter 71) as well as monoclonal antibodies that target the receptor (*ramucirumab*) have been approved for clinical use. The inhibition of endothelial function by these different approaches results in a similar spectrum of mostly cardiovascular adverse effects.

Bevacizumab

Mechanism of Action

Bevacizumab is a humanized monoclonal IgG1 antibody that binds to VEGF; the antibody contains the antigen-recognition domain of a murine antibody inserted into a human IgG1. *Bevacizumab* prevents the interaction of VEGF with its receptors on the surface of endothelial cells and inhibits receptor signaling that normally increases vascular permeability and angiogenesis. *Bevacizumab* delays progression of renal cell carcinoma (RCC) and, in combination with cytotoxic chemotherapy, is approved in the treatment of patients with mCRC, non–small cell lung cancer (NSCLC), and ovarian or cervical cancer, and also following initial therapy, in patients with glioblastoma, and in combination with *atezolizumab* (anti-PD-L1, see below) for the treatment of unresectable or metastatic hepatocellular carcinoma.

ADME

Bevacizumab is administered intravenously as a 30- to 90-min infusion every 2 weeks in patients with metastatic colon cancer and, in conjunction with combination chemotherapy, in patients with nonsquamous metastatic NSCLC every 3 weeks with chemotherapy. The antibody has a plasma half-life of about 20 days (range, 11–50 days).

Therapeutic Use in Cancer

Bevacizumab is indicated as treatment of various cancers, frequently in combination with other drugs. In metastatic colon cancer, the addition of *bevacizumab* to either FOLFOX (*folinic acid* [*leucovorin*], *5FU*, and *oxaliplatin*) or FOLFIRI (see Chapter 70) increases patient median survival by about 5 months (Hurwitz et al., 2004). In NSCLC, the addition of *bevacizumab* to *carboplatin* and *paclitaxel* increases median survival by about 2 months. Also approved are combinations of *bevacizumab* with cytotoxic chemotherapy in patients with cervical or ovarian cancer and used rarely as a combination with interferon (IFN)-α for the treatment of metastatic RCC. For glioblastoma, *bevacizumab* is approved as a single agent following prior therapy.

Other Therapeutic Uses: Wet Macular Degeneration

Vascular endothelial growth factor is an important mediator of pathologic vascular permeability. Intravitreous administration of anti-VEGF–targeted therapy has become a standard treatment of wet macular degeneration (see also Chapter 74). *Bevacizumab* and a modified fragment of *bevacizumab*, *ranibizumab*, are used. *Ranibizumab* is derived from the *bevacizumab* IgG1 by deletion of the Fc domain and changes in six amino acids in the antigen-recognition domain to increase affinity for VEGF. *Brolucizumab*, a humanized single-chain antibody fragment against VEGF, is also used for the treatment of wet macular degeneration and delivered by intravitreal injection.

Because these drugs are administered by injection into the vitreous cavity of the eye, few systemic adverse effects are observed. A direct comparison of intravitreous *bevacizumab*, *ranibizumab*, and *aflibercept* (the VEGF trap protein; see discussion that follows) in patients with diabetic macular edema showed that all three treatments improved vision, although *aflibercept* appeared more effective in patients with worse baseline levels of visual impairment (Wells et al., 2015).

Adverse Effects

Bevacizumab causes a wide range of serious class-related adverse effects that include hypertension, gastrointestinal perforation, thromboembolic events, and hemorrhage including epistaxis. A major concern is the potential for vessel injury and bleeding in patients with lung cancer. *Bevacizumab* is contraindicated for patients with a history of hemoptysis, brain metastasis, or a bleeding diathesis. In appropriately selected patients, the predicted rate of life-threatening pulmonary hemorrhage is less than 2%, and for arterial thromboembolism (stroke, myocardial infarction) observed during treatments that include *bevacizumab*, the predicted rate is less than 4%.

Other adverse effect characteristics of drugs that target the VEGF pathway include hypertension and proteinuria. A majority of patients require antihypertensive therapy, particularly those receiving higher doses and more prolonged treatment. *Bevacizumab* can be associated with congestive heart failure, probably secondary to hypertension, and with reversible posterior leukoencephalopathy in patients with poorly controlled hypertension. Gastrointestinal perforation, a potentially life-threatening complication, has been observed in up to 11% of patients with ovarian cancer. In patients with colon cancer, colonic perforation occurs infrequently (<1%) during *bevacizumab* treatment but increases in frequency in patients with intact primary colonic tumors, peritoneal carcinomatosis, peptic ulcer disease, chemotherapy-associated colitis, diverticulitis, or prior abdominal radiation treatment. Following colon cancer surgery, patients on *bevacizumab* have a higher rate (13% vs. 3.4%) of serious wound-healing complications. Because of the long $t_{1/2}$ of *bevacizumab*, elective surgery should be delayed for at least 4 weeks from the last dose of antibody, and treatment should not be resumed for at least 4 weeks after surgery.

Ramucirumab

Mechanism of Action

Ramucirumab is a human IgG1 monoclonal antibody that binds to VEGFR2, blocking the binding of VEGFR ligands and thereby inhibiting ligand-induced activity in endothelial cells.

ADME

Ramucirumab is administered intravenously every 2 to 3 weeks. Its mean half-life is 14 days.

Therapeutic Uses

Ramucirumab is used in combination with chemotherapy for the treatment of patients with mCRC with disease progression on or after prior therapy; as a single agent or in combination with *paclitaxel* for treatment of patients with advanced gastric adenocarcinoma with disease progression on or after prior chemotherapy; and in combination with *docetaxel* for treatment of patients with metastatic NSCLC with disease progression on or after chemotherapy.

Adverse Effects

The most common adverse effects are hypertension and diarrhea. Other severe adverse effects overlap with those of bevacizumab (anti-VEGF; see previous discussion): increased risk of hemorrhage, gastrointestinal perforation, and impaired wound healing.

Aflibercept

Mechanism of Action

Ziv-aflibercept acts as a trap for VEGFR ligands. It is a recombinant fusion protein that contains the extracellular VEGF-binding domain of human VEGFR1/2 fused to the Fc portion of human IgG1. *Ziv-aflibercept* acts as a soluble receptor that binds to human VEGFR ligands with high affinity (K_ds of 0.5–40 pM), reducing their plasma concentrations to levels that are too low for significant activation of their cognate receptors.

ADME

The elimination half-life is about 6 days (range, 4–7 days). *Ziv-aflibercept* is administered by intravenous infusion over 1 h every 2 weeks.

Therapeutic Uses

In combination with FOLFIRI, *ziv-aflibercept* is indicated for patients with mCRC that is resistant to or has progressed following an *oxaliplatin*-containing regimen.

Adverse Effects

The most common adverse effects are hypertension and diarrhea. Other severe adverse effects overlap with those of *bevacizumab* (anti-VEGF; see previous discussion): increased risk of hemorrhage, gastrointestinal perforation, and impaired wound healing.

III. Activating Immune Cells

HISTORY

The discovery of the T-cell receptor (TCR) and of costimulatory signal pathways in the period from 1982 to 1992 defined essential requirements for T-cell activation during antigen presentation: In addition to the antigen-TCR interaction ("signal 1"), CD28 on T cells binds to B7 proteins on antigen-presenting cells (APCs) ("signal 2"; Figure 72–3; see also Figures 38–2, 38–3, and 38–4). The finding, in the mid-1990s, of coinhibitory molecules that can dampen T-cell activation revealed a potential mechanism for drugs to modulate immune checkpoints and alter anticancer immune activity. Tasuku Honjo and James Allison shared the 2018 Nobel Prize in Physiology or Medicine for "for their discovery of cancer therapy by inhibition of negative immune regulation." TCR activation induces expression of the inhibitory cytotoxic T lymphocyte–associated protein 4 (CTLA-4), a CD28 homolog with higher affinity for the B7 ligands than CD28. CTLA-4 inhibits T-cell activation to control autoimmune tissue damage. Programmed cell death 1 (PD-1), an inhibitory molecule expressed on T cells after activation, and PD-L1, the ligand for PD-1, were described in the early 2000s. It became obvious that T cells present in human tumors were being inhibited by factors in the tumor tissues: T cells isolated from cancer patients and activated exogenously showed antitumor efficacy, paving the way for targeting immune inhibition. Improved survival of patients with metastatic melanoma after anti–CTLA-4 treatment led to FDA approval of *ipilimumab* in 2011; anti–PD-1 drugs *pembrolizumab* and *nivolumab* were approved in 2014 for the treatment of patients with metastatic melanoma. Clinical trials in multiple types of cancer have been ongoing since then and have led to the approval of immune checkpoint inhibitors for more than 20 additional indications as described in this section. Milestones in the discoveries and more details can be found in a review by Korman et al. (2021).

Immune Checkpoint Inhibitors

Recent striking successes of cancer immunotherapy have given credence to the long-held hope that the immune system may be unleashed to treat cancer. The conceptual breakthrough is based on the discovery of inhibitory signals that limit T cell activation, so-called immune checkpoints. The discovery of receptors, ligands, and their function in the control of immune cell activity during the past two decades led to the development of clinically effective monoclonal antibodies that enable antitumor T cells to eradicate cancer cells with acceptable adverse effects (Figure 72–3).

Blocking immune checkpoints on cytotoxic T cells enables them to eradicate cancer cells that express antigens recognized by the T cells. Sustained eradication of metastatic melanoma in a significant number of patients was an impressive outcome of initial clinical trials with a monoclonal antibody, *ipilimumab*, which is an immune checkpoint inhibitor (Robert et al., 2013). Note that immune checkpoint inhibition is distinct from conventional approaches such as vaccination with a known antigen (to generate T cells that recognize cells expressing the antigen); nonspecific stimulation of T cells; isolation, *ex vivo* expansion, and administration of tumor-infiltrating lymphocytes; or approaches using antigen-responsive T cells that express a specific chimeric antigen receptor (CAR). The different approaches are described next.

Mechanisms of T Cell Activation

Antigen-mediated activation of T cells is initiated by engagement of the TCR with antigen presented on major histocompatibility complex (MHC) protein on the surface of an APC, "signal 1." In addition, engagement of CD28 costimulatory molecules on T cells is required for effective T-cell activation that includes cell proliferation, migration, and production of cytokines. This "signal 2" is provided by B7 surface proteins on APCs, for example, CD80 (B7-1) or CD86 (B7-2) (see Figures 38–4 and 72–3). T-cell activation is tightly controlled by immune-suppressive cells and cytokines as well as by coinhibitory molecules present on T cells themselves, such as CTLA-4 or PD-1 (see Figure 72–3). CTLA-4 is expressed by activated CD4 and CD8 T cells and competes with costimulatory CD28 for binding to the B7 protein ligands. Binding of CTLA-4 to B7 proteins interrupts the initial CD28 costimulatory signals and serves as an early negative regulator of the T-cell activation responses, that is, an *immune checkpoint*. Additional control of T-cell activity is provided by later inhibitory signals through other molecules, such as PD-1 that binds the ligand PD-L1. PD-1 is mainly expressed by activated CD4 and CD8 T cells as well as B cells and macrophages.

Blocking monoclonal antibodies to CTLA-4, PD-1, or PD-L1 are currently used to neutralize coinhibitory receptors during T-cell priming (CTLA-4), to maintain cancer antigen-specific T-cell function, and to provide a sustained T-cell response (PD-1) that includes increased production of cytokines such as tumor necrosis factor-α and IFN-γ, as well as granzyme B. Antibodies that target different checkpoint molecules are discussed in the following sections: *ipilimumab* (anti–CTLA-4); *atezolizumab, avelumab, durvalumab* (anti–PD-L1); *nivolumab, pembrolizumab, cemiplimab, dostarlimab* (anti–PD-1); and *relatlimab* (anti-LAG3).

Inhibitors of CTLA-4

CTLA-4 is upregulated during the antigen priming of T cells and binds B7 on APCs to attenuate the T-cell response and thus reduce the risk for chronic autoimmune-dependent inflammation (see Figure 72–3).

Ipilimumab

Ipilimumab is a human IgG1 monoclonal antibody that binds to CTLA-4 and was the first immune checkpoint inhibitor approved for cancer treatment.

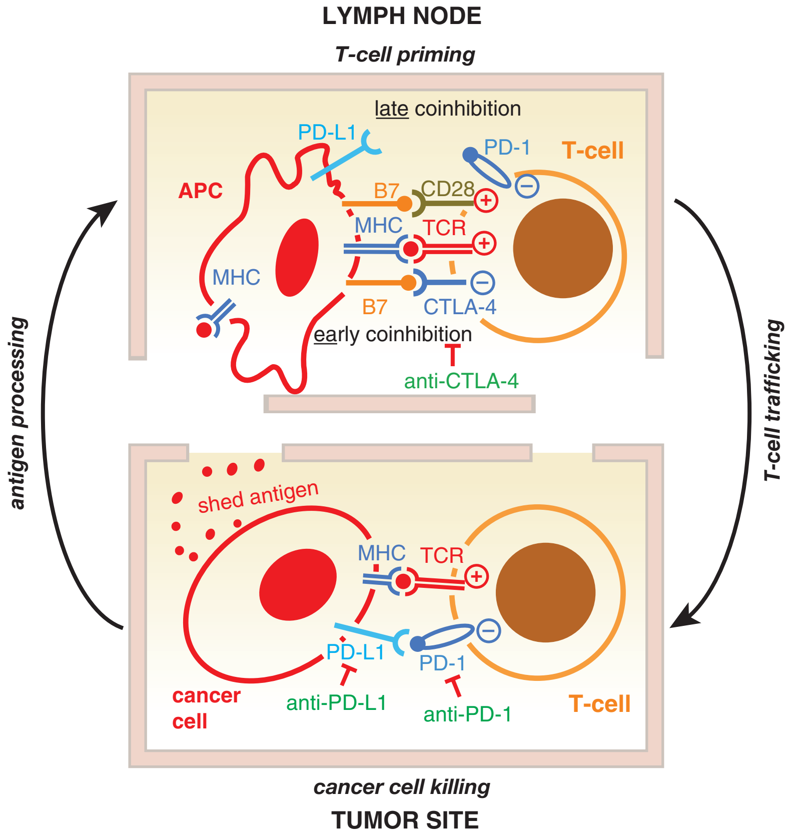

Figure 72–3 *Targeting of immune checkpoints.* Antigens shed from dead cancer cells are captured and processed by APCs. APCs travel to the regional lymph nodes and present the antigen bound to MHC to stimulate TCRs (+). Costimulatory signals leading to T-cell activation are provided by the interaction between CD28 on T cells and B7 (CD80, CD86) on APCs. Inhibitory molecules, including cytotoxic T lymphocyte–associated protein 4 (CTLA-4) and programmed cell death 1 (PD-1), are induced during immune responses and represent a "checkpoint" to dampen T-cell hyperactivation. Sequence homology within the extracellular binding domains of CTLA-4 and CD28 allows binding to the B7 ligands. Higher affinity and avidity of CTLA-4 for the ligands leads to a dampening of the immune response. Human PD-1 is expressed on T cells after TCR stimulation and binds the B7 homologues PD-L1 (B7-H1) and PD-L2 (B7-DC) on APCs but can be induced in nonhematopoietic cells (e.g., by cytokines). Activated T cells also upregulate PD-1 on their surface. (Figures 38–3 and 38–4 show additional details of T-cell activation.)

Activated effector T cells capable of killing cancer cells traffic from the lymph node to the tumor site, where they recognize cancer cells by the interaction of the TCR with the MHC-antigen complex and kill cancer cells through cytolysis (release of perforin and granzyme). This triggers additional antigen release that can induce subsequent rounds of anticancer immunity. Release of IFN-γ upon TCR recognition of the MHC-neoantigen complex induces upregulation of PD-L1 on the surface of tumor cells. PD-L1 binds to PD-1 on the surface of activated T cells, dampening the immune response in the tumor microenvironment.

T-cell checkpoint inhibitors can enhance T-cell priming through blockade of CTLA-4 (*ipilimumab*) or by T-cell activation in the tumor via blocking the PD-1/PD-L1 interaction with antibodies to PD-1 (*cemiplimab, dostarlimab, nivolumab, pembrolizumab*) or PD-L1 (*atezolizumab, avelumab, durvalumab*). Antibodies that block inhibitory immune checkpoints allow a sustained T-cell response that also includes an increased production of cytokines and T-cell proliferation. (Reviewed in Waldman et al. [2020].)

Mechanism of Action

Ipilimumab blocks the interaction of CTLA-4 with B7 ligands on APCs and thereby augments T-cell activation (see Figure 72–3). Inhibition of CTLA-4 signaling can also expand the T-cell repertoire (Cha et al., 2014) and inhibit regulatory T-cell function that dampens cytotoxic T-cell activity and thus increases the antitumor immune response further (Mellman et al., 2011) as well as induce T-cell memory (Felix et al., 2016) that prolongs the immune response.

ADME

Ipilimumab is administered intravenously and shows linear pharmacokinetics in the dose range of 0.3 to 10 mg/kg, with a terminal half-life of 15.4 days.

Therapeutic Uses

A pivotal trial in patients with metastatic melanoma showed improved median overall survival after *ipilimumab* treatment (Hodi et al., 2010). This trial led to FDA approval of *ipilimumab* for the treatment of patients with metastatic melanoma. Further studies showed that some patients had clinical responses that lasted for 10 or more years (Schadendorf et al., 2015). The tumor response can take several months to manifest, and tumors may increase in size during this period, in part due to the evolving inflammatory reaction (Mellman et al., 2011). *Ipilimumab* is approved for treatment of patients with advanced melanoma as a single agent or in combination with *nivolumab*; other approved indications in combination with *nivolumab* are advanced RCC; colorectal cancer with high microsatellite instability (MSI-H) or mismatch repair deficient; pretreated hepatocellular carcinoma; metastatic NSCLC with no EGFR or anaplastic lymphoma kinase (ALK) genomic aberrations; and malignant pleural mesothelioma. Recommended dosing of *ipilimumab* is 3 mg/kg intravenously over 90 min every 3 weeks for a maximum of four doses.

Adverse Effects

Blockade of CTLA-4 compromises immune tolerance to some normal tissue antigens and provokes inflammatory toxicities, mostly in the skin, pituitary gland, thyroid, intestine, and liver. The activation of T-cell responses by *ipilimumab* treatment leads to immune-related toxicities in the majority of patients (73.6%), with 18.6% reaching grade 3 to 4 toxicity (Callahan et al., 2016). Adverse effects on the skin (pruritus, rash, vitiligo) and gastrointestinal tract (diarrhea, colitis) are the most frequent, whereas immune-mediated hepatitis, hypophysitis, and hypo- or hyperthyroidism are less frequent. Withholding treatment because of moderate adverse reactions until return to baseline and high-dose corticosteroids for severe immune-mediated reactions are recommended (Dougan et al., 2021; Esfahani et al., 2020).

Tremelimumab

Tremelimumab (formerly *ticilimumab*) is a human IgG2 monoclonal antibody that targets CTLA-4; it is under investigation in several clinical trials and received orphan drug designation by the FDA for the treatment of malignant mesothelioma.

Inhibitors of PD-1

Activation of the PD-1 checkpoint pathway in T cells by PD-L1 or PD-L2 evokes a negative regulatory immune response and inactivates T cells (see Figure 72–3). Tumor cells can exploit this pathway by presenting the PD-L1 ligand to tumor-infiltrating T cells and thus shield the tumor from T cell–mediated destruction. Presentation of PD-L1 to activated T cells results in T-cell exhaustion, whereas antibody-mediated blockade of PD-1 can restore or maintain T-cell antitumor response.

Nivolumab

Mechanism of Action

Nivolumab is a human monoclonal IgG4 antibody that blocks the interaction between PD-1 and its ligands.

ADME; Clinical Use

Nivolumab is administered as an intravenous infusion every 2 (or 4) weeks until the time of disease progression or unacceptable toxicity. The drug has an elimination half-life of 26.7 days. *Nivolumab* is FDA approved as a single agent for the treatment of a variety of cancers, including advanced or resected high-risk melanoma, previously treated advanced NSCLC, RCC, advanced head and neck cancer, MSI-H or mismatch repair–deficient colorectal cancer, hepatocellular carcinoma, and relapsed/refractory classical Hodgkin lymphoma. Quite strikingly, *nivolumab* provided an overall response rate of 87% in patients with Hodgkin lymphoma who had failed treatment with *brentuximab vedotin* (see discussion that follows).

Adverse Effects

The most common adverse effect of treatment in patients with melanoma is a rash (>20%); in patients with advanced squamous NSCLC, the adverse effects are fatigue, dyspnea, pneumonitis, musculoskeletal pain, decreased appetite, cough, nausea, and constipation. Immune-mediated adverse reactions are much less common than with CTLA-4 antibodies; corticosteroids are recommended based on the severity of the reaction. For pneumonitis, colitis, hepatitis, nephritis, and renal dysfunction, withhold treatment if the adverse effects are moderate and discontinue treatment if the effects are severe (Dougan et al., 2021; Esfahani et al., 2020).

Pembrolizumab

Pembrolizumab (formerly called *lambrolizumab* or MK-3475) is a humanized monoclonal IgG4κ isotype antibody that blocks interaction between PD-1 and its ligands. This humanized antibody has a mouse variable region grafted onto a human antibody framework and shows high affinity toward PD-1 receptors of humans and other primates but no appreciable affinity for mouse or rat PD-1.

ADME; Clinical Use

The recommended treatment schedule for *pembrolizumab* is 2 mg/kg as an intravenous infusion over 30 min every 3 weeks (or a flat dose of 200 mg every 3 weeks or 400 mg every 6 weeks). The elimination half-life is 26 days. In clinical trials, *pembrolizumab* demonstrated an overall response rate of 26% in patients with *ipilimumab*-pretreated and refractory advanced melanoma (Hamid et al., 2013; Robert et al., 2014). *Pembrolizumab* is more effective and less toxic than *ipilimumab* in patients with either treatment-naïve or BRAF inhibitor–pretreated melanoma. *Pembrolizumab* is currently approved for the treatment of 18 different cancers that include advanced and high-risk and intermediate risk resected melanoma, NSCLC (either prior to or after platinum-containing chemotherapy), head and neck cancer, urothelial cancer, Merkel cell carcinoma, Hodgkin lymphoma, triple-negative breast cancer and intermediate-high risk RCC. The approval for *pembrolizumab* includes any cancer with DNA mismatch repair deficiency or MSI-H that leads to a higher mutation load followed by the generation of mutant protein antigens by cancer cells that can activate the immune system. This first cancer tissue–agnostic approval extends immune checkpoint inhibitor treatment to subsets of patients with hereditary colorectal cancer due to Lynch syndrome (MSI-H) and up to 20% of patients with sporadic colon cancer. The frequency is less in endometrial, ovarian, and pancreatic cancers and requires testing of each patient's tumor to assess suitability of the treatment. More recently, the tissue-agnostic approval for *pembrolizumab* was broadened to advanced cancers with high tumor mutational burdens of 10 or more mutations per megabase, which includes a large fraction of solid tumors, as described in Chapter 69 (see Figure 69–3).

Adverse Effects

The most common adverse effects, experienced by more than 20% of patients, are fatigue, cough, nausea, pruritus, rash, decreased appetite, constipation, arthralgia, and diarrhea. Serious adverse events include immune-mediated inflammation, specifically pneumonitis, colitis, hepatitis, hypophysitis, and both hyper- and hypothyroidism. Withholding treatment and administration of systemic corticosteroids for grade 2 or higher grade adverse events or discontinuing treatment with more severe adverse effects is recommended. Because human IgG4 can cross the placenta, the benefit from *pembrolizumab* treatment should be balanced with the potential fetal risk in a pregnant woman.

Cemiplimab

Cemiplimab is a human IgG4 monoclonal antibody that binds to PD-1 and blocks the pathway. It was approved in 2018 for treating patients with advanced cutaneous squamous and in 2021 for the treatment of patients with basal cell carcinoma as well as first-line treatment of those with locally advanced or metastatic NSCLC with high PD-L1. Adverse effects are typical for PD-1 blockade and include immune-related inflammation of different organs.

Dostarlimab

Dostarlimab is a humanized IgG4 monoclonal PD-1–blocking antibody approved in 2021 for the treatment of patients with mismatch repair–deficient chemotherapy-resistant endometrial cancer.

Antagonists of PD-L1

Programmed cell death 1 has two known ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC), each with a distinct expression profile. The PD-L1 ligand is expressed on APCs, T cells, B cells, and nonhematopoietic cells, which can include cancer cells (see Figure 72–3). Expression of PD-L2 is restricted to APCs, macrophages, myeloid dendritic cells, and mast cells. PD-L1 is expressed in many cancers and thus can suppress the activation of cytotoxic T cells that enter the tumor. Anti–PD-L1 antibodies can block this inhibitory effect and promote an effective antitumor response. *Atezolizumab*, *durvalumab*, and *avelumab* are approved antibody inhibitors of PD-L1 with similar profiles.

Atezolizumab

Mechanism of Action

Atezolizumab, also known as MPDL3280A, is a humanized IgG1 monoclonal antibody to PD-L1 approved in 2016.

ADME

Atezolizumab is administered as an intravenous infusion over 60 min every 3 weeks. The terminal half-life is 27 days.

Clinical Use

Atezolizumab is approved for the treatment of different advanced or metastatic cancers: urothelial cancer, NSCLC, small cell lung cancer (with *carboplatin* and *etoposide*, see Chapter 70), triple-negative breast cancer (with *paclitaxel*, see Chapter 70), hepatocellular carcinoma (with *bevacizumab*), and melanoma (with *cobimetinib* and *vemurafenib*, see Chapter 71). High PD-L1 expression is a prerequisite for several of these indications.

Adverse Effects

The most common adverse effects in 20% or more of patients with metastatic NSCLC are fatigue, decreased appetite, dyspnea, cough, nausea, musculoskeletal pain, and constipation. Patients with urothelial carcinoma may report urinary tract infection. Possible immune-related adverse effects include hepatitis, colitis, hypophysitis, thyroid disorders, adrenal insufficiency, and rarely, type 1 diabetes mellitus, pancreatitis, myasthenia gravis, Guillain-Barré syndrome, and ocular inflammation; these may require discontinuation of treatment. Due to potential embryofetal toxicity, female patients should be advised accordingly.

Durvalumab

Durvalumab is a human IgG1κ monoclonal antibody to PD-L1 and was initially approved in 2017 for the treatment of metastatic urothelial cancer. It is currently approved for advanced NSCLC and for first-line treatment of small cell lung cancer (with *etoposide* and either *carboplatin* or *cisplatin*, see Chapter 70).

Avelumab

Avelumab is a human IgG1κ monoclonal antibody to PD-L1 and was approved in 2017 for the treatment of metastatic urothelial and Merkel cell cancer. It is also approved for RCC as first-line treatment in combination with *axitinib*, a VEGFR kinase inhibitor (see Chapter 71).

Combination of Anti–PD-1 and Anti–CTLA-4

Because anti–CTLA-4 and anti–PD-1 target distinct immune checkpoints during T-cell activation, preclinical studies have shown that concurrent targeting of CTLA-4 and PD-1 significantly improves therapeutic efficacy when compared to effects of single agents (Curran et al., 2010). In patients with advanced melanoma, the combination of *ipilimumab* and *nivolumab* produces a tumor response in more than 50% of the patients, and 5-year overall survival is highest in the combination treatment group, with 52% survival versus 44% with *nivolumab* only and 26% with *ipilimumab* only (Larkin et al., 2019). Immune-related adverse effects are similar, but more frequent than with the single agent, although combination therapy increases the rate of rare but fatal myocarditis (combination 0.17%; nivolumab <0.01%) (Johnson et al., 2016). Autoimmune reactions can be managed by dose delays, glucocorticoid treatment, and immunosuppressant treatment reviewed by Dougan et al. (2021) and Esfahani et al. (2020). Immune checkpoint inhibitors also induce regression of melanoma metastases at intracranial and visceral sites in over half of the patients, and integration of immune checkpoint inhibitors with other treatment approaches for melanoma can further improve this (Atkins et al., 2021; Curti and Faries, 2021). Combinations of immune checkpoint inhibitors with other cancer therapies are being evaluated in numerous international clinical trials and are becoming the standard of care in solid and hematological malignancies (Balogh and Nass, 2019).

Immune Checkpoint Targeting Beyond CTLA-4 and PD-1: Anti-LAG3

LAG3 (lymphocyte activation gene 3), TIM3 (T cell immunoglobulin and mucin domain-3), and TIGIT (T cell immunoreceptor with Ig and ITIM domains) are other cell surface receptors that negatively regulate the responsiveness of immune cells and are thus potential targets for immune modulatory therapies (Kraehenbuehl et al., 2022). LAG3 targeting enhanced anti-PD-1 efficacy in preclinical models and was evaluated recently in a Phase III trial in patients with untreated advanced melanoma. Addition of *relatlimab* (anti-LAG3, human IgG4) to *nivolumab* (anti-PD-1) treatment improved median progression-free survival of the patients from 4.6 to 10.1 months. Adverse events increased in frequency and matched those known for anti-PD-1 treatment (Tawbi et al., 2022). This establishes the validity of dual immune checkpoint inhibition beyond the combination of anti-PD-1 and anti-CTLA-4 discussed above.

Impact of the Microbiome on Immune Checkpoint Inhibitor Therapy

Most recently, the impact of the composition of the gut microbiome on the efficacy of immune checkpoint inhibitor treatment of cancer has become an active area of research. Fecal transplants are one tool applied in ongoing clinical trials and are reviewed by Woelk and Snyder (2021); see also Chapter 6.

Cytokines to Stimulate Immune Responses

Interleukin-2

Of the 70 or so proteins and glycoproteins that are classified as cytokines, only IFN (see Chapters 38 and 39) and interleukin (IL)-2 are in routine clinical use, often for actions against cancer.

Activated CD4$^+$ T cells are the primary producers of IL-2. The functions of IL-2 are myriad; among other roles, IL-2 promotes the growth of T cells and natural killer (NK) cells, stimulates the proliferation of and antibody production by B cells, and drives the development of regulatory T cells (mediators of tolerance and suppression). As noted by Liao and colleagues (2013), "IL-2 has broad essential biological actions, not only driving T cell proliferation and modulating effector cell differentiation, but also limiting potentially dangerous autoimmune reactions." This statement provides a general rationale for the clinical use of IL-2 and the likelihood that IL-2 administration might also engender adverse effects.

Mechanism of Action

Interleukin-2 stimulates the proliferation of activated T cells and the secretion of cytokines from NK cells and monocytes. IL-2 stimulation increases cytotoxic killing by T cells and NK cells.

Interleukin-2 is a 133–amino acid glycoprotein (molecular weight ~15 kDa) produced by activated T cells and NK cells; it promotes activated T-cell proliferation and enhanced killing by NK cells. Responsiveness depends on expression of the IL-2 receptor (IL-2R). The receptor comprises three independently expressed membrane monospans, α, β, and γ, which can assemble in several combinations to produce working receptors of varying affinities. These monospans are variously expressed in lymphohematopoietic cells, lack C-terminal protein kinase activities, and transduce signals via coupling to Jak proteins (Figure 72–4). In contrast, nearly all types of cancer cells lack receptor expression and are unresponsive to IL-2.

ADME

Interleukin-2 (as recombinant IL-2, *aldesleukin*) is administered intravenously. The serum $t_{1/2}$ of IL-2 after intravenous administration has an

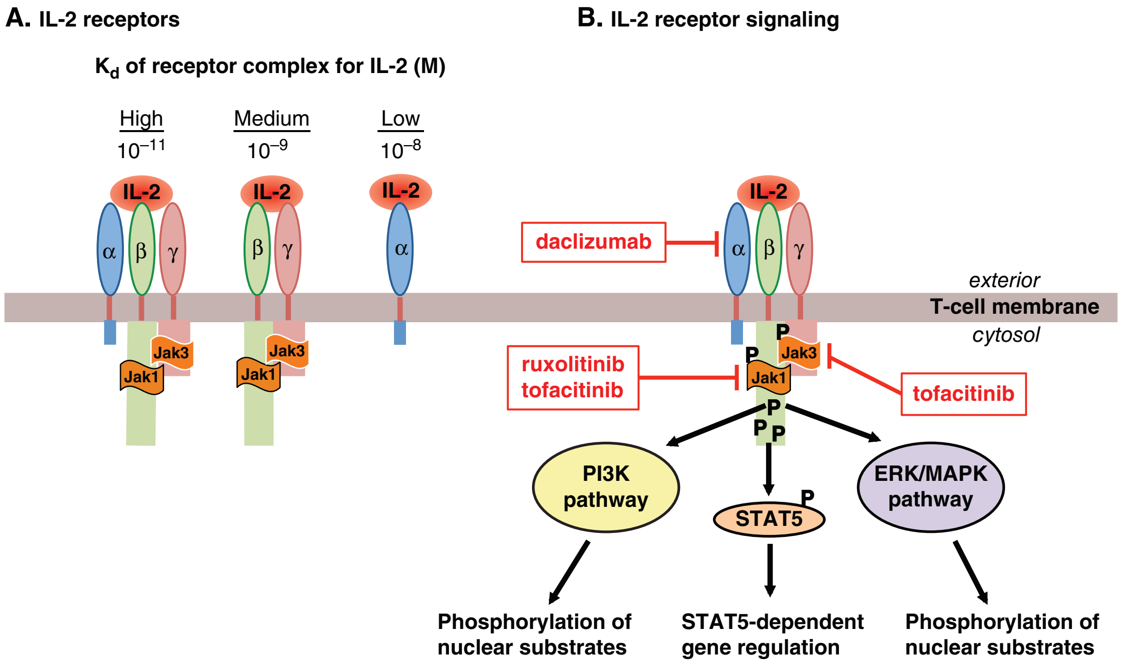

Figure 72–4 *A pharmacologist's view of IL-2 receptors, their cellular signaling pathways, and their inhibition.* **A.** *IL-2 receptors.* The IL-2 receptor has three components: an α chain, a 55-kDa protein (CD25) involved mainly in IL-2 binding; a β chain, a 75-kDa protein that binds to Jak1; and a γ chain, a 64-kDa protein that signals via Jak3. These components combine as shown to produce receptors of varying affinities and with different capacities for intracellular signaling. The γ chain is a component of many cytokine receptors (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21). Resting T cells have a low density of IL-2Rα; however, activation of the TCR (see Figure 38–2) and IL-2, itself, induce IL-2Rα expression. IL-2Rα can also be induced by a large variety of cytokines, viruses, and activators of protein kinase C. The β and γ monospans are constitutively expressed on many lymphohematopoietic cells, and their expression can also be regulated. Tumor cells generally lack receptor expression and are unresponsive to IL-2. **B.** *IL-2 receptor signaling.* The formation of the quaternary complex of IL-2 and the α, β, and γ units of IL-2R suffice to activate intracellular signaling. The IL-2R monospans shown in **A** lack the protein tyrosine kinase activities common to many monospanning hormone receptors. Rather, these receptors respond to IL-2 binding at the extracellular domain by differentially recruiting Jak1 and Jak3 to their cytosolic domains, Jak1 at IL-2Rβ and Jak3 at IL-2Rγ. Heterodimerization of β and γ cytosolic domains leads to activation of Jak1 and Jak3. The Jaks transactivate and also phosphorylate key tyrosines on IL-2Rβ. These phosphotyrosines (**P**) permit important protein-protein interactions that direct downstream signaling:

- Binding of an SHC adapter/scaffolding protein that is the platform for activation of the Ras/MAPK pathway and the PI3K pathway
- Recruitment and phosphorylation of STAT5 (to a lesser extent STATs 1 and 3), leading to STAT-dependent gene regulation

IL-2/IL-2R signaling can be therapeutically targeted by inhibitors that interact with IL-2Rα, Jak1, and Jak3 (red boxes and T bars), as is discussed in Chapters 39, 71, and 75.

α phase of about 13 min and a β phase of about 90 min. IL-2 is excreted in the urine as an inactive metabolite.

Therapeutic Uses

Aldesleukin possesses the biological activities of native human IL-2. The drug is approved for use in patients with metastatic RCC and metastatic melanoma. High-dose IL-2 produces an overall response rate of 15% to 25% in patients with RCC; 8% achieve a complete response. In patients with metastatic melanoma, high-dose IL-2 induces an overall response rate of 16% to 22%, and 6% achieve a complete response. In both instances, responses last a median of about 5 years. Low-dose IL-2 also produces responses, but few are complete responses, and the duration appears less than with high-dose IL-2.

Adverse Effects

Interleukin-2 toxicities are dominated by the capillary leak syndrome, in which intravascular fluid leaks into the extravascular space, producing hypotension, edema, respiratory difficulties, confusion, tachycardia, oliguric renal failure, and electrolyte problems. Other adverse effects include fever, chills, malaise, nausea, vomiting, and diarrhea. Laboratory abnormalities include thrombocytopenia, abnormal liver function tests, lymphopenia and eosinophilia. Most patients develop a pruritic skin rash. Hypothyroidism may occur. Cardiac arrhythmias are a rare complication. These toxicities can be life threatening, yet nearly all are reversible within 24 to 48 h of discontinuing therapy. Patients should have normal renal and hepatic function, adequate respiratory reserve (FEV1 > 2 L), and a normal exercise tolerance test before beginning therapy and should be closely supervised in an inpatient facility capable of care at the intensive care unit level during drug administration.

IV. Targeting Cancer Cell Surface Molecules to Engage Immune Cells

HISTORY

The first therapeutic antibody approved for oncology patients in 1997, *rituximab*, is a chimeric mouse/human monoclonal antibody that binds to the CD20 surface antigen on B cells and has improved outcomes for patients with B-cell malignancies. CD20 is a glycosylated tetra-transmembrane phosphoprotein with intracellular N- and C-terminal regions and a small and a large extracellular loop expressed on developing B cell and many B-cell malignancies. *Rituximab* binds to the large extracellular loop, whereas *ofatumumab*, approved in 2009, binds to a distinct epitope on the large extracellular loop as well as sites on the small loops. *Obinutuzumab*, approved in 2013, is derived from *rituximab* with modifications of its glycosylation to improve immune effector effects. *Bi*specific *T*-cell–*e*ngaging (BiTE) antibody targeting of cancer cell surface molecules is based on antibody engineering concepts that won first approval with *blinatumomab* in 2014: *Blinatumomab* bridges cytotoxic T cells and cancer cells independent of MHC-bound antigen recognition. BiTE antibodies are under development for the treatment of a range of cancers; other bifunctional antibody approaches under development have been reviewed by Goebeler and Bargou (2020). See also Chapter 38.

CD19, CD20, CD52, CD38, CCR4, GD2, and SLAMF7

Rituximab (CD20)

Rituximab is a chimeric murine/human monoclonal IgG1 antibody that targets the CD20 B-cell surface antigen. On binding to the large extracellular loop of CD20, *rituximab* mediates B-cell lysis mostly through CDC and ADCC. In patients with non-Hodgkin lymphoma (NHL), administration of *rituximab* depletes circulating and tissue-based B cells within the first 3 weeks, with sustained depletion for 6 to 9 months after treatment in a majority of patients. Median B-cell levels return to normal by 12 months.

ADME

The drug is administered by intravenous infusion both as a single agent and in combination with chemotherapy. *Rituximab* has a $t_{1/2}$ of about 22 days. As a single agent, it is given weekly for 4 weeks, with maintenance dosing every 3 to 6 months. In combination regimens, the drug may be administered every 3 to 4 weeks, with chemotherapy, for up to eight doses. The rate of infusion should be increased slowly to prevent serious hypersensitivity reactions. Pretreatment with antihistamines, *acetaminophen*, and glucocorticoids decreases the risk of hypersensitivity reactions. Patients with large numbers of circulating tumor cells (as in chronic lymphocytic leukemia [CLL]) are at increased risk for tumor lysis syndrome; in these patients, the initial dose should be no more than 50 mg/m^2 on day 1 of treatment, and patients should receive standard tumor lysis prophylaxis.

Therapeutic Uses

Rituximab is approved as a single agent for relapsed indolent lymphomas and significantly enhances response and survival in combination with chemotherapy for the initial treatment of diffuse large B-cell lymphoma. *Rituximab* improves response rates when added to combination chemotherapy for other indolent B-cell NHLs, including CLL, mantle cell lymphoma, Waldenström macroglobulinemia, and marginal zone lymphomas. Maintenance of remission with *rituximab* delays time to progression and improves overall survival in indolent NHL. It is increasingly used for treatment of autoimmune diseases such as rheumatological disease, thrombotic thrombocytopenic purpura, autoimmune hemolytic anemias, cryoglobulin-induced renal disease, and multiple sclerosis.

Resistance and Adverse Effects

Resistance to *rituximab* may emerge through downregulation of CD20, impaired antibody-dependent cellular cytotoxicity, decreased complement activation, limited effects on signaling and induction of apoptosis, and inadequate blood levels. Polymorphisms in two of the receptors for the antibody Fc region responsible for complement activation may predict the clinical response to *rituximab* monotherapy in patients with follicular lymphoma but not in CLL. *Rituximab* infusion reactions can be life threatening, but with pretreatment, reactions are usually mild and limited to fever, chills, throat itching, urticaria, and mild hypotension. All respond to decreased infusion rates and antihistamines. Uncommonly, patients may develop severe mucocutaneous skin reactions, including Stevens-Johnson syndrome. *Rituximab* may cause reactivation of hepatitis B virus or, rarely, JC (John Cunningham) virus (with progressive multifocal leukoencephalopathy). Hypogammaglobulinemia and autoimmune syndromes (idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura, autoimmune hemolytic anemia, pure red cell aplasia, and delayed neutropenia) may appear 1 to 5 months after administration.

Ofatumumab (CD20)

Ofatumumab is a human IgG1 that binds to CD20 at sites on the large and small extracellular loops of CD20, distinct from the site targeted by *rituximab*. Antibody binding results in B-cell lysis via CDC and ADCC. *Ofatumumab* is approved for treating patients with CLL after failure of *fludarabine* and *alemtuzumab* (see below). A complex dosing scheme is used, beginning with small (300-mg) doses on day 1, followed by higher doses (up to 2 g per week) later. *Ofatumumab*'s primary toxicities consist of immunosuppression and opportunistic infection, hypersensitivity reactions during antibody infusion, and myelosuppression. Blood counts should be monitored during treatment. Rarely, patients may develop reactivation of viral infections. The drug should not be administered to patients with active hepatitis B infection; liver function should be monitored in hepatitis B carriers.

Obinutuzumab (CD20)

Obinutuzumab is a humanized IgG1 that binds to CD20. Antibody binding mediates B-cell lysis through CDC and ADCC. Glycosylation of the Fc portion of *obinutuzumab* is altered to enhance binding of effector immune cells. This is the first glycoengineered antibody approved by the FDA and is used for the treatment of patients with CLL and follicular lymphoma in combination with chemotherapy.

Alemtuzumab (CD52)

Alemtuzumab is a humanized IgG1 that binds to CD52 found on the surface of a subset of normal neutrophils and on all B and T lymphocytes, on testicular elements and sperm, and on most B and T cell lymphomas. Consistently high levels of CD52 expression on lymphoid tumor cells and the lack of CD52 modulation with antibody binding make this antigen a potential target for unconjugated monoclonal antibodies. *Alemtuzumab* can induce tumor cell death through ADCC and CDC.

ADME

Alemtuzumab is administered intravenously in dosages of 30 mg/day, three times per week. Premedication with *diphenhydramine* and *acetaminophen* should precede drug infusion. Dosing should begin with a low-dose infusion, followed by an increased dose 2 days later and, if well tolerated, the highest dose 2 days later. The drug has an initial mean $t_{1/2}$ of 1 h, but after multiple doses, the $t_{1/2}$ extends to 12 days, and steady-state plasma levels are reached at approximately week 6 of treatment.

Therapeutic Uses

Alemtuzumab is approved as a single agent for the treatment of B-cell CLL. Clinical activity has been demonstrated in both B and T cell low-grade lymphomas and CLL, including patients with disease refractory to purine analogues. In chemotherapy-refractory CLL, overall response rates are about 40%, with complete responses of 6% in multiple series. Response rates in treatment-naïve patients with CLL are higher (overall response rates of 83% and complete responses of 24%). *Alemtuzumab* is also approved for the treatment of patients with relapsing forms of multiple sclerosis under a different label.

Adverse Effects

Serious toxicities include acute infusion reactions and depletion of normal neutrophils and T cells. Myelosuppression, with depletion of all blood lineages, occurs in the majority of patients and may represent either direct marrow toxicity or autoimmune responses. Immunosuppression leads to a significant risk of fungal, viral, and other opportunistic infections, particularly in patients who have previously received purine analogues. Patients should receive prophylaxis against *Pneumocystis carinii* and herpesvirus during treatment and for at least 2 months following therapy with *alemtuzumab*. Because reactivation of cytomegalovirus infections may follow antibody use, patients should be monitored for symptoms and signs of viremia, hepatitis, and pneumonia. CD4⁺ T-cell counts may remain profoundly depleted (<200 cells/μL) for 1 year.

Tafasitamab (CD19)

Tafasitamab is directed to CD19, a B-cell surface antigen, and carries a modified Fc domain (IgG1/2 hybrid) to enhance Fc-mediated cytotoxic function (ADCC and ADCP) toward B-cell malignancies. It was approved in 2020 for use in combination with *lenalidomide* (see Chapter 71).

Naxitamab (GD2)

Naxitamab is a humanized IgG1 that binds to the sialic acid–containing glycosphingolipid GD2 (disialoganglioside) overexpressed on the surface of neuroblastoma and other peripheral and central nervous system neuroectodermal cells. Binding to GD2 induces cell death through CDC and ADCC. *Naxitamab* was approved in 2020 for the treatment of relapsed neuroblastoma in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF). Severe neurotoxicity, including neuropathic pain, is a potential adverse effect.

Dinutuximab (GD2)

Dinutuximab is a chimeric human/mouse IgG1 that targets GD2 and has a similar activity and adverse effect profile as *naxitamab* (above). It is used in combination with GM-CSF, IL-2, and retinoic acid (RA) for the treatment of neuroblastoma.

Daratumumab (CD38)

Daratumumab is a human IgG1 that binds to CD38, a transmembrane glycoprotein expressed on the surface of hematopoietic cells, including multiple myeloma (MM); binding to CD38-expressing tumor cells induces cell death through CDC and ADCC. *Daratumumab* was approved for the treatment of patients with MM in combination with other agents such as *lenalidomide* or *bortezomib* and *dexamethasone* (see Chapter 71). The most frequent adverse effects are upper respiratory tract infection, neutropenia, and thrombocytopenia.

Isatuximab (CD38)

Isatuximab is a chimeric human/mouse IgG1 that binds to CD38 with an activity profile and adverse effects similar to *daratumumab* (see above). It was approved in 2020 for the treatment of patients with MM.

Elotuzumab (SLAMF7)

Elotuzumab is a humanized IgG1 that targets SLAMF7 (CD319), a cell surface glycoprotein expressed on MM and NK cells. Binding of *elotuzumab* to the antigen on myeloma cells induces ADCC-dependent tumor cell lysis. ADCC is enhanced by stimulation of the SLAMF7 pathway in NK cells by *elotuzumab*. It is approved for the treatment of patients with MM who have received one to three prior therapies and used in combination with *lenalidomide* and *dexamethasone*.

Mogamulizumab (CCR4)

Mogamulizumab is a humanized IgG1 antibody that targets CCR4, a G protein–coupled receptor that regulates trafficking of lymphocytes and is expressed in some T-cell malignancies. It was approved in 2018 for treatment of relapsed or refractory mycosis fungoides and Sézary disease, types of cutaneous T-cell lymphomas. Adverse effects include moderate to severe rashes and potential bone marrow suppression.

Bispecific Antibodies: CD19 and CD3; EGFR and MET

The introduction of *blinatumomab*, discussed below, opened a distinct category—BiTE antibody targeting of cancer cell surface molecules. Notably, *blinatumomab* bridges cytotoxic T cells and cancer cells independent of MHC-bound antigen recognition. *Amivantamab* is a recently approved bispecific antibody that targets two receptors on cancer cells, EGFR and MET (mesenchymal-epithelial transition factor), and is discussed further below. Bifunctional antibody approaches under development are reviewed by Goebeler and Bargou (2020).

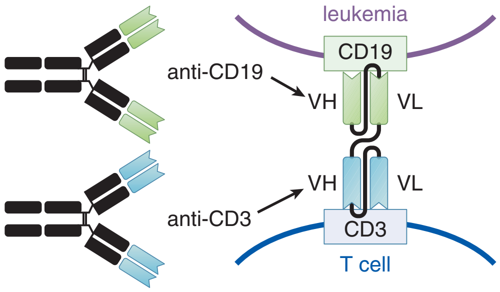

Figure 72–5 *The bispecific T-cell–engaging (BiTE) fusion antibody, blinatumomab.* *BiTE* consists of the VL and VH domains of an anti-CD19 IgG joined via an amino acid link with the VL and VH domains of an anti-CD3 IgG, resulting in a molecular mass of 54 kDa (IgGs are ~150 kDa). The bispecific antibody generates a "synapse" between cytotoxic T cells and leukemia cells that express CD19 on their surface.

Blinatumomab (CD19 and CD3)

Blinatumomab is a first-in-class bispecific antibody that recognizes sites on the surfaces of B cells and T cells, thereby enabling a patient's T cells to recognize malignant B cells (Figure 72–5). To generate this fusion protein, the VH and VL antigen binding domains in IgG1s raised against CD3 (part of the TCR) and against CD19 were isolated. The domains that bind to CD3 were linked to the domains that recognize CD19 on B cells. Due to this bispecific targeting, *blinatumomab* can mediate the formation of a cytotoxic connection between CD19-expressing tumor cells and T cells, resulting in the lysis of CD19-positive cells, release of inflammatory cytokines, and proliferation of T cells. *Blinatumomab* was approved in 2014 for the treatment of acute lymphoblastic leukemia (ALL) including minimal residual disease. Major adverse effects are cytokine release syndrome, neurological toxicities, neutropenic fever, and sepsis.

Amivantamab (EGFR and MET)

Amivantamab is a bispecific IgG1 that binds to the extracellular domains of EGFR and MET. Binding can disrupt ligand signaling through the receptors and initiate targeting of cancer cells by the immune system through ADCC. *Amivantamab* was approved in 2021 for the treatment of patients with locally advanced or metastatic NSCLC with EGFR exon 20 insertion mutations. Adverse effects include pneumonitis, dermatological reactions, and ocular toxicity.

V. Cytotoxin Conjugates With Antibodies or Cytokines

HISTORY

The concept of a "magic bullet," the translation of "Zauberkugel," was introduced by Paul Ehrlich in 1913 to describe his goal of developing cytotoxic drugs that selectively target and destroy cancer cells without impacting healthy tissues. Anticancer drugs developed in the century since Ehrlich's work still aim for this goal (Strebhardt and Ullrich, 2008). The drugs discussed below follow this concept: Cytotoxic drugs are linked to monoclonal antibodies to form antibody-drug conjugates (ADCs) that direct toxic payloads to cancer cells that overexpress the antibody target. Binding of an ADC to the surface antigen on targeted cells can lead to its internalization, intracellular cleavage of the conjugate, and the release of the cytotoxic payload. This will result in the death of the targeted cancer cells. As a "bystander" effect, the release of the cytotoxic payload may impact cells in the immediate tumor microenvironment for additional therapeutic benefit (Jabbour et al., 2021). For details of ADCs, see Figure 38-10 and Table 38-2.

Antibody-Drug Conjugates (ADCs)

Gemtuzumab Ozogamicin (CD33)

Mechanism of Action

Gemtuzumab ozogamicin is a first-generation humanized monoclonal antibody against CD33 covalently linked to a semisynthetic derivative of *calicheamicin*, a potent antitumor antibiotic that induces double-strand DNA breaks and causes cell death. The CD33 antigen is present on most hematopoietic cells, on more than 80% of acute myelocytic leukemia (AML) cells, and on most myeloid cells in patients with myelodysplastic syndrome. Other normal cell types lack CD33 expression, making this attractive for targeted therapy. CD33 has no known biological function, although monoclonal antibody cross-linking inhibits normal and myeloid leukemia cell proliferation. Following its binding to CD33, *gemtuzumab ozogamicin* undergoes endocytosis; cleavage of *calicheamicin* from the antibody takes place within the lysosome. The potent toxin then enters the nucleus, binds in the minor groove of DNA, and causes double-strand DNA breaks and cell death. *Ozogamicin* is conjugated to *gemtuzumab* through surface lysine residues (see Figure 72–1) at an average ratio of two to three cytotoxic molecules per antibody.

ADME

The antibody conjugate produces a 30% complete response rate in patients with relapsed AML when administered at a dose of 9 mg/m^2 for up to three doses at 2-week intervals. The $t_{1/2}$ of total and unconjugated *calicheamicin* are 41 and 143 h, respectively. Following a second dose, the $t_{1/2}$ of drug-antibody conjugate increases to 64 h. Most patients require two to three doses to achieve remission.

Therapeutic Use

The drug was initially approved in 2000, withdrawn due to toxicity, and approved in 2020 for CD33-positive AML. Primary toxicities include myelosuppression and hepatocellular damage in 30% to 40% of patients, manifested by hyperbilirubinemia and enzyme elevations. This agent also causes a syndrome that resembles hepatic veno-occlusive disease when patients subsequently undergo myeloablative therapy or when treatment follows high-dose chemotherapy.

Ado-trastuzumab Emtansine (HER2)

Ado-trastuzumab emtansine is a first-generation antibody-cytotoxic drug conjugate that was approved in 2013. *Ado-trastuzumab-DM1* combines the HER2-targeted properties of *trastuzumab* with the antimicrotubule agent DM1 (derived from *maytansine*), allowing preferential intracellular cytotoxic drug delivery to cancer cells in the treatment of HER2-positive breast cancer. The complex binds to HER2 and enters the cell by receptor-mediated endocytosis. The DM1 (*emtansine*) is conjugated to surface lysine residues on the antibody (see Figure 72–1) and released into the cytosol as the complex is cleaved by proteases in lysosomes. The DM1 disrupts microtubule-dependent events, causing mitotic arrest, disruption of intracellular trafficking, and apoptosis. The adverse effects of treatment include cardiac dysfunction as described for unconjugated *trastuzumab*. Hepatotoxicity is an additional risk as well as bone marrow suppression.

Fam-trastuzumab Deruxtecan (HER2)

Fam-trastuzumab deruxtecan is an HER2-targeted antibody conjugated to a *camptothecin* derivative (topoisomerase I inhibitor) via the hinge cysteine residues (see Figure 72–1). It was approved for the treatment of patients with locally advanced or metastatic HER2-positive breast or gastric cancer in 2019 and 2021, respectively. Interstitial lung disease and pneumonitis, including fatal cases, have been reported as serious adverse effects as well as cardiotoxicity.

Inotuzumab Ozogamicin (CD22)

Inotuzumab ozogamicin is a humanized anti-CD22 antibody-cytotoxic drug conjugate approved in 2017 for the treatment of relapsed or refractory ALL. After binding to CD22-positive tumor cells and cellular uptake, the cytotoxic is released via hydrolytic cleavage. Ozogamicin, a derivative of calicheamicin, binds to the minor grove in DNA, induces double-strand breaks, and causes cell death; conjugation to the antibody is via surface lysine residues (see Figure 72–1). Adverse effects include hepatotoxicity, including hepatic veno-occlusive disease, and bone marrow suppression.

Brentuximab Vedotin (CD30)

Brentuximab vedotin is an anti-CD30 IgG1 monoclonal antibody linked with the microtubule-disrupting agent monomethyl auristatin E (MMAE). CD30 is expressed on a number of malignant cells and is especially prevalent in Hodgkin and anaplastic lymphoma. Binding of the antibody to CD30-expressing cells is followed by internalization and the intracellular release of MMAE via proteolytic cleavage. MMAE disrupts the microtubule network, inducing cell cycle arrest and apoptotic cell death. MMAE is mostly metabolized by CYP3A, and coadministration with CYP3A4 inhibitors will increase exposure to the MMAE toxin. Coadministration with CYP3A4 inducers (e.g., *rifampicin*) reduces exposure. The most common adverse reactions are neutropenia, peripheral sensory neuropathy, fatigue, nausea, anemia, upper respiratory tract infection, diarrhea, pyrexia, rash, thrombocytopenia, cough, and vomiting.

Belantamab Mafodotin (BCMA)

Belantamab mafodotin is a humanized IgG1 targeted to B-cell maturation antigen (BCMA) and conjugated via cysteine residues (see Figure 72–1) to the cytotoxic monomethyl auristatin F (MMAF). Upon binding to BCMA, *belantamab mafodotin* is internalized and MMAF is released. MMAF can bind to tubulin and disrupt the microtubule network and cause cell death. It was approved 2020 for the treatment of patients with relapsed or refractory MM. Adverse effects include damage to the corneal epithelium that impacts vision and may cause loss of vision.

Polatuzumab Vedotin (CD79b)

Polatuzumab vedotin is a CD79b-directed antibody conjugated with the antimitotic MMAE via cysteine residues (see Figure 72–1). It was approved in 2019 for the treatment of patients with relapsed or refractory diffuse large B-cell lymphoma in combination with *bendamustine* (see Chapter 70) and *rituximab*.

Enfortumab Vedotin (Nectin4)

Enfortumab vedotin is a Nectin4-directed antibody conjugated with the antimitotic MMAE via cysteine residues (see Figure 72–1). It was approved in 2019 for the treatment of locally advanced or metastatic urothelial cancer.

Sacituzumab Govitecan (Trop-2)

Sacituzumab govitecan is a Trop-2–directed antibody conjugated to a *camptothecin* derivative (topoisomerase I inhibitor) via the hinge cysteine residues (see Figure 72–1). It was approved in 2020 for the treatment of metastatic triple-negative breast cancer and metastatic urothelial cancer. It can cause severe neutropenia and severe diarrhea.

Moxetumomab Pasudotox (CD22)

Moxetumomab pasudotox is a CD22-directed antibody fused to a truncated form of the *Pseudomonas* exotoxin A. Upon entering cells, the exotoxin inhibits protein synthesis through ADP-ribosylation of elongation factor 2 and causes cell death. It was approved in 2018 for the treatment of relapsed or refractory hairy cell leukemia.

Radioimmunoconjugates (CD20)

Radioimmunoconjugates provide targeted delivery of radionuclides to tumor cells. ^{131}Iodine (^{131}I) is a favored radioisotope because it is readily available, relatively inexpensive, and easily conjugated to a monoclonal antibody. The γ particles emitted by ^{131}I can be used for both imaging and therapy, but protein-iodine conjugates have the drawback of releasing free ^{131}I and ^{131}I-tyrosine into the blood and thus present a health hazard to people in contact with the patient. The β-emitter ^{90}Y (yttrium) has emerged as an alternative to ^{131}I, based on its higher energy and longer path length. Thus, it may be more effective in tumors with larger diameters. It also has a short $t_{1/2}$ and remains conjugated, even after endocytosis, providing a safer profile for outpatient use. Currently available radioimmunoconjugates consist of murine monoclonal antibodies against CD20 conjugated with ^{131}I (tositumomab) or ^{90}Y (ibritumomab). Both drugs have shown response rates in relapsed lymphoma of 65% to 80%. Adverse effects include antibody-related hypersensitivity, bone marrow suppression, and secondary leukemias. Both radioconjugates targeting CD20 on lymphomas have been approved; however, concerns over toxicity have limited their use.

Cytokine-Cytotoxin Conjugates

Tagraxofusp

Tagraxofusp is a fusion protein of human IL-3 and truncated diphtheria toxin that is directed to cells expressing the IL-3 receptor (CD123), where it will inhibit protein synthesis and cause cell death. CD123 is overexpressed in leukemic stem and more differentiated cells from ALL, AML, hairy cell leukemia, and Hodgkin lymphoma as well as plasmacytoid dendritic cell neoplasm, the malignancy it was FDA-approved in 2018. Potentially life-threatening capillary leak syndrome is the major adverse event.

VI. Cellular Therapy and Vaccines

RATIONALE

To meet the challenge of poorly immunogenic cancers, the potential of T-cell–based therapies is harnessed by genetically engineering T cells to redirect them to tumor cell surface antigens (Carpenito et al., 2009). The engineered T cells contain a chimeric antigen receptor (CAR) that consists of the antigen-binding domain of a monoclonal antibody to confer recognition of the targeted tumor antigen coupled to intracellular domains capable of activating T cells (Figure 72–6). When expressed in T cells harvested from the patient, these CARs recognize cell surface antigens and activate T cells independent of antigen presentation by an MHC molecule. In initial studies, CAR-expressing T cells were targeted to NY-ESO-1, a tumor antigen of the cancer/testis family that is highly expressed on many poor-prognosis melanoma cells. The CAR T cells showed long-lasting efficacy (>3 years) in some patients with metastatic melanoma. CAR targeting CD19, a B-cell antigen, also resulted in striking efficacy in patients with B-cell leukemias (Klebanoff et al., 2016). The FDA approved CD19 CAR T cells (*tisagenlecleucel-T*; previously named CTL019) in 2017 based on data showing >80% remission rate for relapsed or refractory B-cell ALL during treatment. Modification of CAR constructs to improve antigen recognition, efficacy, and safety of engineered T cells is an area of active research (Hou, 2021). In addition to this genetic engineering of T cells, the technique of isolating and expanding immune cells that recognize cancer antigens is used in adoptive, cell-based immunotherapy (Restifo et al., 2012).

The names for CAR T-cell therapies contain the syllables "gen" for genetic modification, "leu" for leukocytes as the targeted cell type, and "cel" for cell-based therapy (e.g., tisa*gen*lec*leucel*).

CAR T Cells

Five CAR T therapies have been approved by the FDA, with four of them targeting CD19 in relapsed or refractory B-cell ALL or B-cell NHL and one targeting BCMA. CAR T-cell treatment is not approved for the treatment of central nervous system lymphoma.

Tisagenlecleucel

Tisagenlecleucel, FDA-approved in 2017, was the first CD19 CAR-expressing autologous T cell–based immunotherapy (see Figure 72–6) for the treatment of patients with ALL that is refractory or in second or later relapse. The CAR is composed of a murine single-chain antibody fragment that recognizes CD19 and is fused to intracellular signaling domains from 4-1BB (CD137) and CD3 zeta. The CD3 zeta component is critical for initiating T-cell activation and antitumor activity, while 4-1BB enhances the cell expansion and survival (Ellis et al., 2021). Other CD19-directed CAR T immunotherapies approved since 2017 are *lisocabtagene maraleucel*, *brexucabtagene autoleucel*, and *axicabtagene ciloleucel*.

Adverse Effects

The most common adverse effects of CAR T therapy are cytokine release syndrome (indicated by fever, nausea, headache, rapid heartbeat, low blood pressure, and dyspnea) and potentially life-threatening neurological toxicities. *Tocilizumab*, a humanized IgG1 against the IL-6 receptor, blocks signaling of the IL-6 cytokine that is implicated in the pathogenesis of cytokine release syndrome. IL-6 plays an important role in inflammatory disease and is discussed in Chapter 39; *tocilizumab* is approved for treatment of CAR T cell–induced severe cytokine release syndrome and may be used in combination with corticosteroids.

Resistance to Anti-CD19 CAR T Treatment

Loss of the CD19 antigen is a frequent cause of treatment resistance. Because CD22 expression is typically retained after the loss of CD19 from the tumor cell therapy with anti-CD19, CAR T cells can be used to overcome the resistance (Fry et al., 2018).

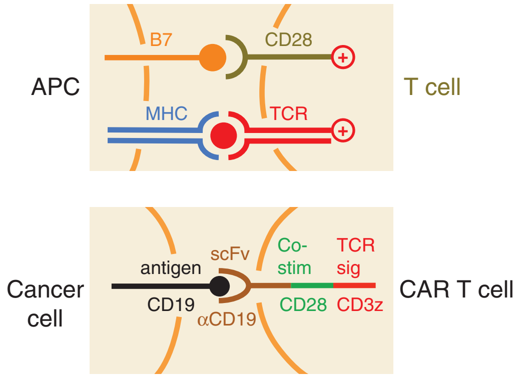

Figure 72–6 *Chimeric antigen receptor (CAR) T-cell activation by a target antigen on cancer cells.* T cells are harvested from a cancer patient and transduced with a CAR expression vector. Elements in a CAR include an extracellular, single-chain antibody fragment of the variable domain (scFv) that recognizes the targeted antigen; intracellular costimulatory domain (e.g., from CD28); and intracellular signaling domain of the TCR. CD19 is shown as an antigen example. APC-mediated stimulation of T cells is depicted for comparison (see Figure 72–3).

Idecabtagene Vicleucel

Idecabtagene vicleucel is an anti-BCMA CAR T immunotherapy that was FDA-approved in 2021 for the therapy of MM.

Sipuleucel-T

Sipuleucel-T is a cell-based approach designed to induce an immune cell response against prostate acidic phosphatase (PAP), which is expressed in most prostate cancers. This cellular immunotherapy is designed to harness a T-cell response from a patient's peripheral blood cells, which are isolated by leukapheresis. The cells are exposed to a human recombinant protein, PAP–GM-CSF. APCs present among the blood cells are thought to take up and process PAP and direct the immune response toward the antigen. A patient receives the treated cells by infusion on three occasions at 2-week intervals. The approach is modeled on adoptive immunotherapy (Restifo et al., 2012). The treatment is approved for patients with minimally symptomatic, hormone-refractory, metastatic prostate cancer. Adverse effects include acute infusion reactions, which can be reduced by premedication with acetaminophen and antihistamine.

Lifileucel

Lifileucel is an adoptive cellular therapy approach that uses tumor-infiltrating lymphocytes isolated from patients. The approach is technically challenging, and clinical trials in patients with melanoma and other cancers in combination with immune checkpoint inhibitors as well as IL-2 are ongoing (Curti and Faries, 2021).

Cancer Vaccines

Vaccination against cancer antigens remains an area of active research. mRNA-based vaccines that code for neoantigens prevalent in a given cancer type or are tailored to the neoantigen composition of each patient's cancer hold significant promise, especially in combination with immune checkpoint inhibitor treatment (Sahin and Türeci, 2018; Sahin et al., 2020). It is noteworthy that the development of mRNA-based approaches in cancer immunotherapy over the past decade (Pastor et al., 2018) was the basis for the rapid generation of highly effective vaccines against COVID-19 that helped to blunt the worldwide pandemic. Currently, only an oncolytic herpesvirus, *T-VEC*, that functions as a cancer vaccine is FDA approved, in this case for the treatment of patients with metastatic melanoma.

T-VEC

T-VEC (*talimogene laherparepvec*) is an oncolytic herpesvirus that replicates within tumors and expresses GM-CSF. Tumor antigens are released after virally induced cell death, and the presence of GM-CSF can promote an antitumor immune response. T-VEC was approved in 2015 for the local treatment of unresectable but injectable cutaneous and nodal lesions in patients with melanoma. T-VEC may synergize with immune checkpoint inhibitor treatment; the combination therapy is under study. Adverse effects are influenza-like symptoms and injection site pain.

VII. Other Proteins

Colony-Stimulating Factors

Many agents used for cancer chemotherapy suppress the production of multiple types of hematopoietic cells, and bone marrow suppression can limit the delivery of chemotherapy on schedule and at prescribed doses. The availability of recombinant growth factors for erythrocytes (i.e., erythropoietin), granulocytes (i.e., G-CSF), and granulocytes and macrophages (i.e., GM-CSF) has advanced the ability to use combination therapy or high-dose cytotoxic therapy with diminished complications such as febrile neutropenia (see Chapter 70) or erythropenia when treating von Hippel-Lindau (VHL)-related disease using the HIF-2 inhibitor *belzutifan* (see Chapter 71). Chapter 45 presents the basic pharmacology of hematopoietic growth factors.

Asparaginase

L-Asparaginase

Bacterial *L-asparaginase* hydrolyzes the amino acid asparagine. It has been used in the treatment of ALL since initial approval in 1978. Some leukemic cells depend on exogenous asparagine because they lack asparagine synthase and cannot survive asparagine depletion, whereas most normal cells can synthesize asparagine from glutamine. Treatment reduces circulating asparagine levels by greater than 90%. Available formulations of *asparaginase* are isolated from *Escherichia coli*, including a polyethylene glycol modified enzyme with prolonged duration of activity, and the enzyme from *Erwinia chrysanthemi* (approved June 2021) for patients who have developed hypersensitivity to *E. coli*–derived *asparaginase*. Adverse reactions include hepatic toxicity and pancreatitis.

Drug Facts for Your Personal Formulary: *Pathway-Targeted Therapies*

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Section I: Growth Factors and Receptors		
Epidermal Growth Factor Receptor (EGFR)		
Monoclonal Antibody EGFR Inhibitors: Intravenous Administration		
Cetuximab *(chimeric human/mouse IgG1)*	• Metastatic colorectal cancer with wild-type KRAS in combination with chemotherapy • HNSCC in combination with radiation or cisplatin	• Skin rash, diarrhea, interstitial lung disease • Rare: infusion reaction, cardiopulmonary arrest, hypomagnesemia
Panitumumab *(human IgG2)*	• Metastatic colorectal cancer with wild-type KRAS in combination with chemotherapy	• Side effects similar to those of cetuximab
Necitumumab *(human IgG1)*	• Metastatic NSCLC in combination with chemotherapy	• Side effects similar to those of cetuximab
Human Epidermal Growth Factor Receptor 2 (HER2)		
Monoclonal Antibody HER2 Inhibitors: Intravenous Administration		
Trastuzumab *(humanized IgG1)*	• HER2-positive breast cancer and gastric cancer • Combination with taxanes possible as chemotherapy	• Congestive heart failure (<5% reduced LVEF; <1% symptomatic); ↑ to 20% in combination with doxorubicin due to cardiotoxicity; monitor LVEF during and after • Acute infusion reaction, nausea, dyspnea, rashes
Pertuzumab *(humanized IgG1)*	• HER2-positive breast cancer in combination with trastuzumab and taxane	• Targets different HER2 domain than trastuzumab; prevents dimerization with other HERs • Side effects similar to those of trastuzumab
Platelet-Derived Growth Factor Receptor Inhibitors		
Olaratumab *(human IgG1)*	• Soft-tissue sarcoma in combination with doxorubicin	• Nausea, fatigue, gastrointestinal toxicity • Neutropenia, thrombocytopenia, elevated activated partial thromboplastin time, hypokalemia, hypophosphatemia
Section II: Tumor Angiogenesis		
Vascular Endothelial Growth Factor Inhibitors		
Bevacizumab *(humanized IgG1)*	• Metastatic colorectal cancer combined with chemotherapy (FOLFOX or FOLFIRI) • NSCLC combined with carboplatin and paclitaxel • Ovarian cancer combined with chemotherapy • RCC combined with interferon-α • Glioblastoma following prior therapy	• Hypertension, related congestive heart failure: monitor blood pressure and treat hypertension • Impaired wound healing: delay elective surgery for 1 month after the last dose; do not resume treatment for at least 1 month after surgery • Spontaneous gastrointestinal perforation
Ramucirumab *(human IgG1 to VEGFR2)*	• Metastatic colorectal cancer, advanced gastric adenocarcinoma, and NSCLC with disease progression on or after prior therapy as a single drug or in combination with chemotherapy	• Hypertension, diarrhea • Hemorrhage, gastrointestinal perforations • Impaired wound healing
Aflibercept *(extracellular domain of VEGFR1/2 fused to Fc portion of human IgG1)*	• Metastatic colorectal cancer in combination with FOLFIRI chemotherapy following FOLFOX	• Soluble trap for VEGF receptor ligands • Hypertension, diarrhea, impaired wound healing • Increased risk of hemorrhage, gastrointestinal perforation • Pathophysiology: increased circulating extracellular domain of VEGFR and decreased VEGF are hallmarks of preeclampsia
Section III: Activating Immune Cells		
Immune Checkpoint Inhibitors		
Ipilimumab *(anti–CTLA-4 human IgG1)*	• Metastatic melanoma as single agent or in combination with nivolumab (anti-PD-1) • Clinical trials with different cancers are ongoing	• Autoimmune inflammatory toxicities in majority of patients (>70%) • Adverse effects: skin (pruritus, rash, vitiligo), GI tract (diarrhea, colitis) • Less frequent: hepatitis, pneumonitis, hypophysitis, hypo- or hyperthyroidism, myocarditis
Tremelimumab *(anti–CTLA-4 human IgG2)*	• Clinical trials with different cancers are ongoing	• See above

Drug Facts for Your Personal Formulary: *Pathway-Targeted Therapies (continued)*

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Nivolumab *(anti–PD-1 human IgG4)*	• Advanced melanoma that progressed after ipilimumab (anti–CTLA-4) • Previously treated NSCLC • Advanced RCC • Relapsed/refractory Hodgkin lymphoma	• Adverse effects: rash, fatigue, dyspnea, musculoskeletal pain, decreased appetite, cough, nausea, constipation • Immune-related serious adverse effects include pneumonitis, colitis, hepatitis, nephritis, renal dysfunction, hypophysitis, hypo- and hyperthyroidism
Pembrolizumab *(anti–PD-1 humanized IgG4)*	• Advanced melanoma that progressed after ipilimumab (anti–CTLA-4) • NSCLC that expresses PD-L1 and progressed under chemotherapy • NSCLC with wild-type EGFR and ALK and disease progression after chemotherapy • HNSCC with disease progression after chemotherapy	• Adverse effects: rash, fatigue, dyspnea, musculoskeletal pain, decreased appetite, cough, nausea, constipation • Immune-related serious adverse effects include pneumonitis, colitis, hepatitis, nephritis, renal dysfunction, hypophysitis, hypo- and hyperthyroidism
Atezolizumab *(anti–PD-L1 human IgG1)*	• NSCLC that is treatment resistant • Urothelial cancer that is locally advanced or metastatic	• Adverse effects: fatigue, decreased appetite, dyspnea, cough, nausea, musculoskeletal pain, constipation • In patients with urothelial cancer: urinary tract infections • Immune-related pneumonitis, colitis, hepatitis, nephritis, renal dysfunction, hypo- and hyperthyroidism, hypophysitis, adrenal insufficiency, pancreatitis, Guillain-Barré syndrome, severe infections
Section IV: Targeting Cancer Cells to Engage Immune Cells		
Antibodies Targeting Cell Surface Antigens		
Rituximab *(chimeric human/mouse IgG1 anti-CD20)*	• NHL • CLL • Rheumatological and other autoimmune disease, including multiple sclerosis	• Infusion-related toxicity with fever, rash, and dyspnea; B-cell depletion; late-onset neutropenia; risk of hypersensitivity reaction: use slow increase in infusion rate and antihistamines • Rare: severe mucocutaneous skin reaction, including Stevens-Johnson syndrome • Risk of tumor lysis syndrome in patients with high tumor burden in the circulation: use lower dose initially • Reactivation of hepatitis B virus or JC polyomavirus
Ofatumumab *(human IgG1 anti-CD20)*	• CLL after treatment failure	• Immunosuppression and opportunistic infections, hypersensitivity reaction during infusion and myelosuppression: monitor blood counts during treatment
Obinutuzumab *(humanized IgG1 anti-CD20)*	• CLL in combination with chemotherapy	• Frequent adverse effects: cytopenia, fever, cough, musculoskeletal disorders
Alemtuzumab *(Campath* or *Lemtrada; humanized IgG1 anti-CD52)*	• CLL (label: *Campath*) • Multiple sclerosis (label: *Lemtrada*)	• Infusion-related toxicity, T-cell depletion with increased infection; myelosuppression with pancytopenia • Antibiotic prophylaxis
Dinutuximab *(chimeric human/mouse anti-GD2)*	• High-risk neuroblastoma	• Infusion reaction • Nerve damage
Daratumumab *(human IgG1 anti-CD38)*	• MM in combination with lenalidomide or bortezomib	• Infusion reactions • Peripheral sensory neuropathy, upper respiratory tract infection
Elotuzumab *(humanized IgG1 anti-CD319)* (SLAMF7)	• MM after one to three prior therapies	• Infusion reaction
Blinatumomab *(bispecific anti-CD19 and anti-CD3)*	• Ph-negative relapsed or refractory B-cell precursor ALL	• Cytokine release syndrome, neurological toxicity, neutropenic fever

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Chapter 73

Hormones, Hormone Receptor Antagonists, and Related Agents in the Therapy of Cancer

Claudine Isaacs, Kerry L. Burnstein, and Anna T. Riegel

INTRODUCTION TO HORMONE-REGULATED CANCERS

ENDOCRINE THERAPY OF BREAST CANCER

ANDROGEN DEPRIVATION THERAPY OF PROSTATE CANCER

USE OF GLUCOCORTICOIDS IN HEMATOLOGICAL MALIGNANCIES

ENDOCRINE THERAPY FOR OTHER MALIGNANCIES

DRUGS THAT TARGET GNRH/LHRH

- GnRH Agonists
- GnRH Antagonists

DRUGS THAT TARGET THE ESTROGEN RECEPTOR

- Estrogen Antagonists
- Drugs That Decrease Estrogen Levels

DRUGS THAT TARGET THE PROGESTERONE RECEPTOR

- Drugs Combined With Agents That Antagonize the Estrogen Receptor

DRUGS THAT TARGET THE ANDROGEN RECEPTOR

- AR Antagonists
- Drugs That Inhibit Androgen Synthesis
- Prostate Cancer Chemoprevention (5α-Reductase Inhibitors)
- Bipolar Androgen Deprivation Therapy

DRUGS THAT TARGET THE GLUCOCORTICOID RECEPTOR

A Note on Treatment Regimens

Cancer treatment regimens change to reflect continuous advances in basic and clinical science: new drugs, both small molecules and biologicals; improved methods of targeting and timing of drug delivery; agents with altered pharmacokinetic properties and selectivities; the use of rational multidrug combinations; and greater knowledge of the basic cell biology of tumorigenesis, metastasis, and immune function, among other advances. As a consequence, this chapter presents relatively few detailed treatment regimens; rather, we refer the reader to the web-based resources of the U.S. FDA (drugs@fda) and the NCCN (National Comprehensive Cancer Network). Table 71–1 provides the details and focuses on the complexities of treatment of two cancers.

Introduction to Hormone-Regulated Cancers

The growth of a number of cancers is hormone-dependent or regulated by hormones. Therapies that employ estrogen and androgen receptor antagonists, steroid hormone synthesis inhibitors, and gonadotropin-releasing hormone (GnRH) analogues or antagonists extend survival and delay or prevent tumor recurrence of both breast and prostate cancer. These molecules interrupt the normal feedback controls regulating steroid hormone synthesis, inhibit androgen and estrogen production, or inhibit binding of these hormones to their cognate receptors, which are ligand-activated transcription factors. By inhibiting the activation and actions of androgen and estrogen receptors, these drugs block or reduce expression of genes and gene networks that ultimately promote tumor growth and survival. Glucocorticoids are used for their antiproliferative and lympholytic properties in hematologic malignancies and, in other oncological settings, to mitigate untoward responses to other treatments as well as some cancer-related symptoms.

The pharmacology of the estrogens and androgens is described in detail in Chapters 48 and 49. Decreasing the actions of these steroid hormones is the therapeutic goal in certain cancers, most notably those of the prostate and breast, because these organs are dependent on steroid hormones for their growth, function, and morphological integrity.

Endocrine Therapy of Breast Cancer

The presence of the estrogen receptor (ER) and/or progesterone receptor (PR) (ER+/PR+) in female breast cancer tissue identifies the subset of hormone receptor–positive cancers with a greater than 60% likelihood of responding to endocrine therapy. The response rate to antiestrogen treatment is somewhat lower in the subset of patients with tumors that are ER+ or PR+ but also positive for human epidermal growth factor receptor (HER2)/neu amplification. In contrast, ER-negative and PR-negative (ER–/PR–) carcinomas do not respond to endocrine therapy. Antiestrogen approaches for the therapy of ER+/PR+ breast cancer include the use of selective estrogen receptor modulator (SERMs), selective estrogen receptor downregulators (SERDs), and aromatase inhibitors (AIs) (Table 73–1 and Figure 73–1A). Historically, high doses of estrogen have been recognized as effective treatment of breast cancer. The growth inhibitory effect of estrogens may be related to their ability to induce apoptosis in endocrine-resistant breast cancer (reviewed in Jordan, 2015). However, interruption of estrogen-induced signaling with SERMs, SERDs, and drugs that reduce estrogen production such as AIs and GnRH/luteinizing hormone–releasing hormone (LHRH) analogues are more effective and better tolerated. These drugs have largely replaced estrogens or progestins for the treatment of breast cancer, although estrogen and progesterone are used occasionally. Male breast cancer is rare and predominantly (>90%) ER+/PR+. Treatment is directed at inhibiting ER with *tamoxifen*. There is a paucity of data on the use of AIs or SERDs in males.

Initial or acquired resistance to endocrine therapies (*tamoxifen* or AIs) frequently occurs. Multiple mechanisms contribute to endocrine resistance in breast cancer (reviewed in Fan and Jordan, 2019); these mechanisms include loss of ER expression, changes in transcriptional coregulator expression or activity, and hormone-independent activation of the ER by stress kinase or growth factor–activated cellular kinase

Abbreviations

ADT: androgen deprivation therapy
AI: aromatase inhibitor
ALL: acute lymphoblastic leukemia
AR: androgen receptor
AUC: area under the curve
BAT: bipolar androgen deprivation therapy
BCRP: breast cancer resistance protein
CDK: cyclin-dependent kinase
CLL: chronic lymphocytic leukemia
CRPC: castration-resistant prostate cancer
CYP: cytochrome P450
ER: estrogen receptor
ERE: estrogen-response element
FSH: follicle-stimulating hormone
GnRH: gonadotropin-releasing hormone
GR: glucocorticoid receptor
HER: human epidermal growth factor
HL: Hodgkin lymphoma
HR: hormone receptor
LH(RH): luteinizing hormone (–releasing hormone)
MM: multiple myeloma
mTOR: mechanistic (or mammalian) target of rapamycin
NHL: non-Hodgkin lymphoma
OATP: organic anion transporting polypeptide
Pgp: P-glycoprotein
PR: progesterone receptor
PROTAC: proteolysis targeting chimeras
SERD: selective estrogen receptor downregulator
SERM: selective estrogen receptor modulator
UGT: UDP-glucuronosyltransferase

pathways. In particular, cross talk between the ER and the HER2/neu pathway has been implicated in *tamoxifen* resistance. In a significant portion of metastatic breast cancers, estrogen deprivation therapy can lead to selection of cancer cells expressing an ER with mutations that allow hormone-independent activation. Acquired ER mutations also contribute to endocrine therapy–resistant breast cancer growth (reviewed in Jeselsohn et al., 2017). Targeting endocrine-resistant disease harboring ER mutations with SERDs and SERMs is an active area of drug investigation and development (reviewed in McDonnell et al., 2021).

Androgen Deprivation Therapy of Prostate Cancer

Androgens are essential for the development and maintenance of the prostate gland. The critical role of androgens in promoting prostate cancer growth was demonstrated in 1941 and led to the awarding of a Nobel Prize in 1966 to Dr. Charles Huggins. Huggins's findings established androgen deprivation therapy (ADT) as the mainstay of treatment for patients with advanced prostate cancer, an approach still used today. ADT is accomplished by surgical castration (bilateral orchiectomy) or "medical" castration, using long-acting GnRH agonist formulations to downregulate GnRH receptors in the anterior pituitary or with GnRH antagonists that directly block GnRH receptor-ligand interaction to achieve more rapid suppression of the major circulating androgen, testosterone (Table 73–2 and Figure 73–1B). ADT results in castrate levels (≤50 ng/dL) of testosterone and hence subsequent reduction of the testosterone metabolite, dihydrotestosterone, which binds with highest affinity to the androgen receptor (AR). Thus, ADT limits the action of the AR in prostate and other sensitive tissues (see Chapter 49).

In localized, high-risk or advanced prostate cancer (prior to metastasis or after metastatic progression), the combination of ADT and AR antagonists or androgen synthesis inhibitors is the standard of care but not a curative treatment. Disease progression commonly occurs despite this "combined androgen blockade" and signifies the development of castration-resistant prostate cancer (CRPC). In CRPC, the target of combined androgen blockade, AR, is typically "reactivated" through a variety of mechanisms (reviewed in Desai et al., 2021; Einstein et al., 2019; Luo et al., 2018; Nakazawa et al., 2017) including increased AR levels, expression of constitutively active AR splice variants that lack the ligand binding domain, or acquisition of somatic *AR* gene gain-of-function mutations that render AR promiscuous for activation by other steroid hormones (including weak adrenal androgens or glucocorticoids) and, in rare cases, by AR antagonists. Increased expression or activity of AR coactivators and pioneer transcription factors (e.g., FOXA1) or decreased AR corepressor proteins may also participate in continued AR activity in CRPC. Another mechanism that may drive persistent AR signaling in CRPC is local production of sufficient quantities of androgens by CRPC tumors to activate AR. Therefore, second-generation, highly potent AR antagonists and inhibitors of androgen synthesis are employed concurrently with GnRH agonists or antagonists as frontline or second-line therapies following progression to CRPC (reviewed in Mitsiades and Kaochar, 2021). Combining ADT with a newer generation AR antagonist or an androgen synthesis–directed drug is associated with improvements in symptoms and prolongation of survival.

Use of Glucocorticoids in Hematological Malignancies

Glucocorticoids act by binding to a specific glucocorticoid receptor (GR) that is a member of the nuclear receptor family of transcription factors. Agonist-liganded GR translocates to the nucleus and induces complex gene expression changes (see Chapter 50) that lead to antiproliferative and apoptotic responses in sensitive cells. Because of their lympholytic effects and their ability to suppress mitosis in lymphocytes, glucocorticoids are used as cytotoxic agents in the treatment of acute leukemia in children and malignant lymphoma in children and adults. In acute lymphoblastic or undifferentiated leukemia of childhood, glucocorticoids may produce prompt clinical improvement and objective hematological remissions in 30% of children. However, the duration of remission is brief. Remissions occur more rapidly with glucocorticoids than with antimetabolites, and there is no evidence of cross-resistance to unrelated agents. Thus, therapy is initiated with *prednisone* and *vincristine* (see Chapter 70), often followed by an anthracycline or *methotrexate* (see Chapter 70), and *l-asparaginase* (see Chapter 72). Glucocorticoids are a valuable component of curative regimens for other lymphoid malignancies, including Hodgkin disease, non-Hodgkin lymphoma, multiple myeloma, and chronic lymphocytic leukemia (CLL). Glucocorticoids are extremely helpful in controlling autoimmune hemolytic anemia and thrombocytopenia associated with CLL.

Endocrine Therapy for Other Malignancies

Endocrine therapies are occasionally used in other malignancies. *Tamoxifen* and *medroxyprogesterone acetate* have activity in metastatic endometrial carcinoma, particularly in low-grade tumors. Use of endocrine therapy in ovarian cancer has also been considered.

Drugs That Target GnRH/LHRH

GnRH Agonists

Synthetic GnRH analogues (e.g., *triptorelin*, *goserelin*, *leuprolide*, *histrelin*, and *nafarelin*) have amino acid substitutions at key residues of the

TABLE 73–1 ■ ESTROGEN RECEPTOR (ER)–TARGETED THERAPY IN ER+ BREAST CANCER

DRUG (DAILY STANDARD DOSE)	THERAPEUTIC APPROACH IN DISEASE SETTING					
	CHEMOPREVENTION		ADJUVANT THERAPY		METASTATIC DISEASE	
	PREMENOPAUSAL	POSTMENOPAUSAL	PREMENOPAUSAL	POSTMENOPAUSAL	PREMENOPAUSAL	POSTMENOPAUSAL
Tamoxifen (20 mg PO)	Yes (5 years)	Yes (5 years)	Yes (5–10 years)	Yes (before AI for 2–5 years)	Yes	Yes
Raloxifene (60 mg PO)		Yes (5 years)				
Fulvestrant (500 mg IM day 1, day 15, day 29, then once per month)						Yes
Anastrozole (1 mg PO)		Yes (5 years)		Yes (5–10 years) (up front or after tamoxifen)		Yes
Letrozole (2.5 mg PO)				Yes (5–10 years) (up front or after tamoxifen)		Yes
Exemestane (25 mg PO)		Yes (5 years)		Yes (5–10 years) (up front or after tamoxifen)		Yes
GnRH agonist in addition to one of above given monthly			Yes (with tamoxifen or AI)		Yes (with tamoxifen, AI, or fulvestrant)	

naturally occurring decapeptide GnRH that increases analogue binding affinity to the GnRH receptor and reduces susceptibility to enzymatic degradation. These GnRH agonists are 100-fold more potent than the native decapeptide. Administration of long-acting GnRH agonists downregulates GnRH receptors in the anterior pituitary gland in both women and men.

In women, the downregulation of GnRH receptors suppresses the release of the gonadotropins follicle-stimulating hormone (FSH) and LH from the pituitary and prevents follicular maturation in the ovary. Serum estrogen levels are reduced to those seen in postmenopausal women or in women after oophorectomy. GnRH agonists are used in the adjuvant treatment of breast cancer or metastatic disease for women who have functioning ovaries; typically, they are used in combination with either *tamoxifen* or AIs.

In treating prostate cancer, the most common form of ADT involves chemical suppression of pituitary gland function with long-acting GnRH agonists (*leuprolide*, *goserelin*, *buserelin*). ADT is given to patients with localized intermediate- to high-risk prostate cancer in conjunction with radiation therapy or in some cases following surgical removal of the prostate gland. GnRH agonists cause an initial release of both LH and FSH and a subsequent increase in testosterone production termed "testosterone flare" from testicular Leydig cells. After 1 week of therapy, GnRH receptors are downregulated on the gonadotropin-producing cells, causing a decline in the pituitary response. The fall in serum LH leads to a decrease in testosterone production to castrate levels within 3 to 4 weeks of the first treatment. Subsequent treatments maintain testosterone at castrate levels.

ADME

GnRH agonists are administered as an injection once a month or every 3, 4, or 6 months. There is in an initial rise in LH and FSH levels, but after 14 to 21 days of therapy, a sustained decrease in serum LH and estrogen in women or testosterone in men is observed.

Therapeutic Use in Breast and Prostate Cancer. In the adjuvant setting, studies have demonstrated a further reduction in the risk of recurrence when GnRH agonists are given in conjunction with either AIs or *tamoxifen*, when compared to *tamoxifen* alone, in very young or higher-risk premenopausal women (Pagani et al., 2020). In addition, these drugs can be given with *tamoxifen*, AIs, or *fulvestrant* in premenopausal women with metastatic breast cancer. In men with metastatic prostate cancer, ADT may be used in conjunction with chemotherapy (e.g., *docetaxel*). Alternatively, ADT is typically combined with potent newer generation AR antagonists or the androgen synthesis inhibitor *abiraterone acetate* in what is referred to as "combined androgen blockade." The advantage of combined androgen blockade is that the GnRH agonist (or GnRH antagonist) will deplete testicular androgens, while the competitive AR antagonist component competes at the AR with residual androgens made by the adrenal glands. When the steroid synthesis inhibitor *abiraterone* is used for combined androgen blockade, there is a decrease in androgens made by the adrenal glands. Numerous trials showed a benefit in survival in metastatic castration-sensitive prostate cancer following ADT combined with either *abiraterone* or *docetaxel*, but these treatments are associated with higher costs (and, in the case of *docetaxel*, significantly higher toxicities) compared to ADT alone (Wang L et al., 2021).

Toxicity

Side effects in women are generally related to hypoestrogenism (i.e., hot flashes, vaginal dryness, decreased libido, osteoporosis, amenorrhea, and dyspareunia). The side effects of GnRH agonists are often reversible on cessation of therapy. AI use in premenopausal women combined with ovarian suppression with GnRH analogues increases menopausal symptoms and sexual dysfunction, and thus, benefits and risks should be carefully considered with each patient (Burstein et al., 2016).

In men, during the transient rise in LH, the resultant testosterone surge or "flare" may induce acute stimulation of prostate cancer growth

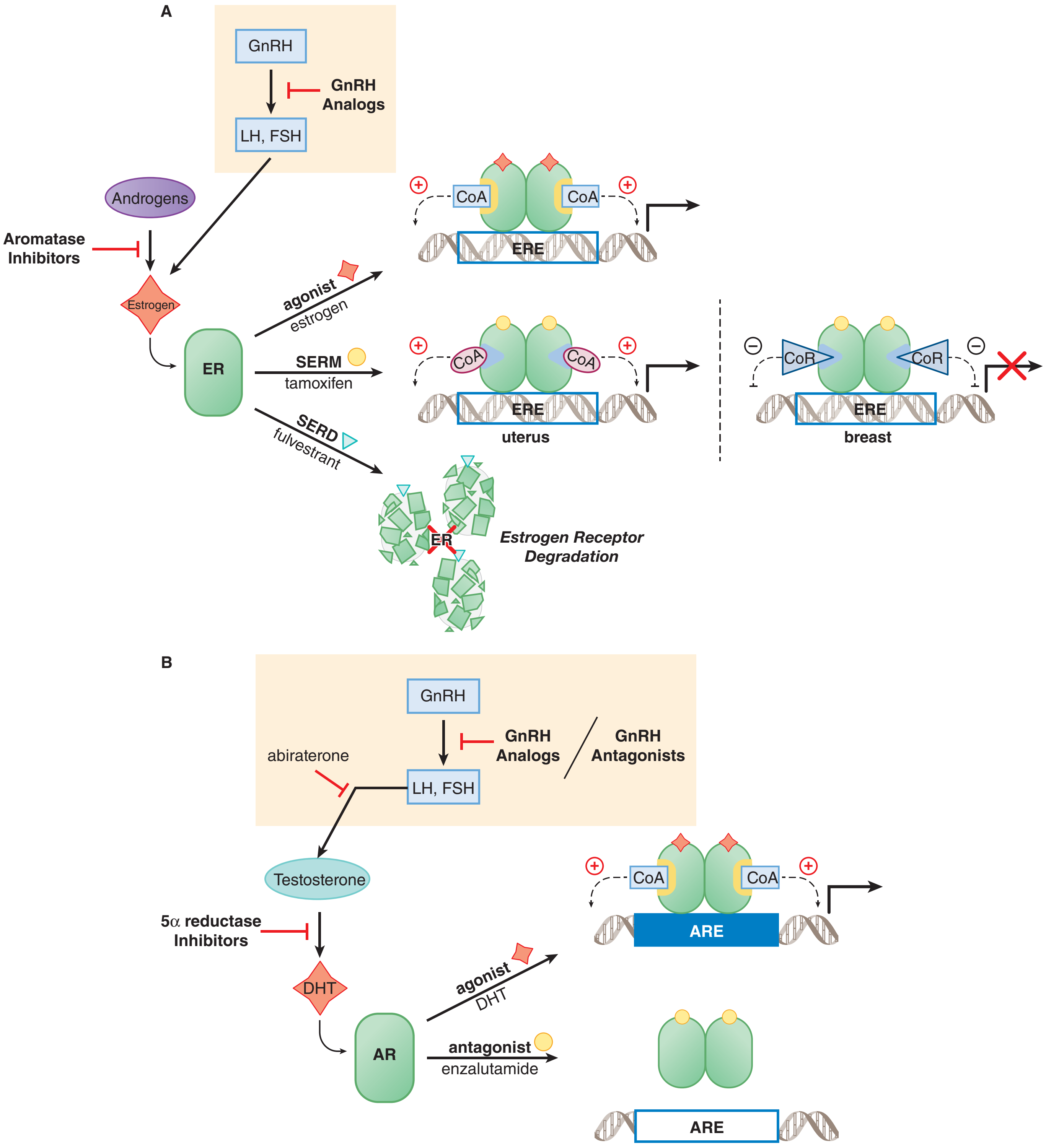
A
GnRH
GnRH Analogs
LH, FSH
Androgens
Aromatase Inhibitors
Estrogen
ER
agonist
estrogen
SERM
tamoxifen
SERD
fulvestrant
CoA
CoA
ERE
CoA
CoA
ERE
uterus
CoR
CoR
ERE
breast
ER
Estrogen Receptor Degradation
B
GnRH
GnRH Analogs
GnRH Antagonists
abiraterone
LH, FSH
Testosterone
5α reductase Inhibitors
DHT
AR
agonist
DHT
antagonist
enzalutamide
CoA
CoA
ARE
ARE

Figure 73–1 **A.** *Targets of agents that interrupt estrogen receptor signaling in females.* GnRH promotes the release of LH and FSH (Figure 46–1), which regulate ovarian production of estrogen and progesterone and can stimulate estrogen production. Aromatase converts the estrogen precursors androstenedione, dihydroepiandrostenedione, and testosterone to estrogens (see Figures 48–1 and 48–2). Estrogen signaling can be interrupted by inhibiting hormone production (GnRH analogues, aromatase inhibitors), by antagonizing the effects of estrogen at the estrogen receptor (ER) with a SERM, or by destabilizing the ER and promoting its degradation with a SERD (e.g., *fulvestrant*). The effects of SERMs (e.g., *tamoxifen*) on organs are dependent on a number of factors including the activity and relative expression levels of transcriptional coactivators (CoA) and corepressors (CoR) in a particular tissue. In the breast, *tamoxifen* is an antagonist; a corepressor effect on gene transcription predominates. On the other hand, in the uterus, *tamoxifen* can drive estrogenic effects, after binding ER, by utilizing coactivators of gene transcription. **B.** *Targets of agents that interrupt androgen receptor signaling in males.* GnRH promotes the release of LH and FSH, which stimulates adrenal and testicular androgen production. GnRH receptors can be inhibited by long-acting GnRH analogues that downregulate GnRH receptors or by GnRH antagonists. *Abiraterone acetate* is a steroid synthesis inhibitor that blocks the conversion of pregnenolone and progesterone to androgen precursors. Androgen signaling is inhibited by androgen receptor (AR) antagonists (e.g., *enzalutamide*), which competitively bind the AR and inhibit binding and activation by androgens, particularly dihydrotestosterone (DHT). AR bound to *enzalutamide* (instead of DHT) has diminished interaction with coactivators and androgen response elements (AREs) on DNA.

TABLE 73–2 ■ ANDROGEN RECEPTOR–TARGETED THERAPY FOR PROSTATE CANCER

	CASTRATION-SENSITIVE		CASTRATION-RESISTANT	
DRUG (DAILY STANDARD DOSE)	NON-METAST	METAST	NON-METAST	METAST
Bicalutamide (one 50-mg tablet daily in combination with ADT)[a]	Yes	Yes	Yes	Yes
Enzalutamide (four 40-mg tablets once daily)		Yes	Yes	Yes
Apalutamide (four 60-mg tablets once daily)		Yes	Yes	
Darolutamide (two 300-mg tablets twice daily)			Yes	
Abiraterone acetate (4×250 mg or 2×500 mg tablets daily) plus prednisone: 5-mg tablet once daily (metastatic castration-sensitive prostate cancer) or 5-mg tablet twice daily (metastatic CRPC)		Yes		Yes
ADT consisting of bilateral orchiectomy, GnRH agonist, or GnRH antagonist in addition to one of above agents	Yes	Yes	Yes	Yes

Metast, metastatic.

[a] Bicalutamide dose as monotherapy is higher.

and symptoms from stimulation of metastatic deposits in bone. Patients may experience an increase in bone pain, spinal cord compression, or obstructive bladder symptoms lasting for 2 to 3 weeks. The flare phenomenon can be effectively counteracted with concurrent administration of 2 to 4 weeks of oral AR antagonist therapy, which may inhibit the action of the increased serum testosterone levels. Common side effects of ADT include vasomotor instability, loss of libido, impotence, gynecomastia, fatigue, anemia, weight gain, decreased insulin sensitivity, altered lipid profiles, osteoporosis and fractures, and loss of muscle mass (Formenti et al., 2021). Skeletal-related events due to ADT may be mitigated by bisphosphonate therapy (see Chapter 52). Studies have shown a small but significant increase in the risk of diabetes and coronary heart disease in patients taking GnRH agonists (Gupta et al., 2018). Because of these concerns, GnRH agonists have undergone FDA review since 2010.

GnRH Antagonists

GnRH antagonists are a class of agents used in the treatment of prostate cancer. They antagonize the GnRH receptor by reversible competition with GnRH, resulting in rapid reduction in LH and FSH secretion from the pituitary and concomitant loss of testosterone production to castrate levels from the testes. *Degarelix* is a small peptide-based GnRH antagonist used in prostate cancer treatment. The recently FDA-approved *relugolix* is an orally available, nonpeptide antagonist used for treatment of prostate cancer (Shore et al., 2020).

ADME

After an initial subcutaneous loading dose (240 mg in two doses of 120 mg), *degarelix* is given as a monthly injection (80 mg). C_{max} is achieved after 2 days, and excretion is mainly through the hepatobiliary system. *Relugolix* is given orally as an initial loading dose (360 mg), followed by one tablet (120 mg) daily for prostate cancer. Steady-state levels are achieved after 7 days. Food can diminish oral bioavailability by 50%.

Absorption and distribution of *relugolix* are influenced by its interaction with the efflux transporter P-glycoprotein (Pgp) and CYP3A4 (the predominant metabolizer of the drug). Oral coadministration with agents that inhibit Pgp (e.g., *erythromycin*) increases the C_{Pmax} and area under the curve (AUC) of *relugolix*; coadministration with *rifampicin* (an inducer of intestinal Pgp and hepatic CYP3A4) will decrease C_{Pmax} and AUC. Metabolites of *relugolix* are excreted mainly in feces.

Degarelix distributes slowly from a subcutaneous depot to total body water. As a peptide, *degarelix* does not interact with Pgp and is not metabolized by hepatic CYPs. Rather, hepatic/biliary hydrolysis of the drug produces peptide fragments that are excreted in the bile/feces (~75%), with approximately 25% of the original dose appearing in the urine.

Therapeutic Use

GnRH antagonists are used in the treatment of advanced and metastatic prostate cancer. Besides avoidance of the initial increase in testosterone seen with GnRH agonists, GnRH antagonists are reported to be more effective at suppressing FSH and have fewer cardiovascular side effects than GnRH agonists (Dearnaley et al., 2020; Shore et al., 2020).

Toxicity

Degarelix is associated with higher rates of injection site reactions compared with GnRH agonists. ADT may cause prolongation of the cardiac QT/QTc interval, a serious side effect of both *relugolix* and *degarelix*. The most common side effects of *relugolix* include hot flushes, increased blood glucose levels, increased triglyceride levels, musculoskeletal pain, anemia, increased liver enzymes, tiredness, constipation, and diarrhea. *Relugolix* causes reproductive and fetal harm in animal toxicity tests; thus, caution is warranted, and male patients with female partners of reproductive potential should use contraception during and for 2 weeks following the last dose of *relugolix*.

The early GnRH antagonists *cetrorelix* and *abarelix* (no longer marketed), although effective, are now rarely used for prostate cancer because of the risk for severe systemic allergic reactions. Later generation GnRH peptide antagonists are not associated with systemic allergic reactions.

Drugs That Target the Estrogen Receptor

Estrogen Antagonists

Selective Estrogen Receptor Modulators

The SERMs bind to the ER and exert either estrogenic or antiestrogenic effects, depending on the specific organ (see Chapter 48). *Tamoxifen* is one of the most widely studied antiestrogenic drugs used in the treatment of breast cancer. *Tamoxifen* also exerts estrogenic agonist effects on nonbreast tissues, which influences the side effect profile of the drug. Therefore, several novel antiestrogen compounds that offer the potential for enhanced efficacy and reduced toxicity compared with *tamoxifen* have been developed. These novel antiestrogens can be divided into *tamoxifen* analogues (e.g., *toremifene*, *droloxifene*, *idoxifene*); "fixed-ring" compounds (e.g., *raloxifene*, *bazedoxifene*, *lasofoxifene*, *arzoxifene*, *miproxifene*, *levormeloxifene*); and the SERDs (e.g., *fulvestrant*), the latter also termed "pure antiestrogens" (McDonnell et al., 2021).

Tamoxifen. *Tamoxifen* was developed as an oral contraceptive but instead was found to induce ovulation and to have antiproliferative effects on estrogen-dependent breast cancer cell lines (reviewed in Abderrahman and Jordan, 2019). *Tamoxifen* is prescribed for the adjuvant therapy of early-stage breast cancer and for the therapy of advanced breast cancer. *Tamoxifen* and other SERMs such as *raloxifene* are also used for the prevention of breast cancer in high-risk patients such as those with a strong family history or prior nonmalignant breast pathology (Visvanathan et al., 2013). The uses, pharmacology, and mechanism of action of *raloxifene* are discussed in Chapter 48.

Mechanism of Action. *Tamoxifen* is a competitive inhibitor of the binding of estrogens (e.g., 17β-estradiol) to the ER and antagonizes estrogen-induced proliferation of human breast cancer.

There are two subtypes of ERs: ERα and ERβ, which have different tissue distributions and can either homo- or heterodimerize. ERα plays a major role in breast cancer progression and is a prognostic marker; the role of ERβ is unclear. Binding of estrogen and SERMs to the estrogen-binding sites of the ERs initiates a change in conformation of the ER, dissociation of the ER from heat-shock proteins, and ER dimerization. Dimerization facilitates the binding of the ER to specific DNA estrogen-response elements (EREs) in the vicinity of estrogen-regulated genes. Coregulator proteins interact with the liganded receptor to act as corepressors or coactivators of gene expression (see Chapter 48). Elegant studies of the crystal structure of ERα bound to different ligands indicate that, when an ER agonist is bound to the ER, a conformational change occurs in the ligand-binding pocket that enables helix 12 in this region to provide a docking site for p160 transcriptional coactivators, thereby increasing transcription of target genes.

Conversely, the interaction of an estrogen antagonist such as 4-hydroxytamoxifen bound to the ligand binding domain of ER causes a structural rearrangement of helix 12. This alteration in structure reduces transcriptional coactivator binding and favors binding of transcriptional corepressors. The net result is inhibition of estrogen-induced gene transcription (reviewed in Legare and Basik, 2016; Nettles and Greene, 2005). Differences in tissue distribution of the ER subtypes and the relative abundance and activities of different transcriptional coactivators and corepressors likely explain the antagonist response to tamoxifen in ER+ breast cancer and its partial agonist activities in noncancerous tissues (see Figure 73–1A) (reviewed in Abderrahman and Jordan, 2019; Green and Carroll, 2007).

Organs displaying agonist effects of *tamoxifen* include the uterine endometrium (endometrial hypertrophy, vaginal bleeding, and endometrial cancer); the coagulation system (thromboembolism); bone metabolism (increase in bone mineral density, which can slow development of osteoporosis); and liver (*tamoxifen* lowers total serum cholesterol, low-density lipoprotein cholesterol, and lipoproteins and raises apolipoprotein A1 levels).

ADME. *Tamoxifen* is given orally once (20 mg) per day. It is readily absorbed following oral administration, with peak concentrations measurable after 3 to 7 h and steady-state levels reached at 4 to 6 weeks. Metabolism of *tamoxifen* is complex and principally involves CYPs 3A4/5 and 2D6 in the formation of *N*-desmethyl tamoxifen and CYP2D6 to form 4-hydroxytamoxifen, a more potent metabolite (Figure 73–2). Both metabolites can be further converted to 4-hydroxy-*N*-desmethyltamoxifen (endoxifen), which retains high affinity for the ER. The parent drug has a terminal $t_{1/2}$ of 7 days. After enterohepatic circulation, glucuronides and other metabolites are excreted in the stool; excretion in the urine is minimal. Polymorphisms in CYP2D6 that reduce its activity lead to lower plasma levels of the potent metabolites 4-OH tamoxifen and endoxifen, but whether this leads to inferior efficacy of *tamoxifen* treatment and higher risk of disease relapse is unclear (reviewed in Hertz and Rae, 2016; Tamura et al., 2020). While drugs that inhibit CYP2D6 activity, such as antidepressants, have been postulated to minimize *tamoxifen* activity in breast cancer, more recent studies do not suggest a clinically significant impact (Haque et al., 2016).

Therapeutic Uses. *Tamoxifen* is used for the treatment of women with ER+ metastatic breast cancer or following primary excision of an ER+ tumor as an adjuvant treatment to prevent recurrence and extend overall survival. For the adjuvant treatment of premenopausal women, *tamoxifen* is given for at least 5 years (see Table 73–1). *Tamoxifen* may also be used for postmenopausal women, but AIs are preferred as they are associated with further reductions in the risk of recurrence (Early Breast

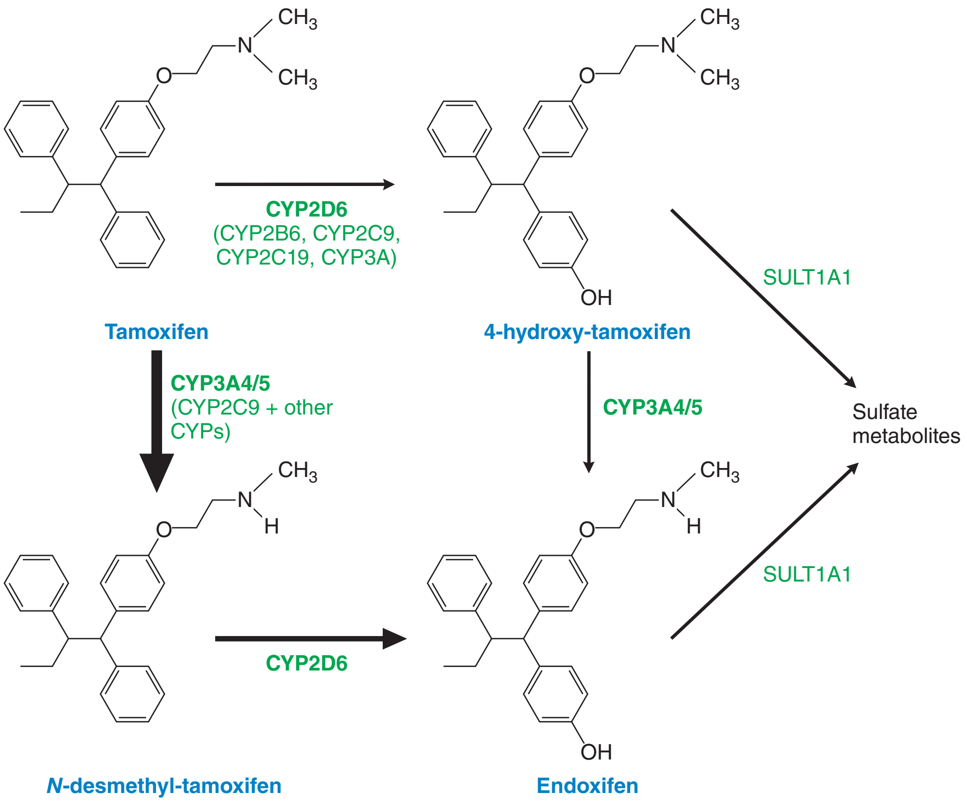

Figure 73–2 *Tamoxifen and its metabolites.*

Cancer Trialists' Collaborative Group, 2015). Recent studies indicate that patients with breast cancer derive modest additional benefit, in terms of disease-free survival, overall survival, and decrease in contralateral breast cancer risk, if *tamoxifen* is taken for up to 10 years or AIs are continued for 5 to 10 years after completion of 5 years of *tamoxifen* (Burstein, 2020; Davies et al., 2013; Goss et al., 2016). Although taken for a finite time, *tamoxifen* has persisting long-term benefits (Ekholm et al., 2016). *Tamoxifen* may be taken as sole adjuvant therapy or after adjuvant chemotherapy (see Table 73–1). Alternative or additional antiestrogen strategies in the adjuvant treatment of premenopausal women with ER+ breast cancer include oophorectomy or suppression of ovarian function with GnRH analogues in combination with either *tamoxifen* or an AI. These combinations, used in premenopausal women, further reduce estrogen stimulation of breast cancer and result in lower rates of disease recurrence in very young women and in higher risk patients receiving chemotherapy (Francis et al., 2015; Pagani et al., 2020). Some studies suggest improved response rates with these combinations in patients with metastatic disease. *Tamoxifen* also is effective (a 40%–50% reduction in tumor incidence) in preventing breast cancer in women at increased risk. In the preventive setting, *tamoxifen* reduces only ER+ tumors, not ER– tumors and does not affect overall mortality. In patients with metastatic cancer, responses to hormonal therapy may not be apparent clinically or by imaging for 8 to 12 weeks. The medication typically should be continued until the disease progresses or unwanted toxicities develop.

Adverse Effects. The common adverse reactions to *tamoxifen* include vasomotor symptoms (hot flashes), atrophy of the lining of the vagina, menstrual irregularities, vaginal bleeding and discharge and pruritus vulvae, these occur with increasing severity in postmenopausal women. The partial agonist activity of *tamoxifen* increases the incidence of endometrial cancer by 2- to 3-fold, particularly in postmenopausal women who receive *tamoxifen* for more than 2 years, although the absolute risk remains low. The increase in risk of thromboembolic events is slight and increases with the age of a patient and during the perioperative period. Hence, it often is advisable to temporarily halt *tamoxifen* prior to elective surgery. *Tamoxifen* is associated with fatty liver disease (although rarely clinically significant), a small increase in risk of cataracts, and, rarely, retinal deposits and decreased visual acuity. The FDA has assigned a boxed warning to *tamoxifen* based on uterine malignancies and thromboembolic events associated with its use, noting that for breast cancer patients, the benefits of *tamoxifen* outweigh these risks.

Toremifene. *Toremifene* is a triphenylethylene derivative of *tamoxifen* and has a similar pharmacological profile, clinical efficacy, and safety. *Toremifene* is used occasionally in the metastatic setting for the treatment of breast cancer in postmenopausal women with tumors that are ER+ or of unknown receptor status. It can also be used for the treatment of desmoid tumors. In rare cases, *toremifene* can cause heart rhythm problems through prolongation of the QT interval.

Selective Estrogen Receptor Downregulators

The SERDS, also termed *pure antiestrogens*, include *fulvestrant* and a number of agents in experimental trials. SERDs, unlike SERMs, are devoid of any estrogen agonist activity.

Fulvestrant. *Fulvestrant* is currently the only FDA-approved SERD, either as a single agent or in combination with *palbociclib*, a CDK4/6 inhibitor (see Chapter 71), for postmenopausal women with ER+/PR+ metastatic breast cancer that has progressed on antiestrogen therapy.

Mechanism of Action. *Fulvestrant* is a steroidal antiestrogen that binds to the ER with an affinity more than 100 times that of *tamoxifen*. The drug not only inhibits the binding of estrogen but also alters the receptor structure and exposes a region that leads to targeting of the protein for proteasomal degradation (see Figure 73–1A); *fulvestrant* also may inhibit receptor dimerization. Unlike *tamoxifen*, which stabilizes or even increases ER expression, *fulvestrant* reduces the number of ER molecules in cells; as a consequence of this downregulation, the drug abolishes ER-mediated transcription of estrogen-dependent genes.

ADME. *Fulvestrant* (500-mg dose) is given intramuscularly with initial biweekly loading doses in the first month followed by once-monthly injections thereafter. Using this dosing regimen, steady-state levels are achieved within the first month (see Table 73–1). C_{Pmax} is reached approximately 7 days after intramuscular administration; the plasma $t_{1/2}$ is approximately 40 days. There is rapid distribution and extensive protein binding of this highly lipophilic drug. Various pathways, similar to those of steroid metabolism (oxidation, aromatic hydroxylation, and conjugation), metabolize *fulvestrant*. CYP3A4 appears to be the major CYP isoenzyme involved in the metabolism of *fulvestrant*. The putative metabolites possess no estrogenic activity, and only the 17-keto compound demonstrates a level of antiestrogenic activity (~22% that of *fulvestrant*). Less than 1% of the parent drug is excreted intact in the urine.

Therapeutic Uses. *Fulvestrant* is used in postmenopausal women or in premenopausal women receiving GnRH agonists as antiestrogen therapy of ER+/PR+ metastatic breast cancer typically after progression on first-line antiestrogen therapy such as *tamoxifen* or an AI. *Fulvestrant* is at least as effective in this setting as the third-generation AI *anastrozole* (Robertson et al., 2016). Endocrine-resistant breast cancer that harbors ERα mutations retains some sensitivity to *fulvestrant*. Newer SERDs are also being developed for the treatment of advanced endocrine-resistant disease (McDonnell et al., 2021). A variety of agents that promote ER degradation such as proteolysis targeting chimeras (PROTACs) are also active areas of investigation (Wang Z et al., 2021).

Toxicity and Adverse Effects. Fulvestrant generally is well tolerated, the most common adverse effects being nausea, asthenia, pain, hot flashes, arthralgias, and headache. The risk of injection site reactions, seen in almost 10% patients, is reduced by giving the injection slowly.

Drugs That Decrease Estrogen Levels

Aromatase Inhibitors

Aromatase converts androgens to estrogens (e.g., androstenedione to estrone). AIs (Figure 73–3) block this enzymatic activity, thereby reducing estrogen production (Figure 73–4). AIs are now considered the standard of care for adjuvant treatment of postmenopausal women with ER+ breast cancer, either as initial therapy or after *tamoxifen* (Dowsett et al., 2010) as well as in higher-risk premenopausal women in combination with GnRH agonists. AIs are also approved in the initial treatment of metastatic ER+/PR+ breast cancer, often in combination with CDK4/6 inhibitors, in postmenopausal women and in combination with GnRH agonists in premenopausal women.

Aromatase (CYP19A1) converts adrenal androgens and gonadal androstenedione and testosterone to the estrogens estrone (E1) and estradiol (E2), respectively (see Figures 73–3 and 73–4; reactions catalyzed by aromatase are noted by a green "A" beside the reaction arrow in Figure 73–4). In postmenopausal women, this conversion occurs in nonovarian tissues (fat, liver, muscle, brain, breast, and breast tumors) and is the primary source of circulating estrogens. In premenopausal women, estrogen is primarily produced in the ovaries. AIs increase gonadotropin production in premenopausal women, which reduces their ability to inhibit ovarian estrogen production. As a result, AIs are not effective in premenopausal women without additional ovarian suppression (e.g., with GnRH analogues; see previous section). In postmenopausal women, AIs suppress most peripheral aromatase activity, leading to profound estrogen deprivation. AIs are classified as first, second, or third generation. In addition, they are further classified as type 1 (steroidal) or type 2 (nonsteroidal) AIs according to their structure and mechanism of action (see Figure 73–3). Type 1 inhibitors are steroidal analogues of androstenedione that bind covalently and irreversibly to the same site on the aromatase molecule. Thus, they commonly are known as aromatase inactivators. Type 2 inhibitors are nonsteroidal and bind reversibly to the heme group of the enzyme, producing reversible inhibition.

Third-Generation Aromatase Inhibitors. First- and second-generation (e.g., *aminoglutethimide*, *formestane*) AIs are no longer used for breast

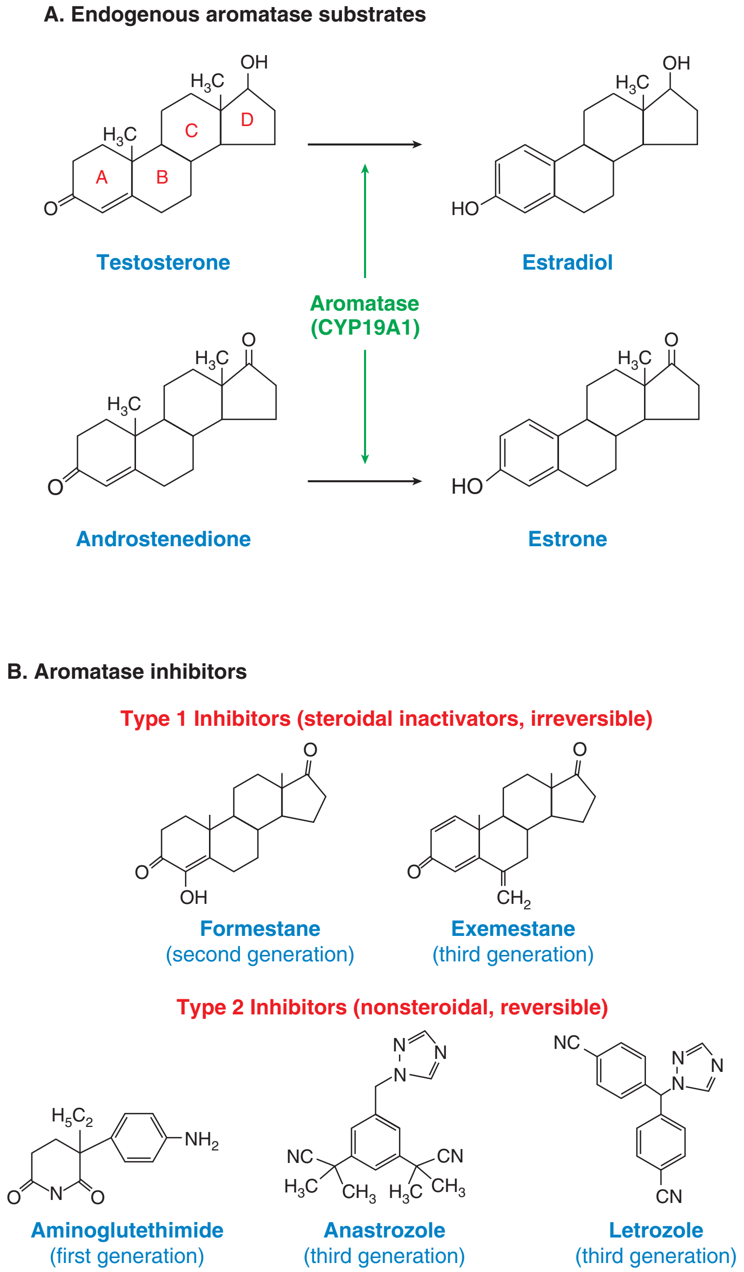
A. Endogenous aromatase substrates
A
B
C
D
Testosterone
Estradiol
Aromatase
(CYP19A1)
Androstenedione
Estrone
B. Aromatase inhibitors
Type 1 Inhibitors (steroidal inactivators, irreversible)
Formestane
(second generation)
Exemestane
(third generation)
Type 2 Inhibitors (nonsteroidal, reversible)
Aminoglutethimide
(first generation)
Anastrozole
(third generation)
Letrozole
(third generation)

Figure 73–3 *Aromatase and its endogenous substrates and inhibitors.* **A.** Aromatization of endogenous substrates. In a multistep reaction, aromatase trihydroxylates the methyl group at C19, eliminating it as formate and aromatizing the A ring of the androgen substrate. Not shown are cofactors (NADPH, O_2) and other reaction products (H_2O, formate). **B.** Aromatase inhibitors (AIs). Type 1 AIs are steroidal analogues of androstenedione that bind covalently and irreversibly to the steroid substrate site on the enzyme and are known as aromatase inactivators. Type 2 inhibitors are nonsteroidal, bind reversibly to the heme group of the enzyme, and produce reversible inhibition.

cancer treatment because of their side effects. Third-generation inhibitors include the type 1 steroidal agent *exemestane* and the type 2 nonsteroidal imidazoles *anastrozole* and *letrozole*; they are FDA approved for use in postmenopausal women. Third-generation AIs are used as part of the treatment of early-stage and advanced breast cancer in postmenopausal women and for chemoprevention (see Table 73–1). The type 1 and 2 AIs have similar clinical efficacy and toxicity profiles (Goss et al., 2013), and these are summarized for AIs using *anastrozole* as a prototype. For *letrozole* and *exemestane*, additional drug-specific information is given. Daily administration of AIs (see dosages in Table 73–1) reduces total-body androgen aromatization by more than 95% after 1 month of treatment. AIs also reduce aromatization within large ER+ breast tumors.

Anastrozole. *Anastrozole* is a potent and selective triazole AI. *Anastrozole*, like *letrozole*, binds competitively and specifically to the heme of the CYP19.

ADME. *Anastrozole* is absorbed rapidly after oral administration; food slows the rate but not the overall extent of absorption. Steady state is attained after 7 days of repeated dosing. *Anastrozole* is metabolized by hepatic *N*-dealkylation, hydroxylation (CYP3A4 mainly), and glucuronidation (UGT1A4); the main circulating metabolite of *anastrozole*, triazole, is inactive. The hepatic metabolites account for 85% of the administered dose and are excreted via bile/feces; approximately 10% of the drug is excreted in the urine as the unmetabolized parent compound. The elimination $t_{1/2}$ is approximately 50 h. The pharmacokinetics of

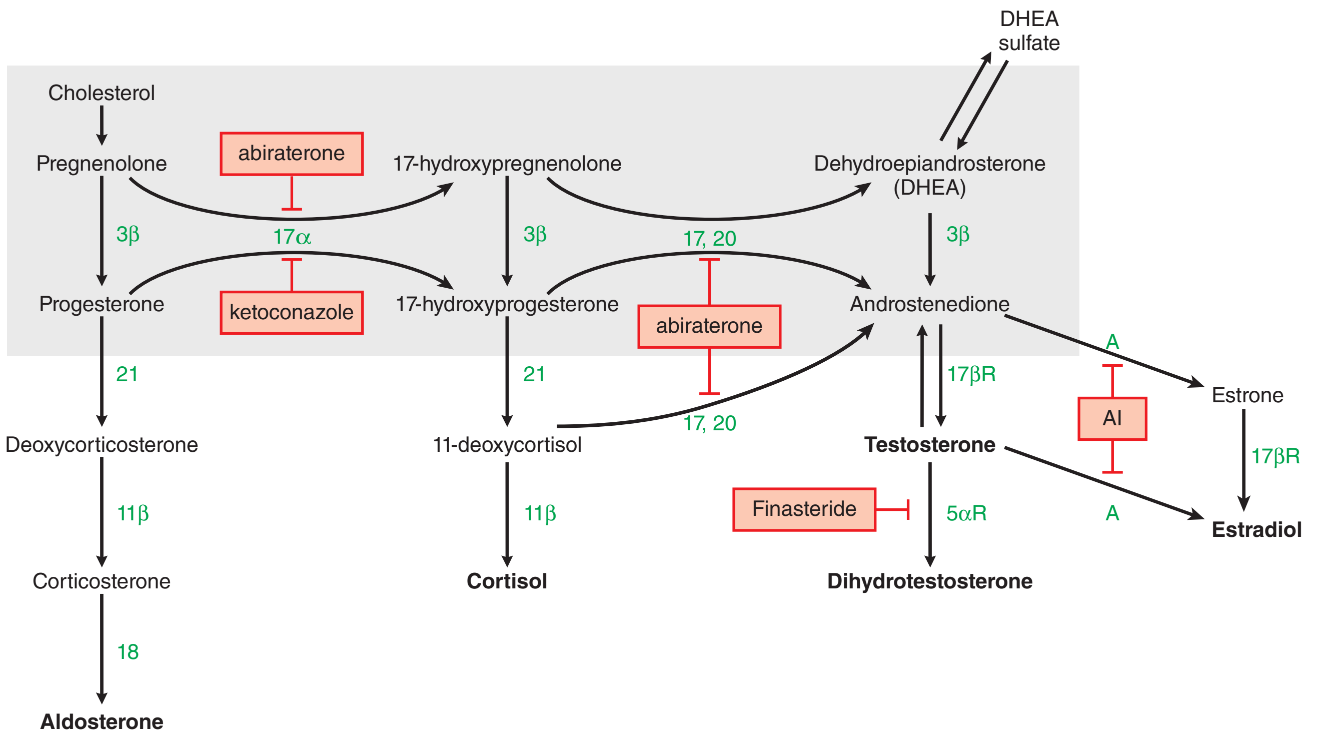

Figure 73–4 *Steroid synthesis pathways.* The shaded area contains the pathways used by the adrenal glands and gonads and clinically used agents that inhibit the pathways. Enzymes are labeled in green, inhibitors in red. Abbreviations pertaining to this figure only: *A*, aromatase; AI, aromatase inhibitor; *3β*, 3β-hydroxysteroid dehydrogenase; *5αR*, 5α-reductase; *11β*, 11β-hydroxylase; *17,20*, 17,20-lyase (CYP17A1); *17α*, 17α-hydroxylase (also CYP17A1); *17βR*, 17β-reductase; *18*, aldosterone synthase; *21*, 21-hydroxylase.

anastrozole, which can be affected by drug interactions via CYP3A4, are decreased 27% by coadministration of *tamoxifen* or *cimetidine*. *Anastrozole* is a substrate for the multidrug resistance transporter (Pgp/ABCB1) but not for the breast cancer resistance transporter (BCRP/ABCG2) (Miyajima et al., 2013).

Therapeutic Uses. AIs are approved for up-front adjuvant hormonal therapy for 5 to 10 years or following prior *tamoxifen* in postmenopausal women with early-stage breast cancer and as treatment of advanced and metastatic breast cancer. In early-stage breast cancer, *anastrozole* is significantly more effective than *tamoxifen* in delaying time to tumor recurrence and decreasing the odds of a new primary contralateral tumor. AIs are combined with ovarian suppression as adjuvant treatment of breast cancer in premenopausal women less than 35 years of age and in those requiring chemotherapy. This combination is associated with significant reduction in risk of recurrence compared to *tamoxifen* alone (Pagani et al., 2020). In postmenopausal women with ER+ or PR+ metastatic breast cancer, AIs are typically used as first-line therapy, as studies have shown them to be superior to *tamoxifen* and *megestrol acetate*. In premenopausal women with advanced disease, AIs are also used but must be given with a GnRH agonist to suppress ovarian function.

Adverse Effects and Toxicity. Most adverse effects are related to estrogen depletion. In postmenopausal women, compared to *tamoxifen*, *anastrozole* causes a lower incidence of hot flashes, vaginal bleeding, vaginal discharge, endometrial cancer, ischemic cerebrovascular events, venous thromboembolic events, and deep venous thrombosis/pulmonary embolism. *Anastrozole* is associated with a higher incidence of symptomatic arthralgias, vaginal dryness, and sexual dysfunction than *tamoxifen*. In addition, the estrogen depletion caused by AIs results in loss of bone mineral density. Compared with *tamoxifen*, treatment with *anastrozole* results in significantly lower bone mineral density in the lumbar spine and total hip and has been associated with increased fracture risk. Bisphosphonates (see Chapter 52) prevent AI-induced loss of bone mineral density in postmenopausal women.

Letrozole. The clinical uses and side effect profile of the type 2 AI *letrozole* are similar to those detailed in the previous section for *anastrozole* (see Table 73–1). *Letrozole*-specific information is as follows.

ADME. *Letrozole* is rapidly absorbed after oral administration, with a bioavailability of 99.9%. Steady-state plasma concentrations of *letrozole* are reached after 2 to 6 weeks of treatment. Following metabolism by CYP2A6 and CYP3A4, *letrozole* is eliminated as an inactive metabolite mainly via the kidneys and has a $t_{1/2}$ of approximately 41 h.

Therapeutic Uses. *Letrozole* is used to treat breast cancer in the same manner as *anastrozole* (see Table 73–1). In addition, improved progression-free survival is observed when ER+/PR+ advanced-stage breast cancer is treated with *letrozole* in combination with a CDK4/6 inhibitor (see Chapter 71) (Finn et al., 2016; Hortobagyi et al., 2016).

Exemestane. *Exemestane* is a more potent, orally administered analogue of the endogenous aromatase substrate androstenedione and lowers estrogen levels more effectively than does its predecessor, *formestane*. *Exemestane* irreversibly inactivates aromatase and is a "suicide substrate" type 1 inhibitor of aromatase. The clinical uses and side effect profile of *exemestane* are similar to those detailed in the section for the AI *anastrozole*.

ADME. Orally administered *exemestane* is rapidly absorbed from the gastrointestinal tract; its absorption is increased by 40% after a high-fat meal. *Exemestane* has a terminal $t_{1/2}$ of approximately 24 h. It is extensively metabolized in the liver, ultimately to inactive metabolites; one metabolite, 17-hydroxyexemestane, has weak androgenic activity that may contribute to antitumor activity. Although metabolites are excreted in the urine, no dosage adjustments are recommended in patients with renal dysfunction.

Therapeutic Uses. The clinical indications for use of *exemestane* in breast cancer treatment are the same as for *anastrozole* (see Table 73–1). In addition, the use of the mechanistic (or mammalian) target of rapamycin (mTOR) inhibitor *everolimus* (see Chapter 71) with *exemestane* has been approved for treatment of advanced-stage breast cancer that has progressed on nonsteroidal type 2 AIs (Piccart et al., 2014).

Toxicity and Adverse Reactions. The side effects are similar to those described for *anastrozole*. *Exemestane* causes fetal toxicities and abortion in preclinical animal experiments and is considered unsafe for administration to pregnant women; women of childbearing potential should use contraception during AI treatment and for a month thereafter.

Drugs That Target the Progesterone Receptor

Progestational agents are mainly used as secondary agents in hormonal therapy for metastatic hormone-dependent breast cancer and are also used in the management of endometrial carcinoma previously treated by surgery and radiotherapy. Progesterone binds to the PR present in target tissues, such as breast and the endometrium. Activation of the PR by progestins in the endometrium is antiproliferative. Chapter 48 covers the mechanisms and uses of hormones and drugs that interact with the PR.

Medroxyprogesterone acetate is available for oral administration; an alternative oral progestational agent is *megestrol acetate*. These agents provide beneficial effects in one-third of patients with endometrial cancer. The response of breast cancer to *megestrol* is predicted by both the presence of ER and PR and the evidence of response to a prior hormonal treatment. The effect of progestin therapy in breast cancer appears to be dose-dependent, with some patients demonstrating second responses following dose escalation of *megestrol*. Clinical use of progestins in breast cancer has been largely superseded by *tamoxifen* and AIs. Additional uses of progestins are as treatment of metastatic endometrial cancer and to stimulate appetite and restore a sense of well-being in cachectic patients with advanced stages of cancer and AIDS.

Drugs Combined With Agents That Antagonize the Estrogen Receptor

The addition of several agents used in cancer (see Chapter 71) when combined with SERMs, SERDs, or AIs improves the therapeutic outcome of breast cancer patients.

CDK4/6 Inhibitors

There are currently three CDK4/6 inhibitors—*palbociclib*, *ribociclib*, and *abemaciclib*—that have been approved for use in combination with ER antagonists (SERDs and AIs). These combinations result in significantly improved outcomes in ER+ HER2-advanced breast cancer (Finn et al., 2016; Goetz et al., 2017; Hortobagyi et al., 2016; Slamon et al., 2020; Sledge et al., 2020; Turner et al., 2018).

A major effect of CDK4/6 inhibitors is to prevent progression through the G_1/S cell cycle checkpoint by inhibiting the activation of D-type cyclins and thereby reducing the production of phosphorylated retinoblastoma-associated protein and E2F transcriptional activity (see Figure 71–3). In ER+ breast cancer, the G_1-to-S checkpoint is a therapeutic target since cyclin D is frequently overexpressed. The presence of the ER in a breast cancer is currently the best predictive marker of response to CDK4/6 inhibitors. While there are questions about the optimal sequence and choice of ER antagonists with CDK4/6 inhibitors, recent trials demonstrate no difference in outcome in the first-line setting whether an AI or *fulvestrant* is used with a CDK4/6 inhibitor (Slamon et al., 2020; Sledge et al., 2020; Turner et al., 2018).

PI3 Kinase (PI3K) and mTOR Inhibitors

The PI3K inhibitor *alpelisib* and the mTOR inhibitor *everolimus* (see Chapter 71) are approved for use in combination with *fulvestrant* and *exemestane*, respectively, in treating advanced-stage breast cancer (Andre et al., 2019; Piccart et al., 2014). The mTOR, a kinase in the phosphoinositide-3-kinase (PI3K)/AKT signaling pathway, controls cell growth and proliferation (see Figure 71–5). In breast cancer, the PI3K/AKT/mTOR pathway modulates the growth of breast cancer through ER-mediated signaling as well as through the HERs (human epidermal growth factor receptors) and mediates the clinical sensitivity to ER antagonists. Breast cancers that harbor activating mutations in PI3K can be resensitized to endocrine therapies by treatment with inhibitors of PI3K or mTOR.

Drugs That Target the Androgen Receptor

AR Antagonists

AR antagonists competitively inhibit the binding of testosterone and dihydrotestosterone to the AR, thereby reducing AR nuclear translocation, chromatin binding, and AR-mediated gene transcription (see Figure 73–1B). Unlike castration, AR antagonist therapy by itself does not decrease LH production; therefore, testosterone levels are normal or increased. Men treated with AR antagonists maintain some degree of potency and libido and do not have the same spectrum of side effects seen with castration. However, AR antagonist therapy is typically given in combination with ADT (described above). AR antagonists include the newer second-generation drugs *enzalutamide, apalutamide*, and *darolutamide*, and the earlier generation antagonists *bicalutamide, flutamide*, and *nilutamide*. Table 73–2 summarizes dosing and conditions for the use of these agents in treating prostate cancer.

Enzalutamide

Enzalutamide is a second-generation, nonsteroidal competitive AR antagonist that has 5- to 8-fold higher binding affinity for the AR compared to the older antagonist *bicalutamide* (Tran et al., 2009). Similar to other AR antagonists, *enzalutamide* prevents binding of androgens to the AR, thereby decreasing receptor-mediated transcriptional activity. *Enzalutamide* is approved for use in nonmetastatic and metastatic CRPC as well as in metastatic castration-sensitive prostate cancer. *Enzalutamide* prolongs survival in patients with metastatic CRPC when given to chemotherapy-naïve patients or after *docetaxel* therapy (Paschalis and de Bono, 2020). *Enzalutamide* combined with ADT, compared with ADT alone, also prolongs metastasis-free and overall survival in men with nonmetastatic castration-resistant disease and rapidly rising circulating prostate-specific antigen (indicative of more aggressive disease and increased risk of progression) (Hussain et al., 2018; Sternberg et al., 2020).

ADME. *Enzalutamide* has improved efficacy and potency compared to older, first-generation AR antagonists. *Enzalutamide* is given orally once daily. Median time to reach C_{Pmax} is 1 h. Its $t_{1/2}$ is approximately 6 days; steady-state levels are achieved in 28 days. CYP2C8 and CYP3A4 metabolize *enzalutamide*. Therefore, concomitant treatment with *enzalutamide* and strong CYP2C8 inhibitors such as *gemfibrozil* or *pioglitazone* may raise levels of *enzalutamide*. Conversely, strong inducers of CYP2C8 or CYP3A4 (e.g., *rifampin*) may reduce plasma *enzalutamide*. *Enzalutamide* can induce multiple hepatic CYPs, including 3A4, 2C9, and 2C19 and thereby has the potential to affect the metabolism of up to 50% of drugs (reviewed in Del Re et al., 2017). Coadministration with substrates of these CYPs (including *fentanyl*, *nifedipine*, *disopyramide*, *quetiapine*, *quinidine*, and *warfarin*) should be avoided as *enzalutamide* may decrease levels of these drugs (Benoist et al., 2018).

Adverse Effects and Toxicity. The most frequently reported side effects are fatigue, back pain, hot flush, constipation, arthralgia, decreased appetite, diarrhea, and hypertension. Fractures, falls, and cognitive impairment have been reported (Fizazi et al., 2020a; Sternberg et al., 2020). A population-based retrospective study showed that *enzalutamide* use is associated with an increased risk of short-term mortality in elderly prostate cancer patients with preexisting cardiovascular comorbidities compared to similar patients without cardiovascular disease (Lu-Yao et al., 2020). *Enzalutamide* crosses the blood-brain barrier; seizures occur infrequently (0.5%–2.2% of patients).

Apalutamide

Apalutamide is a second-generation, nonsteroidal AR antagonist that is approved for use in nonmetastatic castration-resistant and metastatic castration-sensitive prostate cancer (Smith et al., 2021).

ADME. *Apalutamide* is given orally once daily as four tablets, taken with or without food. Mean absolute oral bioavailability is approximately

100%. Median time to achieve peak plasma concentration (t_{max}) is 2 h and may be increased with a high-fat meal. Mean elimination $t_{1/2}$ is 3 to 4 days. Steady state is achieved by approximately 4 weeks. *Apalutamide* is a strong inducer of CYP3A4 and CYP2C19 and a weak inducer of CYP2C9. Concomitant use of *apalutamide* and drugs that are substrates of these CYPs should be avoided as lower exposure to these CYP substrates may result. Coadministration of *apalutamide* and substrates of UDP-glucuronosyltransferase (UGT), Pgp (e.g., *fexofenadine*), breast cancer resistance protein (BCRP), or organic anion transporting polypeptide 1B1 (OAT1B1) (e.g., *rosuvastatin*) may result in decreased exposure to these medications.

Toxicity. *Apalutamide* has a similar toxicity profile as *enzalutamide* and can cause fatigue, hypertension, rash, nausea, hot flush, weight loss, arthralgia, falls, decreased appetite, diarrhea, fracture, and peripheral edema. Hypothyroidism has been reported. *Apalutamide* can increase the QT interval. Seizures occurred in 0.2% of patients receiving *apalutamide*, possibly related to interaction of the drug with the $GABA_A$ receptor.

Darolutamide

Darolutamide is a second-generation, nonsteroidal AR antagonist that is used in the treatment of nonmetastatic CRPC. Addition of *darolutamide* to ongoing ADT significantly increases time to metastasis-free survival in men with nonmetastatic prostate cancer (Fizazi et al., 2020b).

ADME. *Darolutamide* is given orally twice daily and should be given with food. The $t_{1/2}$ is approximately 20 h. C_{Pmax} is reached approximately 4 h after administration of a single 600-mg oral dose. In contrast to *enzalutamide* and *apalutamide*, *darolutamide* does not induce CYPs. Use of *darolutamide* concurrently with inhibitors of Pgp and strong-to-moderate CYP3A inducers may decrease *darolutamide* exposure. Concurrent use of *darolutamide* with inhibitors of Pgp and strong CYP3A4 inhibitors may increase *darolutamide* exposure. *Darolutamide* inhibits BCRP transporter, and therefore, concurrent use of *darolutamide* and BCRP substrates (e.g., *prazosin*, *glyburide*, *cimetidine*, *sulfasalazine*, *rosuvastatin*, and nucleoside and nucleotide analogues such as *zidovudine* and *lamivudine*) may increase BCRP substrate toxicity. *Darolutamide* inhibits OATP1B1 and OATP1B3 transporters. Concomitant use of *darolutamide* and OAT1B1 or OATP1B3 substrates (e.g., statins, taxanes, *cisplatin*) may increase concentrations of drugs that are substrates of OAT1B1 and OATP1B3. Post-hoc analysis of a phase III trial of *darolutamide* reported a low incidence of clinically relevant drug-drug interactions (Shore et al., 2019).

Adverse Effects and Toxicity. *Darolutamide* is structurally distinct from *enzalutamide* and *apalutamide* and is not associated with an increase in adverse events such as falls, seizures, cognitive disorders, mental impairment disorders, and hypertension compared to ADT alone (Fizazi et al., 2020b). The most common adverse reactions are fatigue, pain in extremities, and rash. Significant adverse reactions in patients taking *darolutamide* include ischemic heart disease, heart failure, urinary retention, pneumonia, and hematuria. Of the newer generation AR antagonists, seizures appear to be least frequent with *darolutamide*, which does not penetrate the blood-brain barrier.

Older AR Antagonists

These nonsteroidal agents are still in use but are being supplanted by the more potent second-generation AR antagonists.

Bicalutamide. The agent *bicalutamide* is given once daily in conjunction with a GnRH agonist, usually to combat tumor flare. *Bicalutamide* has a $t_{1/2}$ of 5 to 6 days; it undergoes glucuronidation to inactive metabolites, and the parent compound and metabolites are eliminated in bile and urine. The $t_{1/2}$ of *bicalutamide* is increased in severe hepatic insufficiency and is unchanged in renal insufficiency. *Bicalutamide* is well tolerated at higher doses and has reduced toxicity and improved tolerability and pharmacokinetic profiles relative to *flutamide* and *nilutamide*. Daily *bicalutamide* is significantly inferior compared to surgical or medical castration and should not be used as monotherapy of prostate cancer. *Bicalutamide* inhibits CYP3A4, and therefore, coadministration of *bicalutamide* and CYP3A4 substrates should be monitored. Use of *bicalutamide* in patients receiving anticoagulants requires monitoring since *bicalutamide* may displace coumarin anticoagulants from binding sites.

Nilutamide. *Nilutamide* is given orally once daily. It has an elimination $t_{1/2}$ of 45 h and is metabolized to five products that are all excreted in the urine. Common side effects include mild nausea, alcohol intolerance (5%–20%), and diminished ocular adaptation to darkness (25%–40%); rarely, interstitial pneumonitis occurs.

Flutamide. *Flutamide* is given orally three times per day. It has a $t_{1/2}$ of 5 h; its major metabolite, hydroxyflutamide, is biologically active. Common side effects include diarrhea, breast tenderness, and nipple tenderness. Less commonly, nausea, vomiting, and hepatotoxicity occur. Rare reports of fatal hepatotoxicity have been observed. It is used infrequently as it has the least favorable toxicity profile of the antiandrogens.

Resistance to AR Antagonists, Including to Second-Generation Drugs

Resistance to these agents develops frequently through mechanisms that primarily but not exclusively enhance AR activity, as noted above (reviewed in Schmidt et al., 2021). In an effort to overcome resistance to current antiandrogen therapies, studies are ongoing with a variety of new agents, including those that promote AR degradation such as PROTACs (Salami et al., 2018) and small-molecule receptor degraders (Mohler et al., 2021).

Drugs That Inhibit Androgen Synthesis

As discussed above, even in conditions of castration levels of circulating testosterone, persistent AR activity in CRPC cells may support tumor growth. Synthesis of extragonadal androgens occurs in the adrenal glands or CRPC tumors themselves (see Figure 73–4). Androstenedione, produced by the adrenal glands, is converted to testosterone and dihydrotestosterone in peripheral tissues and prostate tumors. Intratumoral *de novo* androgen synthesis also may provide sufficient androgen for AR-driven cell proliferation. Thus, inhibitors of androgen synthesis combined with ADT have proven useful in reducing AR signaling in CRPC.

Abiraterone Acetate

Abiraterone, with *prednisone*, is used for the treatment of metastatic prostate cancer in patients who are chemotherapy naïve or in those who have received previous *docetaxel*. In both settings, *abiraterone* prolongs survival (reviewed in Paschalis and de Bono, 2020). The combination of *abiraterone* and *prednisone*, in conjunction with ADT, also prolongs overall survival in men with metastatic castration-sensitive prostate cancer (Virgo et al., 2021; Wang L et al., 2021). Resistance to *abiraterone*, similar to that to *enzalutamide*, can occur through a variety of mechanisms mainly involving the AR (discussed above). AR antagonists and abiraterone in additional prostate cancer clinical settings is being evaluated in ongoing clinical trials.

Mechanism of Action. *Abiraterone* is an irreversible inhibitor of 17α-hydroxylase and 17,20-lyase (CYP17A1) activity in testicular, adrenal, and prostatic cancer tissue (see Figure 73–3). Inhibition of CYP17A1 reduces the conversion of pregnenolone and progesterone to their 17α-OH derivatives and their subsequent enzymatic conversion to dehydroepiandrosterone and androstenedione. Thus, circulating levels of testosterone drop to almost undetectable levels after *abiraterone* administration. *Abiraterone* also has some activity as an AR antagonist and inhibitor of other steroid synthetic enzymes and CYPs. Overall, *abiraterone* has greater potency and selectivity than *ketoconazole*, which is described below.

ADME. Abiraterone is the active metabolite of *abiraterone acetate*. With continuous administration, *abiraterone* increases adrenocorticotropic hormone levels, resulting in mineralocorticoid excess. Oral *abiraterone* acetate is administered with *prednisone* to counteract adrenal suppression. *Abiraterone* should be taken on an empty stomach due to the effect of food to increase both C_{max} and AUC of the drug. Alternatively, the National Comprehensive Cancer Network lists low-dose *abiraterone* (250 mg/day) with a low-fat breakfast as an alternative treatment option to 1000 mg/day of *abiraterone* taken on an empty stomach (Szmulewitz et al., 2018)

Abiraterone is metabolized by CYP3A4; thus, concomitant use of drugs that are strong CYP3A4 inducers (e.g., *carbamazepine*, *rifampicin*, St. John's wort, several reverse transcriptase inhibitors) should be avoided. *Abiraterone acetate* inhibits the highly polymorphic CYP2D6 and CYP2C8; thus, concomitant use of drugs that are substrates of these CYPs (e.g., 2D6 substrates: *risperidone*, *metoprolol*, *nebivolol*, *imipramine*-based antidepressants, *fluoxetine*, *tamoxifen*, *codeine*, *hydrocodone*, *oxycodone*, *tramadol*; 2C8 substrates: *amiodarone*, *carbamazepine*, *cerivastatin*, *diclofenac*, *ibuprofen*, *paclitaxel*, *rosiglitazone*) requires careful monitoring for toxicity and possibly dose adjustment.

Toxicity. The most common side effects include fatigue, arthralgia, hypertension, nausea, edema, hypokalemia, hot flush, diarrhea, vomiting, upper respiratory tract infection, cough, and headache. As was observed with enzalutamide in the same study, use of *abiraterone* is associated with an increased risk of short-term mortality in elderly men with preexisting cardiovascular comorbidities (Lu-Yao et al., 2020). Laboratory abnormalities that are most common include anemia, elevated alkaline phosphatase, hypertriglyceridemia, lymphopenia, hypercholesterolemia, hyperglycemia, and hypokalemia.

Ketoconazole

Ketoconazole is an antifungal agent that also inhibits both testicular and adrenal steroidogenesis by blocking CYP17 (17α-hydroxylase), CYP11A, and other P450 cytochrome enzymes. *Ketoconazole* can be administered off-label in conjunction with ADT to reduce adrenal androgen synthesis in CRPC. Oral *ketoconazole* is coadministered with *hydrocortisone* to compensate for inhibition of adrenal steroidogenesis. *Ketoconazole* has limited use in practice due to its toxicity and inferiority compared to *abiraterone acetate*. Chapter 61 presents the basic and clinical pharmacology of the azoles.

Prostate Cancer Chemoprevention (5α-Reductase Inhibitors)

5α-Reductase catalyzes the conversion of testosterone to dihydrotestosterone, the most potent endogenous androgen. Two inhibitors of 5α-reductase, *finasteride* and *dutasteride*, have been studied for prostate cancer chemoprevention. The results have been controversial: two clinical trials indicated decreased risk of low-grade prostate cancer but increased incidence of high-grade disease in the drug treatment arms. However, longer-term follow-up and other studies (Goodman et al., 2019) revealed no elevated incidence for high-grade prostate cancer or mortality from prostate cancer in the *finasteride*-treated subjects; the initial results in higher grade prostate cancer may have resulted from detection bias or may have been related to the study design (reviewed in Chau and Figg, 2018).

Bipolar Androgen Deprivation Therapy

Since CRPC typically becomes resistant to AR-directed therapies such as *enzalutamide* and *abiraterone*, investigators have examined whether bipolar androgen deprivation therapy (BAT) might resensitize tumors to AR-directed drugs. With prolonged deprivation of stimulation by androgens, prostate cancer cells may adapt via upregulation of the AR, thereby becoming resistant to treatment. BAT is rapid cycling between extremes of high and low (castrate) circulating testosterone in order to disrupt the process by which prostate cancer cells adapt to a low androgen milieu. A randomized study compared BAT versus *enzalutamide* in men with CRPC whose disease had progressed following *abiraterone*. Although there was no significant difference between the treatment arms in terms of progression-free survival, BAT did improve the extent and duration of response in the crossover arm in which BAT was the intervening therapy between *abiraterone* and *enzalutamide*. This study underscores the need to define optimal sequencing of BAT and different AR-targeted therapies (Denmeade et al., 2021).

Drugs That Target the Glucocorticoid Receptor

The pharmacology, major therapeutic uses, and toxic effects of the glucocorticoids are discussed in Chapters 50, 74, and 75. Only the applications of these drugs in the treatment of neoplastic disease are considered here.

A number of glucocorticoids are available and at equivalent dosages exert similar effects (see Chapter 50). *Prednisone*, for example, usually is administered orally in doses up to 100 mg for the first few days and gradually reduced to the lowest possible effective dose. Side effects of these agents include glucose intolerance, immunosuppression, osteoporosis, and psychosis (see Chapter 50). *Dexamethasone* is one of the preferred agents for remission induction in multiple myeloma, typically in combination with *bortezomib*, *anthracyclines*, or *lenalidomide*. Glucocorticoids, particularly *dexamethasone*, are used in conjunction with radiotherapy to reduce edema related to tumors in critical areas such as the superior mediastinum, brain, and spinal cord. Frequent lower doses (4–6 mg every 6 h) can have dramatic effects in restoring neurological function in patients with cerebral metastases, but these effects are temporary. However, acute changes in *dexamethasone* dosage can lead to a rapid recrudescence of symptoms. *Dexamethasone* should not be discontinued abruptly in patients receiving radiotherapy or chemotherapy for brain metastases. *Dexamethasone* is also frequently used as part of an antiemetic regimen in patients receiving chemotherapy.

Acknowledgment: *Anton Wellstein contributed to this chapter in the previous edition of this book. We have retained some of his text in the current edition.*

Drug Facts for Your Personal Formulary: *Hormones and Related Agents in Cancer Therapy*

Drug	Therapeutic Use	Clinical Pharmacology and Tips
Glucocorticoid Receptor Agonists		
Dexamethasone Prednisone Others	• Treatment of malignant hematologic disorders (ALL, CLL, MM, HL, NHL) • Symptom palliation in various cancer types (antiemetic; ↓ edema due to spinal cord compression, brain metastases)	• Major toxicities: Cushing syndrome, glucose intolerance, immunosuppression, osteoporosis, psychosis, insomnia • Acute reduction in dosing can lead to recurrence of symptoms • Drug interactions: concomitant use of CYP3A4 inhibitors or inducers. Concomitant use of erythropoietin-stimulating agents or estrogens increases risk of thromboembolism
Selective Estrogen Receptor Modulators: Antiestrogens in breast cancer therapy		
Tamoxifen	• Adjuvant therapy for pre- and postmenopausal women with HR+ breast cancer • Treatment of advanced or metastatic HR+ breast cancer in pre- and postmenopausal women • Breast cancer prevention in pre- and postmenopausal women • Rarely used to treat metastatic endometrial cancer	• SERM with partial agonist and antagonist action. Antagonist of ER in breast. Long $t_{1/2}$. Steady-state levels reached in 3–4 weeks • Some major toxicities due to ER agonism (endometrial carcinoma, thromboembolic events) or ER antagonism (vasomotor symptoms, menstrual irregularities) • Other adverse effects: cataracts • Drug interactions: strong CYP3A/4 inducers (rifampin) reduce AUC and C_{max}. Inhibitors of CYP2D6 increase metabolism (e.g., paroxetine) but it has not been established that this changes clinical efficacy. Increases anticoagulant effect of warfarin
Toremifene	• Postmenopausal metastatic HR+ breast cancer	• Pharmacology, clinical efficacy, and adverse effects similar to those of tamoxifen • Rare: prolongs QT interval, increased risk of torsades de pointes • Drug interactions: with agents that prolong QT. CYP3A/4 inducers/inhibitors change AUC and C_{max}. Use with caution with CYP2C9 substrates (warfarin, phenytoin)
Selective Estrogen Receptor Downregulators: Antiestrogens in breast cancer therapy		
Fulvestrant	• Advanced or metastatic HR+ breast cancer (+/– CDK4/6 inhibitors or +/– PI3K inhibitor alpelisib) in postmenopausal women who have disease progression after antiestrogen therapy	• Binds to ER, blocks estrogen action, and causes degradation of the ER • No estrogen agonist effects • IM loading then monthly dosing; steady state achieved in first month • Side effects: injection site reaction, nausea, weakness, bone and back pain, fatigue, vasomotor symptoms, headache • Drug interactions: no major interactions noted
Aromatase Inhibitors: Antiestrogen in breast cancer therapy		
Anastrozole, letrozole (nonsteroidal, competitive inhibitors) Exemestane (steroidal, irreversible inhibitor)	• Adjuvant treatment of postmenopausal (natural or in conjunction with OFS) women with HR+ breast cancer • Treatment of postmenopausal women (natural or in conjunction with OFS) with HR+ advanced and metastatic breast cancer (+/– CDK4/6 inhibitors) (exemestane +/– mTOR inhibitor) • Breast cancer prevention in postmenopausal women	• AIs significantly lower serum estrogens • Contraindicated in premenopausal women with ovarian function • Major side effects: vasomotor symptoms, arthralgia, loss of bone mineral density, osteoporosis, fractures, vaginal dryness, dyspareunia • Drug interactions: concomitant use with tamoxifen reduces plasma levels 27% (anastrazole). Estrogen coadministration reduces AI efficacy. CYP3A/4 inducers decrease exemestane exposure
Progesterone Receptor Agonists		
Megestrol acetate	• Treatment of endometrial cancer and rarely of breast and prostate cancer • Appetite stimulant in patients with AIDS or cancer-associated cachexia	• Adverse effects: weight gain, nausea, vomiting, edema, breakthrough bleeding, shortness of breath, thrombophlebitis, pulmonary embolism • Drug interactions: coadministration reduces exposure to HIV protease inhibitor indinavir
Medroxyprogesterone acetate	• Management of advanced-stage endometrial carcinoma • Therapy of metastatic hormone-dependent breast cancer	• Adverse effects: hot flashes, weight gain, depression, amenorrhea • With long-term use, bone loss is possible • Drug interactions: CYP3A/4 inducers reduce plasma levels
Gonadotropin-Releasing Hormone Analogues: Chemical castration in cancer therapy		
Prostate Cancer *GnRH agonists*: Leuprolide Goserelin Buserelin Histrelin Triptorelin	• Androgen deprivation therapy: ↓ pituitary release of LH and FSH, ↓ testicular testosterone production • Treatment of all forms of advanced prostate cancer • In combination with radiation therapy or surgery for management of moderate-/high-risk locally confined prostate cancer • In combination with androgen receptor antagonists or androgen synthesis inhibitors for combined androgen blockade	• Can cause initial testosterone surge and tumor flare. Administered with androgen receptor antagonists to reduce initial side effects from testosterone surge • Side effects related to low testosterone: vasomotor symptoms, loss of libido, osteoporosis, fatigue, impotence, gynecomastia, loss of muscle mass • Small but significant increase in risk of diabetes or development of cardiovascular disease • Drug interactions: no major interactions noted

Drug Facts for Your Personal Formulary: *Hormones and Related Agents in Cancer Therapy* (*continued*)

Drug	Therapeutic Use	Clinical Pharmacology and Tips
GnRH antagonists: Degarelix (cetrorelix) Relugolix	• Treatment of all forms of advanced prostate cancer • Cetrorelix less used, requires more frequent injection, higher cost	• No initial testosterone surge; rapid suppression of serum testosterone and prostate-specific antigen levels • Side effect profile in men similar to GnRH agonists above • More effectively decreases FSH compared to GnRH agonists • Relugolix is given orally • Fewer major cardiovascular events (relugolix compared to leuprolide) • Prolonged QT interval with relugolix (rare) • Drug interactions: relugolix interacts with Pgp and CYP3A4. Coadministration with strong CYP3A/4 inducers (rifampin) reduces AUC and C_{max}; Pgp inhibitors (erythromycin) increase AUC and C_{max}
Breast Cancer *GnRH agonist*: Goserelin Leuprolide	• Suppression of ovarian estrogen and progesterone production in pre- and perimenopausal women • With antiestrogens as adjuvant therapy or for metastatic disease	• Adverse effects due to hypoestrogenism: vasomotor symptoms, ↓ libido, osteoporosis, tumor flare, fatigue, vaginal dryness, dyspareunia • Drug interactions: No major interactions noted
Nonsteroidal Androgen Receptor Antagonists: Antiandrogens in prostate cancer therapy		
Enzalutamide	• In conjunction with ADT (or bilateral orchiectomy) for treatment of metastatic castration-sensitive prostate cancer, nonmetastatic and metastatic CRPC	• Adverse effects related to AR antagonism: sexual dysfunction, gynecomastia, breast pain, fatigue, diarrhea, headache, musculoskeletal pain, vasomotor symptoms, hot flashes, hypertension • Increase risk of cardiovascular disease (particularly in elderly men with preexisting conditions) • Rare: seizures (likely due to central "off-target" effects) • Drug interactions: enzalutamide may affect metabolism of many drugs through induction of multiple CYPs. Strong CYP2C8 inhibitors (e.g., the fibrate gemfibrozil) will raise enzalutamide levels. Strong inducers of CYP2C8 or CYP3A4 (e.g., rifampin) will reduce plasma enzalutamide
Apalutamide	• In conjunction with ADT (or bilateral orchiectomy), for treatment of metastatic castration-sensitive prostate cancer and nonmetastatic CRPC (not yet FDA approved for metastatic CRPC)	• Adverse effects related to AR antagonism • Drug interactions: apalutamide strongly induces CYP3A4 and CYP2C19. Concomitant use with substrates of these CYPs should be avoided. Coadministration of apalutamide with substrates of UGT, Pgp, BCRP, and OATP1B1 may decrease exposure to these medications
Darolutamide	• In conjunction with ADT (or bilateral orchiectomy), for treatment of nonmetastatic CRPC	• Adverse effects related to AR antagonism • Drug interactions: concomitant use with Pgp inhibitors and CYP3A inducers may decrease darolutamide exposure. Use with Pgp inhibitors and CYP3A inhibitors may increase darolutamide exposure. BCRP transporter inhibited by darolutamide; coadministration with BCRP substrates may increase substrate toxicity. Darolutamide inhibits OATP1B1 and OATP1B3; concomitant use with OATP substrates may increase concentration of these medications
Bicalutamide	• Older generation AR antagonist used with GnRH analogues to treat all forms of advanced prostate cancer. Current use mainly to ameliorate GnRH agonist flare	• Adverse effects related to AR antagonism • Favorable toxicity and pharmacokinetic profile relative to flutamide or nilutamide • Drug interactions: coadministration of bicalutamide and CYP3A4 substrates should be monitored. Bicalutamide may displace coumarin anticoagulants from binding sites, and therefore, use in patients receiving anticoagulants requires caution
Inhibitors of Steroidogenesis: Antiandrogens in prostate cancer therapy		
Abiraterone	• Treatment of metastatic prostate cancer (castration-sensitive and castration-resistant) • Used in combination with prednisone or dexamethasone (to compensate for adrenal insufficiency induced by abiraterone)	• Irreversibly inhibits CYP17A1, ↓ testosterone and other androgens • Fluid retention, hypertension, hypokalemia, hepatotoxicity, fatigue, joint swelling, vasomotor symptoms, diarrhea, arrhythmia • Increase risk of cardiovascular disease (particularly in elderly men with preexisting conditions) • Should be taken on empty stomach; or reduced dose with low-fat breakfast • Drug interactions: strong CYP3A4 inducers decrease abiraterone plasma levels. Plasma levels of substrates of CYP2D6 and CYP2C8 may increase by coadministration with abiraterone

OFS, ovarian function suppression.

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Section IX

Special Systems Pharmacology

Chapter 74. Ocular Pharmacology / 1453
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Chapter 74

Ocular Pharmacology

Upneet K. Bains, Zeba A. Syed, Jeffrey D. Henderer, and Christopher J. Rapuano

EXTRAOCULAR STRUCTURES

OCULAR STRUCTURES
- Anterior Segment
- Posterior Segment

PHARMACOKINETICS AND TOXICOLOGY OF OCULAR THERAPEUTIC AGENTS
- Drug Delivery Strategies

OPHTHALMIC USES OF DRUGS
- Chemotherapy of Microbial Diseases in the Eye
- Ophthalmic Use of Autonomic Agents, Prostanoids, and Carbonic Anhydrase Inhibitors
- Anti-inflammatory, Immunomodulatory, and Antimitotic Drugs
- Agents Used in Ophthalmic Surgery
- Ocular Side Effects of Systemic Agents
- Agents Used to Assist in Ocular Diagnosis
- Treatment of Retinal Neovascularization, Macular Degeneration, and Vitreomacular Traction
- Anesthetics in Ophthalmic Procedures
- Treatment of Dry Eye and Corneal Edema
- Treatment of the Neurotrophic Cornea

VITAMIN A AND THE VISUAL CYCLE
- Chemistry
- Retinal Cells and the Visual Cycle
- Vitamin A Deficiency
- Vitamin A and Epithelial Structures
- Therapeutic Uses of Vitamin A

The eye is a specialized sensory organ that is relatively secluded from systemic access by the blood-retinal, blood-aqueous, and blood-vitreous barriers. As a consequence, the eye exhibits some unusual pharmacodynamic and pharmacokinetic properties.

Extraocular Structures

The eye is protected by the eyelids and by the orbit, a bony cavity of the skull that has multiple fissures and foramina that conduct nerves, muscles, and vessels (Figure 74–1). In the orbit, connective (i.e., *Tenon's capsule*) and adipose tissues and six extraocular muscles support and align the eyes for vision. The retrobulbar region lies immediately behind the eye (or *globe*). Understanding ocular and orbital anatomy is important for safe periocular drug delivery, including subconjunctival, sub-Tenon, and peribulbar or retrobulbar injections.

The external surface of the eyelids is covered by a thin layer of skin; the internal surface is lined with the palpebral portion of the conjunctiva, which is a vascularized mucous membrane continuous with the bulbar conjunctiva. At the reflection of the palpebral and bulbar conjunctivae is a space called the *fornix*, located superiorly and inferiorly behind the upper and lower eyelids, respectively. Topical medications usually are placed in the inferior fornix, also known as the *inferior cul-de-sac*.

The lacrimal system consists of secretory glandular and excretory ductal elements (Figure 74–2). The secretory system is composed of the main *lacrimal gland*, which is located in the temporal outer portion of the orbit, and accessory glands located in the conjunctiva. The lacrimal gland is innervated by the autonomic nervous system (Table 74–1 and Chapter 10). The parasympathetic innervation is clinically relevant because a patient may complain of dry eye symptoms while taking medications with anticholinergic side effects, such as tricyclic antidepressants (see Chapter 18), antihistamines (see Chapter 43), and drugs used in the management of Parkinson's disease (see Chapter 21). Muscarinic cholinergic and α adrenergic receptors that mediate responses of several pupillary muscles from autonomic nerves also provide means of dilating the pupil for examination of posterior structures.

Tears constitute a functionally trilaminar lubrication barrier covering the conjunctiva and cornea. The anterior tear layer is composed primarily of lipids, produced by the meibomian glands located at the eyelid margin. The middle aqueous layer, produced by the main lacrimal gland and accessory lacrimal glands, constitutes about 98% of the tear film. Adherent to the corneal epithelium, the posterior layer is a mixture of mucins produced by goblet cells in the conjunctiva. Tears also contain nutrients, enzymes, and immunoglobulins to support and protect the cornea. The tear drainage system starts through small puncta located on the medial aspects of both the upper and lower eyelids (see Figure 74–2). With blinking, tears enter the puncta and continue to drain through the canaliculi, lacrimal sac, nasolacrimal duct, and then into the nasal cavity. The nasal cavity is lined by a highly vascular mucosal epithelium; consequently, topically applied medications that pass through this nasolacrimal system have direct access to the systemic circulation.

Ocular Structures

The eye is divided into anterior and posterior segments (Figure 74–3A). Anterior segment structures include the cornea, limbus, anterior and posterior chambers, trabecular meshwork, canal of Schlemm (Schlemm's canal), iris, lens, ciliary zonule, and ciliary body. The posterior segment includes the vitreous, retina, choroid, sclera, and optic nerve.

Anterior Segment

Cornea and Drug Access

The cornea is a transparent and avascular tissue organized into five (or six, depending on definitions used) layers (Figure 74–3B). The hydrophobic epithelial layer is five or six cells thick and is an important barrier to foreign material, including drugs. The basal epithelial cells lie on a basement membrane that is adjacent to Bowman's layer, a distinct sheet of collagen fibers. Constituting about 90% of the corneal thickness, the stroma, a hydrophilic layer, is organized with collagen lamellae synthesized by keratocytes. Beneath the stroma lies Descemet's membrane, the basement membrane of the corneal endothelium. An additional corneal

Abbreviations

AMD: age-related macular degeneration
CAI: carbonic anhydrase inhibitor
CMV: cytomegalovirus
FDA: U.S. Food and Drug Administration
5FU: 5-fluorouracil
ICAM-1: intercellular adhesion molecule 1
IOP: intraocular pressure
LFA-1: lymphocyte function–associated antigen 1
NSAID: non-steroidal anti-inflammatory drug
PDE: phosphodiesterase
PG: prostaglandin
tPA: tissue plasminogen activator
VEGF: vascular endothelial growth factor
WHO: World Health Organization

layer, Dua's layer, has been proposed—a distinct thin, but strong, collagen layer between the stroma and Descemet's membrane (Dua et al., 2013). Lying most posteriorly, the endothelium is a monolayer of cells adhering to each other by tight junctions. These cells maintain corneal dehydration by active transport processes and serve as a hydrophobic barrier. Hence, drug absorption across the cornea requires penetration of the trilaminar hydrophobic-hydrophilic-hydrophobic domains of the various anatomical layers.

At the periphery of the cornea and adjacent to the sclera lies a transitional zone (1- to 2-mm wide) called the *limbus*. Limbal structures include the corneal epithelial stem cells, conjunctival epithelium, Tenon's capsule, episclera, corneoscleral stroma, canal of Schlemm, and trabecular meshwork (see Figure 74–3B). Limbal blood vessels, as well as the tears, provide important nutrients and immunological defense mechanisms for the cornea. The anterior chamber holds about 250 μL of aqueous humor. The peripheral anterior chamber angle is formed by the cornea and the iris root. The trabecular meshwork and canal of Schlemm are located just above the apex of this angle. The posterior chamber, with about 50 μL of aqueous humor, is defined by the boundaries of the ciliary body processes, posterior surface of the iris, and lens surface.

Aqueous Humor Dynamics and Regulation of Intraocular Pressure

Aqueous humor is secreted by the ciliary processes and flows from the posterior chamber, through the pupil, and into the anterior chamber. It exits the eye primarily by the trabecular meshwork and canal of Schlemm, thence to an episcleral venous plexus and into the systemic circulation. This conventional pathway accounts for 80% to 95% of aqueous humor outflow and is the main target for cholinergic drugs used in glaucoma therapy. Another outflow pathway is the uveoscleral route (i.e., fluid flows through the ciliary muscles and into the suprachoroidal space), which is the target of selective prostanoids (see Glaucoma section in this chapter).

The peripheral anterior chamber angle is an important anatomical structure for differentiating two forms of glaucoma: *open-angle glaucoma*, which is by far the most common form of glaucoma in the U.S., and *angle-closure* or *closed-angle glaucoma*. Current medical therapy of open-angle glaucoma is aimed at decreasing aqueous humor production or increasing aqueous outflow. In anatomically susceptible eyes, anticholinergic, sympathomimetic, and antihistaminic drugs can lead to partial dilation of the pupil and a change in the force vectors between the iris and the lens. The aqueous humor is prevented from passing from the posterior chamber through the pupil to the anterior chamber through a ball (lens)-valve (pupil) type mechanism. The change in the lens-pupil relationship leads to an increase in pressure in the posterior chamber, causing an anterior bowing of the iris. The iris root is pushed against the angle wall, thereby covering the trabecular meshwork, closing the filtration angle, and markedly elevating the intraocular pressure (IOP). This can happen slowly over time and result in chronic angle-closure glaucoma, or it can happen suddenly, in which case the result is known as an acute attack of pupillary-block angle-closure glaucoma. The preferred management for angle-closure glaucoma is to create another pathway for aqueous humor to pass from the posterior to the anterior chamber. This equalizes pressure across the pupil, flattens the iris, and if the iris is not adherent to the trabecular meshwork, opens the angle, reestablishes outflow, and lowers the intraocular pressure. This is done by making a hole in the peripheral iris by either laser (laser iridotomy) or incision (surgical iridectomy), but short-term medical management may be necessary to reduce the acute IOP elevation and decrease corneal edema prior to surgery. Long-term surgical IOP reduction may be necessary, especially in chronic angle-closure glaucoma where the peripheral iris has permanently covered the trabecular meshwork.

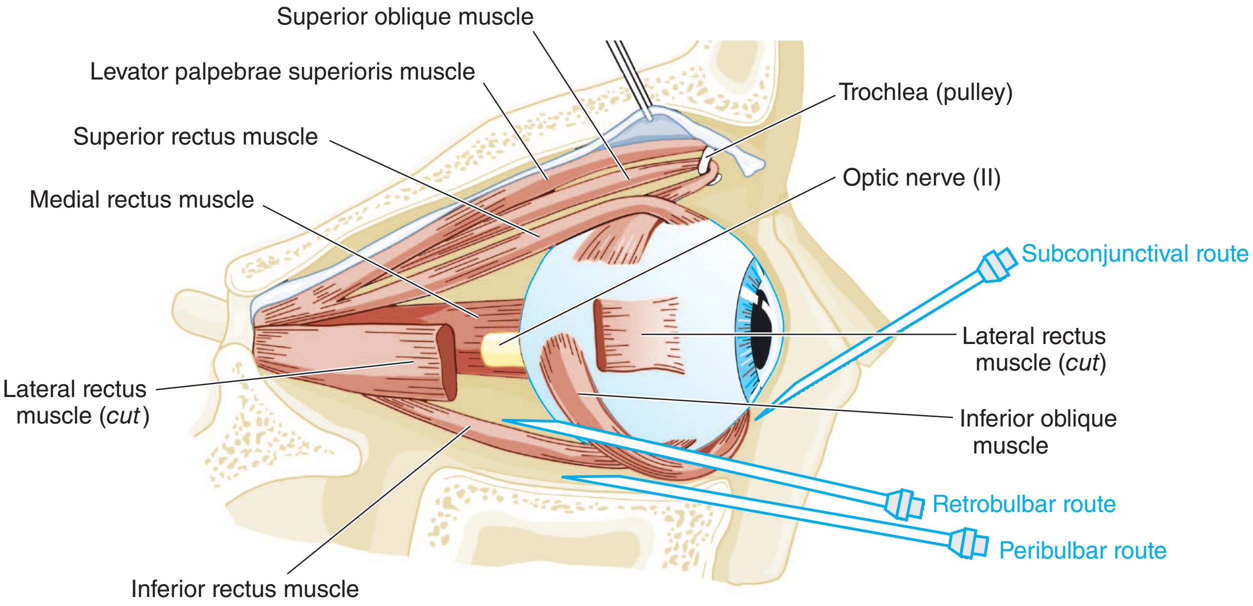


Figure 74–1 *Anatomy of the globe in relation to the orbit and eyelids.* Routes of administration of anesthesia are represented by the blue needles.

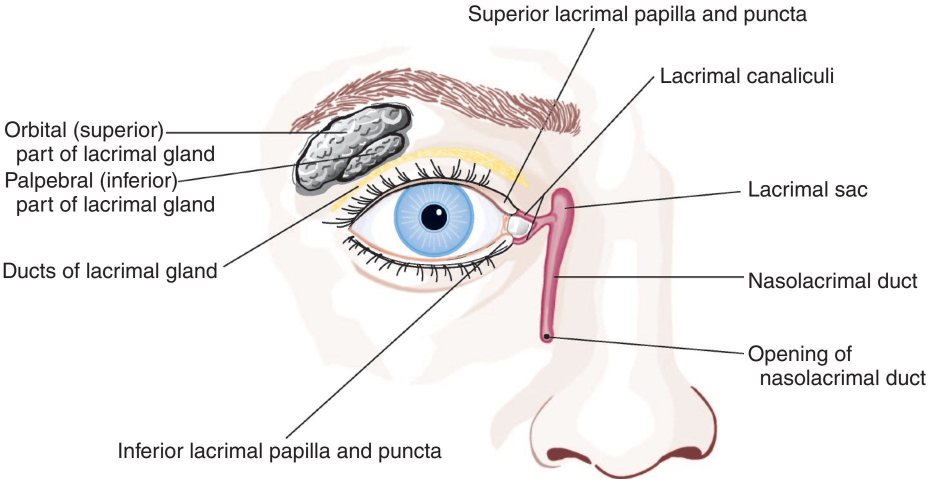

Superior lacrimal papilla and puncta
Lacrimal canaliculi
Orbital (superior) part of lacrimal gland
Palpebral (inferior) part of lacrimal gland
Lacrimal sac
Ducts of lacrimal gland
Nasolacrimal duct
Opening of nasolacrimal duct
Inferior lacrimal papilla and puncta

Figure 74–2 *Anatomy of the lacrimal system.*

Iris and Pupil

The iris is the most anterior portion of the uveal tract, which also includes the ciliary body and choroid. The anterior surface of the iris is the stroma, a loosely organized structure containing melanocytes, blood vessels, smooth muscle, and parasympathetic and sympathetic nerves. Differences in iris color reflect individual variation in the number of melanocytes and their melanosomes located in the stroma. Individual variation may be an important consideration for ocular drug distribution due to drug-melanin binding and side effect profile if melanogenic. The posterior surface of the iris is a densely pigmented bilayer of epithelial cells. Anterior to the pigmented epithelium, the dilator smooth muscle is oriented radially and is innervated by the sympathetic nervous system (Figure 74–4), which causes *mydriasis* (dilation). At the pupillary margin, the sphincter smooth muscle is organized in a circular band with parasympathetic innervation, which, when stimulated, causes *miosis* (constriction). The use of pharmacological agents to dilate normal pupils and to evaluate the pharmacological response of the pupil is summarized in Table 74–2. Agents affecting sympathetic neurotransmission in the eye are also used for the diagnostic evaluation of Horner syndrome and anisocoria.

Ciliary Body

The ciliary body serves two very specialized roles:

- Production and secretion of aqueous humor by the epithelial bilayer
- Accommodation by the ciliary muscle

The anterior portion of the ciliary body (*pars plicata*) comprises 70 to 80 ciliary processes with intricate folds. The posterior portion is the *pars plana*. The ciliary muscle is organized into outer longitudinal, middle radial, and inner circular layers. Coordinated contraction of this smooth muscle apparatus by the parasympathetic nervous system causes the zonular fibers suspending the lens to relax, allowing the lens to become more convex and to shift slightly forward. This process, known as *accommodation*, permits focusing on near objects and may be pharmacologically *blocked* by muscarinic cholinergic antagonists through a process called *cycloplegia*. Contraction of the ciliary muscle also puts traction on the scleral spur and hence widens the spaces within the trabecular meshwork. This last effect accounts for at least some of the IOP-lowering properties of directly acting and indirectly acting parasympathomimetic drugs. Blockade of β adrenergic receptors of the ciliary epithelium decreases production of aqueous humor, as does blockade of carbonic anhydrase enzymes.

Lens

The lens is suspended by the *ciliary zonular fibers,* specialized strands emanating from the ciliary body. The lens is about 10 mm in diameter and is enclosed in a capsule. The bulk of the lens is composed of fibers derived from proliferating lens epithelial cells located under the anterior portion of the lens capsule. These lens fibers are continuously produced throughout life. Aging, in addition to certain medications, such as

TABLE 74–1 ■ AUTONOMIC PHARMACOLOGY OF THE EYE AND RELATED STRUCTURES

	ADRENERGIC RECEPTORS		CHOLINERGIC RECEPTORS	
TISSUE	**SUBTYPE**	**RESPONSE**	**SUBTYPE**	**RESPONSE**
Corneal epithelium	β_2	Unknown	M[a]	Unknown
Corneal endothelium	β_2	Unknown	Undefined	Unknown
Iris radial muscle	α_1	Mydriasis		
Iris sphincter muscle			M_3	Miosis
Trabecular meshwork	β_2	Unknown		
Ciliary epithelium[b]	α_2/β_2	Aqueous production		
Ciliary muscle	β_2	Relaxation[c]	M_3	Accommodation
Lacrimal gland	α_1	Secretion	M_2, M_3	Secretion
Retinal pigment epithelium	α_1/β_2	H_2O transport/unknown		

[a] Although acetylcholine and choline acetyltransferase are abundant in the corneal epithelium of most species, the function of acetylcholine in this tissue is unknown.

[b] The ciliary epithelium also is the target of carbonic anhydrase inhibitors. Carbonic anhydrase isoenzyme II is localized to both the pigmented and nonpigmented ciliary epithelium.

[c] Although β_2 adrenergic receptors mediate ciliary body smooth muscle relaxation, there is no clinically significant effect on accommodation.

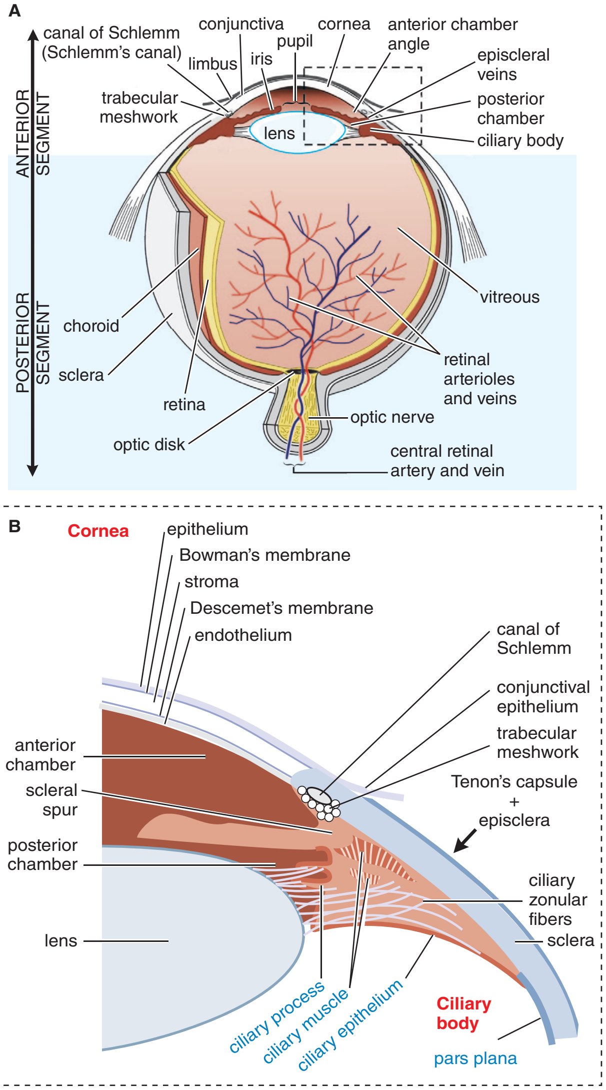

A
canal of Schlemm (Schlemm's canal)
conjunctiva
cornea
anterior chamber angle
pupil
limbus
iris
episcleral veins
trabecular meshwork
posterior chamber
lens
ciliary body
ANTERIOR SEGMENT
vitreous
POSTERIOR SEGMENT
choroid
retinal arterioles and veins
sclera
retina
optic nerve
optic disk
central retinal artery and vein
B
Cornea
epithelium
Bowman's membrane
stroma
Descemet's membrane
endothelium
canal of Schlemm
conjunctival epithelium
trabecular meshwork
anterior chamber
Tenon's capsule + episclera
scleral spur
posterior chamber
ciliary zonular fibers
sclera
lens
ciliary process
ciliary muscle
ciliary epithelium
Ciliary body
pars plana

Figure 74–3 **A.** *Anatomy of the eye.* **B.** *Enlargement of the anterior segment, revealing the cornea, angle structures, lens, and ciliary body.* (Adapted with permission from Riordan-Eva P. Anatomy and embryology of the eye. In: Riordan-Eva P, Whitcher JP, eds. *Vaughan & Asbury's General Ophthalmology*. 17th ed. McGraw-Hill, New York, **2008**. Copyright © 2008 by The McGraw-Hill Companies, Inc. All rights reserved.)

corticosteroids, and certain diseases, such as diabetes mellitus, cause the lens to become opacified, which is termed a *cataract*.

Posterior Segment

Because of the anatomical and vascular barriers to both local and systemic access, drug delivery to the eye's posterior pole is particularly challenging.

Sclera and Choroid

The outermost coat of the eye, the sclera, covers the posterior portion of the globe. The external surface of the scleral shell is covered by an episcleral vascular coat, Tenon's capsule, and the conjunctiva. The tendons of the six extraocular muscles insert collagen fibers into the superficial sclera. Numerous blood vessels pierce the sclera through emissaria to supply and drain the choroid, ciliary body, optic nerve, and iris. Inside the scleral shell, a capillary network (vascular choroid) nourishes the outer retina. The choroid can be a location of abnormal neovascular membranes and is the target of therapy with vascular endothelial growth factor (VEGF) inhibitors. Between the outer retina and the capillary network lie Bruch's membrane and the retinal pigment epithelium, whose tight junctions provide an outer barrier between the retina and the choroid. The retinal pigment epithelium serves many functions, including vitamin A metabolism, phagocytosis of the rod outer segments, and multiple transport processes.

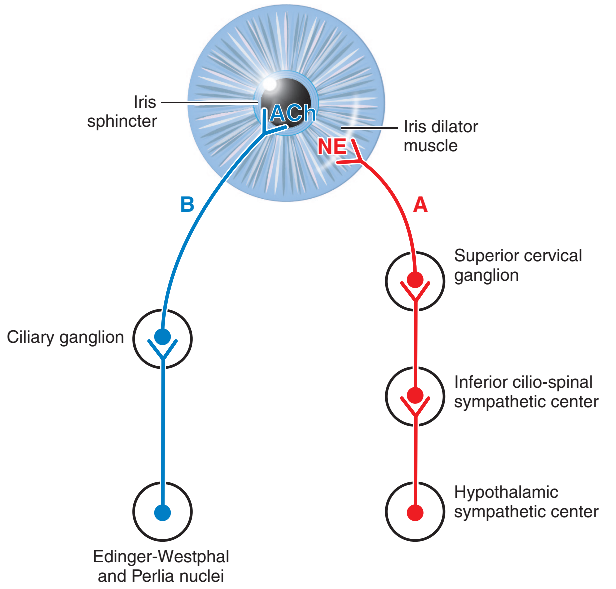

Iris sphincter
ACh
NE
Iris dilator muscle
B
A
Superior cervical ganglion
Ciliary ganglion
Inferior cilio-spinal sympathetic center
Hypothalamic sympathetic center
Edinger-Westphal and Perlia nuclei

Figure 74–4 *Sympathetic* (**A**) *and parasympathetic* (**B**) *efferent pathway of pupil dilation and constriction.* Norepinephrine (NE) and acetylcholine (ACh) are the respective neurotransmitters.

Retina

The retina is a thin, transparent, highly organized structure of neurons, glial cells, and blood vessels; it contains the photoreceptors and the rhodopsin-based G protein signaling system. The elevated IOP of glaucoma damages and causes the death of the retinal ganglion cells whose axons comprise the optic nerve that connect the retina and the brain. Glutamate, acting on *N*-methyl-D-aspartate receptors, can stimulate this process (Sucher et al., 1997). In theory, this offers a target for neuroprotective

TABLE 74–2 ■ EFFECTS OF PHARMACOLOGICAL AGENTS ON THE PUPIL

CLINICAL SETTING	DRUG	PUPILLARY RESPONSE
Normal	Sympathomimetic drugs	Dilation (mydriasis)
Normal	Parasympathomimetic drugs	Constriction (miosis)
Horner syndrome	Cocaine 4%–10% Apraclonidine 0.5%	No dilation Marked dilation[a]
Preganglionic Horner syndrome	Hydroxyamphetamine 1%	Dilation
Postganglionic Horner syndrome	Hydroxyamphetamine 1%	No dilation
Adie's pupil	Pilocarpine 0.05%–0.1%[b]	Constriction
Normal	Opioids (oral or intravenous)	Pinpoint pupils

Topically applied ophthalmic drugs unless otherwise noted.

[a] This is a result of the adrenergic receptor supersensitivity in a Horner pupil that gives a dominant alpha-1 effect in an otherwise alpha-2–dominant medication.

[b] This percentage of pilocarpine is not commercially available and usually is prepared by the physician administering the test or by a pharmacist. This test also requires that no prior manipulation of the cornea (i.e., tonometry for measuring intraocular pressure or testing corneal sensation) be done so that the normal integrity of the corneal barrier is intact. Normal pupils will not respond to this weak dilution of pilocarpine; however, an Adie's pupil manifests a denervation supersensitivity and is responsive to this dilute cholinergic agonist.

agents to protect against cell death, but to date, no treatment other than IOP lowering has demonstrated any benefit in treating glaucoma.

Vitreous

Approximately 80% of the eye's volume is the vitreous, a clear medium containing collagen type II, hyaluronic acid, proteoglycans, glucose, ascorbic acid, amino acids, and a number of inorganic salts. The vitreous is adherent to the retina and optic nerve, which can result in traction on these vital structures, and has been the target of enzymatic lysis.

Optic Nerve

The optic nerve is a myelinated nerve conducting the retinal output to the CNS. It comprises an intraocular portion, visible as the optic disk in the retina; an intraorbital section; an intracanalicular portion; and an intracranial segment. The nerve is sheathed in meninges continuous with the brain. It is susceptible to a variety of insults, ranging from traumatic to toxic (ethambutol, methanol, ethanol) to nutritional (vitamin B_{12} and folic acid deficiency) to infectious, neoplastic, vascular, and inflammatory. At present, pharmacological treatment of optic neuropathies usually is based on management of the underlying disease. For example, arteritic ischemic optic neuropathy (giant cell arteritis) is best treated with systemic glucocorticoids and optic neuritis with intravenous glucocorticoids (Beck and Gal, 2008; Volpe, 2008). Glaucomatous optic neuropathy is medically managed by decreasing IOP.

Pharmacokinetics and Toxicology of Ocular Therapeutic Agents

Drug Delivery Strategies

There are a number of formulations of agents and routes of drug administration that are unique to the eye (Figure 74–1 and Table 74–3).

Several formulations prolong the time a drug remains on the surface of the eye. These include gels, ointments, soft contact lenses, and collagen shields. Prolonging the time a drug remains in the cul-de-sac beneath the eyelid enhances drug absorption. Ophthalmic gels (e.g., pilocarpine 4% gel) release drugs by diffusion following erosion of soluble polymers. Ointments usually contain mineral oil and a petrolatum base and are helpful in delivering antibiotics, cycloplegic drugs, or miotic agents. Drug molecules may also be encapsulated in nanoparticles for controlled release on the ocular surface.

ADME

The ADME pharmacokinetic principles determine the time course of drug action in the eye; however, the routes of ocular drug administration, the flow of ocular fluids, and the architecture of the eye introduce other variables specific to the eye. Most ophthalmic medications are formulated to be applied topically. Drugs also may be injected by subconjunctival, sub-Tenon's, intracorneal, intracameral, intravitreal, and peribulbar or retrobulbar routes.

Absorption. After topical instillation of a drug, the rate and extent of absorption are determined by the dwell time of the drug in the cul-de-sac and precorneal tear film, elimination by nasolacrimal drainage, drug binding to tear proteins, drug metabolism by tear and tissue proteins, and diffusion across the cornea and conjunctiva. A drug's dwell time may be prolonged by changing its formulation or vehicle. Dwell time also may be extended by blocking the egress of tears from the eye by closing the tear drainage ducts with flexible silicone (punctal) plugs. Nasolacrimal drainage contributes to systemic absorption of topically administered ophthalmic medications. Absorption from the nasal mucosa avoids first-pass metabolism by the liver; thus, topical ophthalmic medications can cause significant systemic side effects, especially when used frequently or chronically. Possible absorption pathways of an ophthalmic drug following topical application to the eye are shown schematically in Figure 74–5.

Transcorneal and transconjunctival/scleral absorption are the desired routes for localized ocular drug effects. The drug concentration gradient between the tear film and the cornea and conjunctival epithelium provides the driving force for passive diffusion across these tissues. Other factors that affect a drug's diffusion are the size of the molecule, chemical structure, and steric configuration. Transcorneal drug penetration is a differential solubility process; the cornea resembles a trilaminar "fat-water-fat" structure corresponding to the epithelial, stromal, and endothelial layers, respectively. The epithelium and endothelium represent barriers for hydrophilic substances; the stroma is a barrier for hydrophobic compounds. Hence, an amphipathic agent with both hydrophilic and lipophilic properties is best suited for transcorneal absorption. Drug penetration into the eye is approximately linearly related to its concentration in the tear film. Certain disease states, such as corneal epithelial defects and corneal ulcers, may alter drug penetration. Medication absorption usually is increased when an anatomical barrier is compromised or removed.

Distribution. Topically administered drugs may undergo systemic distribution primarily by nasal mucosal absorption and possibly via local ocular distribution by transcorneal/transconjunctival absorption. Following transcorneal absorption, the aqueous humor accumulates the drug, which then is distributed to intraocular structures and potentially to the systemic circulation via the trabecular meshwork pathway (see Figure 74–3B). Melanin binding of certain drugs is an important factor in some ocular compartments. For example, the mydriatic effect of α adrenergic agonists is slower in onset in humans with darkly pigmented irides compared to those with lightly pigmented irides; drug-melanin binding is a potential reservoir for sustained drug release. Another clinically

TABLE 74–3 ■ SOME CHARACTERISTICS OF OCULAR ROUTES OF DRUG ADMINISTRATION

ROUTE	ABSORPTION PATTERN	SPECIAL UTILITY	LIMITATIONS AND PRECAUTIONS
Topical	Prompt, depending on formulation	Convenient, economical, relatively safe	Compliance, corneal and conjunctival toxicity, nasal mucosal toxicity, systemic side effects from nasolacrimal absorption
Subconjunctival, sub-Tenon's, peribulbar, and retrobulbar injections	Prompt or sustained, depending on formulation	Anterior segment infections, posterior uveitis, cystoid macular edema	Local toxicity, tissue injury, globe perforation, optic nerve trauma, central retinal artery or vein occlusion, direct retinal drug toxicity with inadvertent globe perforation, ocular muscle trauma, prolonged drug effect
Intraocular (intracameral) injection	Prompt	Anterior segment surgery, infections	Corneal toxicity, intraocular toxicity, relatively short duration of action, adjacent tissue injury from injection
Intravitreal injection or device	Absorption circumvented, immediate local effect, potential sustained effect	Endophthalmitis, retinitis, age-related macular degeneration	Retinal toxicity, adjacent tissue injury from injection

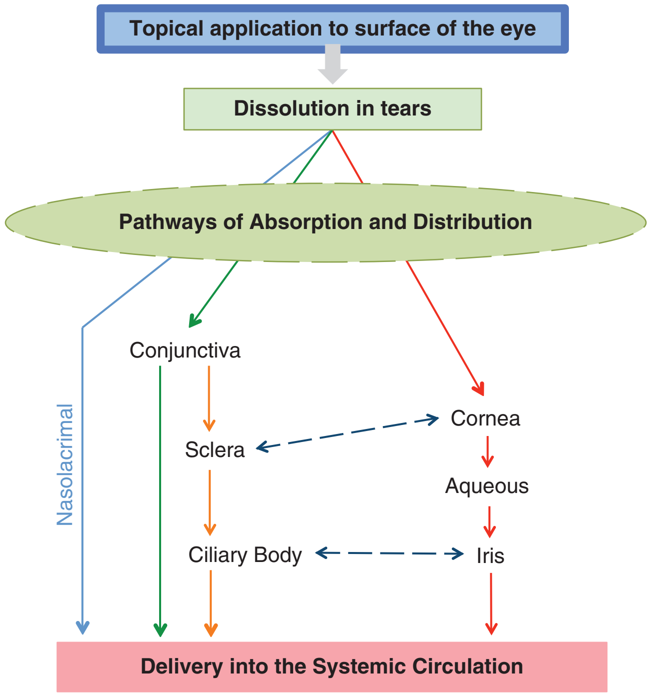

Topical application to surface of the eye
Dissolution in tears
Pathways of Absorption and Distribution
Nasolacrimal
Conjunctiva
Sclera
Cornea
Aqueous
Ciliary Body
Iris
Delivery into the Systemic Circulation

Figure 74–5 *Drugs applied to the surface of the eye have multiple routes to the systemic circulation.*

important consideration for drug-melanin binding involves the retinal pigment epithelium. In the retinal pigment epithelium, accumulation of *chloroquine* (see Chapter 66) causes a toxic retinal lesion known as a "bull's-eye" maculopathy, which is associated with a decrease in visual acuity. To reduce the risk of retinal toxicity from *chloroquine* and related substances (e.g., *hydroxychloroquine*), patients receiving chronic therapy should receive periodic eye exams, and lifetime cumulative doses should generally be limited to 2.3 mg/kg per day of *chloroquine* and 5 mg/kg per day of *hydroxychloroquine* (Melles and Marmor, 2014).

Metabolism. Biotransformation of ocular drugs may be significant; a variety of enzymes, including esterases, oxidoreductases, lysosomal enzymes, peptidases, glucuronide and sulfate transferases, glutathione conjugating enzymes, catechol-*O*-methyltransferase, monoamine oxidase, and 11β-hydroxysteroid dehydrogenase, are found in the eye. The esterases are of particular interest, permitting development of ester prodrugs for enhanced corneal permeability (e.g., *latanoprost* is a prodrug for prostaglandin [PG] $F_{2\alpha}$ used for glaucoma management; *loteprednol* is a *prednisolone* analogue engineered to have limited systemic effects due to metabolic inactivation within the eye).

Excretion. Ocular drugs are excreted from the eye through multiple routes. Lacrimal tearing dilutes and reduces the concentration of medications from the ocular surface. Additionally, nasolacrimal drainage contributes to the elimination of drugs from the ocular surface, redirecting topically administered medications to the nasal cavity and to systemic absorption. Once introduced into the systemic circulation, the drug is distributed to other parts of the body followed by metabolism and excretion.

Toxicity

Most local toxic effects are due to hypersensitivity reactions or to direct toxic effects on the cornea, conjunctiva, periocular skin, and nasal mucosa. Eye drops and contact lens solutions commonly contain antimicrobial preservatives such as benzalkonium chloride, chlorobutanol, chelating agents, and, rarely, thimerosal. Benzalkonium chloride may cause a punctate keratopathy or toxic ulcerative keratopathy. All ophthalmic medications are potentially absorbed into the systemic circulation (see Figure 74–5), so systemic side effects may occur.

Ophthalmic Uses of Drugs

Chemotherapy of Microbial Diseases in the Eye

Bacterial Infections

Infectious diseases of the skin, eyelids, conjunctivae, and lacrimal excretory system are encountered regularly. Periocular skin infections are divided into preseptal and postseptal or orbital cellulitis. Depending on the clinical setting (e.g., preceding trauma, sinusitis, age of patient, relative immunocompromised state), oral or parenteral antibiotics may be administered in addition to topical medications. A number of antibiotics are formulated for topical ocular use (Table 74–4).

Dacryoadenitis, an infection of the lacrimal gland, is most common in children and young adults; it may be bacterial (typically *Staphylococcus aureus, Streptococcus* spp.) or viral (seen in mumps, infectious mononucleosis, influenza, and herpes zoster). When bacterial infection is suspected, systemic antibiotics usually are indicated.

Dacryocystitis is an infection of the lacrimal sac. In infants and children, the disease usually is unilateral and secondary to an obstruction of the nasolacrimal duct. In adults, dacryocystitis and canalicular infections may be caused by *S. aureus, Streptococcus* spp., diphtheroids, *Candida* spp., and *Actinomyces israelii*. Any discharge from the lacrimal sac should be sent for smears and cultures. Systemic antibiotics typically are indicated.

Infectious processes of the eyelids include *hordeolum* and *blepharitis*. A hordeolum, or stye, is an infection of the meibomian, Zeis, or Moll glands in the eyelids. The typical offending bacterium is *S. aureus*, and the usual treatment consists of warm compresses and topical antibiotics (gel, drops, or ointment). Blepharitis is a common bilateral inflammatory process of the eyelids characterized by irritation and burning, usually associated with *Staphylococcus* spp. Local hygiene is the mainstay of therapy, although topical antibiotics frequently are used. Systemic *tetracycline*, *doxycycline*, *minocycline*, *erythromycin*, and *azithromycin* often are effective in reducing severe eyelid inflammation but must be used for weeks to months.

Conjunctivitis is an inflammatory or infectious process of the conjunctiva that varies in severity from mild hyperemia to severe purulent discharge. The more common causes of conjunctivitis include allergies, viruses, environmental irritants, contact lenses, and chemicals. Less common causes include other infectious pathogens, immune-mediated reactions, associated systemic diseases, and tumors of the conjunctiva or eyelid. Commonly reported infectious agents are adenovirus and herpes simplex virus, followed by other viral (e.g., enterovirus, coxsackievirus, measles virus, varicella zoster virus) and bacterial (e.g., *Neisseria* spp., *Streptococcus pneumoniae, Haemophilus* spp., *S. aureus, Moraxella lacunata*, and chlamydial spp.) sources. *Rickettsia*, fungi, and parasites, in both cystic and trophozoite form, are rare causes of conjunctivitis. Effective management is based on selection of an appropriate antibiotic for suspected bacterial pathogens. Unless an unusual causative organism is suspected, bacterial conjunctivitis is treated empirically with a broad-spectrum topical antibiotic without obtaining a culture.

Keratitis, or corneal inflammation or infection, can occur at any level of the cornea. Myriad microbial agents may cause infectious keratitis, including bacteria, viruses, fungi, spirochetes, and parasites. Severe infections with tissue loss (corneal ulcers) generally are treated more aggressively than infections without tissue loss (corneal infiltrates). Mild, small, peripheral, and visually insignificant infections usually are not cultured, and the eyes are treated with frequent broad-spectrum topical antibiotics. In more severe, central, visually significant infections, corneal scrapings for cultures and sensitivities are performed, and the patient is immediately started on intensive hourly, around-the-clock topical antibiotic therapy. When pathogens are not identified using corneal scrapings, the clinician may perform a corneal biopsy. A commercially available broad-spectrum antibiotic may be given, but in severe infections, specially formulated fortified antibiotics are often used. The goal of treatment is to eradicate the infection and reduce the amount of corneal scarring and the chance

TABLE 74–4 ■ TOPICAL ANTIBACTERIAL AGENTS COMMERCIALLY AVAILABLE FOR OPHTHALMIC USE

AGENT	FORMULATION[a]	TOXICITY	INDICATIONS FOR USE
Azithromycin	1% solution	H	Conjunctivitis
Bacitracin	500 units/g ointment	H	Conjunctivitis, blepharitis, keratitis, keratoconjunctivitis, blepharoconjunctivitis, meibomianitis, dacryocystitis
Besifloxacin	0.6% suspension	H	Conjunctivitis, keratitis
Chloramphenicol (not available in the U.S.)	1% ointment	H, BD	Conjunctivitis, keratitis
Ciprofloxacin	0.3% solution, 0.3% ointment	H, D-RCD	Conjunctivitis, keratitis, keratoconjunctivitis, blepharitis, blepharoconjunctivitis, meibomianitis, dacryocystitis
Erythromycin	0.5% ointment	H	Superficial ocular infections involving the conjunctiva or cornea; prophylaxis of ophthalmia neonatorum
Gatifloxacin	0.3% and 0.5% solutions	H	Conjunctivitis, keratitis
Gentamicin	0.3% solution, 0.3% ointment	H	Conjunctivitis, blepharitis, keratitis, keratoconjunctivitis, blepharoconjunctivitis, meibomianitis, dacryocystitis
Levofloxacin	0.5% solution	H	Conjunctivitis, keratitis
	1.5% solution	H	Keratitis
Moxifloxacin	0.5% solution	H	Conjunctivitis, keratitis
Ofloxacin	0.3% solution	H	Conjunctivitis, keratitis
Sulfacetamide	10% solution, 10% ointment	H, BD	Conjunctivitis, other superficial ocular infections
Polymyxin B combinations[b]	Various solutions and ointments	H	Conjunctivitis, blepharitis, keratitis
Tobramycin[c]	0.3% solution, 0.3% ointment	H	External infections of the eye and its adnexa

Toxicity: BD, blood dyscrasia; D-RCD, drug-related corneal deposits; H, hypersensitivity.

[a] For specific information on dosing, formulation, and trade names, refer to the *Physicians' Desk Reference for Ophthalmic Medicines*, which is published annually.

[b] Polymyxin B is formulated for delivery to the eye in combination with bacitracin, neomycin, gramicidin, dexamethasone, hydrocortisone or trimethoprim. See Chapters 56–60 for further discussion of these antibacterial agents.

[c] Tobramycin is formulated for delivery to the eye in combination with dexamethasone or loteprednol etabonate.

of corneal perforation and visual debilitation. The initial medication selection and dosage are adjusted according to the clinical response and culture/sensitivity results. In the case of culture-proven bacterial keratitis that has been treated with appropriate topical antibiotics for several days but still has significant inflammation, judicious topical steroids can be used with close follow-up to decrease corneal scarring.

Endophthalmitis is a potentially severe and devastating inflammatory, and usually infectious, process involving the intraocular tissues. When the inflammatory process encompasses the entire globe, it is called *panophthalmitis*. Endophthalmitis usually is caused by bacteria or fungi or, rarely, by spirochetes. The typical case occurs during the early postoperative course after intraocular surgery, following trauma, or rarely by endogenous seeding in an immunocompromised host or intravenous drug user. Acute postoperative endophthalmitis requires a prompt vitreous tap (often sent for smears and cultures) and empirical injection of intravitreal antibiotics. Systemic antibiotics are helpful in reducing the risk of endophthalmitis following a traumatic open-globe injury (Ahmed et al., 2012). In cases of endogenous seeding, parenteral antibiotics have a role in eliminating the infectious source. Vitrectomy (i.e., specialized surgical removal of the vitreous) is indicated in some cases (Endophthalmitis Vitrectomy Study Group, 1995; Schiedler et al., 2004).

Viral Infections

Antiviral drugs used in ophthalmology are summarized in Table 74–5 (see Chapter 62 for details of these agents). The primary indications for the use of antiviral drugs in ophthalmology are viral keratitis, herpes zoster ophthalmicus, and retinitis. There currently are no antiviral agents indicated for the treatment of viral conjunctivitis caused by adenoviruses, which usually have a self-limited course and typically are treated by symptomatic relief of irritation, but topical *ganciclovir* gel may be of some benefit (Yabiku et al., 2011).

Viral keratitis, an infection of the cornea that may involve the epithelium, stroma, or endothelium, is most commonly caused by herpes simplex type I and varicella-zoster viruses. These viruses also may present with eyelid or periocular skin lesions. Less common viral etiologies include herpes simplex type II, Epstein-Barr virus, and cytomegalovirus (CMV). The topical antiviral agents *trifluridine*, *acyclovir*, and *ganciclovir* are indicated for the treatment of epithelial disease due to herpes simplex infection, but *trifluridine* is more toxic to the corneal epithelium than the other two. Topical glucocorticoids are contraindicated in herpetic epithelial keratitis due to active viral replication. In contrast, for herpetic disciform (or endothelial) keratitis (predominantly a cell-mediated immune reaction), topical glucocorticoids often accelerate recovery. For recurrent herpetic stromal keratitis, there is clear benefit from treatment with an oral antiviral such as *acyclovir* in reducing the risk of recurrence (Herpetic Eye Disease Study Group, 1997, 1998; Young et al., 2010).

Herpes zoster ophthalmicus is a latent reactivation of a varicella-zoster infection in the first division of the trigeminal cranial nerve. Systemic *acyclovir*, *valacyclovir*, and *famciclovir* are effective in reducing the severity and complications of herpes zoster ophthalmicus (Cohen and Kessler, 2016).

Viral retinitis may be caused by herpes simplex virus, CMV, and varicella-zoster virus. With antiretroviral therapy (see Chapters 62 and 64), CMV retinitis does not appear to progress when specific anti-CMV therapy is discontinued, but some patients develop an immune recovery uveitis. Treatment usually involves long-term parenteral administration of antiviral drugs. Intravitreal *ganciclovir*, injected or implanted as an insert, is an effective alternative to systemic use. The *ganciclovir* intravitreal implant provides a *zero-order* rate of delivery by steady-state diffusion, whereby drug is released at a more constant rate over a prolonged period of time rather than as a bolus. Acute retinal necrosis and progressive outer retinal necrosis, most often caused by varicella-zoster virus, can be

TABLE 74–5 ■ ANTIVIRAL AGENTS FOR OPHTHALMIC USE

GENERIC NAME	ROUTE OF ADMINISTRATION	OCULAR TOXICITY	INDICATIONS FOR USE
Trifluridine	Topical (1% ophthalmic solution)	Punctate keratopathy, hypersensitivity	Herpes simplex keratitis and keratoconjunctivitis Dosed initially 9 times a day Corneal toxicity can occur
Ganciclovir	Topical (0.15% ophthalmic gel) Intravenous infusion, intravitreal injection[a]	Punctate keratopathy, conjunctival hyperemia	Herpes simplex keratitis Cytomegalovirus retinitis
Acyclovir	Oral (200-mg capsules, 400- and 800-mg tablets), intravenous	Punctate keratopathy	Herpes zoster ophthalmicus Herpes simplex keratitis, iridocyclitis
Valacyclovir	Oral (500- and 1000-mg tablets)		Herpes simplex keratitis[a] Herpes zoster ophthalmicus
Famciclovir	Oral (125-, 250-, and 500-mg tablets)		Herpes simplex keratitis[a] Herpes zoster ophthalmicus
Foscarnet	Intravenous Intravitreal[a]	Visual disturbance, conjunctivitis, hypersensitivity	Cytomegalovirus retinitis
Valganciclovir	Oral (450-mg tablets)	Retinal detachment, macular edema	Cytomegalovirus retinitis
Cidofovir	Intravenous	Decreased intraocular pressure, uveitis, blindness, cataract, conjunctivitis, corneal lesion, diplopia	Cytomegalovirus retinitis

[a] Off-label use. For additional details, see Chapter 62.

treated by combinations of oral, intravenous, and intravitreal administration of antiviral medications (Newman and Gooding, 2013).

Fungal Infections

The only currently available topical ophthalmic antifungal preparation is *natamycin*, a polyene. Other antifungal agents may be extemporaneously compounded for topical, subconjunctival, intracorneal, intracameral, or intravitreal routes of administration (Table 74–6; see also Chapter 61). As with systemic fungal infections, the incidence of ophthalmic fungal infections has risen with the growing number of immunocompromised hosts. Fungal infections can involve the cornea, sclera, intraocular structures, canaliculi, and orbit. Risk factors for fungal keratitis include trauma, chronic ocular surface disease, contact lens wear, and immunosuppression (including topical steroid use). When fungal infection is suspected, samples of the affected tissues are obtained for smears, cultures, and sensitivities, and this information is used to guide drug selection. Treatment for fungal keratitis is typically prolonged due to the generally poor ocular penetration of these medications and more resistant nature of these pathogens to antimicrobial agents. Consequently, topical antifungal therapy is frequently supplemented with systemic antifungal drugs to improve access to deeper infections.

Protozoal Infections

Parasitic infections involving the eye usually manifest themselves as a form of *uveitis*, an inflammatory process of either the anterior or posterior segments and, less commonly, as conjunctivitis, keratitis, and retinitis. In the U.S., the most commonly encountered protozoal infections include *Acanthamoeba* and *Toxoplasma gondii*. In contact lens wearers who develop keratitis, physicians should be highly suspicious of the presence of *Acanthamoeba*. Risk factors for *Acanthamoeba* keratitis include poor contact lens hygiene, wearing contact lenses in a pool or hot tub, and ocular trauma. Treatment usually consists of a combination of topical agents. The aromatic diamidines—*propamidine isethionate* in topical aqueous and ointment forms (not commercially available in the U.S.)—have been used successfully to treat this relatively resistant infectious keratitis. The cationic antiseptic agent *polyhexamethylene biguanide* also is used in drop form for *Acanthamoeba* keratitis. Alternatively, topical *chlorhexidine* can be used. Both drugs need to be prepared by a specialty compounding pharmacy. Although initially FDA-approved to treat leishmaniasis, oral *miltefosine* has also been used adjunctively in the treatment of *Acanthamoeba* keratitis. Oral imidazoles (e.g., *itraconazole*, *fluconazole*, *ketoconazole*, *voriconazole*) are sometimes used in addition to topical medications. Resolution of *Acanthamoeba* keratitis often requires many months of treatment (Chew et al., 2011; Hoti and Tandon, 2011).

Treatment of *toxoplasmosis* is indicated when inflammatory lesions encroach on the macula and threaten central visual acuity. Several regimens have been recommended with concurrent use of systemic steroids: (1) *pyrimethamine*, *sulfadiazine*, and *folinic acid* (*leucovorin*); (2) *pyrimethamine*, *sulfadiazine*, *clindamycin*, and *folinic acid*; (3) *sulfadiazine* and *clindamycin*; (4) *clindamycin*; and (5) *trimethoprim-sulfamethoxazole* with or without *clindamycin*. Other protozoal infections (e.g., giardiasis, leishmaniasis, malaria) and helminth infections are less common eye pathogens in the U.S. Systemic pharmacological management as well as vitrectomy may be indicated for selected parasitic infections (Maenz et al., 2014; see Chapter 67, *Chemotherapy of Protozoal Infections*).

Ophthalmic Use of Autonomic Agents, Prostanoids, and Carbonic Anhydrase Inhibitors

Autonomic drugs are used extensively for diagnostic and surgical purposes and for the treatment of glaucoma, uveitis, and strabismus. The autonomic agents used in ophthalmology and the responses (i.e., mydriasis, cycloplegia) to muscarinic cholinergic antagonists are summarized in Table 74–7. Table 74–8 presents some characteristics of prostanoids used in ophthalmology.

Glaucoma

Glaucoma is characterized by progressive loss of retinal nerve fiber layer tissue with corresponding visual field loss. The optic nerve acquires a characteristic loss of the neuroretinal rim, frequently referred to as "cupping." The prevalence of the disease is growing as populations age around the world (Tham et al., 2014). Risk factors include increased IOP, positive family history of glaucoma, African American heritage, and possibly myopia, diabetes, and hypertension. Reducing IOP can delay or prevent glaucomatous nerve or field damage. Although markedly elevated IOPs

TABLE 74–6 ■ ANTIFUNGAL AGENTS FOR OPHTHALMIC USE

DRUG CLASS AGENT	METHOD OF ADMINISTRATION	INDICATIONS FOR USE
Polyenes		
Amphotericin B[a]	0.1%–0.5% (typically 0.15%) topical solution	Yeast and fungal keratitis and endophthalmitis
	0.8–1 mg subconjunctival	Yeast and fungal endophthalmitis
	2–10 μg intracorneal and intracameral	Yeast and fungal keratitis
	5–10 μg intravitreal injection	Yeast and fungal endophthalmitis
	Intravenous	Yeast and fungal endophthalmitis
Natamycin	5% topical suspension	Yeast and fungal blepharitis, conjunctivitis, keratitis
Imidazoles		
Fluconazole[a]	Oral, intravenous	Yeast keratitis and endophthalmitis
Itraconazole[a]	Oral	Yeast and fungal keratitis and endophthalmitis
Ketoconazole[a]	Oral	Yeast keratitis and endophthalmitis
Miconazole[a]	1% topical ophthalmic solution	Yeast and fungal keratitis
	5–10 mg subconjunctival	Yeast and fungal endophthalmitis
	10 μg intravitreal injection	Yeast and fungal endophthalmitis
Voriconazole	Oral, intravenous, intravitreal	Yeast and fungal keratitis and endophthalmitis
	50 μg intracorneal and intracameral	Yeast and fungal keratitis

[a] Off-label use. Only natamycin is commercially available and labeled for ophthalmic use. All other antifungal drugs are not labeled for ophthalmic use and must be extemporaneously formulated for the given method of administration. For further dosing information, refer to the *Physicians' Desk Reference for Ophthalmic Medicines*. For additional discussion of these antifungal agents, see Chapter 61.

(e.g., >30 mmHg) usually will lead to optic nerve damage, the optic nerves in certain patients (*ocular hypertensives*) can tolerate IOPs in the mid-to-high 20s. Other patients have progressive glaucomatous optic nerve damage despite having IOPs in the normal range; this form of the disease sometimes is called *normal-tension* or *low-tension* glaucoma. Regardless of the initial level, reducing the IOP delays or prevents disease progression (Collaborative Normal-Tension Study Group, 1998a, 1998b; Ederer et al., 2004; Heijl et al., 2002; Kass et al., 2002; Miglior et al., 2005). At present, the pathophysiological processes involved in glaucomatous optic nerve damage and the relationship to aqueous humor dynamics are not understood. Current pharmacotherapies are targeted at decreasing the production of aqueous humor at the ciliary body and increasing outflow through the trabecular meshwork and uveoscleral pathways.

There is no consensus on the best IOP-lowering algorithm, but a stepwise approach depends on the patient's health, age, and ocular status, with knowledge of systemic effects and contraindications for all medications. For many ophthalmologists, a stepwise medical approach may begin with a *topical PG* analogue (see Table 74–8).

Due to their once-daily dosing, low incidence of systemic side effects, and potent IOP-lowering effect, PG analogues have largely replaced β adrenergic receptor antagonists as first-line medical therapy for glaucoma. The PG analogues consist of *latanoprost* (Figure 74–6), *travoprost*, *bimatoprost*, and *tafluprost*. $PGF_{2\alpha}$ reduces IOP but has intolerable local side effects. Modifications to the chemical structure of $PGF_{2\alpha}$ have produced PG analogues with a more acceptable side effect profile. $PGF_{2\alpha}$ and its analogues (prodrugs that are hydrolyzed to $PGF_{2\alpha}$) bind to receptors for $PGF_{2\alpha}$ (FP receptors, FPr) that link to the $G_{q/11}$-PLC-IP_3-Ca^{2+} pathway. This pathway is active in isolated human ciliary muscle cells. Other cells in the eye also may express FP receptors. Theories of IOP lowering by $PGF_{2\alpha}$ range from altered ciliary muscle tension to effects on trabecular meshwork cells to release of matrix metalloproteinases and digestion of extracellular matrix materials that may impede outflow tracts. *Latanoprostene bunod* is a newer PG analogue with a nitric oxide donating moiety that enhances traditional outflow through the trabecular meshwork by inducing relaxation of cytoskeleton in addition to the effect of the *latanoprost* backbone on the uveoscleral pathway (Cavet and DeCory, 2018).

The *β receptor antagonists* (see Table 74–7) are currently the next most common topical medical treatment. Nonselective β blockers bind to both β_1 and β_2 receptors and include *timolol*, *levobunolol*, and *carteolol*. The β_1-selective antagonist *betaxolol* is available for ophthalmic use but is less efficacious than the nonselective β blockers because the β receptors of the eye are largely of the β_2 subtype. However, *betaxolol* is less likely to cause breathing difficulty due to blockade of pulmonary β_2 receptors. In the eye, the targeted tissues are the ciliary body epithelium and blood vessels, where β_2 receptors account for 75% to 90% of the total population. Production of aqueous humor seems to be activated by a β receptor–mediated cAMP-PKA pathway; β blockade blunts adrenergic activation of this pathway and therefore reduces intracellular cAMP. Another hypothesis is that β blockers decrease ocular blood flow, which decreases the ultrafiltration responsible for aqueous production.

When there are medical contraindications to the use of PG analogues or β receptor antagonists, other agents, such as an *α_2 adrenergic receptor agonist* (see Table 74–7) or a topical *carbonic anhydrase inhibitor* (*CAI*; Table 74–9), may be used as first-line therapy. The α_2 adrenergic agonists appear to decrease IOP by reducing aqueous humor production and by enhancing both conventional (via an α_2 receptor mechanism) and uveoscleral outflow (perhaps via PG production) from the eye. The α_2 adrenergic agonist and *clonidine* derivative *apraclonidine* is a relatively selective α_2 adrenergic agonist that is highly ionized at physiological pH, does not cross the blood-brain barrier, and is thus relatively free of the CNS effects of *clonidine*. *Brimonidine* is a selective α_2 adrenergic agonist that is lipophilic, enabling easy corneal penetration. Both *apraclonidine* and *brimonidine* reduce aqueous production and may enhance some uveoscleral outflow. Both appear to bind to pre- and postsynaptic α_2 receptors. By binding to the presynaptic receptors, the drugs reduce the amount of neurotransmitter release from sympathetic nerve stimulation and thereby lower IOP. By binding to postsynaptic α_2 receptors, these drugs stimulate the G_i pathway, reducing intracellular cAMP production and thereby decreasing aqueous humor production.

The development of a *topical CAI* was prompted by the poor side effect profile of oral CAIs. *Dorzolamide* and *brinzolamide* both work by inhibiting carbonic anhydrase (isoform II), which is found in the ciliary body epithelium. Inhibition of carbonic anhydrase reduces the formation of bicarbonate ions, which reduces fluid transport and thus IOP (see Table 74–9).

Rho kinase inhibitors are the most recent class of medication to be used to treat glaucoma and consist currently of *netarsudil* and *ripasudil* (Table 74–10). Rho kinase is a protein serine-threonine kinase involved in the regulation of the cytoskeleton. In the trabecular meshwork and canal of Schlemm, blockade of this pathway can decrease the density of actin stress fibers. The hypothesis is that these agents lower IOP by augmenting outflow via the trabecular pathway (Tanna and Johnson, 2018).

Any of these drug classes can be used as additive second-line or third-line therapy. In fact, the β receptor antagonist *timolol* has been combined with the CAI *dorzolamide* into a single medication and also with the α_2

TABLE 74–7 ■ AUTONOMIC DRUGS FOR OPHTHALMIC USE

DRUG CLASS	FORMULATION	INDICATIONS	OCULAR SIDE EFFECTS
Cholinergic agonists			
Acetylcholine	1% solution	Miosis in surgery	Corneal edema
Carbachol	0.01% solution	Miosis in surgery, glaucoma	Corneal edema, miosis, induced myopia, decreased vision, brow ache, retinal detachment
Pilocarpine	1%, 2%, and 4% solution	Glaucoma, miosis induction, miosis in surgery	Same as for carbachol
Anticholinesterase agents			
Echothiophate	0.03%, 0.06%, 0.125%, 0.25% solution	Glaucoma, accommodative esotropia	Retinal detachment, miosis, cataract, pupillary block, glaucoma, iris cysts, brow ache, punctal stenosis
Muscarinic antagonists			
Atropine	1% solution, 1% ointment	Cycloplegia, mydriasis,[a] cycloplegic retinoscopy,[b] dilated funduscopic exam,[b] iritis, uveitis, myopia	Photosensitivity, blurred vision; long acting (1 week or longer)
Scopolamine	0.25% solution	Cycloplegia, mydriasis, iritis, uveitis, nystagmus[b]	Same as for atropine but the effect lasts several days
Homatropine	2% and 5% solution	Cycloplegia, mydriasis, iritis,[b] uveitis	Same as for scopolamine but does not last as long
Cyclopentolate	Solution (0.5%, 1%, 2%), also with phenylephrine (0.2%/1%)	Cycloplegia, mydriasis[a]	Same as for atropine but effect lasts ~1 day
Tropicamide	0.5% and 1% solution, also with hydroxyamphetamine	Cycloplegia, mydriasis[a]	Same as for atropine but effect lasts several hours; commonly used for office exams
α Adrenergic agonists			
Phenylephrine	2.5% and 10% solution	Mydriasis, vasoconstriction, decongestion,[b] uveitis, glaucoma	α Adrenergic agonist effect lasts several hours; 2.5% used with tropicamide for office exams
Apraclonidine	0.5% and 1% solution	Glaucoma, ocular hypertension, also used for Horner syndrome diagnosis	α Adrenergic agonist; side effects: allergy (conjunctival hyperemia), dry mouth, sleepiness; used for Horner syndrome diagnosis
Brimonidine (α_2 selective)	Ophthalmic gel (0.33%); solution (0.025%, 0.1%, 0.15%, 0.2%); also with timolol or brinzolamide	Conjunctival injection, glaucoma, ocular hypertension	Same as for apraclonidine; causes respiratory depression in young children
Naphazoline	0.1% solution	Decongestant	Contraindicated in closed-angle glaucoma; safety/efficacy not established in the young (neonate to adolescent); use in infants may result in CNS depression, coma, marked reduction in body temperature; use with caution in patients with hypertension, cardiac disease, hyperthyroidism, diabetes, or infection or trauma in eye
Tetrahydrozoline	0.05% solution	Decongestant	Contraindicated in closed-angle glaucoma; safety/efficacy not established in neonates and children <6 years; use with caution in patients with coronary artery disease, hypertension, hyperthyroidism, or diabetes
β Adrenergic antagonists			
Betaxolol (β_1 selective)	0.25% suspension, 0.5% solution	Glaucoma, ocular hypertension	Reduced incidence of respiratory problems compared to timolol
Timolol	Solution, gel (0.25%, 0.5%); also with brimonidine, dorzolamide	Glaucoma, ocular hypertension	Side effects: exacerbation of respiratory problems (asthma and chronic obstructive pulmonary disease), bradycardia, depression, impotence
Carteolol	1% solution	Glaucoma, ocular hypertension	Same as timolol
Levobunolol	0.5% solution	Glaucoma, ocular hypertension	Same as timolol

[a] Mydriasis and cycloplegia, or paralysis of accommodation, of the human eye occurs after one drop of atropine 1%, scopolamine 0.5%, homatropine 1%, cyclopentolate 0.5% or 1%, and tropicamide 0.5% or 1%. Recovery of mydriasis is defined by return to baseline pupil size to within 1 mm. Recovery of cycloplegia is defined by return to within 2 diopters of baseline accommodative power. The maximal mydriatic effect of homatropine is achieved with a 5% solution, but cycloplegia may be incomplete. Maximal cycloplegia with tropicamide may be achieved with a 1% solution. Times to development of maximal mydriasis and to recovery, respectively, are as follows: for atropine, 30–40 min and 7–10 d; for scopolamine, 20–130 min and 3–7 d; for homatropine, 40–60 min and 1–3 d; for cyclopentolate, 30–60 min and 1 d; for tropicamide, 20–40 min and 6 h. Times to development of maximal cycloplegia and to recovery, respectively, are as follows: for atropine, 60–180 min and 6–12 d; for scopolamine, 30–60 min and 3–7 d; for homatropine, 30–60 min and 1–3 d; for cyclopentolate, 25–75 min and 6 h to 1 d; for tropicamide, 30 min and 6 h.

[b] Off-label use. Refer to *Physicians' Desk Reference for Ophthalmic Medicines* for specific indications and dosing.

TABLE 74–8 ■ PROSTAGLANDIN ANALOGUES FOR OPHTHALMIC USE

AGENT	FORMULATION	INDICATIONS	SIDE EFFECTS
Latanoprost (the most used glaucoma medication)	0.005% solution; also with netarsudil (0.02%)	Glaucoma, ocular hypertension	Once-daily dosing can cause lash growth; may cause allergic conjunctival hyperemia, permanent ↑ iris pigmentation, orbital fat atrophy
Travoprost	0.004% solution		Same as latanoprost but generally less irritating to corneal epithelium
Bimatoprost	0.01% solution, 0.03% solution 10 μg ophthalmic implant		
Tafluprost	0.0015% solution; preservative-free, single-dose droperettes		
Latanoprostene bunod	0.024%		Same as latanoprost

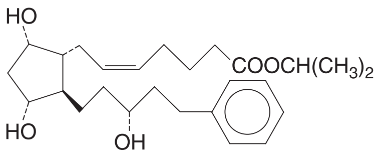
adrenergic agonist *brimonidine*. A *latanoprost/timolol* combination is widely available but not in the U.S. The combination of *brinzolamide/brimonidine* is also an option that is useful when a β receptor antagonist may be contraindicated. More recently, a *latanoprost/netarsudil* combination has become available. Such combinations reduce the number of drops needed and may improve compliance.

Topical miotic agents (see Table 74–7) are less commonly used today because of their numerous side effects and inconvenient dosing. Miotics lower IOP by causing muscarinic-induced contraction of the ciliary muscle, which facilitates aqueous outflow. They do not affect aqueous production. *Pilocarpine* and *carbachol* are cholinomimetics that stimulate muscarinic receptors and are referred to as having a direct mechanism of action. *Echothiophate, an irreversible* organophosphate inhibitor of acetylcholinesterase, enhances muscarinic cholinergic activity by reducing hydrolysis of neurally released acetylcholine (see Chapter 12). *Echothiophate* is relatively stable in aqueous solution and, by virtue of its quaternary ammonium structure, is positively charged and poorly absorbed.

If combined topical therapy fails to achieve the target IOP or fails to halt glaucomatous optic nerve damage, then systemic therapy with a CAI is an option before resorting to laser or incisional surgical treatment. The best-tolerated oral preparation is *acetazolamide* in sustained-release capsules (see Chapter 29), followed by *methazolamide*. The least well tolerated are *acetazolamide* tablets.

The main osmotic drugs for ocular use are *glycerin*, *mannitol*, and *hypertonic saline*. Oral *glycerin* and intravenous *mannitol* are used for short-term management of acute rises in IOP. Sporadically, these agents are used intraoperatively to dehydrate the vitreous prior to anterior segment surgical procedures. Many patients with acute glaucoma do not tolerate oral medications because of nausea; therefore, intravenous administration of *mannitol* or *acetazolamide* may be preferred over oral administration of *glycerin*. These agents should be used with caution in patients with congestive heart failure or renal failure.

Toxicity of Antiglaucoma Agents. Ciliary body spasm is a muscarinic cholinergic effect that can lead to induced myopia and a changing refraction due to iris and ciliary body contraction as the drug effect waxes and wanes between doses. Headaches can occur from the iris and ciliary body contraction. The α_2 agonists, effective in IOP reduction, can cause a vasoconstriction-vasodilation rebound phenomenon, leading to a red eye. Ocular and skin allergies from topical *apraclonidine* and *brimonidine* are common. *Brimonidine* is less likely to cause ocular allergy than *apraclonidine* and therefore is more commonly used. α_2 Agonists can cause CNS depression and apnea in neonates and are contraindicated in children less than 2 years of age. Systemic absorption of α_2 agonists and β adrenergic antagonists can induce all the side effects of systemic administration. Rho kinase inhibitors can have a vasodilating effect that results in conjunctival hyperemia, subconjunctival hemorrhage, blepharitis, and tearing. Rho kinase inhibitors often cause gold-brown pigment deposition in the corneal epithelium (cornea verticillata) and rarely cause reticular corneal epithelial edema. The use of CAIs systemically may give some patients significant problems with malaise, fatigue, depression, paresthesias, and nephrolithiasis; the topical CAIs may minimize these relatively common side effects.



LATANOPROST

Figure 74–5 *Latanoprost.*

Uveitis

Inflammation of the uvea, or uveitis, has both infectious and noninfectious causes, and medical treatment of the underlying cause (if known), in addition to the use of topical therapy, is essential. *Cyclopentolate*, *tropicamide*, or sometimes even longer-acting antimuscarinic agents such as *atropine*, *scopolamine*, and *homatropine* frequently are used to prevent posterior synechia formation between the lens and iris margin and to relieve ciliary muscle spasm that is responsible for much of the pain associated with anterior uveitis.

If posterior synechiae already have formed, an α adrenergic agonist may be used to break the synechiae by enhancing pupillary dilation. Various combinations of an α adrenergic agonist and antimuscarinic agent (e.g., 10% *phenylephrine* with 0.3% *scopolamine*; 1% *hydroxyamphetamine hydrobromide* with 0.25% *tropicamide*; 1% *phenylephrine* with 0.2% *cyclopentolate*; and 2.5% *phenylephrine* with 1% *tropicamide*) are available for the induction of mydriasis. Topical steroids usually are adequate to decrease inflammation but sometimes must be supplemented with local steroid injections or systemic steroids.

Strabismus

Strabismus, or ocular misalignment, has numerous causes and may occur at any age. Besides causing *diplopia* (double vision), strabismus in children may lead to *amblyopia* (reduced vision). Nonsurgical efforts to treat amblyopia include occlusion therapy, orthoptics, optical devices, and pharmacological agents.

An eye with *hyperopia*, or farsightedness, must constantly accommodate to focus on distant images. In some hyperopic children, the synkinetic accommodative-convergence response leads to excessive convergence and a manifest *esotropia* (turned-in eye). The brain rejects diplopia and suppresses the image from the deviated eye. If proper vision is not restored by about 6 to 8 years of age, the brain never learns to process visual information from that eye. The result is that the eye appears structurally normal but does not develop normal visual acuity and is therefore amblyopic. This is a fairly common cause of visual disability. In this setting, *atropine* (1%) instilled in the preferred seeing eye produces cycloplegia and the inability of this eye to accommodate, thus forcing the child to use the amblyopic eye (Pediatric Eye Disease Investigator Group, 2002, 2003). Echothiophate iodide also has been used in the setting of

TABLE 74–9 ■ CARBONIC ANHYDRASE INHIBITORS FOR OPHTHALMIC USE

AGENT	FORMULATION	INDICATIONS	SIDE EFFECTS
Acetazolamide	Tablets (125, 250 mg); extended release (500 mg) Intravenous	Acute ↑ IOP, glaucoma, idiopathic intracranial hypertension	Paresthesias (hands, feet), upset stomach, hypokalemia, allergic rash, kidney stones Rare: aplastic anemia
Methazolamide	Tablets (25, 50 mg)	Glaucoma	Same as acetazolamide but generally lesser paresthesias and upset stomach
Dorzolamide	2% solution; also in combo with timolol (0.5%)	Glaucoma, ocular hypertension	Causes burning, stinging, and a metallic taste
Brinzolamide	1% suspension; also in combo with brimonidine (0.2%)	Glaucoma, ocular hypertension	Causes less burning, stinging, and metallic taste than dorzolamide

accommodative strabismus. Accommodation drives the near reflex, which includes the triad of miosis, accommodation, and convergence. An irreversible cholinesterase inhibitor such as *echothiophate* causes miosis and an accommodative change in the shape of the lens; hence, the accommodative drive to initiate the near reflex is reduced, and less convergence will occur. It is often used in conjunction with a mydriatic to reduce the formation of iris cysts that are associated with *echothiophate* use.

Myopia

Myopia, or nearsightedness, is a condition in which near objects appear clear, while objects farther away are blurred because light entering the eye is not focused properly onto the retina. This has traditionally been treated with refractive correction such as spectacles, contact lenses, or refractive surgery. The prevalence of myopia has been increasing worldwide and is estimated to reach 5 billion by 2050, while high myopia, defined as more myopia than –5.00 D by the World Health Organization (WHO), will reach a prevalence of 1 billion (Gong et al., 2017). The leading cause of visual impairment worldwide is unaddressed refractive error, and high myopia is associated with sight-threatening complications such as chorioretinal atrophy and choroidal neovascularization; therefore, myopia prevention has become an international effort (WHO, 2017). Low-dose *atropine* ranging from 0.01% to 0.5% can be used to slow and even reverse myopia progression in children, although the exact mechanism of action remains unclear. Ocular side effects are dose dependent and include allergic reactions, dilated pupils requiring sunglasses, and loss of accommodation.

Surgical and Diagnostic Uses

For certain surgical procedures and for clinical funduscopic examination, it is desirable to maximize the view of the retina and lens. Muscarinic cholinergic antagonists and sympathomimetic agents frequently are used singly or in combination for this purpose (see Table 74–7). Intraoperatively, there are circumstances in which miosis is preferred, and two cholinergic agonists are available for intraocular use, *acetylcholine* and *carbachol*. Patients with myasthenia gravis may first present to an ophthalmologist with complaints of double vision (diplopia) or eyelid droop (ptosis). Patients with Horner syndrome may present to their ophthalmologist complaining of ptosis, and the reversal of anisocoria that occurs with *apraclonidine* is helpful in diagnosing these patients (see Table 74–2).

Anti-inflammatory, Immunomodulatory, and Antimitotic Drugs

Glucocorticoids

Glucocorticoids have an important role in managing ocular inflammatory diseases (Tables 74–11 and 74–12). The chemistry and pharmacology of glucocorticoids are described in Chapter 50.

Therapeutic Uses. The glucocorticoids formulated for topical administration to the eye are *dexamethasone*, *prednisolone*, *fluorometholone*, *loteprednol*, and *difluprednate*. Because of their anti-inflammatory effects, topical corticosteroids are used in managing significant ocular allergy, dry eye syndrome, external eye inflammatory diseases associated with some infections, ocular cicatricial pemphigoid, anterior uveitis, and postoperative inflammation following refractive, corneal, and intraocular surgery. After glaucoma filtering surgery, topical steroids can delay the wound-healing process by decreasing fibroblast infiltration, thereby reducing potential scarring of the surgical site. Steroids commonly are given systemically and by sub-Tenon's capsule injection to manage posterior uveitis. Intravitreal injection of steroids is used to treat diabetic retinopathy and cystoid macular edema. An intravitreal *triamcinolone* formulation is approved for ocular inflammatory conditions unresponsive to topical corticosteroids and for visualization during vitrectomy. Parenteral steroids followed by tapering oral doses is the preferred treatment of optic neuritis. Two ophthalmic implants, *fluocinolone* and *dexamethasone*, are marketed for the treatment of chronic, noninfectious uveitis; a *dexamethasone* implant is also indicated for the treatment of macular edema.

Toxicity. Ocular complications include the development of posterior subcapsular cataracts, secondary infections, and secondary open-angle glaucoma. There is a significant increase in the risk for developing secondary glaucoma when there is a positive family history of glaucoma. In the absence of a family history of open-angle glaucoma, only about 5% of normal individuals respond to topical or long-term systemic steroids with a marked increase in IOP. With a positive family history, however, moderate-to-marked steroid-induced IOP elevations may occur in up to 90% of patients. Some topical steroids (e.g., *loteprednol*) have been developed that reduce, but do not eliminate, the risk of elevated IOP. Certain corneal infections can be worsened by topical steroids, especially from herpes simplex virus, fungus, and acanthamoeba.

TABLE 74–10 ■ RHO KINASE INHIBITORS FOR OPHTHALMIC USE

AGENT	FORMULATION	INDICATIONS	SIDE EFFECTS
Netarsudil	0.02%; also in combination with latanoprost (0.005%)	Glaucoma, endothelial cell repopulation after Descemet stripping only	Conjunctival hyperemia, subconjunctival hemorrhage, blepharitis, tearing, cornea verticillata, rarely reticular corneal epithelial edema
Ripasudil (not available in the U.S.)	0.4%	Glaucoma, endothelial cell repopulation after Descemet stripping only	Same as netarsudil

TABLE 74–11 ■ GLUCOCORTICOIDS FOR TOPICAL APPLICATION TO THE EYE

AGENT	FORMULATION	INDICATIONS
Dexamethasone	0.1% suspension; 0.1% (sodium phosphate) solution; 0.4 mg intracanalicular insert; 0.7 mg intravitreal implant	Steroid-responsive inflammatory conditions of the palpebral and bulbar conjunctiva, cornea, and anterior segment; infective conjunctivitis to reduce edema and inflammation when hazard of steroid use is acceptable; corneal injury from chemical, radiation, or thermal burns or penetration of foreign object; postoperative inflammation
Difluprednate	0.05% emulsion	Ocular pain; postoperative ocular inflammation; uveitis
Fluorometholone	0.1%, 0.25% suspension; 0.1% ointment	Allergic conjunctivitis; giant papillary conjunctivitis; keratitis; ocular burns; postoperative ocular inflammation; uveitis; vernal keratoconjunctivitis
Loteprednol etabonate	0.2%, 0.25%, 0.5% suspension; 0.38%, 0.5% gel; 0.5% ointment	Allergic conjunctivitis; cyclitis; giant papillary conjunctivitis; iritis; keratitis; ocular pain; postoperative ocular inflammation, uveitis[a]
Prednisolone acetate Prednisolone sodium phosphate	0.12%, 1% suspension 1% solution	Allergic conjunctivitis and marginal corneal ulcer; anterior segment inflammation; bacterial conjunctivitis; chorioretinitis; cyclitis; endophthalmitis[a]; Graves' ophthalmopathy; herpes zoster ocular infection (with antiviral therapy); iritis; nonspecific and superficial punctate keratitis; postoperative ocular inflammation; optic neuritis; sympathetic ophthalmia; diffuse posterior uveitis; vernal keratoconjunctivitis; corneal injury from chemical, radiation, thermal burns; or penetration of foreign objects. Commonly used after ocular surgery

[a] Off-label use.

Nonsteroidal Anti-inflammatory Agents

Pharmacological properties of non-steroidal anti-inflammatory drugs (NSAIDs) are presented in Chapter 42. Five topical NSAIDs are FDA-approved for ocular use: *flurbiprofen*, *ketorolac*, *diclofenac*, *bromfenac*, and *nepafenac*.

The NSAIDs are supplied as solutions and suspensions for topical ocular use to reduce ocular inflammation and cystoid macular edema. *Flurbiprofen* is used to counter unwanted intraoperative miosis during cataract surgery. *Ketorolac* is given for seasonal allergic conjunctivitis. A combination *ketorolac/phenylephrine* intraocular solution is available to add to intraoperative ophthalmic irrigation solutions to decrease miosis during cataract surgery. *Diclofenac* is used for postoperative inflammation and pain. Both *ketorolac* and *diclofenac* are effective in treating cystoid macular edema occurring after cataract surgery and in controlling pain after corneal refractive surgery. *Bromfenac* and *nepafenac* are indicated for treating postoperative pain and inflammation after cataract surgery. Topical NSAIDs occasionally have been associated with sterile corneal melts and perforations, especially in older patients with ocular surface disease such as dry eye syndrome or autoimmune conditions affecting the ocular surface.

Antihistamines and Mast Cell Stabilizers

H_1 antagonists (see Chapter 43) and mast cell stabilizers are used to treat the manifestations of ocular allergies. *Pheniramine* and *antazoline* (discontinued in the U.S.) are formulated in combination with *naphazoline*, a vasoconstrictor, for relief of allergic conjunctivitis; *emedastine difumarate* also is used. *Cromolyn sodium* has found some use in treating conjunctivitis that is thought to be allergen mediated, such as vernal conjunctivitis. The mast cell stabilizer *lodoxamide tromethamine* is available for ophthalmic use for the treatment of ocular inflammatory states such as vernal conjunctivitis and keratitis. *Nedocromil*, primarily a mast cell stabilizer with some antihistaminic properties, is also used. Likewise, *olopatadine hydrochloride, ketotifen fumarate, bepotastine, azelastine*, and *alcaftadine* are H_1 antagonists with mast cell–stabilizing properties. *Epinastine* antagonizes H_1 and H_2 receptors and exhibits mast cell–stabilizing activity.

Immunosuppressants

Topical *cyclosporine* (Table 74–13) is approved for the treatment of chronic dry eye associated with inflammation. Use of *cyclosporine* is associated with decreased inflammatory markers in the lacrimal gland, increased tear production, and improved vision and comfort. Topical *lifitegrast* (Table 74–13) is approved to treat the signs and symptoms of dry eye disease. *Lifitegrast* inhibits an integrin, lymphocyte function–associated antigen 1 (LFA-1), from binding to intercellular adhesion molecule 1 (ICAM-1), which is thought to downregulate inflammation mediated by T lymphocytes. *Interferon* α_{2b} is used off-label in the treatment of conjunctival papilloma and certain conjunctival tumors.

Antimitotic Agents

In glaucoma surgery, the antineoplastic agents *5-fluorouracil* (5FU) and *mitomycin* (Table 74–13; see details of mechanism in Chapter 70) improve the success of filtration surgery by limiting the postoperative wound-healing process.

Therapeutic Uses. *Mitomycin* is used intraoperatively as a single subconjunctival application at the trabeculectomy site. 5FU may be used intraoperatively at the trabeculectomy site or subconjunctivally during the postoperative course (Fluorouracil Filtering Surgery Study Group, 1996). Both agents work by limiting the healing process; sometimes this can result in thin, ischemic, avascular tissue that is prone to breakdown. The resultant leaks can cause hypotony (low IOP) and increase the risk of infection. In corneal disease, both *mitomycin* and 5FU are used in the treatment of certain corneal and conjunctival tumors. *Mitomycin* can be used to reduce the risk of scarring after procedures to remove corneal opacities and prophylactically to prevent corneal scarring after photorefractive and phototherapeutic keratectomy. *Mitomycin* also is used to decrease recurrence after pterygium excision. Caution is advocated when using *mitomycin* in light of the potentially serious delayed ocular complications, including limbal stem cell deficiency and corneal or scleral melt.

Agents Used in Ophthalmic Surgery

Presurgical Antiseptics

Povidone iodine is formulated as a 5% sterile ophthalmic solution for use prior to surgery to prepare periocular skin and irrigate ocular surfaces, including the cornea, conjunctiva, and palpebral fornices. Following irrigation, the exposed tissues are flushed with sterile saline. *Povidone iodine* can cause local irritation and should be avoided in patients with a history of prior reaction to it. *Hypochlorous acid* is an effective skin preparation agent and can be used for ophthalmic surgery when *povidone iodine* is contraindicated.

Viscoelastic Substances

The viscoelastic substances are agents that assist in ocular surgery by maintaining spaces, moving tissue, and protecting surfaces. These substances are prepared from hyaluronate, chondroitin sulfate and

TABLE 74–12 ■ GLUCOCORTICOIDS FOR SUB-TENON'S, INTRAVITREAL, AND SYSTEMIC USE

AGENT	FORMULATION	METHOD OF ADMINISTRATION	INDICATION
Triamcinolone	Solutions and suspensions for injection, 3–40 mg/mL (only triamcinolone acetonide injectable suspension 40 mg/mL is FDA-approved for intravitreal injection)	Sub-Tenon's,[a] intracameral,[a] intravitreal	Ocular inflammation, ocular surgery, uveitis
Betamethasone	Suspension for IM injection (6 mg/mL)	Sub-Tenon's[a]	Corticosteroid-responsive ophthalmic disorders: allergic conjunctivitis and marginal corneal ulcer, anterior segment inflammation, chorioretinitis, conjunctivitis, endophthalmitis,[a] Graves' ophthalmopathy, herpes zoster ocular infection, iritis, keratitis, postoperative ocular inflammation, optic neuritis, diffuse posterior uveitis, vernal keratoconjunctivitis
Dexamethasone	Oral concentrate (1 mg/mL); tablets (0.5–6 mg); injectable solution (4 mg/mL, 10 mg/mL)	Oral, intravenous, intramuscular	Allergic conjunctivitis, allergic marginal corneal ulcer, anterior segment inflammation, chorioretinitis, cyclitis, endophthalmitis,[a] Graves' ophthalmopathy, giant papillary conjunctivitis, herpes zoster ocular infection, iritis, keratitis, superficial punctate keratitis, postoperative ocular inflammation, optic neuritis, diffuse choroiditis, sympathetic ophthalmia, vernal keratoconjunctivitis, or corneal injury (corneal abrasion)
Dexamethasone	0.4 mg ophthalmic implant	Intravitreal	Ocular inflammation due to macular edema following retinal vein occlusion, including branch retinal vein occlusion or central retinal vein occlusion and for the treatment of noninfectious uveitis affecting the posterior segment of the eye
Fluocinolone acetonide	0.18 mg ophthalmic implant	Intravitreal	Diabetic macular edema in patients who had no clinically significant rise in intraocular pressure when treated with corticosteroids
Fluocinolone acetonide	0.59 mg ophthalmic implant	Intravitreal	For the treatment of chronic noninfectious uveitis affecting the posterior segment of the eye
Methylprednisolone	Tablets (2–32 mg): 20 mg/mL, 40 mg/mL, suspension for IM injection (80 mg/mL); sodium succinate solution for IM/IV injection (40–2000 mg)	Oral, intravenous, intramuscular	For the systemic treatment of ophthalmic disorders, including allergic conjunctivitis, allergic marginal corneal ulcer, anterior segment inflammation, chorioretinitis, endophthalmitis,[a] Graves' ophthalmopathy, herpes zoster ocular infection, iritis, keratitis, postoperative ocular inflammation, optic neuritis, diffuse posterior uveitis, or vernal keratoconjunctivitis
Prednisone	1 mg/mL, 5 mg/mL oral solution; 1, 2.5, 5, 10, 20, 50 mg tabs; 1, 2, 5 mg delayed-release tabs	Oral	Inflammatory conditions such as endophthalmitis,[a] optic neuritis, allergic conjunctivitis, keratitis, allergic corneal ulcer, iritis, chorioretinitis, anterior segment inflammation, uveitis, choroiditis, or sympathetic ophthalmia
Prednisolone	Orally disintegrating tablets (10–30 mg); syrup and oral solution (1–5 mg/mL); injection 125 mg/mL	Oral, injection (IV/IM)	Systemic treatment of corticosteroid-responsive eye disorders

IM, intramuscular; IV, intravenous.

[a] Off-label use.

hydroxypropyl methylcellulose and share varying degrees of the following important physical characteristics: viscosity, shear flow, elasticity, cohesiveness, and coatability. Complications associated with viscoelastic substances are related to transient elevation of IOP after surgery with retained intraocular material.

Ophthalmic Glue

Cyanoacrylate tissue adhesive, while not FDA-approved for the eye, is widely used in the management of corneal ulcerations and perforations. *Fibrin sealants* are increasingly being used on the ocular surface to secure tissue such as conjunctiva, amniotic membrane, and lamellar corneal grafts.

Intraoperative Visualization

Intraoperatively, *trypan blue* is marketed as 0.06% and 0.15% ophthalmic solutions to facilitate visualization of the anterior lens capsule during cataract surgery, for staining of Descemet's membrane that is inserted into the eye during Descemet's stripping endothelial keratoplasty, and

TABLE 74–13 ■ IMMUNOSUPPRESSIVE AND ANTIMITOTIC AGENTS FOR OCULAR USE

AGENT	FORMULATION	INDICATION	COMMENTS
Cyclosporine	0.05% or 0.09% emulsion	Dry eye	Dosed in preservative-free individual droperettes; Can be compounded up to 2%
Lifitegrast	5% solution	Dry eye	Dosed in preservative-free individual droperettes
Interferon α_{2B}[a]	Topical solution (1 million IU/mL); subconjunctival injection (3 million IU/0.5 mL)	Conjunctival tumors Conjunctival papilloma	Used in the treatment of ocular surface squamous neoplasia
5-Fluorouracil (5FU)	50 mg/mL solution	Conjunctival tumors Conjunctival papilloma Glaucoma surgery Epithelial downgrowth	Used intraoperatively and postoperatively to prevent subconjunctival scarring Inhibits corneal epithelial healing; cannot be used if there is corneal abrasion
Mitomycin	0.2 mg/mL for topical application	Conjunctival tumors Glaucoma surgery Pterygium surgery Corneal scarring and surface ablation surgery	Generally used as a subconjunctival application intraoperatively to prevent scarring More commonly used than 5FU in glaucoma surgery; not for injection Used topically on the cornea

[a]Off-label use

for staining the retinal surface during surgical vitrectomy to guide the excision of tissue.

Anterior Segment Gases

Sulfur hexafluoride (SF_6) and *perfluoropropane* (C_3F_8) gases are used as vitreous substitutes during retinal surgery. In the anterior segment, SF_6 is used in a nonexpansile concentration primarily after endothelial keratoplasty to help the lamellar graft adhere to the posterior cornea. Patients are typically advised to lay face up for 3 to 5 days after surgery to maximize graft attachment. SF_6 may also be used to treat Descemet's detachments, typically after cataract surgery. These detachments can cause mild-to-severe corneal edema. The gas is injected into the anterior chamber to push Descemet's membrane up against the stroma, where ideally it reattaches and clears the corneal edema.

Vitreous Substitutes

Several compounds, including gases, perfluorocarbon liquids, and silicone oils, are available as vitreous substitutes (Table 74–14). Their primary use is reattachment of the retina following vitrectomy and membrane-peeling procedures for complicated proliferative vitreoretinopathy and traction retinal detachments. The use of expansile gases carries the risk of complications from elevated IOP, subretinal gas, corneal edema, and cataract formation. The gases are absorbed over a period of days (for air) to 2 months (for *perfluoropropane*).

TABLE 74–14 ■ VITREOUS SUBSTITUTES[a]

AGENT	DURATION OR VISCOSITY
Gases	
Air	Duration, 3–7 days
Sulfur hexafluoride (SF_6)[b]	Duration, 10–14 days
Perfluoropropane (C_3F_8)[b]	Duration, 55–65 days
Liquids	
Silicone oil (SO)	Viscosity: 1000–5000 cs
SO-PFA combinations	

cs, centistoke (unit of viscosity); PFA, partially fluorinated alkanes

[a]For further information, see Alovisi et al. (2017).

[b]Expansile gas

The liquid perfluorocarbons (specific gravity 1.76–1.94) are denser than vitreous and are helpful in flattening the retina when vitreous is present. Their use is limited to intraoperative settings due to long-term toxicity to the retina. Silicone oil (polydimethylsiloxanes) is used for long-term tamponade of the retina. Complications from silicone oil use include glaucoma, cataract formation, corneal edema, corneal band keratopathy, and retinal toxicity. Combinations of silicone oil and partially fluorinated alkanes have been approved for clinical use, which may have benefit over silicone oil alone for certain types of retinal breaks (Alovisi et al., 2017).

Surgical Hemostasis and Thrombolytic Agents

Hemostasis has an important role in most surgical procedures and usually is achieved by temperature-mediated coagulation. Intravitreal administration of thrombin can assist in controlling intraocular hemorrhage during vitrectomy. When used intraocularly, a potentially significant inflammatory response may occur that can be minimized by thorough irrigation after hemostasis is achieved.

During intraocular surgeries to assist evacuation of a hyphema, subretinal clot, or nonclearing vitreous hemorrhage, *tissue plasminogen activator* (tPA; see Chapter 36) has been used off-label. tPA also has been administered subconjunctivally and intracamerally (i.e., controlled intraocular administration into the anterior segment) to lyse blood clots obstructing a glaucoma filtration site. The main complication related to the use of tPA is bleeding.

Botulinum Toxin Type A in the Treatment of Strabismus, Blepharospasm, and Related Disorders

Several botulinum toxin type A preparations are marketed in the U.S. with similar indications: *onabotulinumtoxinA*, *abobotulinumtoxinA*, *prabotulinumtoxinA*, and *incobotulinumtoxinA*. These agents are used for the treatment of strabismus and blepharospasm associated with dystonia, facial wrinkles (glabellar lines), bladder dysfunction and urinary incontinence, spasticity, axillary hyperhidrosis, spasmodic torticollis (cervical dystonia), and chronic migraine, among others. By preventing acetylcholine release at the neuromuscular junction, botulinum toxin A usually causes a temporary paralysis of the locally injected muscles. Complications related to this toxin include double vision (diplopia), eyelid droop (ptosis), and rarely, potentially life-threatening distant spread of toxin effect from the injection site hours to weeks after administration.

Agents Used to Treat Blind and Painful Eyes

Retrobulbar injection of either absolute or 95% ethanol may provide relief from chronic pain associated with a blind and painful eye when topical steroids and cycloplegics fail. Retrobulbar *chlorpromazine* also has been used off-label. This treatment is preceded by administration of local anesthesia. Local infiltration of the ciliary nerves provides symptomatic relief from pain, but other nerve fibers may be damaged. This may cause paralysis of the extraocular muscles, including those in the eyelids, or neuroparalytic keratitis. The sensory fibers of the ciliary nerves may regenerate, and repeated injections sometimes are needed to control pain.

Ocular Side Effects of Systemic Agents

Certain systemic drugs have ocular side effects. These can range from mild and inconsequential to severe and vision threatening (Li et al., 2008; Pula et al., 2013). Examples are listed in the sections that follow.

IOP and Glaucoma

The antiseizure drug *topiramate* can cause choroidal effusions leading to angle-closure glaucoma. Inhaled, systemic, or ocular steroids can cause elevated IOP and glaucoma. If the steroids cannot be stopped, glaucoma medications, and even filtering surgery, often are required.

Retina

Numerous drugs have toxic side effects on the retina. The antiarthritis and antimalarial medicines *hydroxychloroquine* and *chloroquine* can cause central retinal toxicity by an unknown mechanism. With normal dosages, toxicity does not appear until about 6 years after the drug is started. Stopping the drug will not reverse the damage but generally will prevent further toxicity. *Tamoxifen* can cause crystalline maculopathy, whereas *cisplatin* and *carmustine* cause a pigmentary retinopathy. The antiseizure drug *vigabatrin* causes progressive and permanent bilateral concentric visual field constriction in a high percentage of patients.

Optic Nerve

The phosphodiesterase (PDE) 5 inhibitors *sildenafil*, *vardenafil*, and *tadalafil* inhibit PDE5 in the corpus cavernosum to help achieve and maintain penile erection (see Chapter 49). They are also used to treat pulmonary arterial hypertension (see Chapter 35). The drugs also mildly inhibit PDE6, which controls the levels of cyclic GMP in the retina (see Figure 74–9), causing a bluish haze or light sensitivity. Multiple medications, including *ethambutol*, *chloramphenicol*, and *rifampin*, can cause toxic optic neuropathy characterized by gradually progressive bilateral central scotomas and vision loss.

Anterior Segment

Steroids have been implicated in cataract formation. *Tamoxifen*, among others, has also been associated with cataracts. *Rifabutin*, if used in conjunction with *clarithromycin* or *fluconazole* for treatment of *Mycobacterium avium* complex opportunistic infections in human immunodeficiency virus–positive persons, is associated with an iridocyclitis and even hypopyon. These conditions resolve with steroids or by stopping the medication.

Ocular Surface

Isotretinoin has a drying effect on mucous membranes and is associated with dry eye and severe dysfunction of the meibomian gland.

Cornea, Conjunctiva, and Eyelids

The cornea, conjunctiva, and eyelids can be affected by systemic medications. One of the most common drug deposits found in the cornea is from the cardiac medication *amiodarone*. It deposits in the inferior and central cornea in a whorl-like pattern termed *cornea verticillata*, appearing as fine tan or brown pigment in the epithelium. The deposits seldom affect vision and are rarely a cause to discontinue the medication. The deposits disappear slowly when the medication is stopped. Other medications, including *indomethacin*, *atovaquone*, *chloroquine*, and *hydroxychloroquine*, can cause a similar pattern. The phenothiazines, including *chlorpromazine* and *thioridazine*, can cause brown pigmentary deposits in the cornea, conjunctiva, and eyelids. They typically do not affect vision. The ocular deposits generally persist after discontinuation of the medication and can even worsen. Gold- and silver-containing medications can be used to treat rheumatoid arthritis and can cause pigmented deposits in the cornea and conjunctiva. Certain chemotherapeutic agents, such as *cytarabine*, can cause temporary corneal toxicity. Tetracyclines can cause a yellow discoloration of the light-exposed conjunctiva. Systemic *minocycline* can induce a blue-gray scleral pigmentation that is most prominent in the interpalpebral zone. *Dupilumab*, a monclonal antibody that inhibits pro-inflammatory signaling by IL-4 and IL-13, is used to treat moderate to severe eczema; its use has been associated with conjunctivitis, keratitis, blepharitis, and dry eye.

Agents Used to Assist in Ocular Diagnosis

A number of agents are used in an ocular examination (e.g., mydriatic agents, topical anesthetics, dyes to evaluate corneal surface integrity); to facilitate intraocular surgery (e.g., mydriatic and miotic agents, topical and local anesthetics); and to help in making a diagnosis in cases of anisocoria and retinal abnormalities (e.g., intravenous contrast agents). The autonomic agents have been discussed previously. The diagnostic and therapeutic uses of topical and intravenous dyes and of topical anesthetics are discussed in the material that follows.

Anterior Segment and External Diagnostic Uses

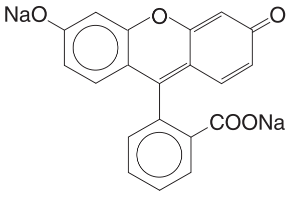
Epiphora (excessive tearing) and surface problems of the cornea and conjunctiva are commonly encountered external ocular disorders. The dyes *fluorescein, rose bengal*, and *lissamine green* are used in evaluating these problems. *Fluorescein* (Figure 74–7) is available as 10% and 25% solutions for injection and as an impregnated paper strip, and reveals epithelial defects of the cornea and conjunctiva and aqueous humor leakage that may occur after trauma or ocular surgery. In the setting of epiphora, *fluorescein* is used to determine the patency of the nasolacrimal system. In addition, this dye is used in *applanation tonometry* (IOP measurement) and to assist in determining the proper fit of rigid and semirigid contact lenses. *Fluorescein* in combination with *proparacaine* or *benoxinate* is available for procedures in which a disclosing agent is needed in conjunction with a topical anesthetic. *Fluorexon*, a high-molecular-weight fluorescent solution, is used when *fluorescein* is contraindicated (as when soft contact lenses are in place). *Rose bengal* and *lissamine green* (available as saturated paper strips) stain devitalized tissue on the cornea and conjunctiva.

Posterior Segment Diagnostic Uses

The integrity of the blood-retinal and retinal pigment epithelial barriers may be examined directly by retinal angiography using intravenous administration of either *fluorescein sodium* or *indocyanine green*. These agents commonly cause nausea and pruritis and may precipitate serious allergic reactions in susceptible individuals.

Treatment of Retinal Neovascularization, Macular Degeneration, and Vitreomacular Traction

The medical treatment of retinal neovascularization has been changing rapidly in the past several decades and will likely continue to do so (Agarwal et al., 2015). Current treatment employs agents that inhibit the actions of VEGF (Table 74–15).



FLUORESCEIN SODIUM

Figure 74–7 *The diagnostic dye fluorescein.*

TABLE 74–15 ■ ANTI-VEGF AGENTS FOR OCULAR USE

AGENT	FORMULATION	INDICATION	COMMENTS
Verteporfin	2 mg/mL reconstituted solution for intravenous infusion	Retinal neovascularization associated with macular degeneration	Dosed according to body surface area Activated by a cool laser as it circulates in the retinal circulation Causes photosensitization and propensity for sunburn
Pegaptanib	0.3 mg/0.09 mL intravitreal injection	Same as verteporfin	Binds to the 165 isoform of VEGF
Aflibercept	2 mg/0.05 mL intravitreal injection	Same as verteporfin *plus* DME or for the treatment of diabetic retinopathy in patients with DME	A decoy receptor for VEGF-A
Bevacizumab[a]	1.25 mg/0.05 mL intravitreal injection	Same as aflibercept	First anti-VEGF antibody commercially available; generally used off-label as first-line therapy. Made by compounding pharmacies
Ranibizumab	0.3 mg/0.05 mL intravitreal injection	Same as aflibercept	A variant of bevacizumab
Brolucizumab	6 mg/0.05 mL intravitreal injection	Same as aflibercept	Single-chain antibody fragment Longer duration Rarely, severe inflammatory reaction

[a] Off-label use high risk due to possible contamination in compounding pharmacies.

Verteporfin is approved for photodynamic therapy of the exudative form of age-related macular degeneration (AMD) with predominantly classic choroidal neovascular membranes. *Verteporfin* also is used in the treatment of predominantly classic choroidal neovascularization caused by conditions such as pathological myopia and presumed ocular histoplasmosis syndrome. *Verteporfin* is administered intravenously; once it reaches the choroidal circulation, the drug is light activated by a nonthermal laser source. Activation of the drug in the presence of O_2 generates free radicals, which cause vessel damage and subsequent platelet activation, thrombosis, and occlusion of choroidal neovascularization. The $t_{1/2}$ of the drug is 5 to 6 h; it is eliminated predominantly in the feces. Potential side effects include headache, injection site reactions, and visual disturbances. The drug causes temporary photosensitization; patients must avoid exposure of the skin or eyes to direct sunlight or bright indoor lights for 5 days after receiving *verteporfin*.

Pegaptanib, a selective VEGF antagonist, is approved for neovascular (wet) AMD. VEGF165 induces angiogenesis and increases vascular permeability and inflammation; these actions likely contribute to the progression of the neovascular (wet) form of AMD, a leading cause of blindness. *Pegaptanib* inhibits VEGF165 binding to VEGF receptors. *Pegaptanib* (0.3 mg) is administered once every 6 weeks by intravitreal injection into the eye to be treated. Following the injection, patients should be monitored for elevation in IOP and for endophthalmitis. Rare cases of anaphylaxis/anaphylactoid reactions have been reported.

Aflibercept is a recombinant fusion protein, consisting of portions of human VEGF receptors 1 and 2, that acts as a soluble decoy receptor for VEGF-A. It is approved for the neovascular (wet) form of AMD as well as macular edema following retinal vein occlusion or associated with diabetic retinopathy. Depending on the underlying disease, *aflibercept* (2 mg) is administered once every month by intravitreal injection into the eye for 3 to 5 months, followed by 2 mg once every 8 weeks. Serious side effects may include eye pain or redness, swelling, vision problems, photosensitivity, headaches, sudden numbness on one side of the body, confusion, and problems with speech and balance. The drug is contraindicated in patients who have an active eye infection or active ocular inflammation.

Bevacizumab is a monoclonal murine antibody that targets VEGF-A and thereby inhibits vascular proliferation and tumor growth (see Chapter 72). *Ranibizumab* is a variant of *bevacizumab* that has had the Fab domain affinity matured. Both drugs are delivered by intravitreal injection and often are used on a monthly basis for maintenance therapy. Both have been associated with the risk of cerebral vascular accidents. The enormous cost difference between these similar antibodies has sparked a debate about the cost-effectiveness of treatment strategies (Shaikh et al., 2015; Stein et al., 2014), especially in light of recent data indicating similar visual acuity outcomes for each medicine (CATT Research Group, 2011).

Brolucizumab, a more recent therapy for macular degeneration, also targets VEGF-A but is a single-chain antibody fragment and therefore smaller with the potential for increased duration. *Brolucizumab* was shown to be noninferior to *aflibercept* with a similar safety profile, but with a higher incidence of uveitis. It also required less frequent dosing (Dugel et al., 2020), which has significant implications given the cost and frequency of visits associated with treating retinal neovascularization.

Ocriplasmin is a proteolytic enzyme that is used to treat vitreomacular traction. It works to dissolve the vitreous and relieve traction on the macula in the center of the retina. An interesting side effect is the possibility of lens subluxation from zonular dehiscence.

Anesthetics in Ophthalmic Procedures

Topical anesthetic agents used clinically in ophthalmology include *proparacaine* and *tetracaine* drops, *lidocaine* gel (see Chapter 25), and intranasal *cocaine*. *Cocaine* may be used intranasally in combination with topical anesthesia for cannulating the nasolacrimal system. *Lidocaine* and *bupivacaine* are used for infiltration and retrobulbar block anesthesia for surgery. Potential complications and risks relate to allergic reactions, globe perforation, hemorrhage, and inadvertent vascular and subdural injections. Both preservative-free *lidocaine* (1%), which is introduced into the anterior chamber, and ophthalmic *lidocaine* jelly (3.5%), which is placed on the ocular surface during preoperative patient preparation, are used for cataract surgery performed under topical anesthesia. Most inhalational agents and CNS depressants are associated with a reduction in IOP. An exception is *ketamine*, which has been associated with an elevation in IOP. In the setting of a patient with a ruptured globe, the anesthesia should be selected carefully to avoid agents that depolarize the extraocular muscles, which may result in expulsion of intraocular contents.

Treatment of Dry Eye and Corneal Edema

The current management of dry eyes usually includes instilling artificial tears and other ophthalmic lubricants. In general, tear substitutes are hypotonic or isotonic solutions composed of electrolytes, surfactants, preservatives, and some viscosity-increasing agent that prolongs the residence time in the cul-de-sac and precorneal tear film.

Common viscosity agents include *cellulose polymers, polyvinyl alcohol, polyethylene glycol, polysorbate, mineral oil, glycerin*, and *dextran*. The tear substitutes are available as preservative-containing or preservative-free preparations. The viscosity of the tear substitute depends on its exact formulation and can range from watery to gel like. Some tear formulations also are combined with a vasoconstrictor, such as *naphazoline*, *phenylephrine*, or *tetrahydrozoline*. The lubricating ointments are composed of a mixture of white petrolatum, mineral oil, liquid or alcohol lanolin, and sometimes a preservative. These highly viscous formulations cause considerable blurring of vision; consequently, they are used primarily at bedtime, in critically ill or sedated patients, or in very severe dry eye conditions. A *hydroxypropyl cellulose* ophthalmic insert that is placed in the inferior cul-de-sac and dissolves during the day is available to treat dry eyes.

Local eye diseases such as blepharitis, ocular rosacea, ocular pemphigoid, or chemical burns may alter the ocular surface and change the tear composition. Appropriate treatment of the symptomatic dry eye includes treating the accompanying disease and possibly the addition of tear substitutes, punctal plugs (see Absorption), ophthalmic *cyclosporine*, or ophthalmic *lifitegrast* (see Immunosuppressants). There also are a number of systemic conditions that may manifest themselves with symptomatic dry eyes, including Sjögren syndrome, rheumatoid arthritis, vitamin A deficiency, Stevens-Johnson syndrome, and trachoma. Treating the systemic disease may not eliminate the symptomatic dry eye complaints; chronic therapy with tear substitutes, ophthalmic *cyclosporine*/*lifitegrast*, insertion of punctal plugs, placement of dissolvable collagen implants, or surgical occlusion of the lacrimal drainage system may be indicated. Ophthalmic *cyclosporine*/*lifitegrast* can be used to increase tear production in patients with ocular inflammation associated with keratoconjunctivitis sicca. *Doxycycline* (see Bacterial Infections) is often used to treat blepharitis due to its anti–matrix metalloproteinase activity. In severe cases, autologous serum tears are formulated using blood drawn from the patient and prepared in specialty laboratories. Because serum contains a mixture of growth factors, proteins, antioxidants, and lipids, it provides a more effective tear replacement than manufactured tears.

Corneal edema is a clinical sign of corneal endothelial dysfunction, and topical osmotic agents may effectively dehydrate the cornea. NaCl is available in either aqueous or ointment formulations. Topical *glycerin* also is available; however, because it causes pain on contact with the cornea and conjunctiva, its use is limited to clinical evaluation. In general, when corneal edema occurs secondary to acute glaucoma, the use of an oral osmotic agent to help reduce IOP is preferred over topical *glycerin*, which simply clears the cornea temporarily. Reducing the IOP will help clear the cornea more permanently to allow both a view of the filtration angle by gonioscopy and a clear view of the iris as required to perform laser iridotomy.

Rho kinase inhibitors (see Glaucoma), including *netarsudil* and *ripasudil*, promote adhesion, survival, and proliferation of corneal endothelial cells (Macsai and Shiloach, 2019). These medications have been used in conjunction with *Descemet's stripping only* surgery in patients with Fuchs endothelial corneal dystrophy limited to the central 5 mm of the cornea, to stimulate endothelial expansion and possibly proliferation into the area of excised Descemet's membrane/endothelium.

Treatment of the Neurotrophic Cornea

The cornea is the most densely innervated tissue in the human body, and corneal nerves play a critical role in corneal homeostasis. Corneal nerves mediate protective blinking and tearing reflexes and also provide trophic support, releasing neuropeptides to promote corneal epithelial proliferation, migration, and adhesion. Neurotrophic keratopathy can result from multiple underlying causes including herpetic infection, chemical injury, long-term diabetes, prior ocular surgery, long-term contact lens use, or an intracranial mass or neurosurgery. Typical treatment options for nonhealing epithelial defects from neurotrophic disease may include artificial tear lubrication, autologous serum tears, tarsorrhaphy, or amniotic membrane transplantation (Bonini et al., 2018). *Cenegermin*, a recombinant human nerve growth factor topical eye drop, was approved for the treatment of neurotrophic keratopathy in the U.S. in 2018.

Vitamin A and the Visual Cycle

Vitamin deficiencies can alter eye function, especially a deficiency of vitamin A (Table 74–16). In vision, the functional form of vitamin A is *retinal*; its deficiency interferes with vision in dim light, contributing to a condition known as *night blindness* (nyctalopia).

Chemistry

Retinoid refers to the chemical entity *retinol* and other closely related naturally occurring derivatives. Retinoids, which exert most of their effects by binding to specific nuclear receptors and modulating gene expression, also include structurally related synthetic analogues that need not have retinol-like (vitamin A) activity. The purified plant pigment carotene (provitamin A) is a protean source of vitamin A. β-Carotene is the most active carotenoid found in plants. The structural formulas for β-carotene and the vitamin A family of retinoids are shown in Figure 74–8.

TABLE 74–16 ■ OPHTHALMIC EFFECTS OF SELECTED VITAMIN DEFICIENCIES AND ZINC DEFICIENCY

DEFICIENCY	EFFECTS IN ANTERIOR SEGMENT	EFFECTS IN POSTERIOR SEGMENT
Vitamin		
A (retinol)	Conjunctiva (Bitot spots, xerosis)	Retina (nyctalopia, impaired rhodopsin synthesis), retinal pigment epithelium (hypopigmentation)
	Cornea (keratomalacia, punctate keratopathy)	
B_1 (thiamine)	—	Optic nerve (temporal atrophy with corresponding visual field defects)
B_6 (pyridoxine)	Cornea (neovascularization)	Retina (gyrate atrophy)
B_{12} (cyanocobalamin)	—	Optic nerve (temporal atrophy with corresponding visual field defects)
C (ascorbic acid)	Lens (? cataract formation)	—
E (tocopherol)	—	Retina and retinal pigment epithelium (? macular degeneration)
Folic acid	—	Vein occlusion
K	Conjunctiva (hemorrhage)	Retina (hemorrhage)
	Anterior chamber (hyphema)	
Zinc	—	Retina and retinal pigment epithelium (? macular degeneration)

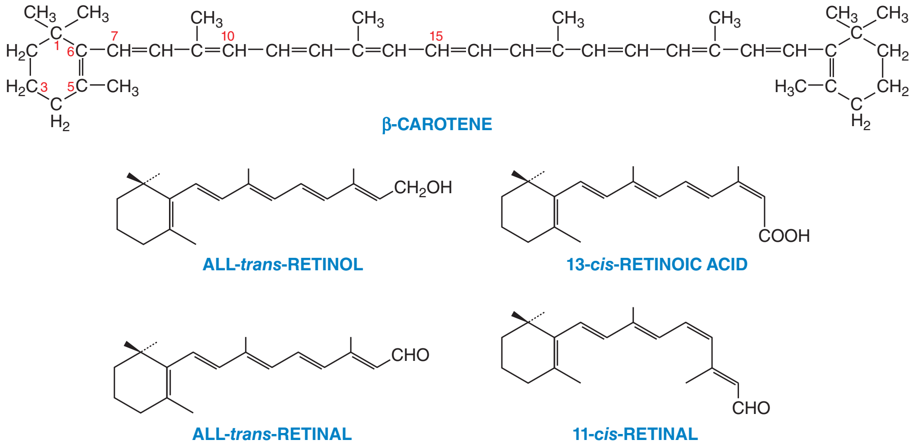

β-CAROTENE
ALL-*trans*-RETINOL
13-*cis*-RETINOIC ACID
ALL-*trans*-RETINAL
11-*cis*-RETINAL
CH₂OH
COOH
CHO
CHO

Figure 74–8 *β-Carotene and some members of the vitamin A family of retinoids.*

Retinal Cells and the Visual Cycle

Photoreception is accomplished by two types of specialized retinal cells, *rods* and *cones*. Rods are especially sensitive to light of low intensity; cones act as receptors of high-intensity light and are responsible for color vision. The chromophore of both rods and cones is 11-*cis*-retinal. The holoreceptor in rods is termed *rhodopsin*—a combination of the protein opsin and 11-*cis*-retinal attached as a prosthetic group. The three different types of cone cells (red, green, and blue) contain individual, related, photoreceptor proteins and respond optimally to light of different wavelengths. This basic scheme was elucidated by Ragnar Granit, Haldan Hartline, and George Wald, who shared the 1967 Nobel Prize in Physiology/Medicine "for their discoveries concerning the primary physiological and chemical visual processes in the eye." Several more recent articles have summarized the process of photoreception (Kefalov, 2012; Saari, 2016). Figure 74–9 summarizes the signaling pathway initiated by absorption of a photon by 11-*cis*-retinal in rods.

Vitamin A Deficiency

Vitamin A is an essential nutrient with multiple functions in the body, including the eye (Sommer and Vyas, 2012). Humans deficient in vitamin A lose their ability for dark adaptation. Rod vision is affected more than cone vision. On depletion of retinol from liver and blood, usually at plasma concentrations of retinol of less than 0.2 mg/L (0.70 µM), the concentrations of retinal and rhodopsin in the retina fall. Unless the deficiency is overcome, opsin, lacking the stabilizing effect of retinal, decays, and anatomical deterioration of the rod outer segment occurs.

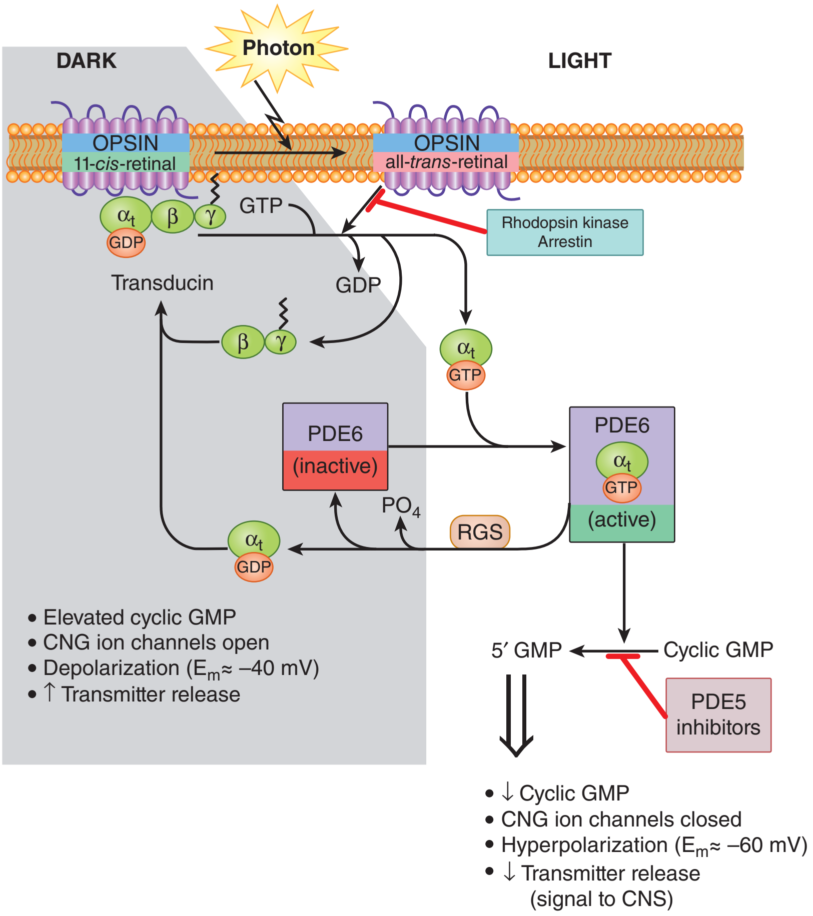

Photon
DARK
LIGHT
OPSIN
11-*cis*-retinal
OPSIN
all-*trans*-retinal
αt
β
γ
GDP
GTP
Transducin
GDP
Rhodopsin kinase
Arrestin
β
γ
αt
GTP
PDE6
(inactive)
PDE6
αt
GTP
(active)
PO₄
RGS
αt
GDP
• Elevated cyclic GMP
• CNG ion channels open
• Depolarization (Em≈ –40 mV)
• ↑ Transmitter release
5′ GMP
Cyclic GMP
PDE5 inhibitors
• ↓ Cyclic GMP
• CNG ion channels closed
• Hyperpolarization (Em≈ –60 mV)
• ↓ Transmitter release (signal to CNS)

Figure 74–9 *Pharmacologist's view of photoreceptor signaling.* CNG, cyclic nucleotide gated; RGS, regulator of G protein signaling.

Vitamin A and Epithelial Structures

Retinoic acid can influence gene expression via interaction with nuclear receptors. There are two families of retinoid receptors, RARs and RXRs (Figure 3–23 and Table 3–3). In the presence of retinol or retinoic acid, basal epithelial cells are stimulated to produce mucus. Excessive concentrations of the retinoids lead to the production of a thick layer of mucin, the inhibition of keratinization, and the display of goblet cells. In the absence of vitamin A, goblet mucous cells disappear and are replaced by basal cells that have been stimulated to proliferate. These undermine and replace the original epithelium with a stratified, keratinizing epithelium. The suppression of normal secretions leads to irritation and infection. Reversal of these changes is achieved by the administration of retinol, retinoic acid, or other retinoids. Common causes of vitamin A deficiency include malnutrition and bariatric surgery.

Therapeutic Uses of Vitamin A

Nutritional vitamin A deficiency causes *xerophthalmia*, a progressive disease characterized by *nyctalopia* (night blindness), *xerosis* (dryness), and *keratomalacia* (corneal thinning), which may lead to corneal perforation (McLaren and Kraemer, 2012). Vitamin A therapy can reverse xerophthalmia; however, rapid, irreversible blindness ensues once the cornea perforates. Vitamin A also is involved in epithelial differentiation and may have a role in corneal epithelial wound healing. The current recommendation for retinitis pigmentosa is to administer 15,000 IU of vitamin A palmitate daily under the supervision of an ophthalmologist and to avoid high-dose vitamin E. Clinical studies suggested a reduction in the risk of progression of some types of AMD by high doses of vitamin C (500 mg), vitamin E (400 IU), β-carotene (15 mg), cupric oxide (2 mg), and zinc (80 mg) (Age-Related Eye Disease Research Group, 2001a, 2001b; Chew et al., 2014), although substituting lutein/zeaxanthin for β-carotene may be more appropriate (Age-Related Eye Disease Study Research Group, 2007; Age-Related Eye Disease Study 2 [AREDS2] Research Group, 2014).

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Chapter 75

Dermatological Pharmacology

Matthew J. Sewell and Dean S. Morrell

PRINCIPLES OF DERMATOLOGICAL PHARMACOLOGY

- Structure of Skin
- Mechanisms of Percutaneous Absorption
- Pharmacological Implications of Epidermal Structure

GLUCOCORTICOIDS

- Topical Glucocorticoids
- Systemic Glucocorticoids

RETINOIDS

- Topical Retinoids
- Systemic Retinoids

VITAMIN ANALOGUES

- Calcipotriene
- β-Carotene

PHOTOCHEMOTHERAPY

- PUVA: Psoralens and UVA
- Photopheresis
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SUNSCREENS

- Inorganic Agents
- Organic Filters

ANTIHISTAMINES

ANTIMICROBIAL AGENTS

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- Antifungal Agents
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- Agents Used to Treat Infestations
- Antimalarial Agents

CYTOTOXIC AND IMMUNOSUPPRESSANT DRUGS

- Antimetabolites
- Alkylating Agents
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- Azathioprine and Mycophenolate Mofetil
- Calcineurin Inhibitors
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IMMUNOMODULATORS AND ANTI-INFLAMMATORY AGENTS

TARGETED IMMUNOTHERAPIES FOR PSORIASIS AND ATOPIC DERMATITIS

- Tumor Necrosis Factor Inhibitors
- IL-12/23 Inhibitors
- IL-17 Inhibitors
- Phosphodiesterase-4 Inhibitors
- Janus Kinase Inhibitors
- IL-4 and IL-13 Inhibitors

INTRAVENOUS IMMUNOGLOBULIN

TARGETED ANTINEOPLASTIC AGENTS

- Targeted Therapies for Basal Cell Carcinoma
- Targeted Therapies for Squamous Cell Carcinoma
- Targeted Therapies for Cutaneous T-Cell Lymphoma
- Targeted Therapies for Melanoma

TREATMENT OF PRURITUS

DRUGS FOR HYPERKERATOTIC DISORDERS

DRUGS AFFECTING HAIR GROWTH

- Androgenic Alopecia
- Other Agents

TREATMENT OF HYPERPIGMENTATION

MISCELLANEOUS AGENTS

WOUND HEALING AND SCAR FORMATION

Principles of Dermatological Pharmacology

The skin is a biologically active, multifunctional, and multicompartment organ. Medications can be applied to the skin for two purposes: to directly treat disorders of the skin and to deliver drugs to other tissues. Effective and safe use of topical pharmacological therapies requires an understanding of skin physiology and factors influencing percutaneous drug absorption and metabolism (Hwa et al., 2011; Wolff et al., 2008). General features of skin structure and percutaneous absorption pathways are outlined in Figure 75–1.

Nonpharmacological therapy is also used for treatment of skin diseases. This includes the use of parts of the electromagnetic spectrum applied by various sources, such as lasers, X-rays, visible light, and infrared light. These approaches may be used alone or to enhance the penetration or alter the nature of drugs and prodrugs. Freezing and ultrasound are other physical therapies that alter epidermal structure for direct treatment or to enhance percutaneous absorption of drugs. Chemicals are also used to decrease the effect of various wavelengths of UV (ultraviolet) light and ionizing radiation on the skin.

Structure of Skin

Stratum Corneum

The stratum corneum is the major barrier to percutaneous absorption of drugs and to the loss of water from the body. It may be considered the "nonliving" portion of the epidermis. The stratum corneum differs in thickness at different body sites: The palm and sole are the thickest followed by the general body stratum corneum, the facial and postauricular area, and the eyelid and scrotum.

Abbreviations

APC: antigen-presenting cell
AUC: area under the curve
BCC: basal cell carcinoma
cAMP: 3′,5′-cyclic adenosine monophosphate (cyclic AMP)
CBC: complete blood cell count
CCR4: C-C chemokine receptor 4
CTCL: cutaneous T-cell lymphoma
CTLA4: cytotoxic T lymphocyte–associated protein 4
DEET: diethyltoluamide (*N*,*N*-diethyl-*m*-toluamide)
DHT: dihydrotestosterone
ECP: extracorporeal photopheresis
En1: engrailed-1
EMA: European Medicines Agency
FDA: U.S. Food and Drug Administration
5FU: 5-fluorouracil
GI: gastrointestinal
GM-CSF: granulocyte-macrophage colony-stimulating factor
G6PD: glucose-6-phosphate dehydrogenase
GRASE: generally recognized as safe and effective
HPV: human papillomavirus
HSV: herpes simplex virus
IFN: interferon
IL: interleukin
IVIG: intravenous immunoglobulin
JAK: Janus kinase
MRGPR: Mas-related G protein-coupled receptor
MRSA: methicillin-resistant *Staphylococcus aureus*
mTOR: mammalian (or mechanistic) target of rapamycin
NSAID: nonsteroidal anti-inflammatory drug
ODC: ornithine decarboxylase
OTC: over the counter
PABA: *p*-aminobenzoic acid
PD-1: programmed death-1
PDE: cyclic nucleotide phosphodiesterase
PDT: photodynamic therapy
PUVA: psoralen and UVA
RAR: retinoic acid receptor
REMS: risk evaluation and mitigation strategy
RXR: retinoid X receptor
S1PR1: sphingosine-1-phosphate receptor 1
SPF: sun protection factor
SSD: silver sulfadiazine
SSTI: skin and soft-tissue infection
STAT: signal transducer and activator of transcription
TNFα: tumor necrosis factor α
TPMT: thiopurine S-methyltransferase
TRPV1: transient receptor potential vanilloid type 1
TYK2: tyrosine kinase 2
UV: ultraviolet
VZV: varicella-zoster virus

A drug may partition into the stratum corneum and form a reservoir that will diffuse into the rest of skin even *after* topical application of the drug has ceased.

Living Epidermis

The "living" layers of the epidermis (stratum basale, stratum spinosum, and stratum granulosum) have metabolically active cells and comprise a layer about 100 μm thick (see Figure 75–1). Intercalated in the living epidermis are pigment-producing cells (melanocytes), neuroendocrine cells (Merkel cells), dendritic antigen-presenting cells (APCs) (epidermal Langerhans cells), and other immune cells (γ-δ T cells). In diseased epidermis, many additional immunological cells, including lymphocytes and polymorphonuclear leukocytes, may be present and be directly affected by applied drugs.

Dermis and Its Blood Vessels

The dermis provides mechanical strength and flexibility to the skin. It is composed primarily of fibroblasts and an extracellular matrix, including collagen, proteoglycans, glycoproteins, and, in the upper dermis, elastic fibers. Cells within the dermis that may be targets for drugs include mast cells (permanent residents and producers of many inflammatory mediators) and infiltrating immune cells producing cytokines. The dermis also contains networks of nerves, blood vessels, and adnexal structures. The superficial capillary plexus between the epidermis and dermis is the site of the majority of the systemic absorption of cutaneous drugs (see Figure 75–1). There are large numbers of lymphatics as well. Hair follicles form a lipid-rich pathway for drug absorption but constitute only 0.1% of the total skin area, so follicles are not the primary route of absorption. Sweat glands are not a known pathway for the absorption of drugs, but some drugs (e.g., *griseofulvin*) are excreted to the skin by this route. Below the dermis is the hypodermis or subcutaneous tissue, which provides insulation, cushioning, and an energy reservoir.

Mechanisms of Percutaneous Absorption

Percutaneous absorption occurs primarily through a tortuous intercellular route, with transcellular or appendageal routes playing a much smaller role. Passage through the stratum corneum is the rate-limiting step for percutaneous absorption. Preferable characteristics of topical drugs include:

- Low molecular mass (≤500 Da)
- Adequate solubility in both oil and water
- A high partition coefficient so the drug will selectively partition from the vehicle to the stratum corneum (Hwa et al., 2011; Tran, 2013)

Except for very small particles, water-soluble ions and polar molecules do not penetrate significantly through intact stratum corneum. The exact amount of drug entering or leaving the skin in clinical situations usually is not measured; rather, the clinical end point (e.g., reduction in inflammation) usually is the desired effect.

A hydrated stratum corneum allows more percutaneous absorption. This is often achieved through the selection of drugs formulated in occlusive vehicles such as ointments and through physical occlusive measures such as the use of plastic films, wraps, or bags for the hands and feet or shower or bathing caps for the scalp. Alternatively, medications that are impregnated on patches or tapes may be used. Occlusion may be associated with increased growth of bacteria and resultant infection (folliculitis) or maceration and breakdown of the epidermis. Absorption of most drugs across the skin is a passive process. Percutaneous absorption may be increased using heat, ultrasonic energy, electric currents (iontophoresis), minimally invasive microneedling, and fractional or ablative lasers (Tran, 2013).

Transdermal drug delivery bypasses hepatic first-pass metabolism; however, the epidermis contains a variety of enzyme systems capable of metabolizing drugs that reach this compartment. A specific CYP isoform, CYP26A1, metabolizes retinoic acid and may control its level in the skin (Baron et al., 2001). In addition, transporter proteins that influence influx (OATP [organic anion-transporting polypeptide]) or efflux (MDR [multidrug resistance transporter], P-glycoprotein) of certain xenobiotics are present in human keratinocytes (Baron et al., 2001; also see Chapter 4). Genetic variants of enzymes that regulate the cellular influx and efflux of *methotrexate* have been associated with toxicity and effectiveness in patients with psoriasis (Warren et al., 2008).

Pharmacologic Implications of Epidermal Structure

When proposing topical application of medications, the healthcare practitioner must consider multiple factors, including proper dosage

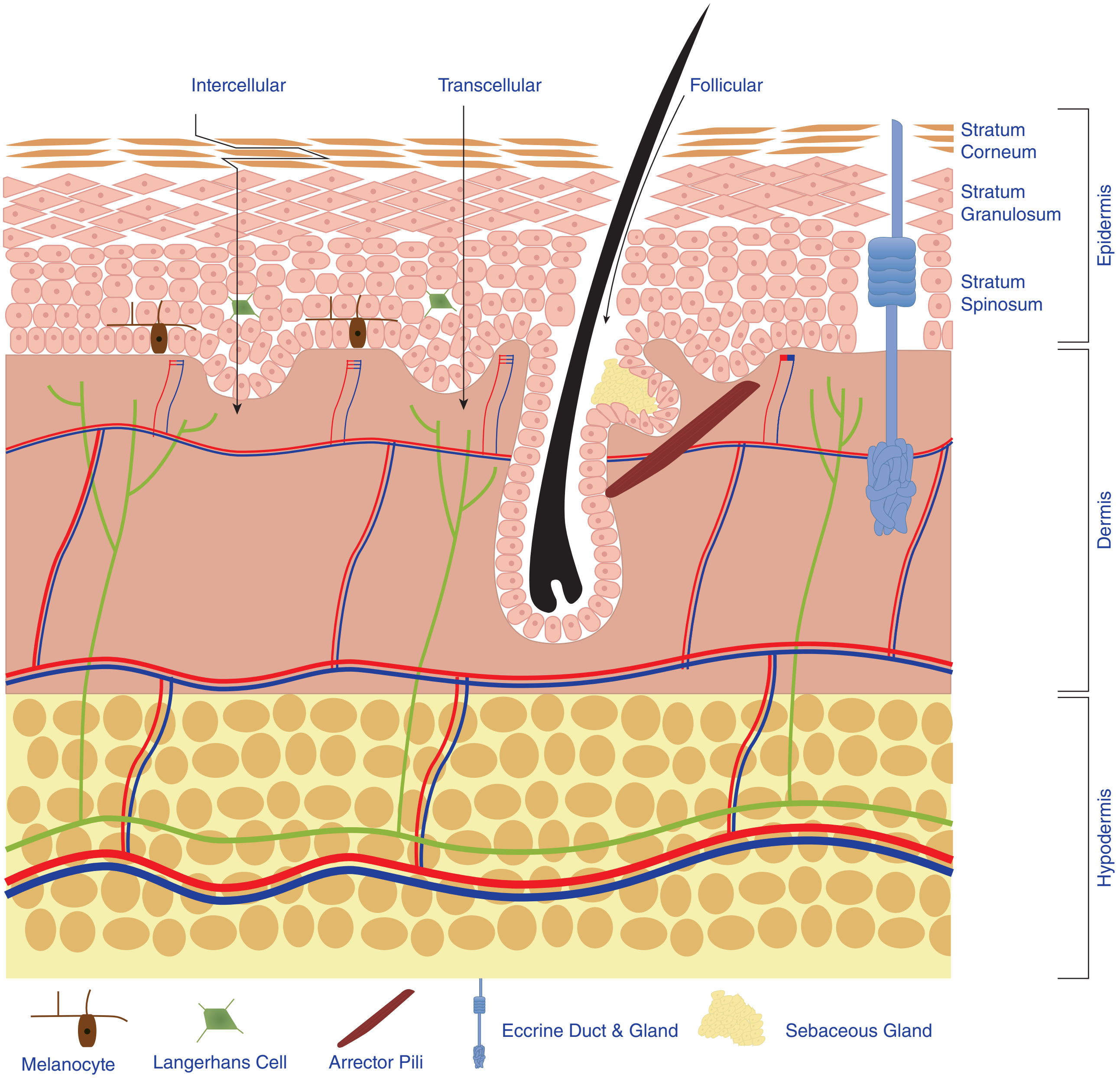


Figure 75–1 *Cutaneous drug absorption.* After application of a drug to the surface of the skin (stratum corneum), evaporation and structural/compositional alterations may occur that affect the drug's ultimate bioavailability. The stratum corneum limits drug diffusion into the lower layers and thence into the body. A number of absorptive routes are possible, singly or in combination: between the cells of the stratum corneum (*intercellular*), across the corneal cellular layer (*transcellular*), and into the concavity of a hair follicle (*follicular*) with its associated sebaceous glandular cells and arrector pili muscle that is innervated by the sympathetic branch of the autonomic nervous system. Melanocytes and Langerhans cells are accessible in the lower epidermis. In the epidermal and dermal layers, drugs may also reach the eccrine glands (sweat glands) and their ducts. Permeation to the dermis brings a drug in contact with lymphatics (in green) and cutaneous vessels carrying arterial and venous blood (red and blue, respectively). These vessels provide an absorptive route into the general circulation. Deeper permeation to the hypodermis may also occur.

and frequency of application, extent and condition of the permeability barrier, patient age and weight, physical form of the preparation to be applied, and whether intralesional or systemic administration should be used (Table 75–1). Various drug vehicles have specific advantages and disadvantages (Table 75–2). Newer vehicles or delivery systems, such as liposomes and microgel formulations, can enhance solubilization of certain drugs, thereby enhancing topical penetration and diminishing irritancy (Rosen et al., 2014). *Children have a greater ratio of surface area to mass than adults do, so the same amount of topical drug can result in greater systemic exposure. Preterm infants have a markedly impaired barrier function until the epidermis keratinizes completely* (Hwa et al., 2011). *Many dermatological diseases also compromise barrier function, leading to increased percutaneous absorption.*

TABLE 75–1 ■ IMPORTANT CONSIDERATIONS WHEN A DRUG IS APPLIED TO THE SKIN

What are the absorption pathways of intact and diseased skin?
How does the chemistry of the drug affect the penetration?
How does the vehicle affect the penetration?
How much of the drug penetrates the skin?
What are the intended pharmacological targets?
What host and genetic factors influence drug function in the skin?
What are the predicted adverse effects (local, systemic)?

TABLE 75–2 ■ VEHICLES FOR TOPICALLY APPLIED DRUGS[a]

	OINTMENT	CREAM	GEL	LOTION/SOLUTION/ FOAM/SPRAY
Physical basis	Solid or liquid dispersed in nonaqueous base	Oil-in-water emulsion	Water-soluble emulsion with gelling agent	Lotion—suspended drug Solution/spray—dissolved drug base Foam—drug with surfactant as foaming agent and propellant
Solubilizing medium	Anhydrous to <20% water	20%–80% water	Contains water-soluble polyethylene glycols May have alcoholic solvent	May be aqueous or alcoholic
Pharmacological advantage	Protective oil film on skin	Leaves concentrated drug at skin surface	Concentrates drug at surface after evaporation	
Advantages for patient	Spreads easily Slows water evaporation	Spreads and removes easily No greasy feel	Nonstaining Greaseless Typically clear appearance	Low residue on scalp or other hairy areas May have cooling effect when evaporates
Locations on body	Avoid intertriginous areas	Most locations	Scalp and other hairy locations	Scalp and other hairy locations
Disadvantages	Greasy Stains clothes	Needs preservatives	Needs preservatives Can be drying, especially if high alcohol content	Can be drying May burn if alcohol content Foams tend to be more expensive due to more complex delivery system
Occlusion	Moderate to high Increases skin moisture	Low	None	None
Composition notes	Oleaginous base (e.g., white petrolatum) or absorption base (e.g., hydrophilic petrolatum)	Requires humectants (glycerin, propylene glycol, polyethylene glycols) to keep moist when applied Long-chain alcohol (e.g., stearyl alcohol) in oil phase for stability and smooth feel	Microspheres or microsponges can be formulated in gels	

[a]Some agents may also be supplied on tapes or patches; in powders, emollients, and lacquers; and in liposomes and nanoparticles.

Glucocorticoids

Glucocorticoids have immunosuppressive and anti-inflammatory properties. They are administered locally through topical and intralesional routes and systemically through intramuscular, intravenous, and oral routes. Mechanisms of glucocorticoid action are discussed in Chapter 50.

Topical Glucocorticoids

Topical glucocorticoids have been grouped into seven classes in order of decreasing potency (Table 75–3). Potency traditionally is measured using a vasoconstrictor assay in which an agent is applied to skin under occlusion and the area of skin blanching is assessed. Other assays of glucocorticoid potency involve suppression of erythema and edema after experimentally induced inflammation and the psoriasis bioassay, in which the effect of steroid on psoriatic lesions is quantified.

Therapeutic Uses

Many inflammatory skin diseases respond to topical or intralesional administration of glucocorticoids. Absorption varies among body sites and with the vehicle of the formulation. The steroid is selected based on its potency, the site of involvement, and the severity of the skin disease. Often, a more potent steroid is used initially, followed by a less potent agent. Twice-daily application of topical glucocorticoids suffices; more frequent application does not improve response. Moderately potent to very potent topical corticosteroids are effective with once-daily versus twice-daily application in atopic dermatitis, and patient adherence may be improved with once-daily regimens (Williams, 2007). In general, only nonfluorinated glucocorticoids should be used on the face or in occluded areas such as the axillae or groin. Intralesional preparations of glucocorticoids include insoluble preparations of *triamcinolone acetonide* and *triamcinolone hexacetonide*, which solubilize gradually and therefore have a prolonged duration of action.

Toxicity

Adverse effects may occur with topical corticosteroid use, including skin atrophy, striae, telangiectasias, purpura, and acneiform eruptions. Fluorinated compounds should not be used on the face because perioral dermatitis and rosacea can develop after their use. Topical corticosteroid use may induce hypothalamic-pituitary-adrenal axis suppression, especially with high-potency corticosteroids, chronic use, application to large body surface areas, or occlusion.

Systemic Glucocorticoids

Systemic glucocorticoid therapy is used for severe or extensive dermatological illnesses such as allergic contact dermatitis to plants (e.g., poison ivy), vesiculobullous dermatoses (e.g., pemphigus vulgaris and bullous pemphigoid), vasculitis, autoimmune connective tissue diseases, and neutrophilic dermatoses (e.g., pyoderma gangrenosum). Chronic administration of oral glucocorticoids is problematic, given the side effects associated with their long-term use (see Chapter 50).

TABLE 75–3 ■ POTENCY OF SELECTED TOPICAL GLUCOCORTICOIDS

	CLASS OF DRUG	GENERIC NAME, FORMULATION
Most potent	1	• Augmented betamethasone dipropionate: 0.05% gel, lotion, ointment • Clobetasol propionate: 0.05% cream, ointment, gel, solution, foam, spray, emulsion, lotion • Diflorasone diacetate: 0.05% ointment • Fluocinonide: 0.1% cream • Flurandrenolide: 4 μg/cm^2 impregnated tape • Halobetasol propionate: 0.05% cream, ointment, lotion, foam
	2	• Amcinonide: 0.1% ointment • Augmented betamethasone dipropionate: 0.05% cream • Betamethasone dipropionate: 0.05% ointment • Clobetasol: 0.025% cream • Desoximetasone: 0.05% gel; 0.25% cream, ointment, spray • Fluocinonide: 0.05% cream, ointment, gel, solution • Halcinonide: 0.1% cream, ointment, solution • Halobetasol propionate: 0.01% lotion
	3	• Amcinonide: 0.1% cream • Betamethasone dipropionate: 0.05% cream • Betamethasone valerate: 0.1% ointment; 0.12% foam • Desoximetasone: 0.05% cream, ointment • Diflorasone diacetate: 0.05% cream • Fluocinonide: 0.05% cream in aqueous emollient base • Fluticasone propionate: 0.005% ointment • Mometasone furoate: 0.1% ointment • Triamcinolone acetonide: 0.5% cream, ointment
	4	• Betamethasone dipropionate: 0.05% spray • Clocortolone pivalate: 0.1% cream • Fluocinolone acetonide: 0.025% ointment • Flurandrenolide: 0.05% ointment • Hydrocortisone valerate: 0.2% ointment • Mometasone furoate: 0.1% cream, lotion • Triamcinolone acetonide: 0.1% cream, ointment
	5	• Betamethasone dipropionate: 0.05% lotion • Betamethasone valerate: 0.1% cream • Desonide: 0.05% gel, ointment • Fluocinolone acetonide: 0.025% cream • Flurandrenolide: 0.05% cream, lotion • Fluticasone propionate: 0.05%: cream, lotion • Hydrocortisone butyrate: 0.1% cream, lotion, solution, ointment • Hydrocortisone valerate: 0.2% cream • Prednicarbate: 0.1% ointment • Triamcinolone acetonide: 0.05% ointment; 0.025% ointment; 0.1% lotion
	6	• Alclometasone dipropionate: 0.05% cream, ointment • Betamethasone valerate: 0.1% lotion • Desonide: 0.05% cream, lotion, foam • Fluocinolone acetonide: 0.01% cream, solution, shampoo, oil • Triamcinolone acetonide: 0.025% cream, lotion
Least potent	7	• Hydrocortisone: 0.5% cream, lipstick; 0.75% lotion; 1% cream, ointment, foam, gel, liquid, lotion, shampoo, spray; 2% lotion; 2.5% cream, lotion, ointment, solution

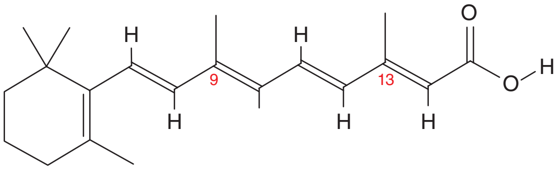
Daily morning dosing with prednisone generally is preferred, although divided doses occasionally are used to enhance efficacy. Fewer side effects are seen with alternate-day dosing; if chronic therapy is required, prednisone usually is tapered to every other day as soon as practical. Pulse therapy using intravenous doses of *methylprednisolone sodium succinate* is an option for severe resistant pyoderma gangrenosum, pemphigus vulgaris, systemic lupus erythematosus with multisystem disease, dermatomyositis, and linear morphea. When used as high-dose "pulse" therapy, the dose of intravenous *methylprednisolone* is usually 15 to 30 mg/kg/dose (maximum, 1000 mg/dose) daily for 3 days in pediatric patients or 7 to 15 mg/kg/dose (generally 500–1000 mg/dose) in adults daily for 3 to 5 days. The infusion is usually given over 2 to 3 h; more rapid infusion has been associated with increased rates of hypotension, electrolyte shifts, and cardiac arrhythmias.

Toxicity and Monitoring

Oral glucocorticoids have numerous systemic effects, as discussed in Chapter 50. Most side effects are dose and duration dependent.

Retinoids

Retinoids comprise natural and synthetic compounds that exhibit vitamin A–like biological activity or bind to nuclear receptors for retinoids. Characteristics of topical and systemic retinoids are summarized in Tables 75–4 and 75–5, respectively.



Tretinoin (all-*trans*-retinoic acid; vitamin A acid)

First-generation retinoids include *retinol* (vitamin A), *tretinoin* (all-*trans*-retinoic acid), *isotretinoin* (13-*cis*-retinoic acid), and *alitretinoin* (9-*cis*-retinoic acid). *Second-generation retinoids*, also known as aromatic retinoids, include *acitretin* and *etretinate*. *Third-generation retinoids* were designed to optimize receptor-selective binding and include *tazarotene*, *bexarotene*, and *adapalene*. A *fourth-generation retinoid, trifarotene*, further optimizes receptor-selective binding.

- ***Mechanism of Action.*** Retinoids exert their effects on gene expression by activating two families of nuclear receptors, *retinoic acid receptors* (RARs) and *retinoid X receptors* (RXRs), which are members of the steroid receptor superfamily. Both retinoid receptor families have three isoforms (α, β, and γ), which are expressed in unique combinations in individual tissues and cells. On binding to a retinoid, RARs and RXRs form heterodimers that subsequently bind specific DNA sequences called retinoic acid response elements (RAREs) that activate transcription of genes whose products produce both the desirable pharmacological effects and the unwanted side effects of these drugs (see Tables 75–4 and 75–5; see also Table 5–5 and Figure 5–13).
- ***Targeted Therapeutic Actions.*** Retinoids that target RARs predominantly affect cellular differentiation and proliferation, whereas retinoids that target RXRs predominantly induce apoptosis. Hence, *tretinoin*, *adapalene*, and *tazarotene*, which target RARs, are used in acne, psoriasis, and photoaging (disorders of differentiation and proliferation), whereas *bexarotene* and *alitretinoin*, which target RXRs, are used in cutaneous T-cell lymphoma (CTCL) and Kaposi sarcoma, respectively, to induce apoptosis of malignant cells.
- ***Retinoid Toxicity.*** Acute retinoid toxicity is similar to vitamin A intoxication. Side effects of systemic retinoids include dry skin, nosebleeds from dry mucous membranes, conjunctivitis, reduced night vision, hair loss, alterations in serum lipids and transaminases, hypothyroidism, inflammatory bowel disease flare, musculoskeletal pain, pseudotumor cerebri, and mood alterations. RAR-selective retinoids are more associated with mucocutaneous and musculoskeletal symptoms, whereas RXR-selective retinoids induce more physiochemical changes. Because all oral retinoids are potent teratogens, they should not be used during pregnancy and should be used cautiously in females of childbearing potential.

TABLE 75–4 ■ TOPICAL RETINOIDS[a]

DRUG	FORMULATION	RECEPTOR SPECIFICITY	LEVEL OF IRRITATION	INDICATION[b]
Adapalene	Cream: 0.1%, 0.3% Gel: 0.1%, 0.3% Lotion: 0.1%	RAR-β, -γ	+	Acne
Tretinoin	Cream: 0.02%, 0.025%, 0.05%, 0.1% Gel: 0.01%, 0.025% Microsphere gel: 0.04%, 0.06%, 0.08%, 0.1% Lotion: 0.05%, 0.1%	RAR-α, -β, -γ	++	Acne; facial fine wrinkles and mottled pigmentation
Trifarotene[c]	Cream: 0.005%	RAR-γ	+	Acne
Tazarotene	Cream: 0.05%, 0.1% Gel: 0.05%, 0.1% Foam: 0.1% Lotion: 0.045%	RAR-α, -β, -γ	++++	Acne; plaque psoriasis; facial fine wrinkles and mottled pigmentation
Alitretinoin[d] (9-cis-retinoic acid)	Gel: 0.1%	RAR-α, -β, -γ RXR-α, -β, -γ	++	Cutaneous AIDS-related Kaposi sarcoma
Bexarotene[e]	Gel: 1%	RXR-α, -β, -γ	+++	Cutaneous T-cell lymphoma

[a] These agents should not be ingested; oral retinoids should not be used during pregnancy or by females of childbearing potential.

[b] FDA-approved indication may vary for different preparations or brand names.

[c] Human studies have not identified a pregnancy risk with topical use; animal reproduction studies with high doses (exposure 800 times that due to maximal recommended human dose) resulted in fetal adverse effects.

[d] Gel could cause fetal harm if significant absorption were to occur in a pregnant woman.

[e] Gel may cause fetal harm when administered to a pregnant female.

TABLE 75–5 ■ SYSTEMIC RETINOIDS[a]

DRUG	RECEPTOR SPECIFICITY	DOSING RANGE	$t_{1/2}$
Isotretinoin	No clear receptor affinity	Standard or Lidose: 0.5–2 mg/kg/day Micronized Lidose: 0.4–1.6 mg/kg/day	10–20 h
Etretinate[b]	RAR-α, -β, -γ	0.25–1 mg/kg/day	80–160 days
Acitretin	RAR-α, -β, -γ	0.5–1 mg/kg/day	50 h[c]
Bexarotene	RXR-α, -β, -γ	300 mg/m^2/day	7–9 h

[a] These agents must not be used by women who are pregnant, planning to become pregnant, or breastfeeding.

[b] Not currently available in the U.S. Warning in footnote c applies.

[c] When combined with ethanol, acitretin is esterified to etretinate, which has a $t_{1/2}$ >80 days. Female patients of childbearing potential should avoid pregnancy for 3 years after receiving acitretin or etretinate to avoid retinoid-induced embryopathy.

Topical Retinoids

Through incompletely understood mechanisms, topical retinoids correct abnormal follicular keratinization, reduce *Propionibacterium acnes* counts, and reduce inflammation, thereby making them the cornerstone of acne therapy. Topical retinoids are first-line agents for noninflammatory (comedonal) acne and are often combined with other agents in the management of inflammatory acne.

Fine wrinkles and dyspigmentation, two important features of photoaging, are also improved with topical retinoids. Within the dermis, this is believed to result from inhibition of AP-1 (activator protein-1), which normally activates synthesis of matrix metalloproteinases in response to UV irradiation (Thielitz and Gollnick, 2008). In the epidermis, retinoids induce epidermal hyperplasia in atrophic skin and reduce keratinocyte atypia.

Toxicity and Monitoring

Adverse effects of all topical retinoids include erythema, desquamation, xerosis, burning, and stinging (see relative irritancy in Table 75–4). These effects often decrease with time and are lessened by concomitant use of emollients. Patients also may experience photosensitivity reactions (enhanced reactivity to UV radiation) and have a significant risk for severe sunburn. Although there is little systemic absorption of topical retinoids and no alteration in plasma vitamin A levels with their use, topical retinoids are not recommended for use during pregnancy.

Available Agents; Clinical Use

Tretinoin (all-*trans*-retinoic acid) is photolabile and should be applied once nightly for acne and photoaging. Benzoyl peroxide also inactivates *tretinoin* and should not be applied simultaneously. Formulations with copolymer microspheres or prepolyolprepolymer 2 that gradually release *tretinoin* to decrease irritancy are available and are less susceptible to oxidation by benzoyl peroxide and photodegradation.

Adapalene is approved for the treatment of acne vulgaris. It has similar efficacy to *tretinoin*. Unlike *tretinoin*, it is stable in sunlight or the presence of benzoyl and is also more lipophilic, which allows rapid penetration through follicular openings.

Tazarotene is approved for the treatment of psoriasis, photoaging, facial wrinkles, and acne vulgaris. *Tazarotene* gel, applied once daily, may be used as monotherapy or in combination with other medications, such as topical corticosteroids, for the treatment of localized plaque psoriasis. Topical corticosteroids improve the efficacy of therapy and reduce the side effects of burning, itching, and skin irritation that are commonly associated with *tazarotene*.

Trifarotene is approved for the treatment of acne vulgaris on the face and trunk. It is a fourth-generation retinoid with selective binding to RAR-γ receptors, which are abundantly expressed in skin.

Alitretinoin is a retinoid that binds all types of retinoid receptors, both RARs and RXRs. It is approved for treatment of cutaneous lesions of Kaposi sarcoma, with its application titrated up from twice daily to three or four times daily as tolerated. *Alitretinoin* should not be applied concurrently with insect repellants containing diethyltoluamide (DEET) because it may increase DEET absorption.

Bexarotene is approved for early-stage (IA and IB) CTCL. Its application is titrated up from every other day to two to four times daily over several weeks to improve patient tolerance. Concurrent application of *bexarotene* with insect repellants containing DEET is not recommended because it may increase DEET absorption.

Systemic Retinoids

Systemic retinoids (see Table 75–5) are approved for the treatment of acne, psoriasis, and CTCL (Desai et al., 2007).

Therapeutic Uses and Contraindications

Off-label uses include ichthyosis, Darier disease, pityriasis rubra pilaris, rosacea, hidradenitis suppurativa, chemoprevention of malignancy, lichen sclerosus, subacute lupus erythematosus, and discoid lupus erythematosus.

Absolute contraindications include use by women who are pregnant, planning to become pregnant, or breastfeeding. Relative contraindications include leukopenia, alcoholism, hyperlipidemia, hypercholesterolemia, hypothyroidism, and significant hepatic or renal disease.

Toxicity and Monitoring

Acute toxicities may include mucocutaneous or laboratory abnormalities. Mucocutaneous side effects may include cheilitis, xerosis, blepharoconjunctivitis, cutaneous photosensitivity, photophobia, myalgia, arthralgia, headaches, alopecia, nail fragility, and increased susceptibility to staphylococcal infections. Some patients develop a “retinoid dermatitis” characterized by erythema, pruritus, and scaling. Very rarely, patients may develop pseudotumor cerebri, especially when systemic retinoids are combined with tetracyclines. Bony changes may occur after chronic use at high doses. There are reports that chronic administration at higher doses can cause diffuse idiopathic skeletal hyperostosis syndrome, premature epiphyseal closure, and other skeletal abnormalities (Desai et al., 2007).

Systemic retinoids are highly teratogenic. There is no safe dose during pregnancy. Prescribing of *isotretinoin* in the U.S. is restricted via the risk-mitigation iPLEDGE system.

Serum lipid elevation is the most common laboratory abnormality. Less common laboratory abnormalities include elevated transaminases, decreased thyroid hormone, and leukopenia. Laboratory monitoring for *acitretin* and *bexarotene* generally includes a baseline evaluation of serum lipids, serum transaminases, and complete blood cell count (CBC) with subsequent monitoring monthly for the first 3 to 6 months then every 3 months thereafter. Thyroid function testing is also included for *bexarotene*. Less frequent laboratory monitoring is needed during *isotretinoin* use for acne in otherwise healthy patients, and a CBC is not required (Takeshita et al., 2020). Two negative pregnancy tests on separate occasions are required for females of childbearing potential prior to starting *acitretin* or *isotretinoin*; *bexarotene* requires one negative pregnancy test within the week prior to initiating therapy. Monthly pregnancy tests should be obtained for females of childbearing potential.

Available Agents and Clinical Use

Isotretinoin is approved for the treatment of recalcitrant and nodular acne vulgaris. The drug has remarkable efficacy in severe acne and may induce prolonged remissions after a single course of therapy. Clinical effects generally are noted within 1 to 3 months of starting therapy. Approximately one-third of patients will relapse, usually within 3 years of stopping therapy. Although most relapses are mild and respond to conventional management with topical and systemic antiacne agents, some may require a second course of *isotretinoin*. Systemic absorption is improved when taken with a high-fat meal. An *isotretinoin*-Lidose formulation (a proprietary eutectic mixture of drug and lipids in a hard gelatin capsule) may be given without respect to meals; there is a micronized *isotretinoin*-Lidose formulation available as well. Non-Lidose formulations of *isotretinoin* may be administered with a high-fat meal to achieve higher systemic exposure to the drug.

Acitretin is approved for use in the cutaneous manifestations of psoriasis. Clinical effect may begin within 4 to 6 weeks, with the full clinical benefit occurring at 3 to 6 months. *Acitretin* has a $t_{1/2}$ of about 50 h; however, when combined with alcohol, *acitretin* is esterified *in vivo* to produce etretinate, which has a $t_{1/2}$ of 80 to 160 days. It is not known how much alcohol is required to induce this conversion, and it must be considered that sources of alcohol ingestion may include mouthwash, cough syrup, or other alcohol-based medications. Thus, female patients of childbearing potential should avoid pregnancy for 3 years after receiving *acitretin* to avoid retinoid-induced embryopathy.

Bexarotene is a retinoid that selectively binds RXRs. Formulations of *bexarotene* are approved for use in patients with CTCL (topical for early stage; oral for refractory CTCL). *Bexarotene* reportedly induces apoptosis of malignant cells (Jawed et al., 2014). Because CYP3A4 metabolizes *bexarotene*, inhibitors of CYP3A4 (e.g., imidazole antifungals, macrolide antibiotics) will increase *bexarotene* plasma levels; conversely, inducers of CYP3A4 (e.g., *rifamycins*, *carbamazepine*, *dexamethasone*, *efavirenz*, *phenobarbital*) will decrease *bexarotene* plasma levels. Side effects are more common than with other retinoids, with an increased incidence of significant lipid abnormalities and hypothyroidism secondary to a reversible RXR-mediated suppression of thyroid-stimulating hormone gene expression, pancreatitis, leukopenia, and gastrointestinal (GI) symptoms. Thyroid function should be measured before initiating therapy and periodically thereafter.

Vitamin Analogues

Calcipotriene

Calcipotriene is a topical vitamin D analogue that is approved for the treatment of psoriasis.

Mechanism of Action

Calcipotriene exerts its effects through the vitamin D receptor (see Chapter 52). On binding the vitamin D receptor, the drug-receptor complex associates with the RXR-α and binds to vitamin D response elements on DNA, increasing expression of genes that modulate epidermal differentiation and inflammation, leading to improvement in psoriatic plaques (Menter et al., 2009a).

Therapeutic Use

Calcipotriene is generally applied twice daily to psoriasis on the scalp or body, often in combination with topical corticosteroids. Hypercalcemia and hypercalciuria may develop when the cumulative weekly dose exceeds the recommended 100 g/week limit and resolves within days of discontinuation of *calcipotriene* (Menter et al., 2009a). *Calcipotriene* also causes perilesional irritation and mild photosensitivity. Concomitant administration of topical corticosteroids will reduce the irritation. *Calcipotriene* may be inactivated by concomitant use of acidic topical agents such as salicylic acid or lactic acid. *Calcipotriene* has been used off label for multiple conditions, including morphea, vitiligo, congenital ichthyoses, and in combination with *5-fluorouracil* for actinic keratoses.

β-Carotene

β-Carotene is a precursor of vitamin A that is in green and yellow vegetables; it is used as a nutrient supplement and food coloring agent. Dietary supplementation with β-carotene is used in dermatology to reduce skin photosensitivity in patients with erythropoietic protoporphyria. The mechanism of action is not established but may involve an antioxidant effect that decreases the production of free radicals or singlet oxygen.

Photochemotherapy

Phototherapy and photochemotherapy are treatment methods in which UV or visible radiation is used to induce a therapeutic response either alone (phototherapy) or in the presence of an exogenous photosensitizing drug (photochemotherapy) (Table 75–6). Patients treated with these modalities should be monitored for concomitant use of other potential photosensitizing medications, such as phenothiazines, thiazides, sulfonamides, nonsteroidal anti-inflammatory drugs (NSAIDs), sulfonylureas, tetracyclines, and benzodiazepines.

The UV radiation region may be subdivided into UVA1 (340–400 nm), UVA2 (320–340 nm), UVB (290–320 nm), and UVC (100–290 nm). UVC radiation is almost completely absorbed by the ozone layer and atmosphere so that measurable amounts do not reach Earth's surface. UVA and UVB exposure can cause sunburns, photoaging, and skin cancer development. UVB is the most erythrogenic and melanogenic, and it is the major action spectrum for sunburn, tanning, skin cancer, photoaging, and cutaneous vitamin D synthesis. UVA is only a thousandth as erythrogenic as UVB but penetrates more deeply into the skin and contributes substantially to photoaging and photosensitivity diseases. UVA is also able to penetrate clouds and glass, so exposure may occur even in the shade or while indoors. Both UVA and UVB cause photoimmune suppression, an effect that is utilized for therapy of certain dermatological conditions.

PUVA: Psoralens and UVA

The PUVA combines UVA light with photosensitizing compounds called psoralens. Orally administered *methoxsalen* (8-methoxypsoralen) followed by UVA (PUVA) is FDA-approved for the treatment of vitiligo and psoriasis. It is also used off label in several other inflammatory or lymphoproliferative cutaneous disorders (Totonchy and Chiu, 2014).

Chemistry and Mechanism of Action

Psoralens are lipophilic, naturally occurring furocoumarins found in some plants. Synthetic versions are also available. The action spectrum for PUVA is between 320 and 340 nm. Psoralens intercalate into the DNA strand, and two distinct photoreactions take place on UVA exposure. Type I reactions involve the oxygen-independent photoaddition of psoralens to pyrimidine bases in DNA. Type II reactions are oxygen dependent and involve the transfer of energy to molecular oxygen, creating reactive oxygen species. Through incompletely understood mechanisms, these phototoxic reactions stimulate melanocytes and induce antiproliferative, immunosuppressive, and anti-inflammatory effects.

Pharmacokinetics

Formulations of solubilized *methoxsalen* in a gel matrix are absorbed rapidly after oral administration, whereas older microcrystalline forms are absorbed slowly and incompletely. Fatty foods slow absorption and decrease peak blood levels. There is significant, but saturable, first-pass elimination in the liver. Peak photosensitivity varies significantly between individuals but typically is maximal 1 to 2 h after ingestion. *Methoxsalen* has a serum $t_{1/2}$ of about 1 h, but the skin remains sensitive to light for 8 to 12 h.

Therapeutic Uses

Oral *methoxsalen* is available as a single 10-mg strength either as soft gelatin capsules (solubilized) or hard gelatin capsules (micronized crystals).

TABLE 75–6 ■ PHOTOCHEMOTHERAPY METHODS

	PUVA	PHOTOPHERESIS	PHOTODYNAMIC THERAPY
Target	Broad cutaneous area	Peripheral blood leukocytes	Focal cutaneous sites
Photosensitizing agent	Methoxsalen (8-methoxypsoralen)	Methoxsalen (8-methoxypsoralen)	Protoporphyrin IX
Method of administration	Oral Topical lotion Bathwater	To isolated plasma within photopheresis device	Topical cream or solution of a prodrug (aminolevulinic acid or methylaminolevulinate)
FDA-approved indications	Psoriasis Vitiligo	CTCL	Actinic keratosis
Activating wavelength	UVA2 (320–340 nm)	UVA2 (320–340 nm)	Blue light (410–420 nm) and red light (630–635) nm
Adverse effects (acute)	Phototoxic reactions Pruritus Hypertrichosis GI disturbance CNS disturbance Bronchoconstriction Hepatic toxicity HSV recurrence Retinal damage	Phototoxic reactions GI disturbance Hypotension Congestive heart failure	Phototoxic reactions Temporary dyspigmentation
Adverse effects (chronic)	Photoaging Nonmelanoma skin cancer Melanoma[a] Cataracts[a]	Loss of venous access after repeated venipuncture	Potential scarring
Pregnancy category	Risk cannot be ruled out	Evidence of risk[b]	Risk cannot be ruled out

[a] Controversial.
[b] Based on fetal toxicity in pregnant rats. No adequate studies in humans.

The dose is 10 to 70 mg, depending on weight (0.4–0.6 mg/kg), taken about 1.5 to 2 h before UVA exposure. Although administration with food can decrease absorption, it may minimize nausea. Topical application of psoralens followed by UVA exposure (topical PUVA) is also used for treatment. A lotion containing 1% *methoxsalen* is available for topical application for vitiligo. The *methoxsalen* lotion or capsules can be diluted in bathwater and used topically for localized palmoplantar (soak PUVA) or more diffuse (bath PUVA) areas to minimize systemic absorption. An extracorporeal solution is available for CTCL (see Photopheresis).

Approximately 70% to 100% of psoriatic patients have clearing or virtual clearing of skin disease within about 24 treatments with PUVA. Remission typically lasts 3 to 6 months; thus, patients often require maintenance therapy with intermittent PUVA or other agents. Vitiligo typically requires between 150 and 300 treatments. Localized vitiligo can be treated with topical PUVA and more extensive disease with systemic administration. PUVA also is employed off label in the treatment of atopic dermatitis, alopecia areata, lichen planus, and urticaria pigmentosa.

Toxicity and Monitoring

The major side effects of PUVA are listed in Table 75–6. Phototoxicity is characterized by erythema, edema, blistering, and pruritus. Ocular toxicity can be prevented by wearing UVA-blocking glasses on the day of treatment. The risk of nonmelanoma skin cancer is dose dependent, with the greatest risk in those receiving more than 200 to 250 treatments (Totonchy and Chiu, 2014). There is a possible association between extensive PUVA exposure and melanoma but the evidence is conflicting.

Photopheresis

Extracorporeal photopheresis (ECP) is a process in which extracorporeal peripheral blood mononuclear cells are separated using a leukapheresis-based method then exposed to UVA radiation in the presence of *methoxsalen* (Perotti and Sniecinski, 2015). *Methoxsalen* is injected directly into the extracorporeal plasma before radiation and reinfusion. The treated lymphocytes are returned to the patient, undergoing apoptosis over 48 to 72 h. ECP is used for CTCL and off label for various other T-cell–mediated diseases, including graft-versus-host disease, transplantation rejection, and scleroderma. Patients receive ECP therapy on 2 consecutive days every 2 to 4 weeks, and intervals are increased as the patient improves (Jawed et al., 2014). ECP can be combined with adjunctive therapies, including PUVA, topical chemotherapy, systemic chemotherapy, radiation, biological agents, and retinoids.

Photodynamic Therapy

Photodynamic therapy (PDT) combines the use of photosensitizing drugs and visible light for the treatment of dermatological disorders. Two drugs are used for topical PDT: *aminolevulinic acid* is FDA-approved, and *methylaminolevulinate* is approved in a number of other countries. Both are prodrugs that are converted into protoporphyrin IX within living cells (Figure 75–2). In the presence of specific wavelengths of light (see Table 75–6) and O_2, protoporphyrin produces reactive oxygen species that oxidize cell membranes, proteins, and mitochondrial structures, leading to apoptosis. The epidermis, sebaceous glands, and neoplastic cells accumulate more porphyrin than other cutaneous cells and structures, providing some preferential targeting of therapy (Rkein and Ozog, 2014). PDT is approved for use on precancerous actinic keratosis. It is also used

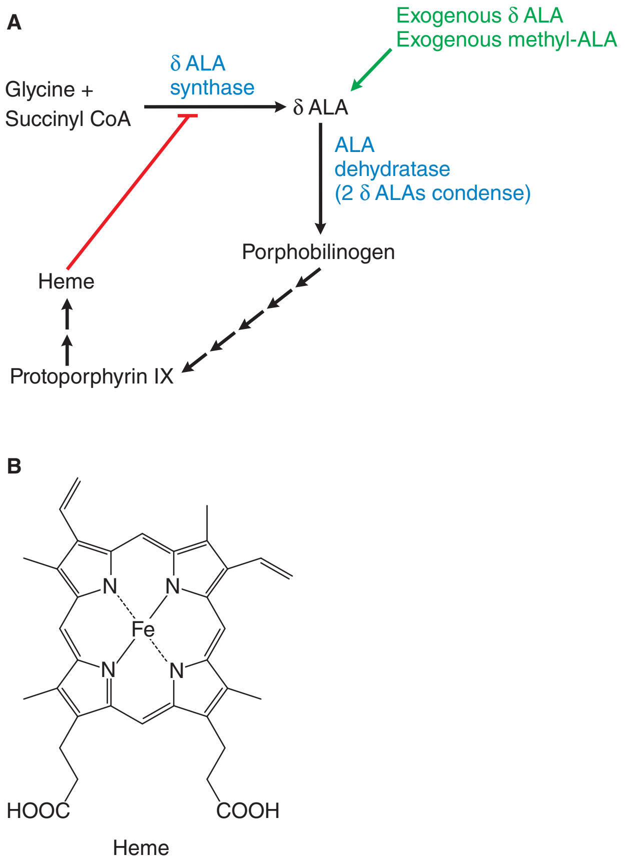


Figure 75–2 *Heme biosynthesis pathway.* **A.** *Under physiological conditions, heme inhibits the enzyme δ-aminolevulinic acid (δ-ALA) synthase by negative feedback*. However, when ALA is provided exogenously, this control point is bypassed, leading to excessive accumulation of protoporphyrin IX and heme. **B.** *Heme.*

off label for superficial nonmelanoma skin cancers, acne, photorejuvenation, and verrucae. Incoherent (nonlaser) and laser light sources have been used for PDT. The wavelengths chosen must include those within the action spectrum of protoporphyrin (see Table 75–6) and ideally those that permit maximum skin penetration. Light sources currently in use emit energy predominantly in the blue portion (maximum porphyrin absorption) or the red portion (better tissue penetration) of the visible spectrum. Because the $t_{1/2}$ of the accumulated porphyrins is about 30 h, patients should protect their skin from sunlight and intense light for at least 48 h after treatment to prevent phototoxic reactions.

Sunscreens

Sunscreens provide temporary photoprotection from the acute and chronic effects of sun exposure. They are an important component of a comprehensive sun protection approach along with minimizing sun exposure and using photoprotective gear. The regular use of sunscreen is efficacious in reducing photocarcinogenesis and photoaging (Mancebo et al., 2014). The sun protection factor (SPF) is defined as the ratio of the minimal dose of incident sunlight that will produce erythema (sunburn) on skin with the sunscreen in place to the dose that evokes the same reaction on skin without the sunscreen. SPF is primarily a measure of UVB protection and does not provide information regarding UVA coverage. In 2011, the FDA published new guidelines for labeling and effectiveness testing of sunscreens (Mancebo et al., 2014). UVA protection is now assessed using the critical wavelength method, and products with a critical wavelength of 370 nm or greater may be labeled as "broad spectrum." Sunscreens providing broad-spectrum coverage with an SPF 15 or greater may include a claim on their labeling that use "decreases risk of skin cancer and early aging caused by the sun." Sunscreens should be applied liberally 15 to 30 min prior to sun exposure and reapplied at least every 2 h. If activities involve swimming or sweating, water-resistant sunscreens are recommended and should be reapplied every 40 or 80 min depending on their labeling.

The major active ingredients of available sunscreens include organic agents ("chemical blockers") that absorb UV radiation in the UVB or UVA ranges and then convert it to heat energy and inorganic agents ("physical blockers") that contain particulate materials that act by scattering or reflecting visible, UV, and infrared radiation to reduce its transmission to the skin. The current FDA-approved active sunscreen ingredients are listed in Table 75–7.

Inorganic Agents

The two inorganic agents available in the U.S. are *zinc oxide* and *titanium dioxide*. They provide UVA, UVB, and visible light protection. Newer microsize and nanosize particle formulations are less opaque and more cosmetically appealing. Both *zinc oxide* and *titanium dioxide* may become classified as non–generally recognized as safe and effective (GRASE) by the FDA.

Organic Filters

Currently available organic filters in the U.S. include benzophenones (*oxybenzone*, *dioxybenzone*, *sulisobenzone*); dibenzoylmethanes (*avobenzone*); anthralates (*meradimate*); camphors (*ecamsule*); aminobenzoates (PABA [*p-aminobenzoic acid*] and *padimate O*); cinnamates (*cinoxate*, *octinoxate*); salicylates (*trolamine salicylate*, *homosalate*, *octisalate*); *octocrylene*; and *ensulizole*. Additional UVA and UVB organic filters are used in other countries but are not currently available in the U.S. PABA and *trolamine salicylate* may become classified as non-GRASE by the FDA. For PABA, risks include allergic and photoallergic skin reactions as well as cross-sensitization with structurally similar compounds. For *trolamine salicylate*, the risks include anticoagulant effects and salicylate toxicity. There is recent concern about potential toxic effects of organic UV filters, including those used in sunscreens, on coral reefs and other marine life (Schneider and Lim, 2019; Mitchelmore et al., 2021), The U.S. National Academy of Sciences has convened a panel to "review the state of science on use of currently marketed sunscreen ingredients, their fate and effects in aquatic environments, and the potential public health implications associated with changes in sunscreen usage" (NAS, 2022); a report will be forthcoming.

Antihistamines

Histamine (see Chapter 43) is a potent vasodilator, bronchial smooth muscle constrictor, and stimulant of nociceptive itch receptors. Additional chemical itch mediators acting as pruritogens on C fibers include *neuropeptides, prostaglandins, serotonin, acetylcholine*, and *bradykinin*. Furthermore, receptor systems (e.g., vanilloid, opioid, and cannabinoid receptors) on cutaneous sensory nerve fibers may modulate itch. Broad classes of pruritogens appear to cause histamine release via their direct interaction with MRGPRX2, a mast cell–specific member of the Mas-related G protein-coupled receptor (MRGPR) superfamily of eight proteins. Two of these proteins in the X family, MRPGRX1 and MRGPRX2, interact with a variety of positively charged compounds, transducing signals that result in itch and pain (McNeil, 2021). These systems, which act independently of the IgE pathway, offer novel future targets for antipruritic therapy.

Histamine is in mast cells, basophils, and platelets. Human skin mast cells express H_1, H_2, and H_4 receptors but not H_3 receptors. Both H_1 and H_2 receptors are involved in wheal formation and erythema, whereas only the H_1 receptor agonists cause pruritus (see Chapter 43). However, blockade of H_1 receptors does not totally relieve itching, and combination therapy with H_1 and H_2 antagonists may be superior to the use of H_1 antagonists alone.

Oral antihistamines, particularly H_1 receptor antagonists, have anticholinergic activity and are sedating (see Chapter 43), making them useful for the control of pruritus. *First-generation sedating H_1 receptor antagonists* include *hydroxyzine, diphenhydramine, promethazine*, and *cyproheptadine*. *Doxepin* is a good alternative to traditional oral antihistamines for severe pruritus. A 5% topical cream formulation of *doxepin*,

TABLE 75–7 ■ SUNSCREEN ACTIVE INGREDIENTS

		PROTECTIVE SPECTRUM			
CLASS	ACTIVE INGREDIENT	UVB 290–320 nm	UVA2 320–340 nm	UVA1 340–400 nm	VISIBLE 400–800 nm
Inorganic					
	Titanium dioxide	++	++	++	+
	Zinc oxide	++	++	++	+
Organic					
Benzophenones	Dioxybenzone[a]	++	+		
	Oxybenzone[a]	++	++		
	Sulisobenzone[a]	++	+		
Dibenzoylmethanes	Avobenzone[a]		++	++	
Anthralates	Meradimate[a]		++		
Camphors	Ecamsule[a]		++	++	
Aminobenzoates	PABA[b]	++			
	Padimate O[a]	++			
Cinnamates	Cinoxate[a]	++			
	Octinoxate[a]	++			
Salicylates	Trolamine salicylate[b]	++			
	Homosalate[a]	++			
	Octisalate[a]	++			
Others	Octocrylene	++			
	Ensulizole[a]	++			

[a] In the U.S., these compounds are pending determination of generally recognized as safe and effective status by the FDA.
[b] Sunscreens with this compound are not currently marketed in the U.S.

which can be used in conjunction with low- to moderate-potency topical glucocorticoids, is also available. The antipruritic effect from topical *doxepin* is comparable to that of low-dose oral *doxepin* therapy. Allergic contact dermatitis to *doxepin* has been reported. Topical *diphenhydramine* formulations available over the counter (OTC) also carry a significant risk of allergic contact dermatitis. *Second-generation H_1 receptor antagonists* lack anticholinergic side effects and are described as nonsedating largely because they do not readily cross the blood-brain barrier. They include *cetirizine, levocetirizine, loratadine, desloratadine,* and *fexofenadine*. Second-generation H_1 antagonists are suitable for treating urticaria, cause fewer adverse effects, and are superior in safety compared to older first-generation H_1 antagonists (Fein et al., 2019).

Antimicrobial Agents

Antibiotics

Antibiotic agents are frequently used to treat skin and soft-tissue infections (SSTIs). Several antimicrobial agents, such as tetracyclines, macrolides, and *dapsone*, also have anti-inflammatory properties, which make them useful for noninfectious conditions, such as acne vulgaris, rosacea, granulomatous diseases, neutrophilic dermatoses, and autoimmune bullous diseases (Bhatia, 2009).

Topical agents are very effective for the treatment of superficial bacterial infections and acne vulgaris (Drucker, 2012). Systemic antibiotics also are prescribed commonly for acne and deeper bacterial infections. The pharmacology of individual antibacterial agents is discussed in Section VI, *Chemotherapy of Microbial Diseases*. Only the topical and systemic antibacterial agents principally used in dermatology are discussed here.

Acne

Acne vulgaris is the most common dermatological disorder treated with either topical or systemic antibiotics (Eichenfield et al., 2013). The gram-positive anaerobe *Cutibacterium acnes* (formerly *Propionibacterium acnes*) is a component of normal skin flora that proliferates in the obstructed, lipid-rich lumen of the pilosebaceous unit, where O_2 tension is low. *C. acnes* generates free fatty acids from sebum and interacts with toll-like receptors, thereby promoting microcomedo formation and inflammatory lesions (Das and Reynolds, 2014). Suppression of cutaneous *C. acnes* with antibiotic therapy is correlated with clinical improvement.

Topical antimicrobials commonly used in acne include *benzoyl peroxide*, *clindamycin* (topical preparation not marketed in the U.S.), *erythromycin*, and *benzoyl peroxide–erythromycin* or *benzoyl peroxide–clarithromycin* combinations. Topical monotherapy with *clindamycin* or *erythromycin* is not recommended due to a slower onset of action and potential for development of bacterial resistance; therefore, addition of *benzoyl peroxide* or use of combination antibiotic–*benzoyl peroxide* products is recommended to improve efficacy and decrease emergence of antibiotic-resistant bacteria (Eichenfield et al., 2013). Other topical antimicrobials used in treating acne include *azelaic acid, dapsone, metronidazole, minocycline, sulfacetamide*, and *sulfacetamide/sulfur* combinations.

Systemic therapy is prescribed for patients with acne vulgaris that is more extensive or resistant to topical therapy. There are data to support the use of tetracyclines (*doxycycline*, *minocycline*, *tetracycline*, *sarecycline*), macrolides (*azithromycin, erythromycin*), and *trimethoprim-sulfamethoxazole*. Other antibiotics that decrease the extent of the infection or the inflammatory response to *C. acnes* may be effective as well. After initiation of antibiotic treatment for acne, 6 to 8 weeks are required for visible clinical results, with maximum effect sometimes requiring 3 to 6 months (Eichenfield et al., 2013). Doses are tapered after control is achieved. Concomitant use with topical *benzoyl peroxide* may decrease development of antibiotic-resistant bacteria.

The tetracyclines are the most commonly employed antibiotics. They should not be used during pregnancy or in patients younger than 8 years.

Although agents in the tetracycline class are antimicrobials, efficacy in acne may be more dependent on anti-inflammatory activity. *Minocycline*, *doxycycline*, and *sarecycline* have better GI absorption than *tetracycline* and may be given with food to minimize GI side effects. *Minocycline* and *sarecycline* may be less photosensitizing than either *tetracycline* or *doxycycline*. Adverse effects include vestibular toxicity, hyperpigmentation of the skin and mucosa, serum sickness–like reactions, and drug-induced lupus erythematosus. With all the tetracyclines, vaginal candidiasis is a potential complication that is readily treated with administration of antifungal drugs. However, *sarecycline* has a more narrow antimicrobial spectrum compared to the other tetracyclines, which may lead to less disruption of the vulvovaginal and GI microbiome.

Cutaneous Bacterial Infections

Numerous organisms may cause cutaneous infections ranging from benign to life-threatening. Clinical presentation and treatment depend on the depth of cutaneous involvement, immune status of the patient, causative organism, and local antibiotic resistance patterns (Rajan, 2012; Stevens et al., 2014). Gram-positive organisms, including *Staphylococcus aureus* and *Streptococcus pyogenes*, are the most common cause of SSTIs. Patients who are diabetic or immunosuppressed are at risk for severe, recurrent, or atypical infections.

Topical Therapy. *Topical therapy* is frequently used for superficial bacterial infections, wounds, and burns. The use of topical antibiotics on clean surgical wounds without signs of infection is not recommended as it does not reduce the rate of infection compared to nonantibiotic ointment or no ointment, and there is a risk of contact dermatitis to topical antibiotics (Levender et al., 2012) and counterproductive selection of resistant bacterial strains.

Mupirocin. *Mupirocin* inhibits protein synthesis by binding to bacterial isoleucyl-tRNA synthetase. *Mupirocin* is effective for treatment of *S. aureus* and *S. pyogenes*, but it is inactive against normal skin flora or anaerobes. *Mupirocin* is available as a 2% ointment or cream, applied two or three times daily for 5 to 10 days. The recommended regimen for impetigo is twice daily for 5 days (Stevens et al., 2014). A nasal formulation is indicated to eradicate nasal colonization by *methicillin*-resistant *Staphylococcus aureus* (MRSA). It is applied intranasally twice a day for 5 days and often combined with daily *chlorhexidine* or dilute *sodium hypochlorite* washes for the body. Prolonged suppression may be achieved with repeat monthly courses.

Retapamulin. *Retapamulin* selectively inhibits bacterial protein synthesis by interacting at a unique site on the 50S subunit of bacterial ribosomes. It is active against multiple gram-positive organisms, including *S. aureus* and *S. pyogenes*, and against anaerobes. *Retapamulin* ointment 1% is FDA-approved for the topical treatment of impetigo caused by susceptible strains of *S. aureus* or *S. pyogenes* in patients 9 months or older. The recommended regimen for impetigo is twice daily for 5 days (Stevens et al., 2014).

Bacitracin, Neomycin, and Polymyxin B. These agents are sold alone or in various combinations with other ingredients (e.g., *hydrocortisone*, *lidocaine*, or *pramoxine*) in a number of OTC formulations. *Bacitracin* inhibits staphylococci, streptococci, and gram-positive bacilli. *Neomycin* is active against staphylococci and most gram-negative bacilli. *Polymyxin B* is active against aerobic gram-negative bacilli, including *Pseudomonas aeruginosa*. Both *bacitracin* and *neomycin* are relatively common causes of allergic contact dermatitis, especially in patients with stasis dermatitis or otherwise disrupted skin (Drucker, 2012).

Gentamicin. *Gentamicin* is an aminoglycoside that inhibits protein synthesis by binding to the 30S subunit of bacterial ribosomes. It has activity for certain gram-positive bacteria, including *S. aureus*, and gram-negative bacteria, including *P. aeruginosa*. *Gentamicin sulfate* 0.1% cream or ointment may be applied three or four times daily to affected areas.

Ozenoxacin. *Ozenoxacin* is a quinolone with bactericidal activity through inhibition of bacterial DNA gyrase A and topoisomerase IV, thereby inhibiting replication. It is approved for treatment of impetigo due to *S. aureus* or *S. pyogenes* in patients 2 months of age or older. *Ozenoxacin* 1% cream is applied twice a day for 5 days.

Silver Sulfadiazine. *Silver sulfadiazine* (SSD) binds to bacterial DNA and inhibits replication. It is bactericidal against gram-positive bacteria, including MRSA, and gram-negative bacteria, including *P. aeruginosa*. SSD is most frequently used in the treatment of partial-thickness burns or lower extremity venous stasis ulcers. However, recent studies have failed to demonstrate efficacy in these settings (Miller et al., 2012); indeed, SSD may impede re-epithelialization. Rarely, argyria has developed in patients using SSD over extensive areas of the body (Browning and Levy, 2008).

Mafenide. *Mafenide acetate* is a topical sulfonamide, although its mechanism of action may be distinct from other sulfonamides. *Mafenide* is approved as adjunctive therapy for burn wounds and is available as either an 85 mg/g cream or as a 5% topical solution supplied as 50-g packets of powder that must be reconstituted. It exerts broad bacteriostatic action against many gram-positive and gram-negative organisms, including *P. aeruginosa* and some anaerobes. The high solubility of *mafenide* allows it to diffuse easily through eschar and devascularized tissue, which is beneficial for treating subeschar burn wound infection. *Mafenide* and its metabolites inhibit carbonic anhydrase, so treated patients may be at risk for metabolic acidosis, especially if they have renal failure or if *mafenide* is applied to large surface areas. Other potential adverse effects include pain or burning sensation with application, facial edema, allergic contact dermatitis, and rarely, hemolytic anemia in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency.

Systemic Therapy. Deeper or complicated SSTIs typically require systemic administration of antimicrobials. Superficial infections with diffuse involvement or that are nonresponsive to topical therapy may also require systemic antimicrobials. Because SSTIs are most commonly caused by streptococcal and staphylococcal species, penicillins (especially β-lactamase–resistant β-lactams) and cephalosporins are the systemic antibiotics used most frequently in their treatment. A growing concern is the increased incidence of SSTIs with hospital- and community-acquired MRSA and drug-resistant pneumococci. Common outpatient treatment options for MRSA include *clindamycin*, *doxycycline*, and *trimethoprim-sulfamethoxazole*. When coverage for both MRSA and streptococci is needed, *clindamycin* alone or the combination of either *doxycycline* or *trimethoprim-sulfamethoxazole* with a β-lactam may be used (Stevens et al., 2014). *Clindamycin* should not be used as empiric therapy if local resistance rates are greater than 10% to 15% (Stevens et al., 2014). Treatment options for more severe or complicated SSTIs that provide coverage for MRSA include orally administered agents, such as *linezolid*, *tedizolid*, *delafloxacin*, and *omadacycline*, and parenterally administered agents, such as *vancomycin, daptomycin, linezolid, tedizolid, delafloxacin, omadacycline, ceftaroline, dalbavancin, oritavancin*, and *telavancin*.

Antifungal Agents

Fungal infections are among the most common causes of skin disease in the U.S., and numerous effective topical and oral antifungal agents have been developed. The pharmacology, uses, and toxicities of antifungal drugs are discussed in Chapter 61. This section addresses the management of common superficial cutaneous mycoses. Recommendations for cutaneous antifungal therapy are summarized in Table 75–8. Topical antifungal agents used for superficial mucocutaneous mycoses are listed in Table 75–9.

Dermatophyte Infections of the Skin (Tinea Corporis, Cruris, and Pedis)

The dermatophyte infections (tinea corporis, cruris, and pedis) generally respond to topical antifungals, including allylamines (*naftifine*, *terbinafine*), benzylamines (*butenafine*), azoles (*econazole*, *luliconazole*), and *ciclopirox* (Rotta et al., 2013). Allylamines and benzylamines may provide a more sustained clinical cure compared to azole antifungals (Rotta et al., 2013). Bacterial superinfection of tinea pedis can occur, and antifungal agents such as *econazole* and *ciclopirox* that also provide some bacterial coverage may be useful in those situations. Systemic therapy with

TABLE 75–8 ■ RECOMMENDED CUTANEOUS ANTIFUNGAL THERAPY

CONDITION	TOPICAL THERAPY	ORAL THERAPY
Tinea corporis, localized	Azoles, allylamines, benzylamines	—
Tinea corporis, widespread	—	Griseofulvin, terbinafine, itraconazole, fluconazole
Tinea capitis	—	Griseofulvin, terbinafine, itraconazole, fluconazole
Tinea pedis	Azoles, allylamines, benzylamines	Griseofulvin, terbinafine, itraconazole, fluconazole
Onychomycosis	—	Terbinafine, itraconazole, fluconazole
Candidiasis, localized	Azoles, nystatin	—
Candidiasis, widespread and mucocutaneous	—	Itraconazole, fluconazole
Tinea versicolor, localized	Azoles, allylamines	—
Tinea versicolor, widespread	—	Itraconazole, fluconazole

terbinafine, *fluconazole*, *itraconazole*, or *griseofulvin* is used when there is more extensive cutaneous involvement or a poor response to topical therapy. Systemic therapy with *ketoconazole* carries a risk of severe hepatotoxicity or QT prolongation so it is not a preferred treatment for superficial fungal infections.

Dermatophyte Infections of the Hair and Follicles

Dermatophyte infections of the hair (tinea capitis) or follicularly based infections of the skin (Majocchi granuloma) require systemic therapy. *Griseofulvin* has been the traditional medication for tinea capitis, and treatment failure is often related to inadequate dosage or length of treatment. *Terbinafine* may be more effective for *Trichophyton tonsurans*, which is the most common cause of tinea capitis in the U.S. (Elewski et al., 2008). *Fluconazole* and *itraconazole* are also effective (Gupta and Paquet, 2013).

Onychomycosis

Onychomycosis is a fungal infection of the nails that is commonly caused by dermatophytes or *Candida*. Onychomycosis is most effectively treated with systemic therapy (de Sa et al., 2014). Fungal infection should be confirmed prior to initiating oral therapy. There are other causes of nail dystrophy, and approximately one-third of nails with clinically suspected onychomycosis do not have fungal infection (Mehregan and Gee, 1999).

Terbinafine is the most effective treatment of dermatophyte onychomycosis, with typical adult dosing of 250 mg daily for 6 weeks for fingernails and 12 weeks for toenails. *Terbinafine* remains in the nails for several weeks after medication administration has ceased, and some studies have utilized pulsed dosing of *terbinafine* 500 mg daily for 1 week per month for 3 months based on this finding.

Itraconazole is another effective option for dermatophyte onychomycosis and is more effective than *terbinafine* for candidal onychomycosis. *Itraconazole* persists in the nail for 6 to 9 months after administration has ceased. Traditional adult dosing for *itraconazole* is 200 mg daily for 6 weeks for fingernails and 12 weeks for toenails; however, pulsed dosing is an alternative option because of *itraconazole*'s persistence in the nail after administration has ceased. *Itraconazole* pulsed dosing in adults is 400 mg daily for 1 week per month for 2 months for fingernails and 3 months for toenails, which may minimize adverse effects. *Fluconazole* is an alternative option that may be useful in patients who cannot use *terbinafine* or *itraconazole*.

Griseofulvin, used in the past, is less efficacious and requires much longer treatment courses; it is no longer a preferred option. Topical antifungals approved for onychomycosis include *ciclopirox lacquer*, *efinaconazole*, and *tavaborole*. These topical antifungals require a 48-week treatment course. They may be useful for patients with mild-to-moderate onychomycosis not involving the nail matrix or in patients who are not candidates for systemic therapy (Gupta et al., 2014).

Cutaneous Candidiasis

Cutaneous candidiasis is typically treated with topical azole antifungals or *nystatin* when localized. Systemic azole antifungals such as *fluconazole* or *itraconazole* may be needed for more recalcitrant or severe cases.

Malassezia *Infections*

Pityriasis versicolor and seborrheic dermatitis are caused by *Malassezia* (*Pityrosporum*) species and are frequently treated with azole antifungals in cream or shampoo formulations. Additional topical therapies include *ciclopirox*, *terbinafine*, *selenium sulfide*, *pyrithione zinc*, *sodium sulfacetamide*, and *sulfur* with or without *salicylic acid*. Oral *fluconazole* and *itraconazole* are sometimes used for diffuse or resistant pityriasis versicolor. Oral *terbinafine* does not reach adequate concentrations in the superficial stratum corneum to be effective.

Antiviral Agents

Viral infections of the skin by human papillomavirus (HPV), herpes simplex virus (HSV), molluscum contagiosum virus, and varicella-zoster virus (VZV) are common and produce a variety of lesions, including warts or blisters. *Acyclovir*, *famciclovir*, and *valacyclovir* are often used systemically to treat HSV and VZV infections (see Chapter 62). *Cidofovir* and *foscarnet* are useful for treating *acyclovir*-resistant HSV. Topically, *acyclovir*, *docosanol*, *penciclovir*, and combination *acyclovir/hydrocortisone* are available for treating mucocutaneous HSV; however, they are less effective than systemic therapies (Sarnoff, 2014). *Acyclovir* is also available as a 50-mg buccal tablet for herpes labialis to be applied as a single dose to the upper gum just above the incisor tooth within 1 h of prodromal symptoms.

Cidofovir is used off label topically or intralesionally as treatment of anogenital warts caused by HPV. Topical *cidofovir* 1% gel or cream must be obtained through a compounding pharmacy. It is applied once daily for 5 consecutive days every other week for a maximum of six cycles. Additional treatments for genital warts are nonspecific cytodestructive or immunomodulatory therapies, which are discussed in further detail in other sections. Cytodestructive modalities include topical use of cryotherapy, *podophyllin* (podophyllum resin) or *podofilox* (podophyllotoxin), and *trichloroacetic acid*. Topical immunomodulatory therapies include *imiquimod* and sinecatechins. Intralesional immunotherapy for warts has included *Candida* antigen, measles-mumps-rubella vaccine, bacillus Calmette-Guérin vaccine, tuberculin purified protein derivative, trichophyton antigen, and more recently, HPV vaccine (Nabil et al., 2020). Intralesional or systemic interferons α_{2b}, α_{n3}, and β may be useful for treating refractory or recurrent genital warts, but their use is limited by the pain of multiple injections for intralesional use and by the potential for severe side effects with systemic use (Smith et al., 2007).

Agents Used to Treat Infestations

Infestations with ectoparasites such as lice and scabies are common throughout the world. These conditions have a significant impact on public health in the form of disabling pruritus, secondary infection, and, in the case of the body louse, transmission of life-threatening illnesses such as typhus. Topical and oral medications are available to treat these infestations (Diamantis et al., 2009).

- *Permethrin* is a synthetic pyrethroid that interferes with insect Na^+ transport proteins, causing neurotoxicity and paralysis. Resistance due to mutations in the transport protein has been reported and appears to be increasing. A 5% cream is available by prescription for the treatment of scabies, and a 1% lotion or cream rinse is available OTC for the

TABLE 75–9 ■ TOPICAL ANTIFUNGAL AGENTS FOR SUPERFICIAL MUCOCUTANEOUS MYCOSES

TYPE AND GENERIC NAME	FORMULATIONS	FDA-APPROVED INDICATIONS	NOTES
Polyenes			
Nystatin	Topical (0.1 megaunit/g): powder, ointment, cream	Cutaneous and mucocutaneous candidiasis	Orally administered nystatin lacks appreciable systemic absorption; it is used for local treatment of oral cavity and GI tract candidiasis
	Oral (0.1 megaunit/g): suspension, solution, capsule or tablet (0.5 megaunit)	Oral cavity, mucocutaneous, cutaneous candidiasis Nonesophageal GI candidiasis (off-label use)	
Azoles			
Butoconazole	Vaginal cream: 1% and 2%	Vaginal yeast infections	
Clotrimazole	Oral lozenge, 10 mg	Oropharyngeal candidiasis	
	Cream, ointment, solution; 1%	Rx: *Candida albicans*, pityriasis versicolor OTC: tinea	
	Topical: cream, 1%; foam, 1%	Tinea, cutaneous candidiasis, pityriasis versicolor Tinea pedis in patients ≥12 years	
Econazole	Topical: solution, 10%	Toenail onychomycosis	
Efinaconazole	Topical: 2% foam, gel; 1% shampoo; 2% shampoo, gel; 2% cream	Seborrheic dermatitis Pityriasis versicolor Tinea, pityriasis versicolor, seborrheic dermatitis, cutaneous candidiasis	
Ketoconazole	1% cream	Tinea	Shampoo FDA-approved for OTC; other formulations are by prescription
Luliconazole	Topical: 2% cream, lotion, ointment, powder, solution, spray, tincture, paste, liquid	Tinea	
Miconazole	Buccal tablet, 50 mg	Oropharyngeal candidiasis	OTC
	Vaginal suppository, 100 or 200 mg Vaginal cream, 2% or 4% vaginal cream	Vulvovaginal candidiasis	FDA-approved OTC drug
	Topical: 1% cream, lotion	Tinea, pityriasis versicolor	FDA-approved OTC drug
Oxiconazole	Topical: 2% cream, solution	Tinea pedis in patients ≥12 years	
Sertaconazole	Topical: 1% cream, solution	Tinea; pityriasis versicolor	
Sulconazole	Vaginal: 0.4%, 0.8% vaginal cream; 80-mg vaginal suppository	Vulvovaginal candidiasis	
Terconazole	Vaginal: 6.5% vaginal ointment	Vulvovaginal candidiasis	
Tioconazole	Vaginal: 6.5% ointment	Vulvovaginal candidiasis	FDA-approved OTC drug
Allylamines	Topical: 1% or 2% cream, gel	Tinea	
Naftifine	Topical: 1% cream, gel, spray	Tinea; pityriasis versicolor	
Terbinafine	Oral: 250-mg tablet	Onychomycosis	FDA-approved OTC drug
Benzylamines	Topical: 1% cream	Rx: pityriasis versicolor OTC: tinea	
Butenafine	Topical: 1% cream	Rx: pityriasis versicolor OTC: tinea	FDA-approved OTC drug
Hydroxypyridones			
Ciclopirox	Topical: 1% shampoo	Seborrheic dermatitis	
	Topical: 0.77% gel, cream, suspension	Tinea, seborrheic dermatitis	
	Topical: 0.77% gel, cream, suspension	Tinea, seborrheic dermatitis	

(*Continued*)

TABLE 75–9 ■ TOPICAL ANTIFUNGAL AGENTS FOR SUPERFICIAL MUCOCUTANEOUS MYCOSES (*CONTINUED*)

TYPE AND GENERIC NAME	FORMULATIONS	FDA-APPROVED INDICATIONS	NOTES
Oxaboroles			
Tavaborole	5% topical solution	Toenail onychomycosis	
Miscellaneous; available OTC			
Selenium sulfide	Topical: 1% or and 2% topical solutions	Abrasions, minor cuts, surface injuries, superficial fungal infections of the skin	
Gentian violet	Topical: 1% foam, spray, impregnated cloth, gel, liquid, lotion, nail oil, ointment, soap, cream, powder, solution, aerosol	Tinea, pityriasis versicolor	Stains skin and clothing purple
Tolnaftate	Topical: 10%–25% topical powder, cream, solution, soap, others	Tinea	
Undecylenic acid	Topical: 10%–25% powder, cream, solution, soap, others	Tinea	

treatment of lice. *Permethrin* is approved for use in infants 2 months or older. Other OTC agents used in the treatment of lice are pyrethrins plus *piperonyl butoxide* in various formulations and *acetic acid* plus *isopropanol* shampoo.

- *Lindane* is an organochloride compound that induces neuronal hyperstimulation and eventual paralysis of parasites. Due to several cases of neurotoxicity in humans, the FDA has labeled *lindane* as a second-line drug in treating pediculosis and scabies and has highlighted the potential for neurotoxicity in children and adults weighing less than 50 kg and patients with underlying skin disorders such as atopic dermatitis and psoriasis. *Lindane* is contraindicated in premature infants and patients with seizure disorders. *Lindane* carries a boxed warning from the FDA; a Lindane Medication Guide must be given to the patient each time *lindane* lotion or shampoo is dispensed.
- *Malathion* is an organophosphate that inhibits acetylcholinesterase in lice, causing paralysis and death. It is approved for treatment of head lice in children 6 years and older. The current formulation of *malathion* lotion contains 78% isopropyl alcohol and is flammable. Detailed information on *malathion*'s actions and adverse effects may be found in Chapter 12.
- *Benzyl alcohol* 5% lotion is approved for the treatment of lice in patients 6 months and older. *Benzyl alcohol* inhibits lice from closing their respiratory spiracles, which allows the vehicle to obstruct the spiracles and causes the lice to asphyxiate. There may be less potential for development of resistance with this mechanism of action compared to traditional pesticides.
- *Ivermectin* is an anthelmintic drug (see Chapter 68) approved for oral treatment of onchocerciasis and strongyloidiasis. Recently, topical *ivermectin* 0.5% lotion was approved to treat lice in patients 6 months or older, and a 1% cream was approved to treat rosacea. *Ivermectin* is also effective in the off-label treatment of scabies. Because *ivermectin* does not cross the human blood-brain barrier, there is no major CNS toxicity; minor CNS side effects with oral administration may include dizziness, somnolence, vertigo, and tremor. Topical administration may cause local irritant effects, such as conjunctivitis, eye irritation, dry skin, or skin-burning sensation, although these occurred in fewer than 1% of patients during clinical trials. For both scabies and lice, *ivermectin* typically is given at an oral dose of 200 μg/kg, which may be repeated in 1 week. In cases of crusted scabies, longer treatment cycles of oral *ivermectin* may be needed, sometimes in addition to topical keratolytics and scabicides (Ortega-Loayza et al., 2013). It should not be used in children weighing less than 15 kg.
- *Abametapir* is an antiparasitic drug approved for treatment of head lice in patients 6 months or older. The drug exhibits pediculicidal and ovicidal activity through inhibition of metalloproteinases critical for louse survival and egg development. *Abametapir* 0.74% lotion is applied to dry hair and scalp and then left in for 10 min before rinsing. Due to its ovicidal activity, only a single application is needed. Potential adverse effects include skin irritation, eye irritation, pruritus, and hair color changes.
- *Spinosad* 0.9% topical suspension is approved for treatment of head lice in patients 6 months or older. Spinosad causes CNS excitation and involuntary muscle contractions, leading to insect paralysis and death.

Less effective topical treatments for scabies and lice include 10% *crotamiton cream* and lotion and extemporaneously compounded 5% to 10% *precipitated sulfur* in petrolatum. *Precipitated sulfur* may be useful for patients in whom other therapies are contraindicated or not approved; it is typically applied once daily for 3 to 5 consecutive days.

Antimalarial Agents

The antimalarial agents *chloroquine*, *hydroxychloroquine*, and *quinacrine* are used in dermatology to treat dermatoses such as cutaneous lupus erythematosus, cutaneous dermatomyositis, polymorphous light eruption, porphyria cutanea tarda, and sarcoidosis (only *hydroxychloroquine* is FDA-approved for treating lupus erythematosus). The mechanism by which antimalarial agents exert their anti-inflammatory therapeutic effects is uncertain but may involve inhibition of endosomal toll-like receptor signaling, resulting in reduced B-cell and dendritic cell activation (Kalia and Dutz, 2007). The usual dosages of antimalarials are *hydroxychloroquine* 200 mg twice a day (maximum of 6.5 mg/kg per day); *chloroquine* 250 to 500 mg/day (maximum of 3 mg/kg per day); and *quinacrine* 100 to 200 mg/day. Patients with porphyria cutanea tarda require lower doses of antimalarials to avoid severe hepatotoxicity. There is strong evidence that smoking decreases the effectiveness of antimalarials in the treatment of cutaneous lupus erythematosus, so smoking cessation is important (Chasset et al., 2015). Clinical improvement may be delayed for several months. If there is no improvement after 3 months of *hydroxychloroquine*, then *quinacrine* may be added (in the U.S., *quinacrine* must be obtained through compounding pharmacies). Dose-dependent ocular toxicity occurs with *chloroquine* more often than *hydroxychloroquine* and is rare to nonexistent with *quinacrine*. The potential toxic effects of antimalarial agents are described further in Chapter 66.

Cytotoxic and Immunosuppressant Drugs

Cytotoxic and immunosuppressant drugs are used in dermatology for immunologically mediated diseases such as psoriasis, autoimmune blistering diseases, and leukocytoclastic vasculitis. See Table 75–10 for their mechanisms of action.

TABLE 75–10 ■ MECHANISM OF ACTION FOR SELECTED CYTOTOXIC, IMMUNOSUPPRESSIVE, AND IMMUNOMODULATORY DRUGS

Methotrexate	Dihydrofolate reductase inhibitor
Fluorouracil	Blocks methylation in DNA synthesis
Cyclophosphamide	Alkylates and cross-links DNA
Mechlorethamine hydrochloride	Alkylating agent
Carmustine	Cross-links in DNA and RNA
Vinblastine	Inhibits microtubule formation
Podophyllin	Inhibits microtubule formation
Tirbanibulin	Inhibits microtubule formation
Bleomycin	Induction of DNA strand breaks
Azathioprine	Purine synthesis inhibitor
Mycophenolate mofetil	Inosine monophosphate dehydrogenase inhibitor
Cyclosporine	Calcineurin inhibitor
Tacrolimus	Calcineurin inhibitor
Pimecrolimus	Calcineurin inhibitor
Sirolimus	mTOR inhibitor
Everolimus	mTOR inhibitor
Imiquimod	Interferon α induction via toll-like receptor 7
Dapsone	Inhibits neutrophil migration, oxidative burst
Thalidomide	Cytokine modulation

Antimetabolites

Methotrexate is used for moderate-to-severe psoriasis. It suppresses immunocompetent cells in the skin, and it also decreases the expression of cutaneous lymphocyte-associated antigen–positive T cells and endothelial cell E-selectin, which may account for its efficacy. *Methotrexate* is useful in treating a number of other dermatological conditions, including atopic dermatitis, pityriasis lichenoides et varioliformis, lymphomatoid papulosis, sarcoidosis, pemphigus vulgaris, pityriasis rubra pilaris, lupus erythematosus, dermatomyositis, and CTCL.

Methotrexate often is used in combination with phototherapy and photochemotherapy or other systemic agents. Widely used regimens in adults include 10 to 25 mg orally once weekly as a single dose or as three divided doses given at 12-h intervals, with similar effectiveness using either regimen, or intramuscular administration of 10 to 25 mg injected once weekly (maximum of 30 mg/week) (Menter et al., 2009b). FDA approval allows for oral, intravenous, intramuscular, and subcutaneous administration of *methotrexate*.

Doses must be decreased for patients with impaired renal clearance. *Methotrexate should not be administered with probenecid, trimethoprim-sulfamethoxazole, salicylates, or other drugs that can compete with it for protein binding and thereby raise plasma concentrations to levels that may result in bone marrow suppression*. Fatalities have occurred because of concurrent treatment with *methotrexate* and NSAIDs. *Methotrexate* exerts significant antiproliferative effects on the bone marrow; therefore, CBCs should be monitored serially. Clinicians administering *methotrexate* should be familiar with the use of *folinic acid* (*leucovorin*) to rescue patients with hematological crises caused by *methotrexate*-induced bone marrow suppression. Careful monitoring of liver function tests is necessary to assess for *methotrexate*-induced hepatotoxicity. Liver biopsy may be recommended after a 3.5- to 4-g cumulative dose in patients without other risk factors versus 1 to 1.5 g in patients with higher risk (e.g., obesity, diabetes) (Menter et al., 2009b). Patients with abnormal liver function tests, symptomatic liver disease, or evidence of hepatic fibrosis should not use this drug. *Methotrexate* is contraindicated during pregnancy and lactation. Many clinicians routinely administer folic acid to ameliorate side effects of *methotrexate*.

5-Fluorouracil (5FU) is used topically in multiple actinic keratoses, actinic cheilitis, Bowen disease, and superficial basal cell carcinomas (BCCs) not amenable to other treatments (Micali et al., 2014a, b). 5FU is applied once or twice daily for 2 to 8 weeks, depending on the indication. The treated areas may become severely inflamed during treatment, but the inflammation subsides after the drug is stopped. Intralesional injection of 5FU has been used off label for keratoacanthomas, warts, and porokeratoses (Good et al., 2011).

Alkylating Agents

Cyclophosphamide is an effective cytotoxic and immunosuppressive agent. Both oral and intravenous preparations of *cyclophosphamide* are used in dermatology. *Cyclophosphamide* is FDA-approved for treatment of advanced CTCL (Jawed et al., 2014).

Other uses include treatment of pemphigus vulgaris, bullous pemphigoid, cicatricial pemphigoid, paraneoplastic pemphigus, pyoderma gangrenosum, toxic epidermal necrolysis, Wegener granulomatosis, polyarteritis nodosa, Churg-Strauss angiitis, Behçet disease, scleromyxedema, and cytophagic histiocytic panniculitis. The usual oral dosage is 2 to 2.5 mg/kg per day in divided doses, and there often is a 4- to 6-week delay in onset of action (Meurer, 2012). Alternatively, intravenous pulse administration of *cyclophosphamide* may offer advantages, including lower cumulative dose and a decreased risk of bladder cancer. *Cyclophosphamide* has many adverse effects (see Chapter 70), including the risk of secondary malignancy and myelosuppression, and is used only in the most severe, recalcitrant dermatological diseases.

Mechlorethamine hydrochloride and *carmustine* are used topically to treat CTCL (Jawed et al., 2014). It is important to monitor CBCs and liver function tests because systemic absorption can cause bone marrow suppression and hepatitis. Other side effects include allergic contact dermatitis, irritant dermatitis, secondary cutaneous malignancies, and pigmentary changes. *Carmustine* also can cause erythema and posttreatment telangiectasias.

Microtubule Inhibitors

Vinblastine is approved for systemic use in Kaposi sarcoma and advanced CTCL. Intralesional *vinblastine* also is used to treat Kaposi sarcoma (Regnier-Rosencher et al., 2013).

Podophyllin (podophyllum resin) is a mixture of chemicals from the plant *Podophyllum peltatum* (mandrake or May apple) that contains podophyllotoxin (podofilox), which binds to microtubules and causes mitotic arrest in metaphase (Fathi and Tsoukas, 2014). In the U.S., it must be obtained through a compounding pharmacy. *Podophyllum resin* 25% is applied topically by a physician and left in place for no longer than 2 to 6 h weekly for the treatment of anogenital warts. Irritation and ulcerative local reactions are the major side effects. It should not be used in the mouth or during pregnancy. It is recommended that the use of clinician-administered *podophyllum resin* be replaced by the purified podophyllotoxin (*podofilox*) for patient-administered use. *Podofilox* is available as a 0.5% gel or solution for at-home application two times daily for 3 consecutive days, repeated weekly as needed for up to four cycles.

Tirbanibulin is first-in-class agent that was recently FDA-approved for the topical treatment of actinic keratoses on the face or scalp. The drug inhibits microtubule polymerization and Src kinase signaling (Gilchrest, 2021; Smolinski et al., 2018). It is available as tirbanibulin 1% ointment, which is applied once daily for 5 consecutive days to evenly cover up to a 25 cm^2 area. Adverse reactions may include local skin irritation, application site pain, or pruritus.

Other Cytotoxic Agents

Bleomycin is used off label intralesionally for palliative treatment of squamous cell carcinoma and recalcitrant warts and has cytotoxic and proinflammatory effects. Intralesional injection of *bleomycin* into the digits has been associated with a vasospastic response that mimics Reynaud phenomenon. Other potential adverse reactions include local skin necrosis and flagellate hyperpigmentation. Systemic *bleomycin* has been used off label for Kaposi sarcoma (see Chapter 70) (Regnier-Rosencher et al., 2013).

Liposomal anthracyclines (specifically *doxorubicin*) may provide first-line monotherapy for advanced Kaposi sarcoma (Regnier-Rosencher et al., 2013).

Ingenol mebutate gel, an extract from the plant *Euphorbia peplus*, is FDA-approved for actinic keratosis (Micali et al., 2014a, b). In experimental studies, it was reported to rapidly cause mitochondrial swelling and apoptosis of dysplastic keratinocytes. The gel is applied once daily for 2 to 3 consecutive days. Adverse effects may include local skin irritation, pain, pruritus, and infection at application site; periorbital edema; nasopharyngitis; and headache. Cases of allergic contact dermatitis and reactivation of herpes zoster have been reported.

Azathioprine and Mycophenolate Mofetil

Azathioprine is discussed in Chapter 39. In dermatological practice, the drug is used off label as a steroid-sparing agent for autoimmune and inflammatory dermatoses, including pemphigus vulgaris, bullous pemphigoid, dermatomyositis, atopic dermatitis, chronic actinic dermatitis, lupus erythematosus, psoriasis, pyoderma gangrenosum, and Behçet disease.

Azathioprine is cleaved to 6-mercaptopurine, which in turn is converted to additional metabolites that inhibit *de novo* purine synthesis (see Chapter 70). The level of thiopurine *S*-methyltransferase (TPMT) enzyme activity should be measured before initiating *azathioprine* therapy because low levels of TMPT activity are associated with increased drug toxicity (see Chapter 70). In general, the starting dosage is 1 to 2 mg/kg per day. Careful laboratory monitoring is important, especially in those with diminished TPMT activity.

Mycophenolate mofetil and *mycophenolate sodium* are immunosuppressants approved for prophylaxis of organ rejection in patients with renal, cardiac, and hepatic transplants (see Chapter 39). *Mycophenolate mofetil* is a prodrug that is hydrolyzed to mycophenolic acid by plasma esterases; the salt of the acid, *mycophenolate sodium*, is available in an enteric-coated formulation. Mycophenolic acid functions as a specific inhibitor of T- and B-lymphocyte activation and proliferation through inhibition of inosine monophosphate dehydrogenase, thereby reducing GMP synthesis by the *de novo* purine synthetic pathway. Lymphocytes are vulnerable due to their lack of the salvage pathway for purine synthesis. The drug also may enhance apoptosis.

Mycophenolate mofetil is used off label increasingly to treat inflammatory and autoimmune diseases in dermatology; dosages typically range from 2 to 3 g/day orally in adults or 30 to 50 mg/kg per day in pediatric patients, given in divided doses twice daily. This agent is particularly useful as a corticosteroid-sparing agent in the treatment of autoimmune blistering disorders and has been used effectively in the treatment of inflammatory diseases such as psoriasis, atopic dermatitis, and pyoderma gangrenosum. Isolated cases of progressive multifocal leukoencephalopathy and pure red cell aplasia have been reported in solid organ transplant patients receiving *mycophenolate mofetil*. The most common side effects of *mycophenolate mofetil* are dose-dependent GI symptoms (e.g., diarrhea, nausea, and abdominal pain). Enteric-coated *mycophenolate sodium* improves GI tolerability; the enteric coating delays absorption until the medication reaches the small intestine rather than occurring in the stomach.

Calcineurin Inhibitors

The mechanisms of action and clinical pharmacology of calcineurin inhibitors are described in Chapter 39. Figure 39–2 shows their sites of action as immunosuppressants

Cyclosporine is a potent immunosuppressant isolated from the fungus *Tolypocladium inflatum*. *Cyclosporine* is FDA-approved for the treatment of psoriasis. A modified microemulsion formulation has increased bioavailability and more consistent absorption than the original formulation. Other cutaneous disorders that typically respond well to *cyclosporine* are atopic dermatitis, alopecia areata, epidermolysis bullosa acquisita, pemphigus vulgaris, bullous pemphigoid, lichen planus, and pyoderma gangrenosum. The usual oral dosage of the modified formulation is 2.5 to 5 mg/kg per day, often given in two divided doses (Menter et al., 2009b; Sidbury et al., 2014).

Hypertension and renal dysfunction are the major adverse effects associated with the use of *cyclosporine*. These risks can be minimized by monitoring serum creatinine (which should not rise more than 30% above baseline), calculating creatinine clearance or glomerular filtration rate in patients on long-term therapy or with a rising creatinine, maintaining a daily dose of less than 5 mg/kg, and regularly monitoring blood pressure. Alternation with other therapeutic modalities may diminish *cyclosporine* toxicity. Patients with psoriasis who are treated with *cyclosporine* are at increased risk of cutaneous, solid organ, and lymphoproliferative malignancies. The risk of cutaneous malignancies is compounded in patients who have received phototherapy with PUVA.

Tacrolimus is available in a topical form for the treatment of skin disease and also is marketed in oral and injectable formulations. Systemic *tacrolimus* has shown some efficacy in the off-label treatment of inflammatory skin diseases such as psoriasis, pyoderma gangrenosum, and Behçet disease. When *tacrolimus* is administered systemically, the most common side effects are hypertension, nephrotoxicity, neurotoxicity, GI symptoms, hyperglycemia, and hyperlipidemia.

Topical formulations (0.03% and 0.1%) of *tacrolimus* ointment are approved for the treatment of atopic dermatitis in adults and in children 2 years or older for 0.03% ointment and 16 years or older 0.1% ointment. Other uses include for treatment of intertriginous psoriasis, vitiligo, mucosal lichen planus, graft-versus-host disease, allergic contact dermatitis, and rosacea. Ointment is applied to the affected area two times daily and generally is well tolerated. Common side effects at the site of application are transient erythema, burning, and pruritus. Other adverse effects include skin tingling, flu-like symptoms, headache, alcohol intolerance, folliculitis, acne, and hyperesthesia. Systemic absorption generally is very low and decreases with resolution of the dermatitis. Topical *tacrolimus* should be used with extreme caution in patients with Netherton syndrome; these patients may develop elevated blood levels of the drug after topical application (Eichenfield et al., 2014).

Pimecrolimus (as a 1% cream) is approved for the treatment of atopic dermatitis in patients 2 years and older. Its mechanism of action and side effect profile are similar to those of *tacrolimus*. It has low-to-negligible systemic absorption after topical application.

Tacrolimus and *pimecrolimus* are approved as second-line agents for short-term and intermittent treatment of atopic dermatitis in patients unresponsive to, or intolerant of, other treatments. Unlike topical corticosteroids, they do not carry the risk of skin atrophy and may be especially useful as steroid-sparing agents or for use on sensitive skin sites (e.g., eyelids, face, skin folds) or areas with steroid-induced atrophy.

mTOR Inhibitors

Sirolimus causes immunosuppressive and antiproliferative effects through inhibition of mTOR (mammalian target of rapamycin) as part of the PI3 kinase/Akt/mTOR pathway (see Figures 39–2 and 71–5). The mTOR inhibitors are established treatments for posttransplant immunosuppression (see Chapter 39) and as antineoplastic agents for malignancies such as renal and hepatocellular carcinoma (see Chapter 71). Off-label use of mTOR inhibitors in dermatology includes treatment of tuberous sclerosis complex, pachyonychia congenita, complex vascular anomalies, and inflammatory dermatoses such as systemic sclerosis (Fogel et al., 2015). Systemic mTOR inhibitors may be associated with serious side effects, with dermatological side effects among the most common, including stomatitis, mucositis, inflammatory cutaneous eruptions, and nail changes such as paronychia (Fogel et al., 2015). Topical formulations have been used to decrease the risk of side effects associated with systemic therapy. Newer semisynthetic analogues of *sirolimus* such as *everolimus* and *temsirolimus* are available and have improved water solubility and efficacy.

Immunomodulators and Anti-inflammatory Agents

Imiquimod exerts immunomodulatory effects by acting as a ligand at toll-like receptors in the immune system and inducing the cytokines interferon (IFN) α, tumor necrosis factor (TNF) α, and interleukin (IL)-1, IL-6, IL-8, IL-10, and IL-12.

Imiquimod cream is available in 2.5%, 3.75%, and 5% strengths. Approved for the treatment of genital warts, *imiquimod* 5% cream is applied to genital or perianal lesions three times a week until resolution of warts or for up to a 16-week period (and repeated as necessary) (Fathi and Tsoukas, 2014). *Imiquimod* also is approved for the treatment of actinic keratosis, with various regimens used (Micali et al., 2014a, b). No more than 36 single-use packets per 16-week course of therapy should be prescribed for actinic keratoses. The 5% cream is also FDA-approved for the treatment of superficial BCCs at a dosage of five applications per week for 6 weeks. Off-label applications include the treatment of nongenital warts, molluscum contagiosum, extramammary Paget disease, and Bowen disease. Irritant reactions occur in virtually all patients; the degree of inflammation parallels therapeutic efficacy.

Sinecatechins are partially purified green tea extracts containing a mixture of 85% to 95% catechins and other green tea components. They are approved for the topical treatment of external genital and perianal warts in immunocompetent patients more than 18 years of age. The mechanism of action is unclear but may include a combination of antioxidant, antiviral, antiangiogenic, proapoptotic, and immunomodulatory activities. The *sinecatechin* 15% ointment is applied three times daily for up to 16 weeks until clearance of the warts. The most common side effects are local skin reactions, including erythema, pruritus or burning, pain, superficial ulceration, and edema, with the intensity of local reactions peaking between 2 and 4 weeks of use. Local site reactions may be indicative of a positive clinical response, and patients are encouraged to treat through them as tolerated.

Dapsone is used in dermatology for its anti-inflammatory properties, particularly in sterile (noninfectious) pustular diseases of the skin (Zhu and Stiller, 2001). *Dapsone* prevents the respiratory burst from myeloperoxidase, suppresses neutrophil migration by blocking integrin-mediated adherence, inhibits adherence of antibodies to neutrophils, and decreases the release of eicosanoids and blocks their inflammatory effects. *Dapsone* is discussed further in Chapter 65.

Dapsone is approved for oral use in dermatitis herpetiformis and leprosy and for topical use in acne vulgaris. It is also particularly useful in the off-label treatment of linear IgA dermatosis, bullous systemic lupus erythematosus, erythema elevatum diutinum, and subcorneal pustular dermatosis. In addition, reports indicate efficacy in patients with acne fulminans, pustular psoriasis, lichen planus, Hailey-Hailey disease, pemphigus vulgaris, bullous pemphigoid, cicatricial pemphigoid, leukocytoclastic vasculitis, Sweet syndrome, granuloma faciale, relapsing polychondritis, Behçet disease, urticarial vasculitis, pyoderma gangrenosum, and granuloma annulare.

The initial oral dosage is 50 mg/day, followed by increases of 25 mg/day at weekly intervals, titrated to the minimal dosage necessary for effect. Potential side effects of *dapsone* include methemoglobinemia and hemolysis (G6PD level should be checked in all patients). The H_2 blocker *cimetidine* inhibits numerous CYPs and thereby reduces the *N*-hydroxylation of *dapsone* to the hydroxylamine metabolite that causes the methemoglobinemia. Other toxicities of *dapsone* include agranulocytosis, peripheral neuropathy, and psychosis.

Thalidomide is an anti-inflammatory, immunomodulatory, and antiangiogenic agent experiencing resurgence in the treatment of dermatological diseases (Wu et al., 2005). For details of its actions, see Figures 71–8 and 71–9.

Thalidomide is FDA-approved for the treatment of erythema nodosum leprosum. Reports also suggest its efficacy in actinic prurigo, aphthous stomatitis, Behçet disease, Kaposi sarcoma, the cutaneous manifestations of lupus erythematosus, and prurigo nodularis and uremic prurigo. *Thalidomide* has been associated with increased mortality when used to treat toxic epidermal necrolysis. *In utero* exposure can cause limb abnormalities (phocomelia), as well as other congenital anomalies. *Thalidomide should never be taken by women who are pregnant or who could become pregnant while taking the drug. It also may cause an irreversible neuropathy. Because of its teratogenic effects, thalidomide use is restricted and requires both pharmacists and physicians to register in a risk evaluation and mitigation strategy (REMS) program. Newer analogues of thalidomide are available, including lenalidomide and pomalidomide.*

Targeted Immunotherapies for Psoriasis and Atopic Dermatitis

Targeted immunotherapies, including biological agents, are rapidly evolving for the treatment of psoriasis, as the underlying pathogenesis is better understood (Table 75–11). In addition, biological therapy for atopic dermatitis is on the horizon. The appeal of these targeted therapies is that they specifically target the activities of T lymphocytes and cytokines that mediate inflammation, whereas traditional systemic therapies are broadly immunosuppressive or cytotoxic. The pathogenesis of psoriasis and atopic dermatitis is discussed briefly next, followed by the relevant targeted therapies. Targeted therapies are discussed in further detail in Chapters 38 and 39.

Psoriasis is a chronic immune-mediated inflammatory disorder involving the innate and adaptive immune systems, with resultant abnormal keratinocyte proliferation (Figure 75–3). Psoriasis is not limited to the skin, as there can be psoriatic arthritis and other systemic manifestations. Previously thought to be primarily T_H1 mediated, more recent developments demonstrate that the T_H17/IL-17 pathway plays the most significant role (Girolomoni et al., 2012). Mediators of inflammation, such as IL-23, TNFα, and IL-17, are key targets for therapy (Girolomoni et al., 2012; Sobell and Leonardi, 2014). In addition, therapies such as inhibitors of Janus kinases (JAKs) and cyclic nucleotide phosphodiesterases (PDEs) are useful to decrease expression or signal transduction of inflammatory cytokines (Sobell and Leonardi, 2014). Sphingosine-1-phosphate receptor 1 (S1PR1) modulators are also being evaluated as potential therapeutic targets for psoriasis through regulation of lymphocyte trafficking from lymphoid tissue into peripheral circulation and the skin; see Figure 39–3 and associated text.

Atopic dermatitis is a chronic skin disorder characterized by epidermal barrier dysfunction and immune-mediated inflammation. Patients often develop asthma and allergic rhinitis as well. Acute atopic dermatitis is T_H2 cell mediated, associated with cytokines IL-4, IL-5, and IL-13, whereas chronic atopic dermatitis also involves T_H1 cell–mediated activity, associated with cytokines IL-12 and IFNγ . Topical corticosteroids, calcineurin inhibitors, and phosphodiesterase-4 (PDE4) inhibitors are commonly used in patients with mild-to-moderate disease. Several of the traditional immunosuppressants have been used for more severe or resistant disease, and more recently, targeted therapy through IL-4/IL-13 inhibition has proven efficacious with a favorable safety profile (Eichenfield et al., 2014; Sidbury et al., 2014). Additional targeted therapies being evaluated for atopic dermatitis include IL-31 inhibitors, IL-13 inhibitors, IL-22 inhibitors, and S1PR1 modulators (Strowd et al., 2020). The FDA has recently approved several JAK inhibitors for treatment of atopic dermatitis (see JAK Inhibitors, below).

Tumor Necrosis Factor Inhibitors

Blockade of TNFα reduces inflammation, decreases keratinocyte proliferation, and decreases vascular adhesion, resulting in improvement in psoriatic lesions. Because TNFα inhibitors alter immune responses,

TABLE 75–11 ■ BIOLOGICAL AGENTS FOR PSORIASIS

	BINDING SITE	STRUCTURAL CLASS	HALF-LIFE (DAYS)	METHOD OF ADMINISTRATION	ADULT DOSAGE FOR PSORIASIS	PEDIATRIC DOSAGE FOR PSORIASIS
TNFα						
Etanercept	TNFα TNFβ	Human p75 TNF receptor and Fc IgG_1 fusion protein	3–5.5	SC	50 mg twice weekly for 3 months, then 50 mg once weekly thereafter	0.8 mg/kg once weekly (max 50 mg per week)
Adalimumab	TNFα	Human $\text{IgG}_{1\kappa}$ mAb	10–20	SC	80 mg at week 0, 40 mg at week 1, then 40 mg every other week thereafter	
Infliximab	TNFα	Chimeric $\text{IgG}_{1\kappa}$ mAb	7.7–9.5	IV	5 mg/kg at weeks 0, 2, and 6, then every 8 weeks thereafter	
Certolizumab pegol	TNFα	PEGylated humanized Fab’ fragment mAb	14	SC	400 mg every other week If ≤90 kg, alternate dosing of 400 mg at weeks 0, 2, and 4, then 200 mg every other week thereafter may be acceptable	
IL-17						
Bimekizumab*	IL-17A IL-17F	Humanized IgG_1 mAb	17–22	SC	320 mg every 4 weeks for 16 weeks then every 8 weeks thereafter	
Brodalumab	IL-17 receptor A	Human $\text{IgG}_{2\kappa}$ mAb	10.9	SC	210 mg at weeks 0, 1, and 2, then every 2 weeks	
Ixekizumab	IL-17A	Humanized IgG_4 mAb	13	SC	160 mg at week 0, then 80 mg at weeks 2, 4, 6, 8, 10, and 12, and then every 4 weeks thereafter	<25 kg: 40 mg at week 0 and then 20 mg every 4 weeks thereafter 25–50 kg: 80 mg at week 0, then 40 mg every 4 weeks thereafter >50 kg: 160 mg at week 0, then 80 mg every 4 weeks thereafter
Secukinumab	IL-17A	Human $\text{IgG}_{1\kappa}$ mAb	22–31	SC	300 mg at weeks 0, 1, 2, 3, and 4, and every 4 weeks thereafter For some, maintenance of 150 mg every 4 weeks may be acceptable	
IL-12/23						
Ustekinumab	p40 subunit of IL-12 and IL-23	Human $\text{IgG}_{1\kappa}$ mAb	~15–45	SC	If ≤100 kg: 45 mg at weeks 0 and 4, and every 12 weeks thereafter If >100 kg: 90 mg at weeks 0 amd 4, and every 12 weeks thereafter	<60 kg: 0.75 mg/kg at weeks 0 and 4, and every 12 weeks thereafter ≥60 to ≤100 kg: 45 mg at weeks 0 and 4, and every 12 weeks thereafter >100 kg: 90 mg at weeks 0 and 4, and every 12 weeks thereafter
IL-23						
Guselkumab	p19 subunit of IL-23	Human $\text{IgG}_{1\lambda}$ mAb	15–18	SC	100 mg at weeks 0 and 4, and every 8 weeks thereafter	
Risankizumab	p19 subunit of IL-23	Humanized IgG_1 mAb	28	SC	150 mg at weeks 0 and 4, and every 12 weeks thereafter	
Tildrakizumab	p19 subunit of IL-23	Humanized $\text{IgG}_{1\kappa}$ mAb	23	SC	100 mg at weeks 0 and 4, and every 12 weeks thereafter	

*Approved dosage in European Union; not currently FDA-approved.

mAb, monoclonal antibody.

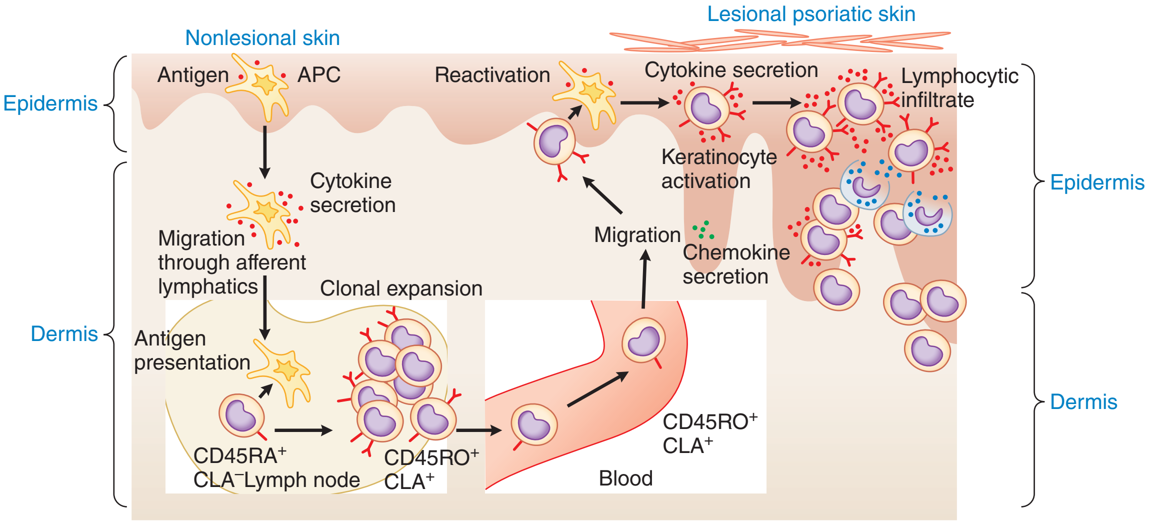


Figure 75–3 *Immunopathogenesis of psoriasis.* Psoriasis is a prototypical inflammatory skin disorder in which specific T-cell populations are stimulated by as-yet-undefined antigen(s) presented by APCs. The dendritic APCs, T cells, keratinocytes, and other cells release proinflammatory chemokines and cytokines, such as TNFα, IFNγ, IL-23, and IL-17, which ultimately induce keratinocyte hyperproliferation and sustain chronic inflammation.

patients on all anti-TNFα agents are at increased risk for serious infection and for malignancies. Other adverse events include exacerbation of congestive heart failure and demyelinating disease in predisposed patients. All patients should be screened for tuberculosis, history of demyelinating disorder, cardiac failure, active infection, or malignancy prior to therapy. TNFα inhibitors include monoclonal antibodies directed against TNFα (*adalimumab, certolizumab, infliximab*) or a soluble TNF receptor that can bind TNFα (*etanercept*) (see Chapter 39). All TNFα inhibitors are able to bind both soluble and membrane-bound TNF, but the monoclonal antibodies against TNFα with an IgG_1 Fc portion are also able to activate complement-dependent cytotoxicity on binding of membrane-bound TNFα. This may explain the increased efficacy of these agents in comparison to *etanercept* for treating granulomatous conditions as well as the higher risk of infection with their use.

Etanercept is a soluble, recombinant, fully human TNF receptor fusion protein consisting of two molecules of the ligand-binding portion of the TNF receptor (p75) fused to the Fc portion of IgG_1. It binds both TNFα and TNFβ. *Etanercept* is approved for plaque psoriasis in adults and pediatric patients 4 years or older and for psoriatic arthritis in adults.

Infliximab, a mouse-human chimeric IgG_1 monoclonal antibody directed against human TNFα, is approved for treatment of plaque psoriasis and psoriatic arthritis in adults. The administration of *infliximab* via intravenous infusion can be a limiting factor; however, compared to other TNFα inhibitors, its onset of action may be faster and efficacy greater. Neutralizing antibodies to *infliximab* may develop and lead to decreased efficacy over time. Concomitant administration of *methotrexate* or glucocorticoids may suppress this neutralizing antibody formation.

Adalimumab is a human IgG_1 monoclonal antibody directed against TNFα; thus, the risk for development of neutralizing antibodies is reduced compared to *infliximab*. *Adalimumab* is approved for treatment of plaque psoriasis and psoriatic arthritis in adults. Safety of *adalimumab* in pediatric patients for psoriasis is supported by its use in other conditions, such as juvenile idiopathic arthritis (Bellodi Schmidt and Shah, 2015). *Adalimumab* is also approved for treatment of hidradenitis suppurativa.

Certolizumab pegol is a PEGylated Fab fragment of a humanized monoclonal antibody against TNFα. PEGylation allows for delayed elimination and therefore an extended half-life. Because *certolizumab pegol* lacks the Fc portion, it is not able to activate complement-mediated cytotoxicity. *Certolizumab pegol* has minimal to no placental transfer or transfer into breast milk, which is primarily due to its lack of an Fc portion (Clowse et al., 2017; Mariette et al., 2018). It is approved for the treatment of plaque psoriasis and psoriatic arthritis in adults.

Golimumab is a TNFα inhibitor currently approved for psoriatic arthritis but not psoriasis. However, studies for psoriatic arthritis also demonstrated improvement of plaque psoriasis. *Golimumab* is a fully human monoclonal antibody against TNFα with a longer $t_{1/2}$ than other TNFα inhibitors.

IL-12/23 Inhibitors

Ustekinumab is a human IgG_1 monoclonal antibody directed against the common p40 subunit of IL-12 and IL-23. IL-12 promotes T_H1 activity and related TNFα and IFNγ production, while IL-23 activates T_H17 cells that produce IL-17A, which regulates tissue inflammation and autoimmune responses. *Ustekinumab* is approved for treatment of plaque psoriasis in adults and pediatric patients 6 years or older and of psoriatic arthritis in adults. After initial doses at weeks 0 and 4, it is administered every 12 weeks for the maintenance phase of therapy, which may be ideal for some patients.

IL-17 Inhibitors

Keratinocytes are the major target for IL-17 in psoriasis, with IL-17 stimulation causing increased keratinocyte expression of inflammatory cytokines and other effects that contribute to epidermal hyperproliferation and barrier dysfunction. Clinical trials have demonstrated that IL-17 inhibitors are very efficacious for plaque psoriasis.

Brodalumab, a human IgG2κ monoclonal antibody against the receptor for IL-17A (IL-17RA), is approved for the treatment of moderate-to-severe plaque psoriasis in adults. Through antagonism of the IL-17RA, brodalumab inhibits IL-17 A, C, D, E, and F signaling. There is a boxed warning for *brodalumab* regarding suicidal ideation and behavior, which requires certification through a REMS program prior to dispensing. Patients with psoriasis often have psychiatric comorbidities such as depression: however, studies have not demonstrated a causal relationship between *brodalumab* and suicidal ideation and behavior (Lebwohl et al., 2018).

Bimekizumab, a humanized IgG_1 monoclonal antibody against IL-17A and IL-17F, is EMA-approved for the treatment of moderate-to-severe plaque psoriasis in adults. Dual inhibition of IL-17A and IL-17F may increase efficacy compared to inhibition of IL-17A alone.

Ixekizumab, a humanized IgG_4 monoclonal antibody against IL-17A, is approved for the treatment of moderate-to-severe plaque psoriasis in adults and pediatric patients 6 years or older and of psoriatic arthritis in adults.

Secukinumab is a fully human $IgG_{1\kappa}$ monoclonal antibody against IL-17A approved for the treatment of moderate-to-severe plaque psoriasis in adults.

The most common adverse effects for IL-17 inhibitors are nasopharyngitis, upper respiratory tract infection, and diarrhea. There is the potential for increased mucocutaneous candidiasis infections, a risk that is greater with dual IL-17A/IL-17F inhibitors. There are reports of the exacerbation of inflammatory bowel disease by IL-17 inhibitors.

Phosphodiesterase-4 Inhibitors

Phosphodiesterase type 4 (PDE4) hydrolyzes intracellular cyclic adenosine monophosphate (cAMP), which is a critical intracellular messenger in numerous cell types, including immune cells. In both psoriasis and atopic dermatitis, lesional skin has subnormal cAMP levels due to increased PDE4 activity, with a resultant increase in the production of cytokines. Inhibition of PDE4 increases intracellular cAMP levels and thereby decreases expression of inflammatory cytokines such as TNFα, IFNγ, IL-17, and IL-23.

Apremilast is an oral PDE4 inhibitor approved for the treatment of moderate-to-severe plaque psoriasis, psoriatic arthritis, and oral ulcers associated with Behçet disease in adults. It has also been used off-label for atopic dermatitis, alopecia areata, hidradenitis suppurativa, lichen planus, cutaneous sarcoidosis, and discoid lupus erythematosus. The most common side effects are weight loss and depression.

Crisaborole is a PDE4B inhibitor approved for the topical treatment of mild-to-moderate atopic dermatitis in adult and pediatric patients 3 months and older. It is available as a 2% topical ointment, which is applied twice daily. The most common side effect is burning or stinging at the application site. *Crisaborole* is a boron-containing benzoxaborole that mimics the phosphate of cAMP and competitively inhibits PDE4 activity. The use of boron also contributes to the low molecular weight of *crisaborole* (251 Da), which facilitates effective penetration through skin.

Roflumilast is currently approved as an oral PDE4 inhibitor treatment for chronic obstructive pulmonary disease (see Chapter 44). It is being studied as a topical treatment for both psoriasis and atopic dermatitis.

Janus Kinase Inhibitors

Janus kinases (JAKs) are non–receptor tyrosine kinases involved in cytokine receptor signaling. Their interaction with STATs (signal transducers and activators of transcription) leads to activation of the transcription of genes involved in many vital events, such as inflammation, hematopoiesis, wound repair, and apoptosis, among others. As a consequence, inhibition of this pathway offers current and potential treatments for myriad diseases, including dermatological conditions. Dozens of growth factors and cytokines, including ILs, IFNs, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor (GM-CSF), erythropoietin, growth hormone, and leptin, interact with membrane receptors and initiate signaling via the JAK-STAT signaling pathway. There are four JAKs: JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2); they interact selectively with seven STATs (see Figure 3–22A).

JAK inhibitors exhibit variable selectivity for the JAK isoforms; thus, not all JAK inhibitors are approved for identical uses; the approved uses and limitations grow and shift as research, clinical trials, and postmarketing information advance. JAK inhibitors are used, alone or with other agents, to treat chronic and progressive inflammatory conditions such as rheumatoid arthritis and psoriatic arthritis; recently, atopic dermatitis has been added to the FDA-approved indications. A study comparing *tofacitinib* with anti-TNF antagonists noted that the use of the JAK inhibitor was associated with an increased risk of blood clots and death. In response, the FDA has recently issued a boxed warning for oral JAK inhibitors used chronically. The essence of the black-box warning for these agents is:

- Increased risk of serious bacterial, fungal, viral, and opportunistic infections leading to hospitalization or death
- Higher rate of all-cause mortality, including sudden cardiovascular death
- Higher rate of certain cancers
- Higher rate of cardiovascular death, myocardial infarction, and stroke
- Thrombosis

These agents are associated with dose-related increases in total cholesterol, triglycerides, and low-density lipoprotein cholesterol. To varying degrees, these agents also share common side effects, including upper respiratory infection, viral infections (herpes simplex, herpes zoster), headache, nausea, nasopharyngitis, and acne. Combining JAK inhibitors or coadministering them with other strong systemic immunosuppressants is generally not recommended. All age-appropriate vaccinations (see Chapter 40) should be completed prior to therapy, including prophylactic herpes zoster vaccinations, and vaccination with live vaccines immediately prior to, during, and immediately after the course of therapy should be avoided. JAK inhibitors should be used with awareness of the latest information from the FDA and with knowledge of the recommended usages and potential adverse reactions.

Abrocitinib

Abrocitinib is a JAK1 inhibitor that is FDA-approved for the treatment of adults with refractory, moderate-to-severe atopic dermatitis that is not adequately controlled with other systemic agents. The drug is administered orally in doses of 100 and 200 mg, with the 200-mg dose being recommended for patients who are not responding to the 100-mg dose (a 50-mg dose is used in patients with moderate kidney failure). The $t_{1/2}$ of *abrocitinib* and its two major active metabolites is 5 to 6 h but is prolonged by inhibitors of CYP2C19 and in patients who are CYP2C19 poor metabolizers (see Chapter 7); *abrocitinib* should not be used in patients with severe hepatic impairment. There are insufficient data to inform pregnancy risk; the drug may, however, impair fertility (reversible with cessation of the drug). Nursing mothers should not breastfeed while using *abrocitinib* and for 1 day after completing therapy. Antiplatelet therapies (except for low-dose *aspirin* [≤81 mg daily]) are contraindicated during the first 3 months of treatment.

A recent study showed that *abrocitinib*, in combination with topical therapy, was also safe and efficacious in treating atopic dermatitis in adolescents (Eichenfield et al., 2021).

Baricitinib

Baricitinib is JAK1/JAK2 inhibitor EMA-approved for moderate-to-severe atopic dermatitis in adults who are candidates for systemic therapy. For this indication, the drug is administered orally at 2 mg daily (may be increased to 4 mg daily if 2 mg daily proves inadequate). Baricitinb is FDA-approved for the treatment of severe alopecia areata and moderate-to-severe active theumatoid arthritis. A high-fat meal decreases the area under the curve (AUC) (11%) and the $C_{\max}$ (18%). The $t_{1/2}$ of *baricitinib* is approximately 12 h. Modest hepatic metabolism is by CYP3A4; the drug is largely excreted in the urine, predominantly as the unchanged drug. Inhibitors of the OAT3 transporter increase exposure to the drug, which is also a substrate for P-glycoprotein. Use is not recommended in patients with liver or kidney impairment. *Baricitinib* can be used in combination with topical steroids and/or topical calcineurin inhibitors. There are insufficient data to inform pregnancy risk; due to the potential for serious adverse reactions in infants, nursing mothers should not breastfeed while using *baricitinib*. Clinical trials are in progress for other indications (e.g., chronic plaque psoriasis, systemic lupus erythematosus).

Upadacitinib

Upadacitinib is JAK1-selective inhibitor FDA-approved for treatment of rheumatoid arthritis and psoriatic arthritis; its indications were recently extended to include moderate-to-severe atopic dermatitis in patients 12 years (>40 kg) and older who have not responded to previous systemic treatments. Dosage is one 15-mg extended-release tablet, taken orally each day (or one 30-mg tablet daily if an adequate response is not achieved with the lower dose). *Upadacitinib* is a substrate for CYP3A4; thus, strong inhibitors (e.g., *ketoconazole*) and strong inducers (e.g., *rifampin*) of CYP3A4 must be avoided since they will alter the drug's AUC with the possibility of increasing the risk of adverse effects or reducing the blood level below the therapeutic range. The drug's elimination $t_{1/2}$ is 8 to 14 h, with unchanged drug being excreted in urine and feces and about a third excreted as metabolites. *Upadacitinib* may cause fetal harm; females of childbearing potential should use effective contraception during treatment and for 4 weeks following the completion of treatment. Breastfeeding is not recommended during treatment and for 6 days thereafter

Ruxolitinib

Ruxolitinib is a JAK1/2 inhibitor approved for systemic therapy of acute and chronic graft-versus-host disease, myelofibrosis, and polycythemia vera. More recently, it has been approved as a topical treatment for the short-term and non-continuous chronic treatment of mild to moderate atopic dermatitis in immunocompetent patients 12 years and older whose disease is not adequately controlled with topical prescription therapies or when those therapies are not advisable. *Ruxolitinib* is available as a 1.5% cream, which is applied in a thin layer to the affected areas twice daily. The maximum recommended application amount is no more than 60 grams per week and should not exceed 20% of the total body surface area (BSA). Use of *ruxolitinib* in combination with therapeutic biologics, other JAK inhibitors, or potent immunosuppressants is not recommended. There are insufficient data to determine safety of use during pregnancy or with breastfeeding.

Deucravacitinib

Deucravacitinib is an oral, selective TYK2 inhibitor currently in clinical trials for the treatment of psoriasis.

Tofacitinib

Tofacitinib is an oral JAK inhibitor approved for use in rheumatoid arthritis, psoriatic arthritis, ulcerative colitis, and polyarticular juvenile arthritis in adults. It has demonstrated efficacy for psoriasis in clinical trials and for atopic dermatitis in a pilot study.

IL-4 and IL-13 Inhibitors

The cytokines IL-4 and IL-13 are important mediators of the Th2 immune response that is involved in acute atopic dermatitis. Transcriptome data of atopic dermatitis lesional skin demonstrates dominant expression of IL-13 compared to IL-4, which suggests that IL-13 may be the more significant driver of inflammation in atopic dermatitis. Therapies targeting IL-13 or IL-4/IL-13 have emerged and demonstrated efficacy for atopic dermatitis with a favorable safety profile.

Dupilumab is a fully human IgG4 monoclonal antibody against the alpha subunit of IL-4 receptor (IL-4Rα) that inhibits both IL-4 and IL-13 signaling as they each exert their action through binding an IL-4Rα/IL-13Rα1 heterodimer receptor signaling complex (Beck et al., 2014). *Dupilumab* is approved for the treatment of moderate to severe atopic dermatitis in adults and pediatric patients 6 years of age or older whose disease is not adequately controlled with topical prescription therapies or when those therapies are not advisable. *Dupilumab* has shown promising results, demonstrating significant improvement in disease severity and fewer skin infections compared to placebo (Beck et al., 2014). The most frequent adverse effects are conjunctivitis, nasopharyngitis, headache, peripheral eosinophilia, and injection site reactions. Some patients may also develop a drug-associated face and neck dermatitis (also called "*dupilumab* facial redness" or "new regional dermatosis") after initiation of *dupilumab*.

Tralokinumab is a human IgG4 monoclonal antibody that selectively binds to IL-13 and blocks its interaction with the IL-13 receptor a1 and a2 subunits. *Tralokinumab* is approved for the treatment of moderate to severe atopic dermatitis in adults that is not adequately controlled with topical prescription therapies or when those therapies are not advisable. It is administered as a subcutaneous injection of 600 mg on week 0 then 300 mg every 2 weeks thereafter. Patients with a body weight < 100 kg who achieve clear or almost clear skin after 16 weeks of therapy may reduce the dosage to 300mg every 4 weeks. The most common adverse effects were upper respiratory infection, headache, injection site reactions, and conjunctivitis. Conjunctivitis may be less common and less severe with *tralokinumab* than *dupilumab*.

Lebrikizumab is a human IgG4 monoclonal antibody that selectively targets IL-13 and prevents formation of the IL-13Rα1/IL- IL-4Rα heterodimer receptor signaling complex. In comparison to *tralokinumab*, *lebrikizumab* does not inhibit binding of IL-13 to the IL-13Ra2, which may function as a decoy receptor for endogenous regulation of IL-13 levels. The clinical relevance of this is unknown at this time. *Lebrikizumab* is currently in clinical trials for the treatment of atopic dermatitis.

Intravenous Immunoglobulin

Intravenous immunoglobulin (IVIG) is prepared from fractionated pooled human sera derived from thousands of donors with various antigenic exposures (see Chapter 40). Preparations of IVIG are composed of more than 90% IgG, with minimal amounts of IgA, soluble CD4, CD8, human leukocyte antigen molecules, and cytokines. In dermatology, IVIG is used off label as an adjuvant or rescue therapy for autoimmune bullous diseases, toxic epidermal necrolysis, connective tissue diseases, vasculitis, urticaria, atopic dermatitis, and graft-versus-host disease (Smith et al., 2007).

Although the mechanism of action of IVIG is not understood fully, proposed mechanisms include suppression of IgG production, accelerated catabolism of IgG, neutralization of complement-mediated reactions, neutralization of pathogenic antibodies, downregulation of inflammatory cytokines, inhibition of autoreactive T lymphocytes, inhibition of immune cell trafficking, and blockage of Fas-ligand/Fas-receptor interactions (Smith et al., 2007). IVIG is contraindicated in patients with severe selective IgA deficiency (IgA <0.05 g/L). These patients may possess anti-IgA antibodies that place them at risk for severe anaphylactic reactions. Other relative contraindications include congestive heart failure and renal failure.

Targeted Antineoplastic Agents

Recent advances in understanding the molecular pathways and genetic alterations underlying cancer development have allowed for the rapid emergence of oncotherapeutic agents targeted against the specific molecules involved in tumor pathogenesis (Iwasaki et al., 2012; Jawed et al., 2014; John and Cowey, 2015). They are often better tolerated than conventional chemotherapeutic medications; however, adverse effects do still occur, with cutaneous toxicities among the most common. These therapies are discussed in further detail in Chapters 71 and 72.

Targeted Therapies for Basal Cell Carcinoma

Basal cell carcinoma is the most common malignancy in humans, and exposure to UV radiation plays a large role in its development, with intermittent, intense exposure increasing risk more than cumulative exposure. Some genetic disorders, such as nevoid BCC syndrome, predispose to BCC development as well. Typically, BCCs exhibit an indolent growth pattern with local invasion and tissue destruction over time, but metastasis is extremely rare. Nearly all BCCs have aberrant *sonic hedgehog* pathway signaling, which leads to persistent activation and uncontrolled proliferation of basal cells (Iwasaki et al., 2012). Therapies have been developed that inhibit signaling through the sonic hedgehog pathway.

Vismodegib and *sonidegib* are *smoothened* inhibitors, which block activation of the hedgehog pathway (Iwasaki et al., 2012). They are approved for use in patients with locally advanced, metastatic, or recurrent disease that cannot be adequately managed with surgery or radiation therapy. They have also been used in patients with nevoid BCC syndrome. The most common side effects are muscle spasms, dysgeusia, alopecia, and diarrhea. The hedgehog pathway is vital to normal fetal development, so extreme caution is needed in women of reproductive potential, and men should be cautioned regarding potential for exposure and fetal harm through semen.

The antifungal agent *itraconazole* also has an inhibitory effect on the hedgehog pathway (Deng et al., 2020) but has more limited efficacy for treatment-refractory BCC.

Cemiplimab is a recombinant human IgG_4 monoclonal antibody that inhibits programmed death-1 (PD-1) to release PD-1 pathway-mediated inhibition of the immune response (see Figure 38–7). It is approved for

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treatment of patients with locally advanced BCC who have previously been treated with a hedgehog inhibitor or for whom a hedgehog inhibitor is not appropriate. The most common side effects are fatigue, musculoskeletal pain, diarrhea, rash, pruritus, and upper respiratory tract infection.

Targeted Therapies for Squamous Cell Carcinoma

Squamous cell carcinoma is the second most common type of skin malignancy. Cumulative exposure to UV radiation plays a large role in its development. Immunosuppression is also a risk factor for development of cutaneous squamous cell carcinoma, such as in organ transplant recipients. The large majority of cutaneous squamous cell carcinomas are treated surgically; however, there may be a need for nonsurgical treatment for those with increased risk for local recurrence or with nodal or distant metastasis.

Cemiplimab and *pembrolizumab* are PD-1 inhibitors approved for the treatment of cutaneous squamous cell carcinoma that is recurrent, metastatic, or locally advanced and is not curable by surgery or radiation.

Additional off-label treatment options for advanced or metastatic cutaneous squamous cell carcinoma include chemotherapy or epidermal growth factor inhibitors.

Targeted Therapies for Cutaneous T-Cell Lymphoma

Histone deacetylase inhibitors approved for treatment of progressive, persistent, or recurrent CTCL refractory to other systemic therapies include *vorinostat* and *romidepsin*. The exact mechanism of antineoplastic effect is not fully characterized; however, inhibition of histone deacetylase may restore expression of tumor suppressor or cell cycle regulatory genes with resultant cell cycle arrest, differentiation, and apoptosis of cancer cells (Jawed et al., 2014). The partial response rate is approximately 30% to 35%, with uncommon complete response. The most common side effects are GI symptoms, fatigue, and hematological abnormalities.

Alemtuzumab is a humanized monoclonal antibody directed against CD52, which is found on immune cells, including T and B cells. Binding of *alemtuzumab* to CD52 causes neutrophil-mediated, antibody-dependent cellular toxicity (Jawed et al., 2014). It has been effectively used off label for CTCL and may be a secondary treatment option for patients with CTCL refractory to other therapies. It is commonly associated with infusion reactions and causes prolonged immunosuppression. Early studies reported opportunistic infections, so antimicrobial prophylaxis is recommended.

Mogamulizumab is a defucosylated humanized $\text{IgG}_{1\kappa}$ monoclonal antibody that selectively binds to the C-C chemokine receptor 4 (CCR4) on target cells, targeting them for antibody-dependent cellular cytotoxicity. The CCR4 chemokine receptor is normally expressed on regulatory T and T_H2 cells and highly expressed on many malignant T cells. The defucosylation leads to a 50-fold higher binding affinity for FcγRIIIa, which results in improved activation of antibody-dependent cellular cytotoxicity. *Mogamulizumab* is approved for the treatment of adult patients with relapsed or refractory mycosis fungoides (CTCL) or Sézary syndrome after at least one prior systemic therapy. *Mogamulizumab* must be administered slowly over at least 60 min with monitoring for infusion reactions, some of which can be severe.

Denileukin diftitox is a fusion protein composed of diphtheria toxin fragments A and B linked to the receptor-binding portion of IL-2. In the U.S., *denileukin diftitox* carried a boxed warning from the FDA, alerting practitioners to serious and fatal reactions of the drug, including capillary leak and vision loss. *Denileukin diftitox* is not currently available in the U.S.

Targeted Therapies for Melanoma

Until recently, therapies for metastatic melanoma had low response rates along with either high toxicity or no effect on long-term survival. There has been rapid development of several new therapeutic options for metastatic or unresectable melanoma, primarily geared toward inhibition of mutated, abnormally activated tyrosine kinases that drive growth or enhance immune function to combat malignant cells by inhibiting immune checkpoints (John and Cowey, 2015). These therapies include BRAF inhibitors (*vemurafenib, dabrafenib, encorafenib*), MEK inhibitors (*trametinib, cobimetinib, binimetinib*), inhibitors of cytotoxic T lymphocyte–associated protein 4 (CTLA4) (*ipilimumab*), and PD-1 inhibitors (*pembrolizumab, nivolumab*). These targeted treatments are discussed further in Chapters 71 and 72. An additional novel therapy for unresectable melanoma is *talimogene laherparepvec* (T-Vec), an oncolytic immunotherapy derived from genetically modified attenuated HSV type 1. It is administered by intralesional injection and is designed to selectively replicate within tumors and produce GM-CSF to enhance systemic antitumor immune responses. *Talimogene* is contraindicated in pregnancy and in immunosuppressed patients.

Treatment of Pruritus

Pruritus (itching) occurs in a multitude of dermatological disorders, including xerosis (dry skin), atopic dermatitis, urticaria, and infestations. Itching also may be a sign of internal disorders, including malignant neoplasms, chronic renal failure, and hepatobiliary disease. In addition to treating the underlying disorder, a general approach to the treatment of pruritus can be made by classifying pruritus into one of four clinical categories, as depicted by Table 75–12.

Drugs for Hyperkeratotic Disorders

Keratolytic agents reduce hyperkeratosis through myriad mechanisms (e.g., breaking of intercellular junctions, increasing stratum corneum water content, increasing desquamation). Common disorders treated with keratolytics include psoriasis, seborrheic dermatitis, xerosis, ichthyoses, and verrucae.

α-Hydroxy acids can reduce the thickness of the stratum corneum by solubilizing components of the desmosome, activating endogenous hydrolytic enzymes, and drawing water into the stratum corneum, thereby facilitating cell separation. They also appear to increase glycosaminoglycans, collagen, and elastic fibers in the dermis and are used in various formulations to reverse photoaging. The FDA requires that cosmetics containing α-hydroxy acids be labeled with a sunburn alert warning that the product may increase sensitivity to the sun. α-Hydroxy acids used include glycolic, lactic, malic, citric, hydroxycaprylic, hydroxycapric, and mandelic.

Salicylic acid functions through solubilization of intercellular "cement," reducing keratinocyte adhesion, and softening the stratum corneum. Salicylism may occur with widespread and prolonged use, especially in children and patients with renal or hepatic impairment. Salicylate toxicity has been reported with application of as little as 1% to 2% *salicylic acid* in neonates when applied multiple times per day over a large body surface area (Madan and Levitt, 2014). *Salicylic acid*, while chemically not a true β-hydroxy acid, often is listed as such on cosmetic labels. Other β-hydroxy acid ingredients in cosmetics include β-hydroxybutanoic acid, δ-tropic acid, and trethocanic acid. Sun protection should accompany the topical application of these agents.

Urea, at low concentrations, increases skin absorption and retention of water, leading to increased flexibility and softness of the skin. At concentrations greater than 40%, *urea* denatures and dissolves proteins and is used to dissolve calluses or avulse dystrophic nails.

Sulfur is keratolytic, antiseptic, antiparasitic, and antiseborrheic. It exerts its keratolytic effect by reacting with cysteine within keratinocytes, producing cystine and hydrogen sulfide (H_2S). H_2S breaks down keratin, causing dissolution of the stratum corneum.

Propylene glycol (as 60%–100% aqueous solutions) increases the water content of the stratum corneum and enhances desquamation. It is most effective in disorders with retention hyperkeratosis.

TABLE 75–12 ■ AGENTS USED FOR THE TREATMENT OF PRURITUS

Pruritoceptive pruritus: Itch originating in the skin due to inflammation or other cutaneous disease

- Emollients—Repair of barrier function
- Coolants (menthol, camphor, calamine)—Counterirritants
- Capsaicin—Counterirritant
- Antihistamines—Inhibit histamine-induced pruritus
- Topical phosphodiesterase 4 inhibitors—Anti-inflammatory and antipruritic effects
- Topical steroids—Direct antipruritic and anti-inflammatory effects
- Other topical immunomodulators—Anti-inflammatory
- Phototherapy—Reduced mast cell reactivity and anti-inflammatory effects
- Thalidomide—Anti-inflammatory through suppression of excessive TNFα
- NK-1 inhibitors (aprepitant, serlopitant, tradipitant)—Neurokinin-1 receptor blockade on dermal mast cells and partial agonist of epidermal growth factor receptor (EGFR) on keratinocytes
- Cannabinoids—Modulate peripheral and central neuronal activity; anti-inflammatory through local modulation of keratinocytes and mast cells
- Botulinum toxin—Inhibition of neurotransmitter release including acetylcholine, glutamate, substance P, calcitonin gene-related peptide
- IL-31 inhibitors—Inhibition of IL-31 receptor signaling on peripheral sensory nerves decreases pruritus and improves skin barrier function (effect on keratinocytes)

Neuropathic pruritus: Itch due to disease of afferent nerves

- Carbamazepine—Blockade of synaptic transmission and use-dependent Na^+ channels
- Gabapentin, pregabalin—Mechanism unclear; thought to decrease transmission of nociceptive sensations by binding to the α-2-δ subunit of voltage-dependent Ca^{2+} channels
- Topical anesthetics (EMLA, benzocaine, pramoxine)—Inhibit nerve conduction via decreased nerve membrane permeability to sodium
- Ketamine—NMDA receptor antagonist
- Cannabinoids—Modulate peripheral and central neuronal activity; anti-inflammatory through local modulation of keratinocytes and mast cells
- Botulinum toxin—Inhibition of neurotransmitter release including acetylcholine, glutamate, substance P, calcitonin gene-related peptide

Neurogenic pruritus: Itch that arises from the nervous system without evidence of neural pathology

- Thalidomide—Central depressant
- Opioid receptor antagonists (naloxone, naltrexone)—Decrease opioidergic tone
- Tricyclic antidepressants—Decrease pruritus signaling through alteration in neurotransmitter concentrations
- SSRIs—Decrease pruritus signaling through alteration in neurotransmitter concentrations

Psychogenic pruritus: Itch due to psychological illness

- Anxiolytics (benzodiazepines)—Relieve stress-reactive pruritus
- Antipsychotic agents (chlorpromazine, thioridazine, thiothixene, olanzapine)—Relieve pruritus with impulsive qualities
- Tricyclic antidepressants—Relieve depression and insomnia related to pruritus
- SSRIs—Relieve pruritus with compulsive qualities

NMDA, *N*-methyl-D-aspartate; SSRIs, selective serotonin reuptake inhibitors.

Drugs Affecting Hair Growth

Androgenic Alopecia

Androgenetic alopecia, commonly known as male and female pattern baldness, is the most common cause of hair loss in adults older than 40 years. The frequency and severity increase with age and may begin during puberty in some patients. It is a genetically inherited trait with variable expression. In susceptible hair follicles, dihydrotestosterone (DHT) binds to the androgen receptor, and the hormone-receptor complex activates the genes responsible for the gradual transformation of large terminal follicles into miniaturized vellus follicles. Treatment of androgenetic alopecia is aimed at reducing hair loss and maintaining existing hair (Varothai and Bergfeld, 2014).

Minoxidil, originally developed as an antihypertensive agent, was noted to be associated with hypertrichosis in some patients. *Minoxidil* enhances follicular size, resulting in thicker hair shafts, and stimulates and prolongs the anagen phase of the hair cycle, resulting in longer and increased numbers of hair. Treatment must be continued or any drug-induced hair growth will be lost. Patients with increased sulfotransferase enzyme activity are most likely to respond to *minoxidil* treatment; this may be a useful predictive test in the future (Roberts et al., 2014). Topical *minoxidil* is available as a 2% solution and a 5% solution or foam. Allergic and irritant contact dermatitis can occur, and care should be taken in applying the drug because hair growth may emerge in undesirable locations. This is reversible on stopping the drug. Patients should be instructed to wash their hands after applying *minoxidil*. Oral low-dose *minoxidil* (0.25 – 5 mg daily) has been used off label for androgenic alopecia with recommended dosages of 0.5 – 2.5 mg daily in females or 2.5 – 5 mg daily in males. The most common side effect with oral low-dose *minoxidil* is hypertrichosis. Additional potential side effects include postural hypotension, lower extremity edema, tachycardia, and headache.

Finasteride inhibits the type II isozyme of 5α-reductase, the enzyme that converts testosterone to DHT (see Chapter 49) and that is found in hair follicles. Balding areas of the scalp are associated with increased DHT levels and smaller hair follicles than nonbalding areas. Orally administered *finasteride* (1 mg/day) variably increases hair growth in men over a 2-year period. *Finasteride* is approved for use only in men but has been used off label for female pattern hair loss (Varothai and Bergfeld, 2014). *Finasteride* use in women is limited by its teratogenicity. Pregnant women should not be exposed to the drug because of the potential for inducing genital abnormalities in male fetuses. Adverse effects of *finasteride* include decreased libido, erectile dysfunction, ejaculation disorder, and decreased ejaculate volume. There have been postmarketing surveillance reports of persistent sexual dysfunction after stopping the medication. As with *minoxidil*, new hair growth will be lost when *finasteride* is discontinued. *Dutasteride* (0.5 mg/day) is a combined type I and type II isozyme 5α-reductase inhibitor that has similar or greater efficacy, but side effects may be more common (Varothai and Bergfeld, 2014).

Spironolactone is an aldosterone antagonist and K^+-sparing diuretic; it also has antiandrogen activity. It is used off label for female pattern alopecia at dosages of 50 to 200 mg/day (Varothai and Bergfeld, 2014). Women of reproductive potential should not receive *spironolactone* without reliable contraception because *spironolactone* can cause feminization of a male fetus.

Other Agents

Eflornithine is an inhibitor of ornithine decarboxylase (ODC) that is approved for the reduction of excessive and unwanted facial hair in women. ODC is the rate-limiting enzyme in the synthesis of polyamines, which are important in cellular migration, differentiation, and proliferation. Levels of ODC activity are higher in proliferating cells, such as in follicular matrix cells in anagen phase, and inhibiting ODC decreases hair growth. *Eflornithine* 13.9% cream is applied twice daily and is intended to be used along with the patient's preferred hair removal method to slow hair regrowth.

Bimatoprost is a prostaglandin analogue approved for topical treatment of hypotrichosis of the eyelashes by increasing their growth, including length, thickness, and darkness. The prostaglandin-mediated increase in eyelash growth was serendipitously noted during the use of intraocular prostaglandin analogues for glaucoma. The proposed mechanism of eyelash growth is by an increase in the fraction of hairs in, and the duration of, the anagen phase. Increased pigmentation of the hairs is thought to occur due to stimulation of melanin production without an increase in the number of melanocytes. Importantly, brown pigmentation of the iris and eyelid skin may occur, and the increased iris pigmentation may be permanent.

Treatment of Hyperpigmentation

The agents discussed are most effective on hormonally or light-induced pigmentation within the epidermis. They have limited efficacy on postinflammatory pigmentation within the dermis. Sun protection or avoidance is a vital component of any treatment regimen (Sheth and Pandya, 2011).

Hydroquinone (1,4-dihydroxybenzene) decreases melanocyte pigment production by inhibiting tyrosinase, the initial enzyme in the melanin biosynthetic pathway. In addition, it causes degradation of melanosomes and destruction of melanocytes by production of reactive oxygen radicals. Formulations containing 1.5% to 2% *hydroquinone* are available OTC; 3% to 4% *hydroquinone* formulations are available by prescription. Penetration enhancers and sunscreen ingredients are added to some formulations. A combination prescription product consisting of *hydroquinone* 4%, *fluocinolone* 0.01%, and *tretinoin* 0.05% is available. Adverse effects may include dermatitis and ochronosis.

Azelaic acid, a dicarboxylic acid isolated from cultures of *Malassezia furfur,* inhibits tyrosinase activity but is less effective than *hydroquinone*. Because it has mild comedolytic, antimicrobial, and anti-inflammatory properties, it also is often used in acne and papulopustular rosacea, especially in patients with postinflammatory hyperpigmentation.

Mequinol (4-hydroxyanisole) is a competitive inhibitor of tyrosinase. It was approved as a 2% prescription product in combination with 0.01% *tretinoin* and *vitamin C* for skin lightening, but it is not currently commercially available.

Monobenzone (monobenzyl ether of hydroquinone) causes *permanent* depigmentation and should *not* be used for routine hormonally induced or postinflammatory hyperpigmentation. A 20% cream is approved for final depigmentation therapy of extensive vitiligo affecting at least more than 50% body surface area; it is rarely used and not currently commercially available.

Glycolic acid is an α-hydroxy acid used in chemical peels for disorders of pigmentation. It is thought to work by inhibiting tyrosinase in a pH-independent manner and to cause exfoliation by decreasing keratinocyte adhesion. Potential side effects are erythema, desquamation, and postinflammatory hyperpigmentation. *Glycolic acid* peels are best used as adjunctive therapy along with other topical therapy in patients with refractory epidermal hyperpigmentation (Sheth and Pandya, 2011).

Tranexamic acid is a synthetic analogue of lysine that exhibits antifibrinolytic activity by competitively inhibiting transformation of plasminogen to plasmin. It is approved for the treatment of heavy menstrual bleeding or to prevent hemorrhage in patients with hemostatic defects undergoing tooth extraction. *Tranexamic acid* is used off label for the treatment of melasma. UV radiation induces plasminogen activator production by keratinocytes, which leads to increased melanogenesis through stimulation of melanocytes by plasmin, arachidonic acid, and fibroblast growth factor, and thence to increased neovascularization via vascular endothelial growth factor stimulation. *Tranexamic acid* mitigates this UV-induced melanogenesis and neovascularization through inhibition of plasminogen activation. *Tranexamic acid* has been used topically as a 2% to 5% formulation twice daily or intradermally at a concentration of 4 mg/mL with injection frequency ranging from once weekly to once monthly. The most common side effect with intradermal injection was injection site burning. *Tranexamic acid* has been used orally at a dosage of 250 to 325 mg twice daily. The most common side effect is abdominal bloating or headache. Patients should be screened for thromboembolic risk factors prior to systemic use.

Miscellaneous Agents

Capsaicin is an alkaloid derived from plants of the *Solanaceae* family (i.e., hot chili peppers). *Capsaicin* interacts with the transient receptor potential vanilloid type 1 (TRPV1) receptor on C-fiber sensory neurons. TRPV1 is a ligand-gated nonselective cation channel of the TRP family, modulated by a variety of noxious stimuli. Chronic exposure to *capsaicin* first stimulates and then desensitizes this channel to *capsaicin* and diverse other noxious stimuli. *Capsaicin* also causes local depletion of substance P, an endogenous neuropeptide involved in sensory perception and pain transmission. *Capsaicin* (cream, lotion, gel, roll-on, and transdermal patch) is FDA-approved for the temporary relief of minor aches and pains associated with backache, strains, and arthritis. It is also approved in patch form by prescription for postherpetic neuralgia and is used for off-label treatment of painful diabetic neuropathy and some forms of pruritus.

Bentoquatam (quaternium-18 bentonite) is a mixture of quaternium-18 (quaternary ammonium chloride salts made from the fatty acids of tallow) and bentonite clay. This organoclay mixture is approved for OTC use as a topical barrier to prevent allergic contact dermatitis to the urushiol resin of poison ivy, oak, or sumac. The 5% topical lotion must be applied prophylactically at least 15 min prior to potential risk for exposure to urushiol and reapplied every 4 h.

Coal tar is a distillation product from coal, a mixture of over 10,000 compounds. It is used in dermatology primarily for the treatment of inflammatory skin conditions such as psoriasis, seborrheic dermatitis, and atopic dermatitis or other forms of eczematous dermatitis. The mechanism of action is unknown, although it is known to suppress DNA synthesis. *Coal tar* has anti-inflammatory, antimicrobial, and antipruritic activity. In addition, it has a photosensitizing effect within the UVA and visible light spectrum between wavelengths of 330 and 550 nm. Multiple formulations and products are available commercially or through compounding, including those containing crude coal tar, coal tar extracts, or coal tar solution (Sandhu and Schwartz, 2011). *Coal tar solution*, also known as liquor carbonis detergens, is an alcohol extract of *coal tar* emulsified with polysorbate 80 to yield a more cosmetically acceptable product. *Coal tar* may be used in combination with UVB phototherapy (e.g., Goeckerman regimen), topical *salicylic acid*, or topical corticosteroids. *Coal tar* products are often poorly tolerated by patients due to its unpleasant odor, messiness, and potential for staining of clothing. It can also cause folliculitis or irritant contact dermatitis. Although occupational exposures to *coal tar* have been associated with malignancies (e.g., scrotal cancer), patients with psoriasis or atopic dermatitis who are treated with topical *coal tar* products have not exhibited increased risk of cancer (Menter et al., 2009a).

Anthralin (dithranol), a synthetic version of chrysarobin, is derived from the bark of the Brazilian araroba tree and is used in the treatment of psoriasis and alopecia areata; its mechanisms of action are unclear (Menter et al., 2009a). Use of *anthralin* has been limited by the potential to cause irritant contact dermatitis and to stain skin, hair, nails, fabrics, and household items.

Brimonidine (0.33% topical gel) is an α_2 adrenergic agonist approved for once-daily treatment of persistent erythema of rosacea. It causes temporary vasoconstriction, and rebound erythema has been reported. *Oxymetazoline* (1% cream) is an α_1 adrenergic agonist approved for once-daily treatment of persistent facial erythema of rosacea in adults. It also causes temporary vasoconstriction; rebound erythema occurs in less than 1% of patients.

Propranolol and *timolol* are nonselective β adrenergic antagonists traditionally used for cardiovascular disease. These agents are also effective in treating infantile hemangiomas and have become the preferred treatment of infantile hemangiomas requiring intervention (Chen et al., 2013). Topical *timolol* drops and gel-forming solution are available but do not adequately treat deeper lesions.

Wound Healing and Scar Formation

In normal wound healing, engrailed-1 (EN1) lineage-positive fibroblasts are activated to produce fibrotic scars that lack normal fiber orientation and normal skin components (e.g., sebaceous glands and hair follicles) and that have reduced mechanical strength. The resulting scars can be unsightly and detrimental to function. Tension-activated signaling via mechano-sensors drives EN1 expression and normal scar formation. Inhibitors of mechano-sensitive signaling pathways have been studied for their capacity to alter wound healing. The results with *verteporfin* and *VS-6062*, an inhibitor of focal adhesion kinase, are very encouraging.

Verteporfin is a benzoporphyrin derivative that is approved for use as a photosensitizer in photodynamic therapy for the treatment of subfoveal choroidal neovascularization. However, *verteporfin* has also been studied in vitro for effects wound healing that are mediated via a non-photoactivated mechanism. *Verteporfin* blocks mechano-transduction signaling through the Hippo pathway by inhibition of Yes-1 associated protein (YAP), which results in decreased En1 lineage-positive fibroblast activation in wounds. At appropriate dosage, *verteporfin* permits a response by En1-lineage negative fibroblasts that results in a regenerative response of normal skin with appropriate strength and ultrastructure without scarring (Mascharak et al., 2021).

Focal adhesion kinase (FAK) facilitates the normal linkage of mechanical forces to inflammatory signaling and fibrosis. *VS-6062* inhibits focal adhesion kinase and disrupts the linkage of mechanical stress to profibrotic differentiation of fibroblasts, resulting in faster wound healing/skin regeneration with reduced fibrosis (reduced scarring) and skin with nearly normal mechanical and structural properties (Chen et al., 2021). The detailed molecular pathways of scarring and regenerative healing are currently being delineated (Mascharak et al., 2022). One can imagine that continued progress in this area will prove important not only in wound healing but also in tissue repair and regeneration.

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Drug Facts for Your Personal Formulary: *Dermatological Agents*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Glucocorticoids—Table 75–3 and Chapter 50		
Topical glucocorticoids	• Psoriasis • Atopic dermatitis • Other inflammatory skin diseases	• Occlusion increases absorption • May cause skin atrophy, striae, periorificial dermatitis, folliculitis • Limit to ≤2–3 weeks of use • Avoid potent corticosteroids on face and genital areas • *Systemic* glucocorticoids for severe disease; see Chapter 50
Retinoids		
Topical retinoids (see Table 75–4)	• Acne • Facial wrinkling and photodamage • Psoriasis • Cutaneous Kaposi sarcoma (alitretinoin) • CTCL (bexarotene)	• Start every-other-night application to reduce likelihood of skin irritation • Decreased activity in presence of sunlight or BPO except adapalene and tazarotene • Avoid concurrent application of DEET due to potential increased DEET absorption
Systemic retinoids (see Table 75–5)	• Psoriasis (acitretin) • Acne (isotretinoin) • CTCL (bexarotene)	• Teratogenic: pregnancy should be avoided during and for 1 month (3 years for acitretin) after cessation of treatment • Multiple potential side effects, including cheilitis, dermatitis, conjunctivitis, myalgias, arthralgias, epistaxis, decreased night vision, hyperlipidemia
Topical Vitamin D Analogues		
Calcipotriene	• Psoriasis	• Potential hypercalcemia and hypercalciuria • May cause lesional or perilesional irritation
Photochemotherapeutic Agents (see Table 75–6)		
Sunscreen Active Ingredients (see Table 75–7)		
Biological Agents for Psoriasis (see Table 75–11)		
Antihistamines for Urticaria (see Chapter 43)		
Topical Antimicrobial Agents for Acne and Rosacea		
Azelaic acid	• Acne • Rosacea	• Comedolytic, antibacterial, anti-inflammatory • Also useful for postinflammatory hyperpigmentation due to acne
Benzoyl peroxide	• Acne	• Antibacterial, mildly comedolytic • Skin irritation with higher concentrations
Clindamycin	• Acne • Rosacea (off label)	• Antibacterial, anti-inflammatory • Bacterial resistance likely if used as monotherapy, use with benzoyl peroxide
Dapsone	• Acne	• Anti-inflammatory • Use with benzoyl peroxide causes orange-brown staining of skin or hair • G6PD testing not needed
Erythromycin	• Acne • Rosacea (off label)	• Antibacterial, anti-inflammatory • Bacterial resistance likely if used as monotherapy; use with benzoyl peroxide
Metronidazole	• Rosacea	• Anti-inflammatory
Minocycline	• Acne	• Antibacterial, anti-inflammatory
Sulfacetamide ± sulfur	• Acne • Rosacea	• Antibacterial, anti-inflammatory; sulfur also keratolytic • Use with benzoyl peroxide causes orange-brown staining of clothing but not skin • Sulfur may have pungent odor
Topical Antimicrobial Agents for Infection		
Bacitracin Neomycin Polymyxin B Gentamicin	• Superficial bacterial skin infections	• See Section VII, Chemotherapy of Microbial Diseases • Topical use restricted for superficial infections • Not indicated in clean surgical wounds • May cause contact dermatitis (especially bacitracin, neomycin)
Mupirocin Retapamulin	• Superficial bacterial skin infections due to *S. aureus* or *S. pyogenes* • Intranasal decolonization of MRSA	

Drug Facts for Your Personal Formulary: *Dermatological Agents (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Mafenide acetate	• Adjunctive therapy for burn wounds	• Inhibits carbonic anhydrase and can cause metabolic acidosis
Ozenoxacin	• Superficial bacterial skin infections due to *S. aureus* or *S. pyogenes*	
Silver sulfadiazine	• Prevention or treatment in partial-thickness burns or venous stasis ulcers	• Studies have failed to demonstrate efficacy for prevention or treatment in partial-thickness burns or venous stasis ulcers • May impede re-epithelialization
Topical Antifungal Agents (see Table 75–8)		
Oral Antifungal Agents (see Chapter 61)		
Topical Antiviral Agents (see Chapter 62)		
Acyclovir Penciclovir	• Orolabial HSV • Mucocutaneous or genital HSV (acyclovir ointment)	• Inhibits viral DNA synthesis and viral replication
Docosanol	• Orolabial HSV	• Prevents viral entry and replication
Cidofovir	• Off-label treatment for warts	• Inhibits viral DNA synthesis
Oral Antiviral Agents (see Chapter 62)		
Acyclovir Famciclovir Valacyclovir	• VZV • HSV	• Inhibits viral DNA synthesis and viral replication
Agents for Infestations		
Abametapir	• Head lice	• Inhibits metalloproteinases critical for louse survival and egg development
Benzyl alcohol	• Head lice	• Inhibits closure of respiratory spiracles; subsequent obstruction by mineral oil vehicle causes asphyxiation of lice
Ivermectin	• Head lice • Scabies (oral)	• Activates glutamate-gated chloride channels, causing hyperpolarization of nerve or muscle cells of parasite
Lindane	• Scabies • Lice	• Causes neuronal hyperstimulation, eventual parasite paralysis • Potential neurotoxicity with prolonged use or in patients with impaired skin barrier (e.g., atopic dermatitis)
Malathion	• Head lice	• Inhibits acetylcholinesterase, causing neuromuscular paralysis • Flammable due to high alcohol content
Permethrin	• Scabies • Lice	• Interferes with Na^+ transport, causing neurotoxicity and paralysis • Approved for infants ≥2 months • May cross-react with sunflower family plants to cause allergic contact dermatitis
Spinosad	• Head lice	• Causes CNS excitation and involuntary muscle contractions, leading to parasite paralysis
Crotamiton	• Scabies	• Mode of action is unknown • Less effective than other agents but has additional antipruritic effect
Precipitated sulfur	• Scabies	• Mode of action is unknown • Poor odor and mild skin irritation • Considered safe in pregnancy and infants
Systemic Cytotoxic, Immunosuppressant, and Immunomodulatory Agents		
Methotrexate	• Psoriasis • Off label for multiple inflammatory dermatoses	• See Chapter 70
Cyclophosphamide	• CTCL • Off label for severe autoimmune blistering dermatoses	• See Chapter 70
Vinblastine	• Kaposi sarcoma • CTCL	• See Chapter 70
Doxorubicin	• Kaposi sarcoma	• See Chapter 70
Azathioprine	• Off label for inflammatory and autoimmune blistering disorders	• Inhibits *de novo* purine synthesis to decrease T-cell and B-cell activation and proliferation • TPMT enzyme activity should be measured before initiation

Drug Facts for Your Personal Formulary: *Dermatological Agents (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Systemic Cytotoxic, Immunosuppressant, and Immunomodulatory Agents (cont.)		
Mycophenolate mofetil and mycophenolic acid	• Off-label for inflammatory and autoimmune blistering disorders	• Inhibits *de novo* purine synthesis to decrease T-cell and B-cell activation and proliferation • Most common side effect is GI upset
Cyclosporine	• Psoriasis • Off label for multiple inflammatory dermatoses	• Calcineurin inhibitor • Potential side effects: hypertension, renal dysfunction, hypertrichosis, gingival hyperplasia, tremor
mTOR inhibitors Sirolimus Everolimus Temsirolimus	• Off-label use in tuberous sclerosis, complex vascular malformations, and inflammatory dermatoses	• Inhibits mTOR • Potential side effects: stomatitis, mucositis, inflammatory cutaneous eruptions, nail changes
Dapsone	• Dermatitis herpetiformis • Leprosy • Neutrophilic dermatoses (off label)	• See Chapter 65
Thalidomide	• Erythema nodosum leprosum • Off label for prurigo nodularis, cutaneous lupus erythematosus, Behçet disease	• See Chapters 39 and 65
Topical or Intralesional Cytotoxic, Immunosuppressant, and Immunomodulatory Agents		
5-Fluorouracil	• Actinic keratoses • Superficial basal cell carcinoma • Warts (off label)	• See Chapter 70
Bleomycin	• Squamous cell carcinoma (off label) • Recalcitrant warts (off label)	• See Chapter 70
Alkylating agents Carmustine Mechlorethamine	• CTCL	• See Chapter 70
Podophyllum resin and podofilox	• Genital warts	• Inhibits microtubule polymerization, ⇒ mitotic arrest in metaphase • Side effects: irritation and ulcerative local reactions
Ingenol mebutate	• Actinic keratoses	• MOA: mitochondrial swelling and apoptosis of dysplastic keratinocytes
Tirbanibulin	• Actinic keratoses	• MOA: microtubule inhibition
Imiquimod	• Genital warts • Actinic keratoses • Superficial basal cell carcinoma	• Activates toll-like receptor 7 (TLR-7), inducing cytokines and upregulating immune response • Potential local skin reaction or systemic flu-like symptoms
Sinecatechins	• Genital warts	• MOA is uncertain • Potential local skin reactions, including erythema, pruritus, and swelling that peaks between 2 and 4 weeks of use
mTOR inhibitors Sirolimus Everolimus Temsirolimus	• Off-label use in tuberous sclerosis, complex vascular malformations, and some inflammatory dermatoses	• Topical mTOR inhibitors not currently commercially available but may be compounded • Topical use may decrease potential for side effects seen with systemic use
Topical calcineurin inhibitors Pimecrolimus Tacrolimus	• Psoriasis • Atopic dermatitis • Other inflammatory skin diseases	• Decreases T-cell activation • No skin atrophy • Useful in sensitive areas such as face and skinfolds • Common application site reactions (e.g., burning) decrease with continued use
Targeted Immunotherapies for Psoriasis and Atopic Dermatitis		
Inhibitors of TNFα, PDE4, JAKs Inhibitors of IL-12/23, IL-17, IL-23	• Psoriasis	• See Chapter 39 • See Table 75–11
Inhibitors of PDE4, JAKs Inhibitors of IL-4, IL-13	• Atopic dermatitis	• See Chapter 39

Drug Facts for Your Personal Formulary: *Dermatological Agents (continued)*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Targeted Antineoplastic Agents		
Smoothened and hedgehog inhibitors Vismodegib Sonidegib *PD-1 inhibitor* Cemiplimab	• Basal cell carcinoma	• See Chapter 72
PD-1 inhibitors Cemiplimab Pembrolizumab	• Squamous cell carcinoma	• See Chapter 72
Alemtuzumab Mogamulizumab Denileukin difitox Histone deacetylase inhibitors	• CTCL	• See Chapter 72
BRAF inhibitors MEK inhibitors CTLA4 inhibitors PD-1 inhibitors Talimogene laherparepvec	• Melanoma	• See Chapters 71 and 72
Topical Agents for Hyperkeratotic Disorders		
α-Hydroxy acids Glycolic acid Lactic acid	• Hyperkeratotic disorders	• Reduces keratinocyte adhesion by promoting degradation of corneodesmosomes • Potential skin irritation
Salicylic acid	• Hyperkeratotic disorders	• Reduces keratinocyte adhesion by affecting desmosomal adhesion proteins • Potential skin irritation • Potential salicylate toxicity with heavy use
Urea	• Hyperkeratotic disorders	• Increases hydration of stratum corneum, enhancing desquamation • Potential skin irritation
Sulfur	• Hyperkeratotic disorders	• May act via interaction with cysteine, causing reduction to hydrogen sulfide with subsequent breakdown of keratin • Pungent odor
Propylene glycol	• Hyperkeratotic disorders	• Increases hydration of stratum corneum, enhancing desquamation
Retinoids	• Hyperkeratotic disorders	• Stimulates keratinocyte turnover • Potential skin irritation
Agents Affecting Hair Growth		
Minoxidil, topical/oral	• Androgenetic alopecia	• Stimulates and prolongs anagen phase • Requires continued use to sustain effect • Oral preparation is used off label
Finasteride, oral Dutasteride, oral	• Androgenetic alopecia • Hirsutism (off label)	• Inhibits 5α reductase to decrease conversion of testosterone to DHT • Side effects: decreased libido, sexual dysfunction, hypotension
Spironolactone, oral	• Hirsutism (off label) • Female pattern alopecia (off label)	• Aldosterone antagonist with antiandrogenic activity • Side effects: breast tenderness, menstrual irregularities, increased urination • Feminization of male fetus
Eflornithine, topical	• Unwanted facial hair in women	• Ornithine decarboxylase inhibitor • Slows hair growth; use in combination with hair removal methods
Bimatoprost, topical	• Hypotrichosis of the eyelashes	• Prostaglandin analogue; increases fraction of hairs in anagen phase • May cause brown pigmentation of eyelid and iris (permanent)

Please note that there are numerous additional off-label uses of medications in dermatological conditions. MOA, mechanism of action.

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Chapter 76

Environmental Toxicology

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ASSESSMENT AND MANAGEMENT OF ENVIRONMENTAL RISK

EPIDEMIOLOGICAL APPROACHES TO RISK ASSESSMENT

- Epidemiological Studies
- Biomarkers

TOXICOLOGICAL APPROACHES TO RISK ASSESSMENT

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- Carcinogenesis
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- Lead
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- Ethylenediaminetetraacetic Acid
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- TCDD
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- PFAS

GI MICROBIOME AND ENVIRONMENTAL TOXICANTS

Humans are exposed to chemicals from their environment on a daily basis. Fortunately, mammals have evolved mechanisms to protect themselves from toxic effects of many exogenous chemicals, including the xenobiotic transport and metabolic mechanisms described in Chapters 4 to 7. While the human body is relatively well adapted to deal with xenobiotics, there are situations in which such environmental agents may cause significant toxicity. The Industrial Revolution and the development of chemical industries have increased human exposures to chemicals that were previously infrequent or absent. Concern about environmental toxicants has stimulated interest and research in environmental toxicology, the study of how chemicals in our environment adversely affect human health; and in occupational toxicology, the study of how chemicals in the workplace affect human health. Many authoritative textbooks are available in these areas. This chapter does not attempt a thorough coverage; rather, it sets forth a few basic principles, discusses carcinogens and chemoprevention and heavy metal intoxication and chelation therapy, and provides an overview of endocrine disruptors and immunotoxicants. The chapter concludes with emerging data on how microbiome-mediated metabolism contributes to xenobiotic biotransformation and toxicity.

Assessment and Management of Environmental Risk

People are exposed to many environmental *xenobiotics* at low doses over long periods of time, which poses challenges for assessing the risks from those exposures. Thus, the focus of environmental risk assessment is on the low end of the dose-response curve, using experiments based on chronic exposures. Unlike drugs, which are given to treat a specific disease and should have benefits that outweigh the risks, environmental toxicants are usually only harmful. In addition, exposures to environmental toxicants usually are involuntary, there is uncertainty about the severity of their effects, and people are much less willing to accept their associated risks.

Epidemiology and *toxicology* provide complimentary approaches to predict the toxic effects of environmental exposures. Epidemiologists monitor health effects in humans and use statistics to associate those effects with exposure to an environmental stressor, such as a toxicant. Toxicologists perform laboratory studies to examine potential toxic mechanisms of a chemical and to predict whether it is likely to be harmful to humans. These approaches provide complimentary information, necessitating integration of data from both fields to inform *environmental risk assessment*. Risk assessment is used to develop management approaches, such as laws and regulations, with the goal of limiting exposures to environmental toxicants to below a level that is considered safe.

Epidemiological Approaches to Risk Assessment

Epidemiologists use a variety of study designs to look for statistical associations between environmental exposures, including chemical exposures, and health outcomes. This approach has the advantage of examining the effects of real-world exposures to humans but can be expensive, subject to biases, confounding effects, and the inherent difficulties of attributing toxicity to one chemical entity when the exposure includes myriad.

Epidemiological Studies

Several types of epidemiological studies are used to assess risks, each with its own set of strengths and weaknesses.

- *Ecological studies* correlate frequencies of exposures and health outcomes between different geographical regions. These studies are relatively inexpensive and are effective for generating hypotheses but are subject to confounders and are not effective for establishing causality.

Abbreviations

AhR: aryl hydrocarbon receptor
AR: androgen receptor
ARE: antioxidant response element
ATSDR: Agency for Toxic Substances Disease Registry
BAL: British anti-Lewisite (dimercaprol)
BLL: blood lead level
BPA: bisphenol A
CaNa$_2$EDTA: calcium disodium ethylenediaminetetraacetic acid
CDC: U.S. Centers for Disease Control and Prevention
COX-2: cyclooxygenase 2
DDE: dichlorodiphenyldichloroethylene
DDT: dichlorodiphenyltrichloroethane
DEHP: di-2-ethylhexyl phthalate
DMPS: sodium 2,3-dimercatopropane sulfonate, dimercaprol
EDC: endocrine-disrupting chemical
EDTA: ethylenediaminetetraacetic acid
EPA: U.S. Environmental Protection Agency
ER: estrogen receptor
FSHR: follicle-stimulating hormone receptor
GI: gastrointestinal
GSH: reduced glutathione
Hg^0: elemental mercury
IARC: International Agency for Research on Cancer
LHR: luteinizing hormone receptor
LOAEL: lowest adverse effect level
MCL: maximum contaminant level
MeHg$^+$: methyl mercury
MMA: monomethylarsenic
NF-κB: nuclear factor-κB
NO: nitric oxide
NOAEL: no adverse effect level
NR: nuclear receptor
PAH: polycyclic aromatic hydrocarbon
PCB: polychlorinated biphenyls
PFAS: per- and poly-fluoroalkyl substances
PFOA: perfluorooctanoic acid
PFOS: perfluorooctane sulfonate
PKC: protein kinase C
PM: particulate matter
PPAR: peroxisome proliferator-activated receptor
RfD: reference dose
ROS: reactive oxygen species
TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin

- *Cross-sectional studies* examine the prevalence of exposures and outcomes at a single point in time. Such studies are an inexpensive way to determine an association but do not provide a temporal relationship and are not effective for establishing causality. They also can be subject to bias, as a health outcome under study might cause someone to eliminate his or her exposure.
- *Case-control studies* start with a group of individuals affected by a disease; this group then is matched to another group of unaffected individuals for known confounding variables. Questionnaires often are used to evaluate past exposures. This method also is relatively inexpensive and is good for examining rare outcomes because the end point is known. However, case-control studies rely on assessments of past exposures, which can be unreliable and subject to bias.
- *Cohort studies* measure exposures in a large group of people and follow that group for a long time to measure health outcomes. These studies are not as susceptible to bias and are better than case-control studies at establishing causality. However, they are expensive, particularly when measuring rare outcomes, because a large study population is required to observe sufficient disease to obtain statistical significance.
- *Clinical trials* (see Chapter 1) cannot be used to directly measure the effects of environmental toxicants (for obvious ethical reasons) but can be used to examine the effectiveness of an interventional strategy for reducing both exposures and disease.

Biomarkers

Because of the difficulties in assessing human exposures and the long times required to clinically observe effects on health, epidemiologists often rely on biomarkers in risk assessment. Different types of biomarkers provide different information useful for risk assessment. Biomarkers aim to be *specific*, only detecting the change in affected individuals, and *sensitive*, detecting the change in all affected individuals.

- *Biomarkers of exposure* provide information about dose or duration of exposures. Blood and urine concentrations of a chemical or its metabolite measure recent exposures, while levels in hair and toenails can measure exposure over a period of months. An example of an unusual exposure biomarker is X-ray fluorescent measurement of bone lead levels, which estimates lifetime exposure to lead.
- *Biomarkers of toxicity* are used to measure toxic effects at a subclinical level. Examples include measurement of liver enzymes in serum, changes in the quantity or contents of urine, and performance on specialized exams for neurological or cognitive function.
- *Biomarkers of susceptibility* are used to predict which individuals are likely to develop toxicity in response to a given chemical. Examples include single-nucleotide polymorphisms in genes for metabolizing enzymes involved in the activation or detoxification of a toxicant.
- Some biomarkers simultaneously provide information on exposure, toxicity, or susceptibility. For example, the measurement in the urine of N7-guanine adducts from aflatoxin B$_1$ provides evidence of both exposure and a toxic effect (in this case, DNA damage).

Toxicological Approaches to Risk Assessment

Toxicologists use model systems, including experimental animals, to examine the toxicity of chemicals and predict their effects on humans. Toxicologists test single chemicals over large dose ranges and at concentrations higher than would be environmentally relevant and over time frames that accommodate laboratory experimentation; this is done in order to get a robust toxic outcome and obtain statistical significance. As a result, there is often uncertainty about the effects of very low doses of chemicals or combinations of chemicals with real-world exposure times measured in decades. To determine the applicability of model systems, toxicologists also study the mechanisms involved in the toxic effects of chemicals, with the goal of predicting whether that mechanism would occur in humans.

To predict the toxic effects of environmental chemicals, toxicologists perform *subchronic studies* (3 months of treatment for rodents) and *chronic studies* (2 years for rodents) in at least two different animal models. Doses for these studies are based on shorter preliminary studies, with the goal of having one concentration that does not have a significant effect, one concentration that results in statistically significant toxicity at the low end of the dose-response curve, and one or more concentrations that will have moderate-to-high levels of toxicity. A theoretical dose-response curve for an animal study is shown in Figure 76–1.

An animal study provides two numbers that estimate the risk from a chemical:

- The *NOAEL* (no adverse effect level) is the highest dose used that does not result in a statistically significant increase in negative health outcomes.
- The *LOAEL* (lowest adverse effect level) is the lowest dose that results in a significant increase in toxicity.

The NOAEL is divided by 10 for each source of uncertainty to determine a reference dose (RfD), which is commonly used as a starting point

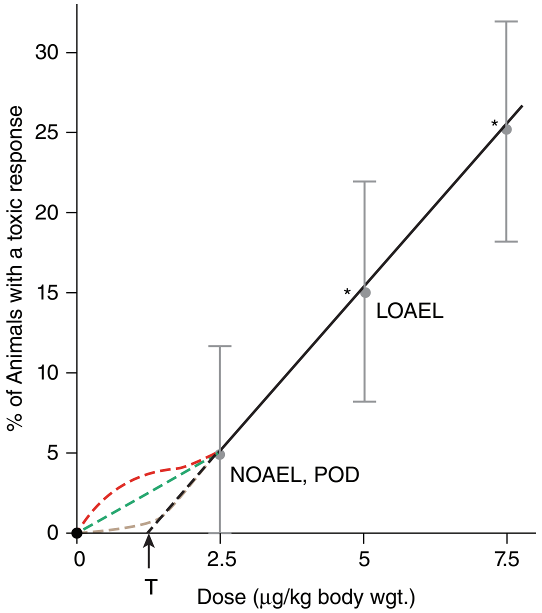

Figure 76–1 *LOAEL and NOAEL.* The theoretical dose-response curve from an animal study demonstrates the *NOAEL* and the *LOAEL*. Below the NOAEL level, there is considerable uncertainty regarding the shape of the response curve; thus, the NOAEL represents the *point of departure* (POD). The dose response could continue linearly below this point to reach a threshold dose (T) where there would be no harmful effects from the toxicant, or it could have a number of different possible inflection points. Each of these curves would have very different impacts on human populations. *Statistically significant toxicity.

for determining regulations on human exposures to chemicals. The modifiers used to determine the RfD are based on the uncertainties between experimental and human exposure. The most common modifiers used are for interspecies variability (human to animal) and interindividual variability (human to human), in which case RfD = NOAEL/100. Other modifiers can be used to account for specific experimental uncertainties, such as the unavailability of data from chronic studies. The use of factors of 10 in the denominator for determination of RfD is an application of the "precautionary principle," which attempts to limit human exposure by assuming a worst-case scenario for each unknown variable.

A major concern with animal studies is that they do not detect effects at low concentrations. Typically, they are designed to obtain statistical significance with a 10% to 15% increase in an outcome. As a result, there is considerable uncertainty about what occurs below that level, as demonstrated in Figure 76–1. Toxicologists often assume that there is a threshold dose below which there is no toxicity. However, the shape of the dose-response curve below the NOAEL is not known with certainty and represents a point of departure. The existence of a threshold dose assumes that there are cellular defenses that prevent toxicity at concentrations below a given level and that they can be overwhelmed. Some toxicants may not exhibit a threshold, while others, for which detoxification mechanisms exist, do have a threshold. Ideally, mechanistic studies should be done to predict which dose-response curve is most likely to fit a given chemical.

Toxicologists perform mechanistic studies to understand how a chemical might cause toxicity. Computer modeling using a compound's three-dimensional structure to determine quantitative structure-activity relationships is commonly performed on both drugs and environmental chemicals. Quantitative structure-activity relationship approaches can predict which chemicals are likely to exhibit toxicities or bind to specific molecular targets. Cell-based approaches in prokaryotes and eukaryotes are used to determine whether a compound damages DNA or causes cytotoxicity. DNA damage and the resulting mutagenesis often are determined with the Ames test, which uses strains of *Salmonella typhimurium* and *e. coli* with specific mutations in genes needed to synthesize essential amino acids (Walker et al, 2020). These strains are treated with chemicals in the presence or absence of a metabolic activating system (usually hepatic xenobiotic metabolizing enzymes). If a compound is a mutagen in the Ames test, it reverts the mutation and allows the bacteria to form colonies on plates that lack the specific essential amino acid targeted by the mutation. At the pathway level, RNA sequencing and other "omic" approaches provide a useful tool to identify the molecular targets and pathways altered in cells or tissues from animals exposed to a toxicant. To determine if a toxicant acts through a specific gene target, knockout cell lines or animal models can help to determine whether the specific knocked-out genes are involved in the mechanism of toxicity.

Carcinogens and Chemoprevention

Carcinogenesis

The International Agency for Research on Cancer (IARC) classifies the carcinogenicity of compounds into groups based on risk assessments using epidemiological, human exposure, animal, and mechanistic data. Chemicals in *group 1* are known human carcinogens; *group 2A* chemicals are chemicals that are probably carcinogenic in humans; *group 2B* are chemicals that are possibly carcinogenic in humans; *group 3* are chemicals for which data are lacking to suggest a role in carcinogenesis; and *group 4* are those for which the data indicate that they are unlikely to be carcinogens. Table 76–1 provides examples of group 1 carcinogens.

The transformation of a normal cell to a malignancy is a multistage process, and exogenous chemicals can act at one or more of these stages. A classic model of chemical carcinogenesis is *tumor initiation* followed by *tumor promotion* and *tumor progression*. In this model, an initiator causes gene mutations that increase the ability of cells to proliferate and avoid apoptosis. A promoter does not directly modify genes but changes signaling pathways or the extracellular environment to increase survival, proliferation, or invasiveness of precancerous cells. Chromosome instability and additional mutations ultimately lead to invasiveness and metastases. Although this model is an oversimplification of the many processes of carcinogenesis, it demonstrates the types of changes that must occur for a normal cell to transform into cancer. Chemical carcinogens cause cancer through genotoxic and nongenotoxic mechanisms (Figure 76–2).

Genotoxic carcinogens initiate tumor formation through damage to DNA. Typically, genotoxic carcinogens undergo metabolism in a target tissue to a reactive intermediate. This reactive intermediate can directly damage DNA via covalent reaction to form a DNA adduct. Alternatively, it can indirectly damage DNA through the formation of reactive oxygen species (ROS), which can oxidize DNA or form lipid peroxidation products that react with DNA. If DNA damage from a genotoxic carcinogen is not repaired prior to DNA replication, a mutation can result. If this mutation is in a key tumor suppressor gene or proto-oncogene, it can provide advantages in proliferation or survival. Alternatively, mutations to DNA repair genes can increase the probability that future mutations will occur. With the right extracellular environment, mutations in key genes will allow a cell to proliferate faster than surrounding normal cells and possibly develop into malignant cancer.

Benzo[*a*]pyrene, a key carcinogen in tobacco smoke, is an example of a genotoxic carcinogen that forms both direct DNA adducts and ROS. Benzo[*a*]pyrene is oxidized by CYPs to a 7,8-dihydrodiol, which represents a proximate carcinogen (a more carcinogenic metabolite). This metabolite either can undergo a second oxidation step by a CYP to form a diol epoxide, which readily reacts with DNA, or can undergo oxidation by aldo-keto reductases to form a catechol, which will redox cycle to form ROS (Conney, 1982; Penning, 2009).

Nongenotoxic carcinogens increase the incidence of cancer without damaging DNA, and most agents in this category are tumor promoters. Many nongenotoxic carcinogens bind to receptors that stimulate proliferation or other tumor-promoting effects, such as tissue invasion or angiogenesis. For example, phorbol esters mimic diacylglycerol and activate protein kinase C (PKC) isoforms. This activation in turn stimulates

TABLE 76–1 ■ EXAMPLES OF IMPORTANT GROUP 1 CARCINOGENS[a]

CARCINOGEN CLASS	EXAMPLE	SOURCE	MECHANISM
Genotoxic			
Nitrosamines	Nicotine-derived nitrosaminoketone (NNK)	Tobacco products	Metabolic activation to form DNA adducts
Polycyclic aromatic hydrocarbons	Benzo[a]pyrene	Fossil fuel combustion, tobacco smoke, charbroiled foods	Metabolic activation to form DNA adducts or ROS
Aromatic amines	2-Aminonaphthalene	Dyes	Metabolic activation to form DNA adducts
Fungal toxins	Aflatoxin B_1	Corn, peanuts, and other foods	Metabolic activation to form DNA adducts
Nongenotoxic			
Liver toxicants	Ethanol	Beverages, environment	Toxicity and compensatory proliferation; depletion of GSH
Phorbol esters	Tetradecanoyl phorbol acetate	Horticulture; rubber and gasoline production	Activation of PKC isoforms
Estrogens	Diethylstilbestrol	Drugs, environment	Activation of estrogen receptor signaling
Metals	Arsenic	Environment, occupation	Inhibition of DNA repair; activation of signal transduction pathways
Irritants	Asbestos	Environment, occupation	Stimulation of inflammation; formation of ROS
Dioxins	2,3,7,8-Tetrachlorodibenzop-dioxin (TCDD)	Waste incineration, herbicides, paperpulp bleaching	Activation of the aryl hydrocarbon (Ah) receptor

[a] Compounds in this table are classified as group 1 carcinogens by IARC, with the exception of the phorbol esters, which have not been examined.

mitogen-active protein kinase (MAPK) pathways, leading to proliferation, invasiveness, and angiogenesis (Chapter 3 presents these signaling pathways). In most normal cells, prolonged activation of this pathway stimulates apoptosis, but cells with defective apoptotic mechanisms due to preceding mutation(s) are resistant to cell death. Estrogenic carcinogens activate estrogen receptor alpha (ERα) and stimulate proliferation and invasiveness of estrogen-responsive cells. Chronic inflammation is another mechanism of nongenotoxic carcinogenesis. Inflammatory cytokines stimulate PKC signaling, leading to proliferation, invasiveness, and angiogenesis. Irritants such as asbestos are examples of carcinogens that work through inflammation. Chronic exposure to hepatotoxic chemicals (or chronic liver diseases) also causes nongenotoxic carcinogenesis by stimulating compensatory proliferation to repair the liver damage. This damage and repair process increases the likelihood of DNA mutations which can select for cells that proliferate faster or are less sensitive to cell death.

Many compounds can also act as complete carcinogens because repeated exposure can cause tumor initiation and promotion. Examples of complete carcinogens include benzo[*a*]pyrene and ultraviolet radiation.

Tumor initiation also may occur through nongenotoxic mechanisms. For example, some heavy metals do not directly react with DNA but interfere with proteins involved in DNA synthesis and repair, increasing the likelihood that an error will be made during replication. Nongenotoxic carcinogens also can cause heritable changes to gene expression by altering the methylation state of cytosines in 5′-CpG-3′ islands of gene promoters. Methylation can silence tumor suppressor genes, while demethylation of proto-oncogenes can increase their expression. These *epigenetic* effects can occur through sustained transcription or silencing of a gene.

Some chemicals enhance the carcinogenesis of other chemicals and thus act as *cocarcinogens*. These chemicals can increase the absorption or change the metabolism of other chemicals, increasing the probability

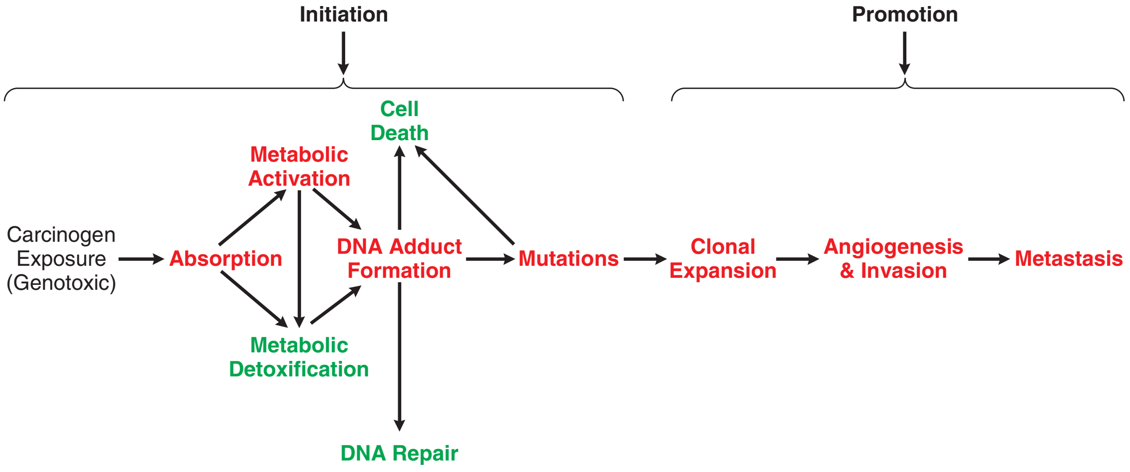

Figure 76–2 *Carcinogenesis: initiation and promotion.* There are multiple steps that occur between the exposure to a genotoxic carcinogen and the development of cancer. Processes in red lead to the development of cancer, while those in green reduce the risk. Nongenotoxic carcinogens act by enhancing steps leading to cancer or inhibiting protective processes. A chemopreventive agent acts by inhibiting steps leading to cancer or by increasing protective processes.

that they form active metabolites in target tissues. Cocarcinogens can also interfere with DNA repair, increasing the occurrence of mutations from a second genotoxic carcinogen. For example, ethanol acts as a solvent and increases the absorption of tobacco carcinogens, increasing the risk of head and neck cancers in people who both smoke and drink. Ethanol also depletes GSH (reduced glutathione), limiting target cells' ability to detoxify reactive metabolites of carcinogens and ROS.

Chemoprevention

Drugs that interfere with the carcinogenic process to prevent cancer before it is diagnosed are termed *chemopreventive agents* (Szabo, 2006; William et al., 2009). The chemoprevention concept was pioneered during the 1960s by Wattenberg and others, who observed that dietary constituents, such as *t*-butyl hydroquinone, could prevent cancer in rodents (Wattenberg, 1966). Chemoprevention strategies often are based on epidemiological studies on nutrition, where there are many examples of clear protective effects of plant-based foods and drinks on the incidence of various types of cancer (Fahey and Kensler, 2007). By isolating the active compounds from protective plants, researchers hope to understand their protective mechanisms and develop drugs to prevent cancer. Chemoprevention is an emerging field; a number of compounds for the prevention of cancer are in clinical trials (Table 76–2). Most chemicals tested for chemoprevention have not exhibited a benefit, and in some cases, they have increased the risk of cancer (Potter, 2014). There are currently no drugs approved for chemoprevention of environmental carcinogenesis, but there are approved drugs to prevent carcinogenesis due to endogenous estrogen (*tamoxifen* and *raloxifene*) and viruses (hepatitis B and human papillomavirus vaccines).

Chemopreventive agents can interfere with initiation or promotion (see Figure 76–2). One mechanism of anti-initiation is prevention of carcinogen activation. Isothiocyanates inhibit CYPs involved in activating many carcinogens and also upregulate genes controlled by the antioxidant response element (ARE); the ARE-responsive genes include γ-glutamylcysteine synthase light chain (which catalyzes the rate-determining step in GSH synthesis) and quinone reductase. Increased expression of ARE-regulated genes is predicted to increase the detoxification of proximate carcinogens. Isothiocyanates also stimulate apoptosis of p53-deficient cells via the formation of cytotoxic DNA adducts.

Compounds that act as antioxidants may provide protection because many carcinogens work through the generation of ROS. Some compounds simultaneously prevent carcinogen activation and act as antioxidants. For example, flavonoids and other polyphenols found in a wide variety of plants are potent antioxidants that also inhibit CYPs and induce expression of ARE-regulated genes. Chlorophyll and other compounds can protect against carcinogens by binding to or reacting with carcinogens or their metabolites and preventing them from reaching their molecular target.

Inflammation is a potential target for chemoprevention through interference with promotion. In phase III studies, the cyclooxygenase 2 (COX-2) inhibitor *celecoxib* demonstrated efficacy at reducing the risk of colorectal cancer. However, this benefit was offset by an increased risk of death due to cardiovascular events, forcing the early termination of the trial (William et al., 2009). Studies examining long-term treatment with *aspirin* for cardiovascular benefits found that *aspirin* also reduces the incidence of colorectal adenomas and overall cancer mortality. Naturally occurring compounds such as α-tocopherol have also been hypothesized to exert chemoprevention by reducing inflammation. However, α-tocopherol actually increased the risk of prostate cancer in a phase III trial (Potter, 2014).

Another approach to chemoprevention is disruption of nuclear receptor signaling. Retinoids reduce the incidence of head and neck cancers and represent one of the first successful uses of chemoprevention in

TABLE 76–2 ■ CHEMOPREVENTIVE AGENTS BEING STUDIED IN HUMANS

CHEMOPREVENTIVE CLASS	EXAMPLE COMPOUND	NATURAL SOURCE OR TYPE OF DRUG	CANCER TYPE(S)	MECHANISM	CURRENT STATUS
Isothiocyanates	Phenethyl isothiocyanate	Cruciferous vegetables (broccoli, cabbage, etc)	Liver, lung, breast, etc.	↓ CYP, ↑ GSH, ↑ NQO1, ↑ apoptosis	Phase II clinical trials
Synthetic drugs that modify metabolism	Oltipraz	Antischistosomal drug	Liver, lung	↓ CYP, ↑ GSH, ↑ NQO1	Beneficial effects on biomarkers in phase II clinical trials
Flavonoids and other polyphenols	Catechin	Green tea, red wine, berries, cacao, etc.	Lung, cervical, etc.	↓ ROS, ↓ CYP, ↑ GSH, ↑ NQO1	Phase II clinical trials
Other plant compounds	Curcumin	Turmeric (curry)	Colorectal, pancreatic, etc.	↓ ROS, ↓ CYP, ↑ GSH, ↑ NQO1	Phase II clinical trials
Other plant compounds	Chlorophyllin	All plants	Liver	Reaction with active intermediates, ↓ ROS, ↓ CYP	Beneficial effects on biomarkers in phase II clinical trials
Other antioxidants	α-Tocopherol (vitamin E)	Food	Prostate	Antioxidant, anti-inflammatory	Phase III clinical trials found ↑ prostate cancer with α-tocopherol
Antihormonal therapies	Tamoxifen	Adjuvant for breast cancer	Breast	Inhibit ERα in breast	FDA-approved for chemoprevention
NSAIDs (see Chapter 42)	Aspirin	Anti-inflammatory drugs	Colorectal, etc.	Inhibit PG formation	Phase III trials of aspirin for prevention of cardiovascular disease observed ↓ cancer; phase III trials for cancer prevention are ongoing
COX-2 selective inhibitors (see Chapter 42)	Celecoxib	Anti-inflammatory drugs	Colorectal, etc.	Inhibit PG formation	Phase III trial found ↓ cancer but unacceptable side effects for prevention

NSAID, nonsteroidal anti-inflammatory drug; PG, prostaglandin.

humans (Evans and Kaye, 1999; William et al., 2009). Retinoids are also effective for the treatment for acute promyelocytic leukemia (see Section VII). However, in large clinical trials, retinoids increased the incidence of lung cancer, particularly among women, and had other unacceptable toxicities (Omenn et al., 1996).

The selective ER modulators *tamoxifen* and *raloxifene* reduced the incidence of breast cancer in high-risk women in large phase III clinical trials and are approved for chemoprevention in these patients (Vogel et al., 2006). The success of selective ER modulators for chemoprevention provides a proof-of-principle that the development of compounds based on mechanistic predictions of antipromotion activity can lead to effective drugs for the prevention of cancer.

Aflatoxin B_1

Agents are being developed as chemopreventants of hepatocarcinogenesis mediated by aflatoxin B_1. Aflatoxins are produced in regions with hot and wet climates by *Aspergillus flavus*, a fungus that is a common contaminant of foods, especially corn, peanuts, cottonseed, and tree nuts. As a result of exposure to aflatoxin, human hepatocellular carcinoma is a serious problem in subtropical and tropical regions of Latin America, Africa, and Southeast Asia. Human exposure to aflatoxin in the U.S. is rare and not thought to have a significant impact on health (IARC, 2002).

ADME

Aflatoxin B_1 is readily absorbed from the gastrointestinal (GI) tract and initially distributed to the liver, where it undergoes extensive first-pass metabolism (Guengerich et al., 1996). Aflatoxin B_1 is metabolized by CYPs 1A2 and 3A4 to yield either an 8,9-epoxide or products hydroxylated at the 9 position (aflatoxin M_1) or 3 position (aflatoxin Q_1; Figure 76–3). While the hydroxylation products are detoxification products, the 8,9-epoxide reacts with DNA and is responsible for aflatoxin carcinogenesis. The 8,9-epoxide is short lived and undergoes detoxification via nonenzymatic hydrolysis or conjugation with GSH. Aflatoxin M_1 enters the circulation and is excreted in urine and milk.

Toxicity

Aflatoxin B_1 primarily targets the liver, although it also is toxic to the GI tract and hematological system. High-dose exposures result in acute necrosis of the liver, leading to jaundice and, in many cases, death. Acute toxicity from aflatoxin is rare in humans and requires consumption of milligram quantities of aflatoxin per day for multiple weeks. Chronic exposure to aflatoxins results in cirrhosis of the liver and immunosuppression.

Carcinogenicity

Based on increased incidence of hepatocellular carcinoma in humans exposed to aflatoxin and on supporting animal and mechanistic data, IARC has classified aflatoxin B_1 as a known human carcinogen (group 1) (IARC, 2002). Aflatoxin exposure and the hepatitis B virus work synergistically to cause hepatocellular carcinoma. Aflatoxin or hepatitis B exposure alone increases the risk of hepatocellular carcinoma 3.4- or 7.3-fold, respectively; those exposed to both have a 59-fold increased risk of cancer compared to unexposed individuals (Groopman et al., 2005).

Aflatoxin primarily forms DNA adducts at deoxyguanosine residues, reacting at either the N1 or N7 position (IARC, 2002); the 8,9-epoxide of aflatoxin B_1 readily reacts with amines. The N7-guanine adduct mispairs with adenine, leading to G → T transversions. Human aflatoxin exposure is associated with hepatocellular carcinomas bearing an AGG-to-AGT mutation in codon 249 of the *p53* tumor suppressor gene, resulting in the replacement of an arginine with cysteine (Hussain et al., 2007).

Several mechanisms for the synergistic interaction between hepatitis B and aflatoxin are under investigation (Sylla et al., 1999; Moudgil et al, 2013). The X gene of the hepatitis B virus encodes a protein, HBx, that binds to and inhibits p53, resulting in suppressed nucleotide excision repair of aflatoxin B_1 adducts (Hussain et al., 2007). Hepatitis B also alters the metabolism of aflatoxin B_1 by upregulating CYP genes, including CYP3A4, and decreases GSH *S*-transferase activity. In addition, hepatocellular proliferation to repair damage done by hepatitis B infection increases the likelihood that aflatoxin-induced DNA adducts will cause mutations. The hepatotoxic and tumor-promoting effects of hepatitis B also could provide a more favorable environment for the proliferation and invasion of initiated cells.

Chemoprevention of Aflatoxin-Induced Hepatocellular Carcinoma

The relationship between aflatoxin metabolism and its carcinogenicity makes it an appealing target for chemopreventive strategies that modify its metabolism (see Figure 76–3). Inhibiting CYP activity or increasing GSH conjugation will reduce the intracellular concentration of the 8,9-epoxide and prevent DNA adduct formation (Groopman et al., 2008; Kensler et al., 2004). *Oltipraz*, an antischistosomal drug, potently inhibits CYPs and induces genes regulated by the ARE that are involved in GSH synthesis. *Oltipraz* increases the excretion of the *N*-acetylcysteine

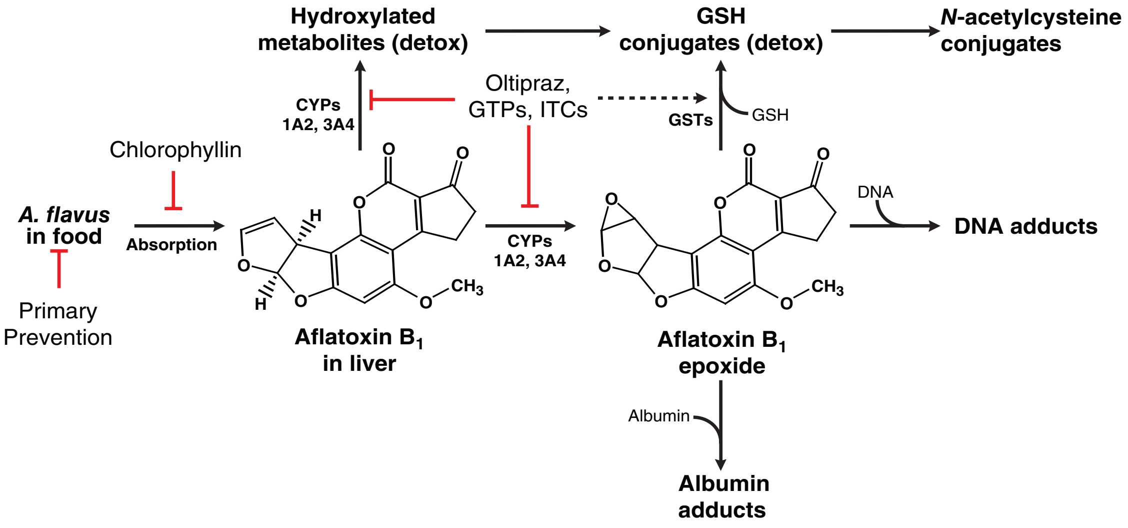

Figure 76–3 *Metabolism and actions of aflatoxin B_1.* Following absorption, aflatoxin B_1 undergoes activation by CYPs to its 8,9-epoxide, which can be detoxified by glutathione *S*-transferases (GSTs) or by spontaneous hydration. Alternatively, it can react with cellular macromolecules such as DNA and protein, leading to toxicity and cancer. *Oltipraz*, green tea polyphenols (GTPs), and isothiocyanates (ITCs) decrease aflatoxin carcinogenesis by inhibiting the CYPs involved in activating aflatoxin and increasing the synthesis of the cofactor GSH for GSTs involved in detoxification.

aflatoxin conjugate, indicating enhanced GSH conjugation of the epoxide. At 500 mg/week, *oltipraz* reduced the levels of aflatoxin M_1, consistent with inhibition of CYP activity.

Green tea polyphenols also have been used to modify aflatoxin metabolism in exposed human populations. Individuals receiving a daily dose of 500 or 1000 mg (equivalent to 1 or 2 L of green tea) demonstrated a small decline in the formation of aflatoxin-albumin adducts and a large increase in the excretion of the *N*-acetylcysteine aflatoxin conjugate, consistent with a protective effect.

Another approach used for the chemoprevention of aflatoxin hepatocarcinogenesis is the use of "interceptor molecules." *Chlorophyllin*, a mixture of water-soluble chlorophyll salts, binds tightly to aflatoxin in the GI tract, forming a complex that is not absorbed. *In vitro*, *chlorophyllin* inhibits CYP activity and acts as an antioxidant. In a phase II trial, administration of 100 mg of *chlorophyllin* with each meal reduced aflatoxin–N7-guanine adduct levels in the urine. Because of the strong interaction between hepatitis B and aflatoxin in carcinogenesis, the hepatitis B vaccine reduces the sensitivity of people to the induction of cancer by aflatoxin. Primary prevention of aflatoxin exposure through hand or fluorescent sorting of crops to remove those with fungal contamination also reduces human exposure. A cost-effective primary prevention approach is to improve food storage to limit the spread of *A. flavus*, which requires a warm and humid environment.

Water and Air Pollution

The U.S. Environmental Protection Agency (EPA) protects the public from water and airborne pollutants by enforcing compliance with the Clean Water Act (protects the public and the environment from water pollution); the Safe Drinking Water Act (ensures the quality of the drinking water); and the Clean Air Act (to protect the public from air pollutants). Under these laws, the EPA combines scientific risk assessment with economic analyses and other factors to develop regulations on the release or presence of specific pollutants to limit their effect on human health and the environment.

Toxicity screening of many water contaminants has led to the development of maximum contaminant levels (MCLs) for drinking water. To protect against potential adverse health effects, a primary MCL sets a level for a contaminant that must not be exceeded. The primary MCL is based on consideration of the toxic dose-response curve and on whether a sufficiently sensitive analytical method exists to measure the contaminant for compliance purposes. A secondary MCL is not enforced but is established for aesthetic value (e.g., taste, color, odor) that may not affect health. Currently, there is concern about pharmaceutical waste in the water supply, but MCLs have not been set for these compounds.

The EPA also monitors six priority air pollutants (CO, Pb, NO_x, O_3, particulate matter, and SO_2) that they have determined play the largest role in human health effects from air pollution. The EPA sets National Ambient Air Quality Standards in which local levels of these pollutants may not be exceeded within a given time frame. Measurements made by stationary air monitors provide daily air quality data and also lead to warnings (e.g., ozone awareness days), which advise citizens to stay indoors. Fine particles, smaller than 2.5 μm in diameter (PM2.5), are particularly concerning as they penetrate deep into the lung and can cause or exacerbate respiratory or cardiovascular illness.

Metals

Metals are an important class of environmental toxicants that come from both natural and anthropogenic sources. The U.S. Centers for Disease Control and Prevention (CDC) lists arsenic, mercury, and lead as the top three pollutants of concern, based on their toxicity and levels of human exposure. Although the toxicity of high levels of exposure to metals has long been known, the effects of low-dose chronic exposure to metals have only recently been appreciated. Many of the toxic metals in the environment also are carcinogens (Table 76–3). In addition, several essential metals also are toxic at high concentrations. Copper and iron are associated with toxicities, primarily targeting the liver through generation of ROS.

Lead

Chronic exposure to very low levels of lead has major deleterious effects, particularly for children.

Exposure

Despite substantial improvements over the past four decades, lead exposure remains a major concern, especially for children. In the U.S., paint containing lead for use in and around households was banned in 1978, while the use of tetraethyl lead in gasoline was phased out between 1976 and 1996. Despite these bans, past use of lead carbonate and lead oxide in paint and tetraethyl lead in gasoline remain the primary sources of lead exposure. Lead is not degradable and remains in dust, soil, and the paint of older homes. Young children are exposed to lead by nibbling sweet-tasting paint chips or ingesting dust and soil in and around older homes. Renovation or demolition of older buildings may cause substantial lead exposure. Removal of lead from gasoline reduced lead levels in air by more than 90% between 1982 and 2002, but lead fallout from air

TABLE 76–3 ■ TOXIC METALS WITH FREQUENT ENVIRONMENTAL OR OCCUPATIONAL EXPOSURE[a]

METAL	CERCLA PRIORITY[b]	COMMON SOURCE OF EXPOSURE	ORGAN SYSTEMS MOST SENSITIVE TO TOXICITY	IARC CARCINOGEN CLASSIFICATION
As	1	Drinking water	CV, skin, multiple other	Group 1, carcinogenic to humans—liver, bladder, lung
Pb	2	Paint, soil	CNS, blood, CV, renal	Group 2A, probably carcinogenic
Hg	3	Air, food	CNS, renal	Group 2B, possibly carcinogenic ($MeHg^+$); group 3, not classifiable (Hg^0, Hg^{2+})
Cd	7	Occupational, food, smoking	Renal, respiratory	Group 1, carcinogenic to humans—lung
Cr^{6+}	18	Occupational	Respiratory	
Be	42	Occupational, water	Respiratory	
Co	49	Occupational, food, water	Respiratory, CV	Group 2B, possibly carcinogenic
Ni	53	Occupational	Respiratory, skin (allergy)	Group 1, carcinogenic (soluble Ni compounds); group 2B, possibly carcinogenic (metallic Ni)—lung

[a] The ATSDR has both detailed monographs and brief summaries for each of these compounds (available at https://www.atsdr.cdc.gov). The IARC also has monographs available (http://monographs.iarc.fr).

[b] CERCLA, Comprehensive Environmental Response, Compensation, and Liability Act.

pollution remains in soils, particularly in urban environments. Lead was also commonly used in plumbing and can leach into drinking water, as evidenced by lead contamination in drinking water in numerous U.S. cities (Fedinick, 2021). As summarized by the Agency for Toxic Substances Disease Registry (ATSDR), lead exposure also has been traced to other sources, such as inhalation of dusts and fumes at firing ranges, retained bullets, artists' paint pigments, ashes and fumes from painted wood, lead-glazed pottery, lead toys, non-Western folk medicines, cosmetics, jewelers' wastes, home battery manufacture, and lead type (ATSDR, 2020). Blood lead levels (BLLs) in children have steadily decreased since the 1970s, when the mean level was 15 μg/dL. Currently, the average BLL in children is 1.3 μg/dL.

The CDC recently updated its recommendations to reflect the modern understanding that *there is no safe level of lead exposure for children* (CDC, 2012). The CDC no longer provides a "level of concern," as any lead exposure to a child is concerning. Instead, the CDC emphasizes primary prevention of lead exposure in all children and recommends that children with a BLL that places them in the top 2.5% (currently ≥5 μg/dL) be identified as in need of exposure reduction and additional screening. They did not change the existing recommendation that physicians consider chelation therapy for children with a BLL above 45 μg/dL.

Occupational exposure to lead also has decreased because of protective regulations. Occupational exposure generally is through inhalation of lead-containing dust and lead fumes. Workers in lead smelters and storage battery factories are at the greatest risk for lead exposure. Other workers at risk for lead exposure are those associated with steel welding or cutting, construction, rubber and plastic industries, printing, firing ranges, radiator repair shops, and any industry where lead is flame soldered (ATSDR, 2020).

Chemistry and Mode of Action

Lead exists in its metallic form and as divalent or tetravalent cations. Divalent lead is the primary environmental form; inorganic tetravalent lead compounds are not found naturally. Organo-lead complexes primarily occur with tetravalent lead and include the gasoline additive tetraethyl lead.

Lead toxicity results from molecular mimicry of other divalent metals (Garza et al., 2006), principally zinc and calcium. Because of its size and electron affinity, lead alters protein structure and can inappropriately activate or inhibit protein function.

ADME

Lead exposure occurs through ingestion or inhalation. GI absorption of lead varies considerably with age and diet. Children absorb a much higher percentage of ingested lead (~40% on average) than adults (<20%). Absorption of ingested lead is drastically increased by fasting. Dietary calcium or iron deficiencies increase lead absorption, suggesting that lead is absorbed through divalent metal transporters. The absorption of inhaled lead (~90%) generally is much more efficient than through dietary intake. Tetraethyl lead is readily absorbed through the skin, but transdermal absorption does not occur with inorganic lead.

About 99% of lead in the bloodstream binds to hemoglobin. Lead initially distributes in the soft tissues, particularly in the tubular epithelium of the kidney and the liver. Over time, lead is redistributed and deposited in bone, teeth, and hair. About 95% of the adult body burden of lead is found in bone. Growing bones will accumulate higher levels of lead and can form lead lines visible by radiography. Bone lead is slowly reabsorbed into the bloodstream, which can be accelerated when calcium levels are depleted, including during pregnancy. Small quantities of lead accumulate in the brain, mostly in gray matter and the basal ganglia. Lead readily crosses the placenta.

Lead is excreted primarily in the urine. The concentration of lead in urine is directly proportional to its free concentration in plasma (~1% of the total BLL). Lead is excreted in milk and sweat and deposited in hair and nails. The serum $t_{1/2}$ of lead is 1 to 2 months, with a steady state achieved in about 6 months. Lead accumulates in bone, where its $t_{1/2}$ is estimated at 20 to 30 years.

Health Effects

Although the effects of high-dose lead poisoning have been known for more than 2000 years, the insidious toxicities of chronic low-dose lead poisoning (BLL <20 μg/dL) have only recently been discovered. Lead is a nonspecific toxicant; the most sensitive systems are the nervous, hematological, cardiovascular, and renal systems (Table 76–4). Uncovering the effects of low-level lead exposure on complex health outcomes, such as neurobehavioral function and blood pressure, has been the subject of extensive research and the object of considerable public concern.

Neurotoxic Effects. The biggest concerns with low-level lead exposure are cognitive delays and behavior changes in children (ATSDR, 2020; Bellinger and Bellinger, 2006). The developing nervous system is very sensitive to the toxic effects of lead, with effects persisting down to the lowest measurable levels of lead (Cranfield et al., 2003; Lanphear et al., 2005).

Lead neurotoxicity primarily results from inhibition of Ca^{2+} transporters and channels and altered activities of Ca^{2+} responsive proteins, including PKC and calmodulin (Bellinger and Bellinger, 2006; Garza et al., 2006). These actions limit the normal activation of neurons in response to Ca^{2+} release and cause inappropriate production or release of neurotransmitters. Lead affects multiple neurotransmitter pathways, including the dopaminergic, cholinergic, and glutaminergic systems. At

TABLE 76–4 ■ HEALTH EFFECTS OF LEAD

BLL (μg/dL)	HEALTH EFFECT
Children	
<10	Inhibition of neural development Increased blood pressure Hearing loss Inhibition of hemoglobin synthesis enzymes Short stature Delayed sexual maturation (girls)
>10	Immunological effects (increased IgE)
>15	Increased erythrocyte protoporphyrin Decreased vitamin D
>30	Depressed nerve conduction velocity
>40	Anemia
>45	Chelation therapy warranted
>60	Colic
>70	Encephalopathy
Adults	
<10	Increased blood pressure Inhibition of hemoglobin synthesis enzymes Decreased glomerular filtration
>20	Increased erythrocyte protoporphyrin
>30	Hearing loss Enzymuria/proteinuria
>40	Peripheral neuropathy Reduced fertility Altered thyroid hormone levels Neurobehavioral effects
>50	Anemia
>60	Colic Chelation therapy warranted
>100	Encephalopathy

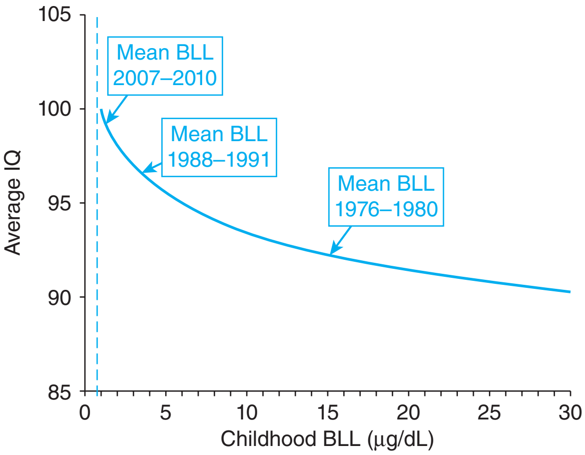

Figure 76–4 *Effect of childhood lead exposure on IQ.* Childhood exposure to lead shows a nonlinear dose-response relationship, with a steeper curve at low concentrations of lead (estimated dose-response curve based on data from Lanphear et al., 2005 and Canfield et al., 2003). For comparison, the average BLL from three iterations of the National Health and Nutritional Examination Survey are shown (Wheeler and Brown, 2013).

high concentrations, lead causes disruption of membranes, including the blood-brain barrier, increasing their permeability to ions.

Lead alters brain development by interfering with the pruning of synapses, neuronal migration, and the interactions between neurons and glial cells. Neurotransmitter release and PKC signaling determine which synapses are maintained and which are lost during brain development; this process is disrupted by lead. Lead-induced alterations in brain development can result in decreased IQ, poor performance on exams, language and motor deficits, and behavioral problems such as distractibility, impulsivity, aggression, short attention span, and inability to follow simple sequences of instructions (ATSDR, 2020; CDC, 2014). There is no evidence for a threshold; associations are evident even at the lowest measurable BLL (Figure 76–4). The cognitive and behavioral changes caused by lead vary considerably among children and may depend on the timing of exposure.

Children with very high BLL (>70 µg/dL) are at risk for encephalopathy. Symptoms of lead-induced encephalopathy include lethargy, vomiting, irritability, anorexia, and vertigo, which can progress to ataxia, delirium, and eventually coma and death. Mortality rates for lead-induced encephalopathy are about 25%, and most survivors develop long-term sequelae such as seizures and severe cognitive deficits.

Adults can also develop encephalopathy from lead exposure, although they are less sensitive than children. Workers chronically exposed to lead can develop neuromuscular deficits, termed *lead palsy*. Symptoms of lead palsy, including wrist drop and foot drop, were commonly associated with painters and other lead-exposed workers during previous eras but are rare today. Lead induces degeneration of motor neurons, usually without affecting sensory neurons. Studies in older adults have shown associations between lead exposure and decreased performance on cognitive function tests, suggesting that lead accelerates neurodegeneration due to aging (ATSDR, 2020).

Cardiovascular and Renal Effects. Elevated blood pressure is a lasting effect of low-level lead exposure (BLL <10 µg/dL). Adults who were exposed to lead during infancy and childhood have elevated blood pressure even in the absence of a recent exposure (ATSDR, 2020). Lead exposure is associated with an increased risk of death due to cardiovascular and cerebrovascular disease (Schober et al., 2006).

In the kidney, low-level lead exposure (BLL <10 µg/dL) depresses glomerular filtration. Higher levels (>30 µg/dL) cause proteinuria and impaired transport, while very high levels (>50 µg/dL) cause permanent physical damage, including proximal tubular nephropathy and glomerulosclerosis. Impaired glomerular filtration and elevated blood pressure are closely interrelated and likely have causative effects on one another (ATSDR, 2020).

The cardiovascular effects of lead are thought to involve the production of ROS, which react with nitric oxide (NO) to prevent vasodilation (Vaziri and Khan, 2007). It is not known how lead reduces glomerular filtration rate, although there is evidence that lead targets kidney mitochondria and interferes with the electron transport chain (ATSDR, 2020).

Other Effects. Lead causes both immunosuppression and increased inflammation, primarily through changes in helper T-cell and macrophage signaling; these effects can occur at low BLLs in children (Dietert and Piepenbrink, 2006). Lead inhibits the activity of several enzymes involved in the biosynthesis of heme; this effect persists down to very low BLL (<10 µg/dL; Figure 76–5). Chronic high-dose lead intoxication is associated with hypochromic microcytic anemia, which is observed more frequently in children and is morphologically similar to iron-deficient anemia. Acute high-dose lead exposure affects the smooth muscle of the gut, producing intestinal symptoms, termed *lead colic*.

Carcinogenesis. The IARC classifies lead in group 2A, "probably carcinogenic to humans" (IARC, 2006). Epidemiological studies show associations between lead exposure and cancers of the lung, brain, kidney, and stomach. Rodents exposed to lead develop kidney tumors, and some rats develop gliomas. Lead is not mutagenic but increases clastogenic events. Lead carcinogenesis may result from inhibition of DNA-binding zinc-finger proteins, including those involved in DNA repair and synthesis. Lead is a good example of a nongenotoxic carcinogen.

Treatment

The most important response to lead poisoning is to remove the source of lead exposure. Supportive measures should be undertaken to relieve symptoms.

Chelation therapy is warranted for children and adults with very high BLLs (>45 µg/dL and >60 µg/dL, respectively) or acute symptoms of lead poisoning (Ibrahim et al., 2006). For children with a BLL above 45 µg/dL but below 70 µg/dL, oral chelation is recommended. A BLL above 70 µg/dL in a child is a medical emergency requiring hospitalization and immediate intravenous chelation (American Academy of Pediatrics, 2005).

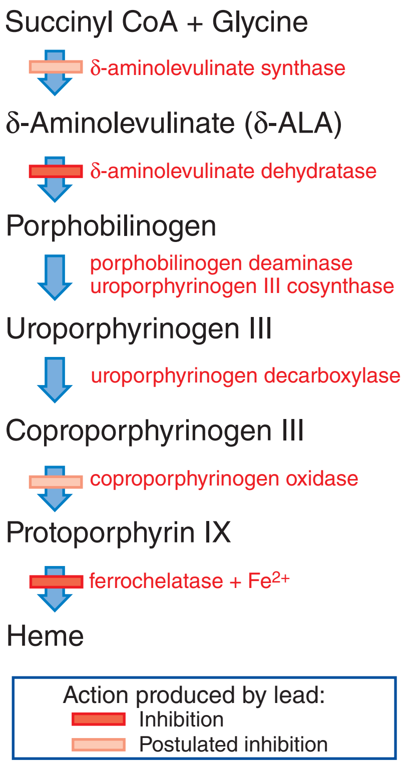
Succinyl CoA + Glycine
δ-aminolevulinate synthase
δ-Aminolevulinate (δ-ALA)
δ-aminolevulinate dehydratase
Porphobilinogen
porphobilinogen deaminase
uroporphyrinogen III cosynthase
Uroporphyrinogen III
uroporphyrinogen decarboxylase
Coproporphyrinogen III
coproporphyrinogen oxidase
Protoporphyrin IX
ferrochelatase + Fe2+
Heme
Action produced by lead:
Inhibition
Postulated inhibition

Figure 76–5 *Actions of lead on heme biosynthesis.* CoA, coenzyme A.

Although chelation therapy is effective at lowering BLL and relieving acute symptoms of lead poisoning, it does not reduce the chronic effects of lead beyond the benefit of lead abatement alone (Rogan et al., 2001). In rats, chelators enhance mobilization of lead from the soft tissues to the brain and may increase the adverse neurodevelopmental effects of lead (Andersen and Aaseth, 2002).

Mercury

Mercury is a unique metal in that it is a liquid at room temperature. Mercury has been used industrially since ancient Greece due to its capacity to amalgamate with other metals, and mercury's toxicity was noted by Hippocrates. Mercury also was used as a therapeutic drug for several centuries. Its use for the treatment for syphilis inspired Paracelsus's observation that "the dose makes the poison," one of the central concepts of toxicology, and also gave rise to the cautionary expression: "A night with Venus, a year with Mercury." The phrase "mad as a hatter" originated from the exposure of hatters to metallic mercury vapor during production of felt for hats using mercury nitrate. While the phrase likely inspired the character of the Mad Hatter in *Alice in Wonderland*, his symptoms are not consistent with mercury exposure.

Exposure

Inorganic mercury cations and metallic mercury are found in the Earth's crust, and mercury vapor is released naturally into the environment through volcanic activity and off-gassing from soils. Mercury also enters the atmosphere through human activities, such as combustion of fossil fuels and gold mining. In 2011, the EPA set the first standards to reduce mercury emissions from coal-fired power plants. Once in the air, metallic mercury is photo-oxidized to inorganic mercury, which can then be deposited in aquatic environments in rain. Microorganisms can then conjugate inorganic mercury to form methyl mercury ($MeHg^+$). $MeHg^+$ concentrates in muscle and other tissues and will bioaccumulate up the food chain (Figure 76–6). As a result, mercury concentrations in aquatic organisms at the top of the food chain, such as swordfish or sharks, are high (ATSDR, 1999).

The primary source of exposure to elemental mercury (Hg^0) in the general population is vaporization of Hg^0 in dental amalgam. Human exposure to organic mercury primarily is through the consumption of fish. Other foods contain inorganic mercury at low levels (ATSDR, 1999).

Workers are exposed to Hg^0 and inorganic mercury, most commonly through exposure to vapors. The highest risk for exposure is in the chloralkali industry (i.e., bleach) and in other chemical processes in which mercury is used as a catalyst. Mercury is a component of many devices, including alkaline batteries, fluorescent bulbs, thermometers, and scientific equipment, and exposure occurs during the production of these devices. Dentists also are exposed to Hg^0 from amalgam. Hg^0 can be used to extract gold during mining, which results in substantial occupational exposure. Mercuric salts are used as pigments in paints (ATSDR, 1999).

Thimerosal is an antimicrobial agent used as a preservative in some vaccines. Its use is controversial because it releases ethyl mercury, which is chemically similar to $MeHg^+$. Some parents have expressed concern that thimerosal might contribute to autism. Even though these concerns were based on a long-discredited report, the American Academy of Pediatrics and the U.S. Public Health Service issued a call for thimerosal's replacement in vaccines to improve the prevalence of vaccination, and thimerosal was removed from childhood vaccines in 2001 (Ball et al., 2001). Concurrently, studies found no association between thimerosal use in vaccines and negative outcomes, and thimerosal is still used in influenza vaccines (Heron and Golding, 2004). The FDA maintains a listing of the thimerosal content in vaccines (http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/VaccineSafety/UCM096228).

Chemistry and Mode of Action

There are three general forms of mercury of concern to human health. Metallic, or elemental, mercury (Hg^0) is the liquid metal found in scientific equipment and dental amalgam; it is volatile, and exposure is often to the vapor. *Inorganic mercury* can be either monovalent (*mercurous*, Hg^{1+}) or divalent (*mercuric*, Hg^{2+}) and forms a variety of salts. *Organic mercury* compounds consist of divalent mercury complexed with one or two alkyl groups. *Methyl mercury* ($MeHg^+$), which is formed environmentally from inorganic mercury by aquatic microorganisms, is of most concern. Both Hg^{2+} and $MeHg^+$ readily form covalent bonds with sulfur, which causes most of the biological effects of mercury. At very low concentrations, mercury reacts with sulfhydryl residues on many proteins and disrupts their functions. Microtubules are particularly sensitive to the toxic effects of mercury, which disrupts their formation and can catalyze their disassembly (Clarkson, 2002). There also may be an autoimmune component to mercury toxicity.

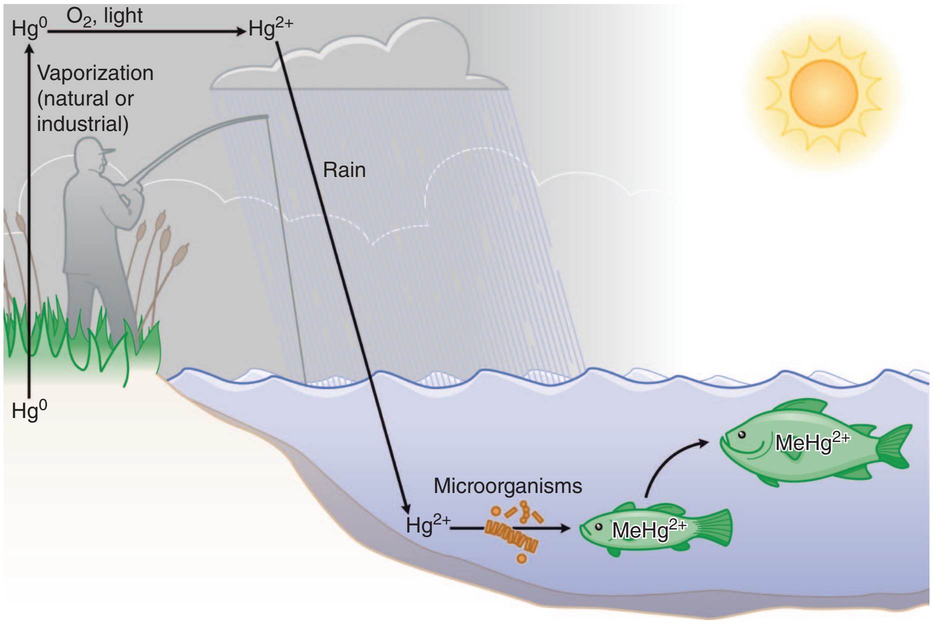

Figure 76–6 *Mobilization of mercury in the environment.* Metallic mercury (Hg^0) is vaporized from Earth's surface both naturally and through human activities such as burning coal. In the atmosphere, Hg^0 is oxidized to form divalent inorganic mercury (Hg^{2+}), which falls to the surface in rain. Aquatic bacteria can methylate Hg^{2+} to form methyl mercury ($MeHg^+$). $MeHg^+$ in plankton is consumed by fish. Because of its lipophilicity, $MeHg^+$ bioaccumulates up the food chain.

ADME

The vapor of Hg^0 is readily absorbed through the lungs (~70%–80%), but GI absorption of Hg^0 is negligible. Once absorbed, Hg^0 distributes throughout the body and crosses membranes, such as the blood-brain barrier and the placenta, via diffusion. Hg^0 is oxidized by catalase in cells to form Hg^{2+}. Shortly after exposure, some Hg^0 is eliminated in exhaled air. After a few hours, distribution and elimination of Hg^0 resemble the properties of Hg^{2+}. Hg^0 vapor can be oxidized to Hg^{2+} in the brain and retained (ATSDR, 1999).

Gastrointestinal absorption of mercury salts varies depending on the individual and on the particular salt and averages about 10% to 15%. Hg^{1+} will form Hg^0 or Hg^{2+} in the presence of sulfhydryl groups. Hg^{2+} is primarily excreted in the urine and feces; a small amount also can be reduced to Hg^0 and exhaled. With acute exposure, the fecal pathway predominates, but following chronic exposure, urinary excretion becomes more important. All forms of mercury also are excreted in sweat and breast milk and deposited in hair and nails. The $t_{1/2}$ for inorganic mercury is approximately 1 to 2 months (ATSDR, 1999).

Complexes between $MeHg^+$ and cysteine resemble methionine and can be recognized by transporters for that amino acid and taken across membranes (Ballatori, 2002). Orally ingested $MeHg^+$ is almost completely absorbed from the GI tract. $MeHg^+$ readily crosses the blood-brain barrier and the placenta and distributes fairly evenly to the tissues, although concentrations are highest in the kidneys (ATSDR, 1999). $MeHg^+$ can be demethylated to form inorganic Hg^{2+}. The liver and kidney exhibit the highest rates of demethylation, but this also occurs in the brain. $MeHg^+$ is excreted in the urine and feces, with the fecal pathway dominating. The $t_{1/2}$ for $MeHg^+$ is about 2 months. The toxicodynamic properties of $MeHg^+$ are thought to result from molecular mimicry.

Health Effects

Metallic Mercury. Inhalation of high levels of Hg^0 vapor over a short duration is acutely toxic to the lung. Respiratory symptoms of Hg^0 exposure start with cough and tightness in the chest and can progress to interstitial pneumonitis and severely compromised respiratory function. Other initial symptoms include weakness, chills, metallic taste, nausea, vomiting, diarrhea, and dyspnea. Acute exposure to high doses of Hg^0 is also toxic to the CNS, with symptoms similar to those of chronic exposure (Figure 76–7).

Toxicity to the nervous system is the primary concern with chronic exposure to Hg^0. Symptoms include tremors (particularly of the hands); emotional lability (irritability, shyness, loss of confidence, and nervousness); insomnia; memory loss; muscular atrophy; weakness; paresthesia; and cognitive deficits. These symptoms intensify and become irreversible with increases in duration and concentration of exposure. Other common symptoms of chronic mercury exposure include kidney damage, tachycardia, labile pulse, severe salivation, and gingivitis.

Inorganic Salts of Mercury. Ingestion of Hg^{2+} salts is intensely irritating to the GI tract, leading to vomiting, diarrhea, and abdominal pain. Acute exposure to Hg^{1+} or Hg^{2+} salts (typically in suicide attempts) leads to renal tubular necrosis, resulting in decreased urine output and often acute renal failure. Chronic exposures also target the kidney, with glomerular injury predominating.

Organic Mercury. The CNS is the primary target of $MeHg^+$ toxicity. Symptoms of high-dose $MeHg^+$ exposure include visual disturbances, ataxia, paresthesia, fatigue, hearing loss, slurring of speech, cognitive deficits, muscle tremor, movement disorders, and following severe exposure, paralysis and death. The developing nervous system exhibits increased sensitivity to $MeHg^+$. Children exposed *in utero* can develop severe symptoms, including intellectual disability and neuromuscular deficits, even in the absence of symptoms in the mother. In adults, $MeHg^+$ causes focused lesions in specific areas of the brain, while the brains of children exposed *in utero* sustain widespread damage (Clarkson, 2002).

The effects of low-dose $MeHg^+$ exposure from routine consumption of fish are difficult to assess due to the opposing beneficial effects of ω-3 fatty acids found in fish oils, and studies have produced discrepant results (Grandjean et al., 1999; Myers et al., 2003).

Treatment

Termination of exposure to Hg^0 is critical, and respiratory support may be required. Emesis may be used within 30 to 60 min of exposure to Hg^{1+} or Hg^{2+}, provided the patient is awake and alert and there is no corrosive injury. Maintenance of electrolyte balance and fluids is important for these patients. Chelation therapy is beneficial in patients with acute inorganic or metallic mercury exposure. There are limited treatment options for $MeHg^+$; chelation therapy does not provide clinical benefits, but nonabsorbable thiol resins may be beneficial by preventing absorption of $MeHg^+$ from the GI tract.

Because of the conflicting effects of mercury and ω-3 fatty acids, there is considerable controversy regarding the restriction of fish intake in women of reproductive age and children. The EPA recommends limiting fish intake to 12 oz (two meals) per week. Many experts feel this recommendation is too conservative. The recommendation that women consume fish that is lower in mercury content (i.e., canned light tuna,

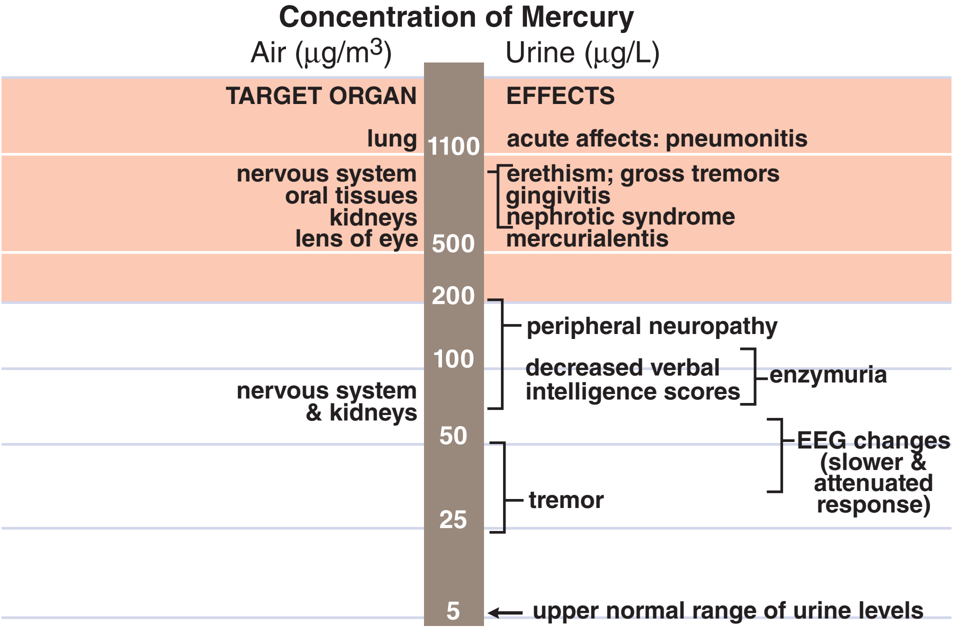

Figure 76–7 *Concentrations of mercury in air and urine are associated with specific toxic effects.*

salmon, pollock, catfish) and avoid top predators, such as swordfish, shark, and tilefish, is not controversial.

Arsenic

Arsenic is a metalloid that is common in rocks and soil. Arsenic compounds have been used for more than 2400 years as both therapeutic agents and poisons. In the late 19th century, Robert Ehrlich coined the terms *magic bullet* and *chemotherapy* to describe his work using the organic arsenic compound *arsphenamine* for the treatment for syphilis. The use of arsenic in drugs has been mostly phased out, but *arsenic trioxide* is still used as an effective chemotherapy agent for acute promyelocytic leukemia (see Chapter 70).

Exposure

The primary source of exposure to arsenic is through drinking water. Arsenic naturally leaches out of soil and rocks into well and spring water (Mead, 2005). Levels of arsenic in drinking water average 2 µg/L (ppb [parts per billion]) in the U.S. but can be more than 50 µg/L (five times the MCL set by the EPA) in private well water. Drinking water from other parts of the world, particularly Taiwan, China, Argentina, Chile, Bangladesh, and eastern India, can be contaminated with much higher levels of arsenic (sometimes several hundred micrograms per liter), and widespread poisonings have resulted (Figure 76–8). Arsenic can enter the environment through human activities, such as the use of arsenic-containing pesticides, mining, and burning of coal. Food, particularly seafood, often is contaminated with arsenic. Arsenic in seafood exists primarily as organic compounds (i.e., arsenobetaine), which are much less toxic than inorganic arsenic. The average daily human intake of arsenic is 10 µg/day, almost exclusively from food and water.

Before 2003, more than 90% of arsenic used in the U.S. was as a preservative in pressure-treated wood, but the lumber industry has voluntarily replaced arsenic with other preservatives. Arsenic-treated wood is thought to be safe unless burned. The major source of occupational exposure to arsenic is in the production and use of organic arsenicals as herbicides and insecticides. Exposure to metallic arsenic, arsine, arsenic trioxide, and gallium arsenide also occurs in high-tech industries, such as the manufacture of computer chips and semiconductors.

Chemistry and Mode of Action

Arsenic exists in its elemental form and trivalent (arsenites/arsenious acid) and pentavalent (arsenates/arsenic acid) states. Arsine (AsH_3) is a gaseous hydride of trivalent arsenic that exhibits toxicities that are distinct from other forms. Organic compounds of either valence state of arsenic are formed in animals. The toxicity of a given arsenical is related to the rate of its clearance from the body and its ability to concentrate in tissues. In general, toxicity increases in the sequence: organic arsenicals $< As^{5+} < As^{3+} < AsH_3$.

Like mercury, trivalent arsenic compounds form covalent bonds with sulfhydryl groups. The pyruvate dehydrogenase complex is particularly sensitive to inhibition by trivalent arsenicals: The two sulfhydryl groups of lipoic acid that participate in acetate transfer to coenzyme A react with arsenic to form a six-membered ring, effectively inhibiting pyruvate dehydrogenase and other lipoamide-containing enzymes. Inorganic arsenate (pentavalent) inhibits the electron transport chain. It is thought that arsenate competitively substitutes for phosphate during the formation of ATP, forming an unstable arsenate ester that is rapidly hydrolyzed.

ADME

The absorption of arsenic compounds is directly related to their aqueous solubility. Poorly water-soluble compounds such as arsenic sulfide, lead arsenate, and arsenic trioxide are not well absorbed. Water-soluble arsenic compounds are readily absorbed via inhalation and ingestion. GI absorption of arsenic dissolved in drinking water is more than 90% (ATSDR, 2016). At low doses, arsenic is fairly evenly distributed throughout the tissues of the body. Nails and hair, due to their high sulfhydryl content, exhibit high concentrations of arsenic. After an acute high dose (i.e., fatal poisoning), arsenic is preferentially deposited in the liver and, to a lesser extent, kidney, with elevated levels also observed in the muscle, heart, spleen, pancreas, lungs, and cerebellum. Arsenic readily crosses the placenta and blood-brain barrier.

Arsenic undergoes biotransformation in humans and animals (Figure 76–9). Trivalent compounds can be oxidized back to pentavalent compounds, but there is no evidence for demethylation of methylated arsenicals. Humans excrete much higher levels of monomethylarsenic (MMA) compounds than most other animals (ATSDR, 2016). Because the pentavalent methylated arsenic compounds have greatly reduced toxicity, the methylation pathway was long thought to be a detoxification pathway. However, the trivalent methylated arsenicals actually are more toxic than inorganic arsenite due to an increased affinity for sulfhydryl groups, and formation of MMA^{III} now is considered a bioactivation pathway (Aposhian and Aposhian, 2006).

Elimination of arsenicals by humans primarily is in the urine, although some is also excreted in feces, sweat, hair, nails, skin, and exhaled air. Compared to most other toxic metals, arsenic is excreted quickly, with a $t_{1/2}$ of 1 to 3 days. In humans, ingested inorganic arsenic that appears in urine is a mixture of 10% to 30% inorganic arsenicals, 10% to 20% monomethylated forms, and 60% to 80% dimethylated forms.

Health Effects

With the exception of arsine gas, the various forms of inorganic arsenic exhibit similar toxic effects. Inorganic arsenic exhibits a broad range of toxicities and has been associated with effects on every organ system tested (ATSDR, 2016). Humans also are exposed to large organic arsenic compounds in fish, compounds that are relatively nontoxic. Humans are the most sensitive species to the toxic effects of inorganic arsenic. Acute exposure to large doses of arsenic (>70–180 mg) often is fatal. Death immediately following arsenic poisoning typically is the result of its effects on the heart and GI tract. Death sometimes occurs later as a result of arsenic's combined effect on multiple organs.

Cardiovascular System. Acute and chronic arsenic exposure causes myocardial depolarization, cardiac arrhythmias, and ischemic heart disease; these are known side effects of arsenic trioxide for the treatment for leukemia. Chronic exposure to arsenic can cause peripheral vascular disease, the most dramatic example of which is "blackfoot disease," a condition characterized by cyanosis of the extremities, particularly the feet, progressing to gangrene. Blackfoot disease is endemic in regions of Taiwan, where well water contains arsenic at levels of 170 to 800 µg/L. Arsenic dilates capillaries and increases their permeability; this causes edema after acute exposures and is likely responsible for peripheral vascular disease following chronic exposure. Methylation of DNA may mediate some of arsenic's deleterious effects on the cardiovascular system (Domingo-Relosso et al., 2022) (see *Carcinogenesis*, below).

Skin. Dermal symptoms often are diagnostic of arsenic exposure. Arsenic induces hyperkeratinization of the skin (including formation of multiple corns or warts), particularly of the palms of the hands and the soles of the feet. It also causes areas of hyperpigmentation interspersed with spots of hypopigmentation. These symptoms can be observed in individuals exposed to drinking water with arsenic concentrations of at least 100 µg/L and are typical in those chronically exposed to much higher levels. Hyperpigmentation can be observed after 6 months of exposure; hyperkeratinization takes years. Children are more likely than adults to develop these effects (ATSDR, 2016; Mead, 2005).

GI Tract. Acute or subacute ingestion of high-dose arsenic is associated with GI symptoms ranging from mild cramping, diarrhea, and vomiting to GI hemorrhaging and death. GI symptoms are caused by increased capillary permeability, leading to fluid loss. At higher doses, fluid forms vesicles that can burst, leading to inflammation and necrosis of the submucosa and then rupture of the intestinal wall. GI symptoms are not observed with chronic exposure to lower levels of arsenic.

Nervous System. The most common neurological effect of acute or subacute arsenic exposure is peripheral neuropathy involving both sensory and motor neurons. This effect is characterized by the loss of sensation in the hands and feet, often followed by muscle weakness. Neuropathy occurs several days after exposure and can be reversible following

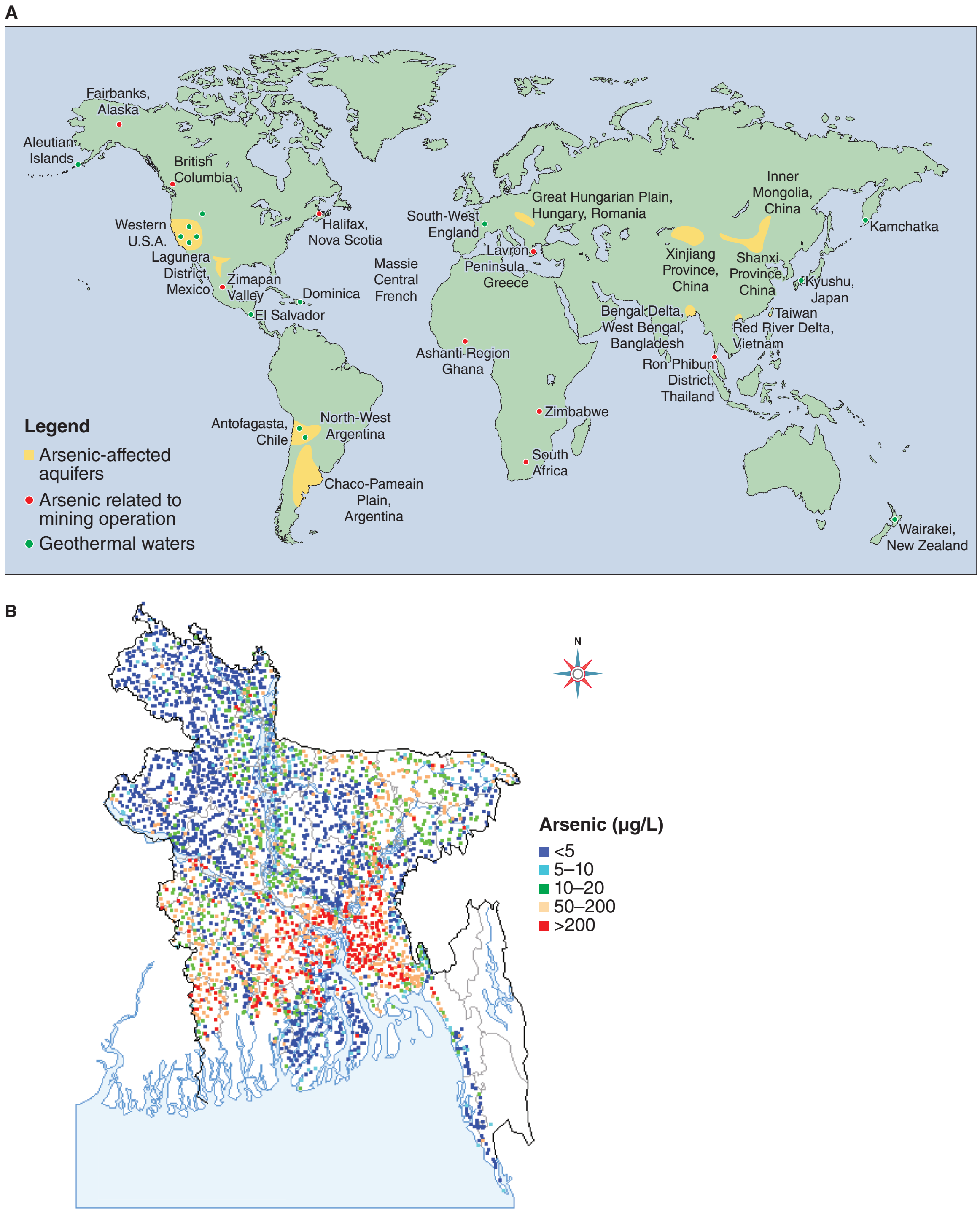
A
Fairbanks, Alaska
Aleutian Islands
British Columbia
Western U.S.A.
Lagunera District, Mexico
Zimapan Valley
Halifax, Nova Scotia
Dominica
El Salvador
Massie Central French
South-West England
Great Hungarian Plain, Hungary, Romania
Lavron Peninsula, Greece
Inner Mongolia, China
Kamchatka
Xinjiang Province, China
Shanxi Province, China
Kyushu, Japan
Taiwan
Red River Delta, Vietnam
Bengal Delta, West Bengal, Bangladesh
Ron Phibun District, Thailand
Ashanti-Region Ghana
Zimbabwe
South Africa
Antofagasta, Chile
North-West Argentina
Chaco-Pameain Plain, Argentina
Wairakei, New Zealand
Legend
🟨 Arsenic-affected aquifers
● Arsenic related to mining operation
● Geothermal waters
B
N
Arsenic (µg/L)
<5
5–10
10–20
50–200
>200

Figure 76–8 *Arsenic in drinking water.* **A.** World map demonstrating regions where there is increased arsenic exposure in drinking water. **B.** Map of Bangladesh demonstrating arsenic concentrations in drinking water in samples from wells across the country. (Adapted from a report produced by the British Geological Survey [2001] and the Department of Public Health Engineering [Bangladesh] undertaking a project funded by the U.K. Department for International Development [DFID]. Any views expressed are not necessarily those of DFID.)

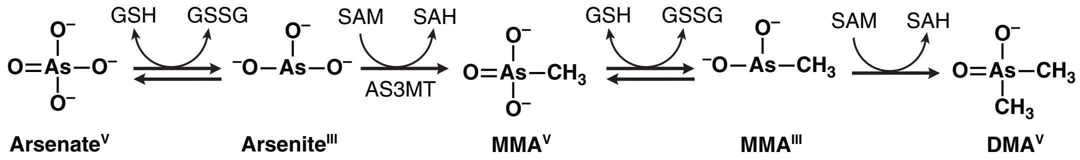
GSH
GSSG
SAM
SAH
AS3MT
GSH
GSSG
SAM
SAH
Arsenate^{V}
Arsenite^{III}
MMA^{V}
MMA^{III}
DMA^{V}

Figure 76–9 *Metabolism of arsenic.* AS3MT, arsenite methyltransferase; DMA^{V}, dimethylarsinic acid; GSH, reduced glutathione; GSSG, oxidized glutathione; MMA^{III}, monomethylarsonous acid; MMA^{V}, monomethylarsonic acid; SAH, S-adenosyl-L-homocysteine; SAM, S-adenosyl-L-methionine.

cessation of exposure, although recovery usually is not complete. Arsenic exposure may cause intellectual deficits in children (Wasserman et al., 2007). Acute high-dose arsenic exposure causes encephalopathy in rare cases, with symptoms that can include headache, lethargy, mental confusion, hallucination, seizures, and coma.

Other Noncancer Toxicities. Acute and chronic arsenic exposures induce anemia and leukopenia, likely via direct cytotoxic effects on blood cells and suppression of erythropoiesis. Arsenic also may inhibit heme synthesis. In the liver, arsenic causes fatty infiltrations, central necrosis, and cirrhosis of varying severity. The action of arsenic on renal capillaries, tubules, and glomeruli can cause severe kidney damage. Inhaled arsenic is irritating to the lungs, and ingested arsenic may induce bronchitis, progressing to bronchopneumonia in some individuals. Chronic exposure to arsenic is associated with an increased risk of diabetes.

Carcinogenesis. Regions with very high arsenic levels in drinking water consistently have substantially higher rates of skin cancer, bladder cancer, and lung cancer. There also are associations between arsenic exposure and other cancers, including liver, kidney, and prostate tumors. Inhalation exposure to arsenic in occupational settings causes lung cancer. IARC classifies arsenic as "carcinogenic to humans (group 1)."

The developing fetus and young children may be at increased risk of arsenic-mediated carcinogenesis and have an elevated risk of developing lung cancer (Smith et al., 2006). Studies in rodents also have observed increased cancer risks from *in utero* exposure and suggest that the second trimester of pregnancy represents a critical susceptibility window (Waalkes et al., 2007).

Arsenic does not directly damage DNA; rather, arsenic is thought to work through changes in gene expression, DNA methylation, inhibition of DNA repair, generation of oxidative stress, or altered signal transduction pathways (Hartwig et al., 2002; Salnikow and Zhitkovich, 2008). Arsenic compounds can act as tumor promoters or cocarcinogens in rodents, particularly when combined with ultraviolet light. In humans, exposure to arsenic potentiates lung tumorigenesis from tobacco smoke. Smokers in regions with high concentrations of arsenic in the drinking water have a 5-fold increased risk of cancer over smokers living in low-arsenic regions (Ferreccio et al., 2000). Arsenic cocarcinogenesis may involve inhibition of proteins involved in nucleotide excision repair (Hartwig et al., 2002; Salnikow and Zhitkovich, 2008). Arsenic also has endocrine-disrupting activities on several nuclear steroid hormone receptors, enhancing hormone-dependent transcription at very low concentrations and inhibiting it at slightly higher levels.

Arsine Gas

Arsine gas, formed by reduction of arsenic in an industrial setting, is a rare cause of poisonings. Arsine induces rapid and often-fatal hemolysis, which probably results from arsine combining with hemoglobin and reacting with O_2. A few hours after exposure, patients can develop headache, anorexia, vomiting, paresthesia, abdominal pain, chills, hemoglobinuria, bilirubinemia, and anuria. Jaundice appears after 24 h. Arsine induces renal toxicities that can progress to kidney failure. Approximately 25% of cases of arsine exposure result in death.

Treatment

Following acute exposure to all forms of arsenic, the patient should be stabilized, and further absorption of the poison should be prevented. Close monitoring of fluid levels is important because arsenic can cause fatal hypovolemic shock. Chelation therapy is effective following short-term exposure to arsenic but has little or no benefit in chronically exposed individuals. Exchange transfusion to restore blood cells and remove arsenic is often warranted following arsine gas exposure (Ibrahim et al., 2006).

Cadmium

Cadmium was discovered in 1817 and first used industrially in the mid-20th century. Cadmium is resistant to corrosion and exhibits useful electrochemical properties, which has led to its use in electroplating, galvanization, plastics, paint pigments, and nickel-cadmium batteries.

Exposure

In the general population, the primary source of exposure to cadmium is through food, with an estimated average daily intake of 50 μg. Cadmium also is found in tobacco and tobacco smoke; a cigarette contains 1 to 2 μg of cadmium (Jarup and Akesson, 2009). Workers in smelters and other metal-processing industries can be exposed to high levels of cadmium, particularly by inhalation.

Chemistry and Mode of Action

Cadmium exists as a divalent cation and does not undergo oxidation-reduction reactions. There are no covalent organometallic complexes of cadmium of toxicological significance. The mechanism of cadmium toxicity is not fully understood. Like lead and other divalent metals, cadmium can replace zinc in zinc-finger domains of proteins and disrupt them. Through an unknown mechanism, cadmium induces formation of ROS, resulting in lipid peroxidation and GSH depletion. Cadmium also upregulates inflammatory cytokines and may disrupt the beneficial effects of NO.

ADME

Cadmium is not well absorbed from the GI tract (1.5%–5%) but is absorbed via inhalation (~10%). Cadmium primarily distributes first to the liver and later the kidney, which together account for 50% of the absorbed dose. Cadmium distributes fairly evenly to other tissues but does not cross the blood-brain barrier or the placenta. Cadmium primarily is excreted in the urine and exhibits a $t_{1/2}$ of 10 to 30 years (ATSDR, 2012b).

Health Effects

Toxicity. Acute cadmium toxicity is due to local irritation along the absorption route. Inhaled cadmium causes respiratory tract irritation with severe, early pneumonitis accompanied by chest pains, nausea, dizziness, and diarrhea. Toxicity may progress to fatal pulmonary edema. Ingested cadmium induces nausea, vomiting, salivation, diarrhea, and abdominal cramps; the vomitus and diarrhea often are bloody.

Symptoms of chronic cadmium toxicity vary by exposure route. The lung is an important target of inhaled cadmium, while the kidney is a major target of cadmium from both inhalation and ingestion.

Cadmium bound to metallothionein is transported to the kidney, where it can be released. Renal toxicity results from increased excretion of low-molecular-weight proteins, especially β_2 microglobulin and retinol-binding protein. Cadmium also causes glomerular injury, with a resulting decrease in filtration. Chronic occupational exposure to cadmium is associated with an increased risk of renal failure and death. There is no evidence for a threshold level for cadmium's effects on the kidney; cadmium levels consistent with normal dietary exposure can cause renal toxicity, including a reduction in glomerular filtration rate and creatinine clearance (Jarup and Akesson, 2009).

Workers with long-term inhalation exposure to cadmium exhibit decreased lung function. Symptoms include bronchitis and fibrosis of the lung, leading to emphysema. The exact cause of cadmium-induced lung toxicity is not known but may result from inhibition of the synthesis of α_1 antitrypsin. Chronic obstructive pulmonary disease causes increased mortality in cadmium-exposed workers.

When accompanied by vitamin D deficiency, cadmium exposure increases the risks for fractures and osteoporosis. This may be an effect of cadmium interfering with calcium and phosphate homeostasis due to its renal toxicity.

Carcinogenicity. Chronic occupational exposure to inhaled cadmium increases the risk of developing lung cancer (IARC, 1993; National Toxicology Program, 2021). The mechanism of cadmium carcinogenesis is not fully understood. Cadmium causes chromosomal aberrations in exposed workers and treated animals and human cells. It also increases mutations and impairs DNA repair in human cells (National Toxicology Program, 2021). Cadmium substitutes for zinc in DNA repair proteins and polymerases and may inhibit nucleotide excision repair, base excision repair, and the DNA polymerase responsible for repairing single-strand breaks

(Hartwig et al., 2002). There is evidence that cadmium also alters cell signaling pathways and disrupts cellular controls of proliferation (Waisberg et al., 2003). Thus, cadmium acts as a nongenotoxic carcinogen.

Treatment

Treatment for cadmium poisoning is symptomatic. Patients who have inhaled cadmium may require respiratory support. Patients with kidney failure as a result of cadmium poisoning may require a transplant. There is no evidence for clinical benefit from chelation therapy following cadmium poisoning, and chelation therapy may result in adverse effects (ATSDR, 2012b).

Chromium

Chromium is an industrially important metal used in a number of alloys, particularly stainless steel, which contains at least 11% chromium. Chromium can be oxidized to multiple valence states, with trivalent (Cr^{III}) and hexavalent (Cr^{VI}) being the two forms of biological importance. Chromium exists almost exclusively as the trivalent form in nature. Trivalent chromium was long thought to be an essential metal involved with glucose metabolism and insulin signaling; more recent analyses refute this view (Bailey, 2014; Vincent, 2017). Cr^{VI} is thought to be responsible for the toxic effects of chromium (ATSDR, 2012a).

Exposure

Exposure to chromium in the general population primarily is through the ingestion of food, although there also is exposure from drinking water and air. Workers are exposed to chromium during chromate production, stainless steel production and welding, chromium plating, ferrochrome alloy and chrome pigment production, and in tanning industries. Exposure usually is to a mixture of Cr^{III} and Cr^{VI}.

Chemistry and Mode of Action

Chromium occurs in its metallic state or in any valence state between divalent and hexavalent. Cr^{III} is the most stable and common form. Cr^{VI} is corrosive and is readily reduced to lower valence states. The primary reason for the different toxicological properties of Cr^{III} and Cr^{VI} is thought to be differences in their absorption and distribution. Hexavalent chromate resembles sulfate and phosphate and can be taken across membranes by anion transporters. Once inside the cell, Cr^{VI} undergoes a series of reduction steps, ultimately forming Cr^{III}, which is thought to cause most of the toxic effects. Trivalent chromium readily forms covalent interactions with DNA. Hexavalent chromium also induces oxidative stress and hypersensitivity reactions.

ADME

Absorption of inhaled chromium depends on its solubility, valence state, and particle size. Smaller particles are deposited deeper in the lungs. Absorption into the bloodstream of hexavalent and soluble forms is higher than the trivalent or insoluble forms, with the remainder often retained in the lungs. Approximately 50% to 85% of inhaled Cr^{VI} particles (<5 μm) are absorbed. Absorption of ingested chromium is less than 10% and varies depending on water solubility. Cr^{VI} crosses membranes by facilitated transport, while Cr^{III} crosses by diffusion. Cr^{VI} is distributed to all of the tissues and crosses the placenta. The highest levels are attained in the liver, kidney, and bone; Cr^{VI} is also retained in erythrocytes. Excretion primarily is through urine, with small amounts also excreted in bile and breast milk and deposited in hair and nails. The $t_{1/2}$ of ingested Cr^{VI} is about 40 h; the $t_{1/2}$ of Cr^{III} is about 10 h (ATSDR, 2012a).

Health Effects

Toxicity. Acute exposure to very high doses of chromium causes death via damage to multiple organs, particularly the kidney. Chronic low-dose chromium exposure causes toxicity at the site of contact. Workers exposed to inhaled chromium develop symptoms of lung and upper respiratory tract irritation, decreased pulmonary function, and pneumonia. Chronic exposure to chromium via ingestion, including after mucociliary clearance of inhaled particles, causes symptoms of GI irritation (oral ulcer, diarrhea, abdominal pain, indigestion, and vomiting). Cr^{VI} is a dermal irritant and can cause ulceration or burns. Low-dose exposure by any route causes some individuals to become sensitized. These individuals will develop allergic dermatitis following dermal exposure to chromium, including products containing metallic chromium. Chromium-sensitized workers often also develop asthma following inhalation exposure (ATSDR, 2012a).

Carcinogenicity. The Cr^{VI} compounds are group 1 known human carcinogens (IARC, 1990). There is insufficient evidence for carcinogenesis from metallic and trivalent chromium (classified in IARC group 3). Workers exposed to Cr^{VI} via inhalation have elevated incidence of and mortality from lung and nasal cancer. Environmental exposure to Cr^{VI} in drinking water increases the risk of developing stomach cancer.

There are multiple potential mechanisms for chromium carcinogenicity (Salnikow and Zhitkovich, 2008). Reduction of Cr^{VI} to Cr^{III} occurs with concomitant oxidation of cellular molecules. Ascorbate is the primary reductant, but other molecules, including GSH, lipids, proteins, and DNA, also can be oxidized. Cr^{III} forms a large number of covalent DNA adducts, primarily at the phosphate diester backbone. The DNA adducts are not very mutagenic and are repaired by nucleotide excision repair. It is thought that the high level of nucleotide excision repair activity following chromium exposure contributes to carcinogenesis, either by preventing repair of mutagenic lesions formed by other carcinogens or through the formation of single-strand breaks due to incomplete repair. Chromium also forms cross-links between DNA and protein. Chronic inflammation due to chromium-induced irritation also may promote tumor formation.

Treatment

There are no standard protocols for treatment for acute chromium poisoning. One approach that has shown promise in rodents is the use of reductants such as ascorbate, GSH, or *N*-acetylcysteine to reduce Cr^{VI} to Cr^{III} after exposure but before absorption to limit bioavailability (ATSDR, 2012a). These compounds and EDTA (ethylenediaminetetraacetic acid) also increase urinary excretion of chromium after high-dose exposure, particularly if given soon enough to prevent uptake into cells. Exchange transfusion to remove chromium from plasma and erythrocytes may be beneficial.

Treatment for Metal Exposure

The most important response to environmental or occupational exposures to metals is to eliminate the source of the exposure. It also is important to stabilize the patient and provide symptomatic treatment.

Treatment for acute metal intoxications often involves the use of chelators. A chelator is a compound that forms stable complexes with metals, typically as five- or six-membered rings. Formation of complexes between chelators and metals should prevent or reverse metal binding to biological ligands. The ideal chelator should *be highly soluble in water, be resistant to biotransformation, reach sites of metal storage, form stable and nontoxic complexes with toxic metals, and be readily excreted as a metal-chelator complex.* A low affinity for the essential metals calcium and zinc also is desirable because toxic metals often compete with these metals for protein binding.

In cases of acute exposure to high doses of most metals, chelation therapy reduces toxicity. However, following chronic exposure, chelation therapy does not show clinical benefits beyond those of cessation of exposure alone and, in some cases, does more harm than good. Chelation therapy may increase the neurotoxic effects of heavy metals and is only recommended for acute poisonings. The structures of the most commonly used chelators are shown in Figure 76–10.

Ethylenediaminetetraacetic Acid

Ethylenediaminetetraacetic acid (EDTA) and its various salts are effective chelators of divalent and trivalent metals. $CaNa_2EDTA$ (*calcium disodium ethylenediaminetetraacetic acid*) is the preferred EDTA salt for metal poisoning, provided that the metal has a higher affinity for EDTA than calcium has. $CaNa_2EDTA$ is effective for the treatment for acute lead

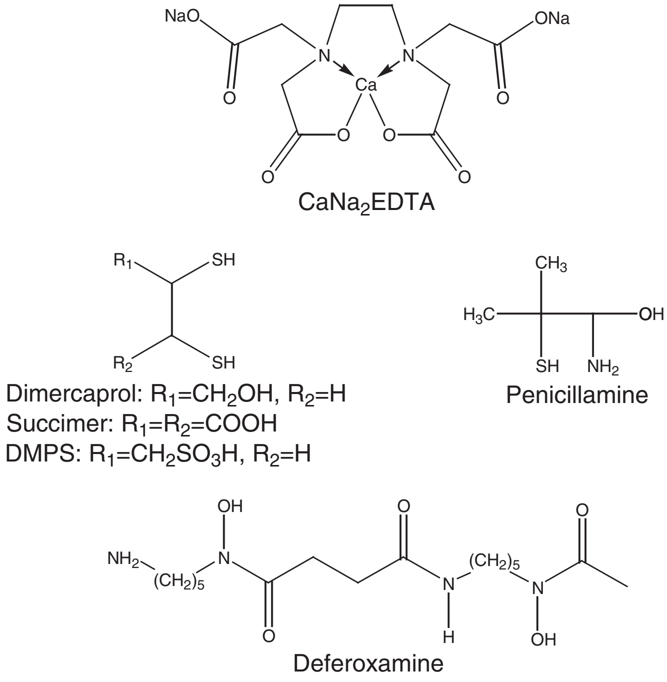

Figure 76–10 *Structures of chelators commonly used to treat acute metal intoxication.*

poisoning, particularly in combination with *dimercaprol*, but is not an effective chelator of mercury or arsenic *in vivo*.

A tetradentate molecule related to EDTA, *ethylene glycol-bis(*β*-aminoethyl ether)-N,N,N,N-tetraacetic acid (EGTA)*, has greater specificity for Ca^{2+} over Mg^{2+} and is used by researchers to buffer Ca^{2+} concentrations in physiologic solutions. Modifications of EGTA led to research that culminated in a Nobel prize (see Box 76–1).

Chemistry and Mode of Action

The pharmacological effects of CaNa$_2$EDTA result from chelation of divalent and trivalent metals in the body. Accessible metal ions with a higher affinity for CaNa$_2$EDTA than Ca^{2+} will be chelated, mobilized, and usually excreted. Because EDTA is charged at physiological pH, it does not significantly penetrate cells. CaNa$_2$EDTA mobilizes several endogenous metallic cations, including zinc, manganese, and iron. Additional supplementation with zinc following chelation therapy may be beneficial. The most common therapeutic use of CaNa$_2$EDTA is for acute lead intoxication. CaNa$_2$EDTA does not provide clinical benefits for the treatment for chronic lead poisoning.

CaNa$_2$EDTA is available as edetate calcium disodium (calcium disodium versenate). Intramuscular administration of CaNa$_2$EDTA results in good absorption, but pain occurs at the injection site; consequently, the chelator injection often is mixed with a local anesthetic or administered intravenously. For intravenous use, CaNa$_2$EDTA is diluted in either 5% dextrose or 0.9% saline and is administered slowly by intravenous drip. A dilute solution is necessary to avoid thrombophlebitis.

BOX 76–1 ■ From EGTA to a Nobel Prize Via Jellyfish

In the early 1980s, Roger Tsien (1952-2016) published two papers (Tsien, 1980; Tsien, 1981) that described modifications of the tetradentate chelator EGTA that ultimately became a family of fluorophores that were membrane-permeant acetoxy-methyl esters, molecules that were hydrolyzed to ionic forms in the cytosol and thereby trapped within cells, enabling real-time assessment of intracellular Ca^{2+} concentrations. Tsien improved these reporters over the years and also developed a family of genetically encoded indicators for a variety of signaling molecules and protein kinases (Zhou et al. 2021), taking advantage of the discovery that the green fluorescent protein of *Aequorea victoria* jellyfish (Chalfie et al,1994; Shimomura, 2009) can function as a genetically encodeable fluorescent tag (Rodriguez et al, 2017). Osamu Shimomura, Martin Chalfie, and Roger Tsien shared the 2008 Nobel Prize in Chemistry "for the discovery and development of the green fluorescent protein, GFP".

ADME

Less than 5% of CaNa$_2$EDTA is absorbed from the GI tract. After intravenous administration, CaNa$_2$EDTA has a $t_{1/2}$ of 20 to 60 min. In blood, CaNa$_2$EDTA is found only in the plasma. CaNa$_2$EDTA is excreted in the urine by glomerular filtration, so adequate renal function is necessary for successful therapy. Altering either the pH or the rate of urine flow has no effect on the rate of excretion. There is very little metabolic degradation of EDTA. The drug is distributed mainly in the extracellular fluids; little gains access to the spinal fluid (5% of the plasma concentration).

Toxicity

The principal toxic effect of CaNa$_2$EDTA is on the kidney. Repeated large doses of the drug can eventually cause degeneration of proximal tubular cells. The early renal effects usually are reversible, and urinary abnormalities disappear rapidly with cessation of treatment. The most likely mechanism of toxicity is chelation of essential metals, particularly zinc, in proximal tubular cells. To minimize nephrotoxicity, adequate urine production should be established prior to and during treatment with CaNa$_2$EDTA. For patients with lead encephalopathy and cerebral edema, CaNa$_2$EDTA can lead to a potentially fatal increase in intracranial pressure following intravenous administration. Intramuscular administration is preferred for these patients.

Rapid intravenous administration of a form lacking calcium, such as Na$_2$EDTA, causes hypocalcemic tetany. However, a slow infusion (<15 mg/min) administered to a normal individual elicits no symptoms of hypocalcemia because of the availability of extracirculatory stores of Ca^{2+}. In contrast, CaNa$_2$EDTA can be administered intravenously with no untoward effects because the change in the concentration of Ca^{2+} in the plasma and total body is negligible.

Other side effects associated with CaNa$_2$EDTA include malaise, fatigue, and excessive thirst, followed by the sudden appearance of chills and fever and subsequent myalgia, frontal headache, anorexia, occasional nausea and vomiting, and rarely, increased urinary frequency and urgency. CaNa$_2$EDTA is teratogenic in laboratory animals, probably as a result of zinc depletion; it should be used in pregnant women only under conditions in which the benefits clearly outweigh the risks (Kalia and Flora, 2005). Other possible effects include sneezing, nasal congestion, and lacrimation; glycosuria; anemia; dermatitis with lesions strikingly similar to those of vitamin B$_6$ deficiency; transitory lowering of systolic and diastolic blood pressures; prolonged prothrombin time; and T-wave inversion on the electrocardiogram.

Dimercaprol

Dimercaprol was developed during World War II as an antidote to lewisite, a vesicant arsenical war gas, hence its alternative name, British anti-Lewisite (BAL). Arsenicals and other heavy metals form a stable and relatively nontoxic chelate ring with *dimercaprol*.

Chemistry and Mode of Action

The pharmacological actions of *dimercaprol* result from formation of chelation complexes between its sulfhydryl groups and metals. Dissociation of *dimercaprol*-metal complexes and oxidation of *dimercaprol* occur *in vivo*. The sulfur-metal bond may be labile in the acidic tubular urine, which may increase the delivery of metal to renal tissue and increase toxicity. The dosage regimen should maintain a concentration of *dimercaprol* in plasma adequate to favor the continuous formation of the more stable 2:1 (BAL-metal) complex. However, because of pronounced and dose-related side effects, excessive plasma concentrations must be avoided. The concentration in plasma therefore must be maintained by repeated dosage until the metal is excreted. *Dimercaprol* is most beneficial when given very soon after exposure to the metal because it is more effective in preventing inhibition of sulfhydryl enzymes than in reactivating them. *Dimercaprol* limits toxicity from arsenic, gold, and mercury, which form mercaptides with essential cellular sulfhydryl groups. It also is used in combination with $CaNa_2EDTA$ to treat lead poisoning. *Dimercaprol* should not be used in iron, cadmium, or selenium poisoning because the resulting metal complexes are more toxic than the metal alone, especially to the kidneys.

ADME

Dimercaprol is given by deep intramuscular injection as a 100-mg/mL solution in peanut oil and should not be used in patients who are allergic to peanuts. Peak *dimercaprol* concentrations in blood are attained in 30 to 60 min. The $t_{1/2}$ is short, and metabolic degradation and excretion essentially are complete within 4 h. *Dimercaprol* and its chelates are excreted in both urine and bile. *Dimercaprol* is contraindicated for use following chronic exposures to heavy metals because it does not prevent neurotoxic effects. There is evidence in laboratory animals that *dimercaprol* mobilizes lead and mercury from various tissues to the brain (Andersen and Aaseth, 2002). This effect may be due to the lipophilic nature of *dimercaprol* and is not observed with its more hydrophilic analogues described later in the chapter (see Succimer and Sodium 2,3-Dimercaptopropane-1-Sulfonate).

Toxicity

Side effects occur in about 50% of subjects receiving 5 mg/kg intramuscularly. *Dimercaprol* causes a rise in systolic and diastolic arterial pressures, accompanied by tachycardia. The pressure rises immediately but returns to normal within 2 h. *Dimercaprol* also can cause anxiety and unrest, nausea and vomiting, headache, a burning sensation in the mouth and throat, a feeling of constriction or pain in the throat and chest, conjunctivitis, blepharospasm, lacrimation, rhinorrhea, salivation, tingling of the hands, a burning sensation in the penis, sweating, abdominal pain, and the occasional appearance of painful sterile abscesses at the injection site. The *dimercaprol*-metal complex breaks down easily in an acidic medium; production of alkaline urine protects the kidney during therapy. Children react similarly to adults, although about 30% also may experience a fever that disappears on drug withdrawal. *Dimercaprol* is contraindicated in patients with hepatic insufficiency, except when this condition is a result of arsenic poisoning.

Succimer

Succimer (2,3-dimercaptosuccinic acid [DMSA]) has been approved in the U.S. for the treatment of children with BLL greater than 45 μg/dL. Because of its oral availability, improved toxicity profile, and selective chelation of heavy metals, *succimer* also is used off label for the treatment of adults with lead poisoning and for the treatment for arsenic and mercury intoxication, although no large clinical trials have been undertaken for these indications.

Chemistry and Mode of Action

Succimer is an orally effective chelator that is chemically similar to *dimercaprol* but contains two carboxylic acids that modify the spectrum of absorption, distribution, and chelation of the drug. It has an improved toxicity profile over *dimercaprol*.

ADME

After absorption, *succimer* is biotransformed to a mixed disulfide with cysteine (Aposhian and Aposhian, 2006). *Succimer* lowers BLL and attenuates lead toxicity. The *succimer*-lead chelate is eliminated in both urine and bile. The fraction eliminated in bile can undergo enterohepatic circulation.

Succimer has several desirable features over other chelators. It is orally bioavailable, and because of its hydrophilic nature, it does not mobilize metals to the brain or enter cells. It also does not significantly chelate essential metals such as zinc, copper, or iron. As a result of these properties, *succimer* exhibits a much better toxicity profile relative to other chelators. Animal studies suggest that *succimer* also is effective as a chelator of arsenic, cadmium, mercury, and other toxic metals (Andersen and Aaseth, 2002; Kalia and Flora, 2005).

Toxicity

Succimer is much less toxic than *dimercaprol*. The most commonly reported adverse effects are nausea, vomiting, diarrhea, and loss of appetite. In a few patients, rashes necessitate discontinuation of therapy. Transient elevations in hepatic transaminases have been observed with *succimer* treatment.

Sodium 2,3-Dimercaptopropane-1-Sulfonate

Another dimercapto compound, *sodium 2,3-dimercatopropane sulfonate* (DMPS), is used for the chelation of heavy metals. DMPS is approved for use in Germany. In the U.S., DMPS is available from compounding pharmacies upon physician request.

Chemistry and Mode of Action

DMPS is a clinically effective chelator of lead, arsenic, and especially mercury. It is orally available and is rapidly excreted, primarily through the kidneys. It is negatively charged and exhibits distribution properties similar to those of *succimer*. DMPS is less toxic than *dimercaprol* but mobilizes zinc and copper and thus is more toxic than *succimer*. In a small clinical trial, DMPS exhibited some clinical benefit for the treatment for chronic arsenic poisoning (Kalia and Flora, 2005), but more thorough clinical studies are needed.

Penicillamine; Trientine

Penicillamine is an effective chelator of copper, mercury, zinc, and lead and promotes the excretion of these metals in the urine. *Penicillamine* is indicated for use in patients with Wilson disease (excess body burden of copper due to diminished excretion) and is used in other heavy metal intoxications. *Penicillamine* is more toxic and is less potent and selective for chelation of heavy metals relative to other available chelation drugs. It is therefore not a first-line treatment for acute intoxication with lead, mercury, or arsenic. However, because it is inexpensive and orally bioavailable, it often is given at fairly low doses following treatment with $CaNa_2EDTA$ or *dimercaprol* to ensure that the concentration of metal in the blood stays low following the patient's release from the hospital.

ADME

Penicillamine is well absorbed (40%–70%) from the GI tract. Food, antacids, and iron reduce its absorption. Peak concentrations in blood are obtained 1 to 3 h after administration. *Penicillamine* is relatively stable *in vivo* compared to its unmethylated parent compound cysteine. Hepatic biotransformation is responsible for degradation of *penicillamine*; very little drug is excreted unchanged. Metabolites are found in both urine and feces. *N*-Acetylpenicillamine is more effective than *penicillamine* in protecting against the toxic effects of mercury, presumably because it is more resistant to metabolism.

Therapeutic Use

Penicillamine is available for oral administration. For chelation therapy, the usual adult dose is 1 to 1.5 g/day in four divided doses, given on an empty stomach to avoid interference by metals in food. *Penicillamine* is used in Wilson disease, cystinuria, and rheumatoid arthritis (rarely). For the treatment for Wilson disease, 1 to 2 g/day usually is administered in four divided doses. The urinary excretion of copper should be monitored to determine whether the dosage of *penicillamine* is adequate.

Toxicity

Penicillamine induces cutaneous lesions, including urticaria, macular or papular reactions, pemphigoid lesions, lupus erythematosus, and dermatomyositis. It also causes dryness and scaling. Cross-reactivity with penicillin may be responsible for some episodes of urticarial or maculopapular reactions with generalized edema, pruritus, and fever that occur in as many as one-third of patients taking *penicillamine*. Hematological reactions may be fatal and include leukopenia, aplastic anemia, and agranulocytosis. Renal toxicity induced by *penicillamine* usually is manifested as reversible proteinuria and hematuria, but it may progress to nephrotic syndrome with membranous glomerulopathy. Rare fatalities have been reported from Goodpasture syndrome. Severe dyspnea, although uncommon, has been reported from *penicillamine*-induced bronchoalveolitis. Myasthenia gravis also has been induced by long-term therapy with *penicillamine*. A specific schedule for physical and laboratory monitoring for skin, blood, kidney, lung, liver, and other toxicities is published in the manufacturer's product labeling.

Penicillamine is a teratogen in laboratory animals, but for pregnant women with Wilson disease, the benefits outweigh the risks. Less serious side effects include nausea, vomiting, diarrhea, dyspepsia, anorexia, and a transient loss of taste for sweet and salt, which is relieved by supplementation of the diet with copper. Contraindications to *penicillamine* therapy include pregnancy in the absence of Wilson disease, renal insufficiency, or a previous history of *penicillamine*-induced agranulocytosis or aplastic anemia. *Penicillamine* increases the requirement for pyridoxine; supplementation with 25 mg daily is advised.

Trientine

Trientine (triethylenetetramine dihydrochloride) is an acceptable alternative for patients with Wilson disease who are intolerant of *penicillamine*. *Trientine* is effective orally. Maximal daily doses of 2 g for adults or 1.5 g for children are taken in two to four divided portions on an empty stomach. Iron deficiency may occur during *trientine* therapy; this can be overcome with short courses of iron therapy, but iron and *trientine* should not be ingested within 2 h of each other.

Deferoxamine; Deferasirox; Deferiprone

Chemistry and Mode of Action

Deferoxamine is isolated as the iron chelate from *Streptomyces pilosus* and is treated chemically to obtain the metal-free ligand. *Deferoxamine* (deferoxamine mesylate) has a remarkably high affinity for ferric iron ($K_a = 10^{31}$ M^{-1}) coupled with a very low affinity for calcium ($K_a = 10^2$ M^{-1}). *In vitro*, it removes iron from hemosiderin and ferritin and, to a lesser extent, from transferrin. Iron in hemoglobin or cytochromes is not removed by *deferoxamine*.

ADME and Therapeutic Use

Deferoxamine is poorly absorbed after oral administration, and parenteral (intravenous, intramuscular, or subcutaneous) administration is required. For acute iron toxicity, intramuscular administration is preferred, except for patients in cardiovascular collapse or shock. For patients in shock, the drug is administered intravenously at 10 to 15 mg/kg/h by constant infusion for the first 1000 mg. If additional treatment is needed beyond 1000 mg, it should be administered at a rate not to exceed 125 mg/h. Faster rates of infusion usually are associated with hypotension. For acute iron poisoning without cardiovascular collapse or shock, *deferoxamine* should be given intramuscularly at a dose of 50 mg/kg with a maximum dose of 1 g. Subsequent doses of 500 mg may be given, if needed, every 4 to 12 h. Hypotension also can occur with the intramuscular route. For chronic iron intoxication (e.g., thalassemia), subcutaneous administration is recommended. Continuous subcutaneous administration should be given to achieve a dose of 1 to 2 g/day. The duration of the infusion (8–24 h) should be individualized to the patient. Poorly compliant patients with thalassemia can be given *deferoxamine* by slow intravenous infusion (rate not to exceed 15 mg/kg/h) prior to or after receiving transfusion. *Deferoxamine* may also be administered via an intramuscular dose of 0.5 to 1.0 g/day. *Deferoxamine* is not recommended in primary hemochromatosis; phlebotomy is the treatment of choice. Off label, *deferoxamine* has also been used for the chelation of aluminum in dialysis patients.

Deferoxamine is metabolized to unknown products by plasma enzymes. The parent drug and its metabolites are readily excreted in the urine.

Toxicity

Deferoxamine can cause a number of allergic reactions, including pruritus, wheals, rash, and anaphylaxis. Other adverse effects include dysuria, abdominal discomfort, diarrhea, fever, leg cramps, and tachycardia. Occasional cases of cataract formation have been reported. *Deferoxamine* may cause neurotoxicity during long-term, high-dose therapy for transfusion-dependent thalassemia major; both visual and auditory changes have been described. A "pulmonary syndrome" has been associated with high-dose (10- to 25-mg/kg/h) *deferoxamine* therapy; tachypnea, hypoxemia, fever, and eosinophilia are prominent symptoms. Contraindications to the use of *deferoxamine* include renal insufficiency and anuria; during pregnancy, the drug should be used only if clearly indicated.

Deferasirox; Deferiprone

Deferasirox and *deferiprone* are oral chelators that are FDA approved to treat chronic iron overload in patients receiving transfusions. *Deferasirox* is also indicated for the treatment for chronic iron overload in non–transfusion-dependent thalassemia syndromes. Because of their oral availability, these drugs may allow for better compliance rates for patients with thalassemia. Both drugs form an uncharged chelate with iron and appear to be more effective than *deferoxamine* at reducing cardiac iron. However, both carry FDA black-box warnings (*deferasirox* for renal failure, hepatic failure, GI hemorrhage; *deferiprone* for agranulocytosis, neutropenia), and there are currently insufficient data to recommend replacing *deferoxamine* as the first-line treatment for chronic iron overload.

Endocrine Disruptors

Endocrine-disrupting chemicals (EDCs) include a broad range of compounds that interfere with the endocrine system through modulating hormone production and/or signaling. Key characteristics defining mechanisms through which compounds act as EDCs are outlined in Figure 76–11. These mechanisms include:

- Directly binding or regulating hormone receptors (e.g., ER, androgen receptor [AR], thyroid hormone receptor), their binding partners, or components of their downstream signaling pathways
- Influencing hormone production, secretion, or fate (La Merrill, 2020)

In addition to affecting reproductive function, endocrine disruptors can also negatively influence development, cognitive function, and metabolism. Endocrine disruptors are ubiquitously found in consumer products, including plastics, toys, cosmetics, and food.

Some EDCs have been banned, including the pesticide dichlorodiphenyltrichloroethane (DDT). Such efforts notwithstanding, many putative endocrine disruptors remain in use. The FDA has identified over 1800 manufactured chemicals that are known to have, or are predicted to have, endocrine-disrupting capacity. An exhaustive list of these chemicals can be found in the FDA's Endocrine Disruptor Knowledge Base (FDA, 2019). The following sections will focus on three classes of ubiquitous environmental compounds with well-studied EDC activity: DDT, phthalates, and bisphenol A (BPA).

DDT

Efficacy, low volatility, high lipid solubility, and slow biotransformation and degradtion made organochlorine compounds especially effective insecticides. Their stability, persistence in the environment, and lipid solubility also were the source of concern by environmentalists. In 1962, Rachel Carson published *Silent Spring* (Carson, 1962), which focused on evidence that DDT and other synthetic pesticides persist in the environment, bioaccumulate up the food chain, and cause observable harm to natural ecological systems, notably to avian reproduction. The book incited public outrage against DDT use. Sweden banned DDT in 1970; the U.S. banned its use in 1972. While most countries worldwide have also banned DDT, the persistence of its metabolite, dichlorodiphenyldichloroethylene (DDE), in both humans and the food chain, and its emergency use in controlling mosquito vectors make the health effects of this EDC still relevant today.

Exposure

During its period of active use (1940s to 1970s), DDT exposure occurred through handling or inhaling the chemical during application or interacting with DDT-sprayed surfaces. Today, DDT exposure most often occurs

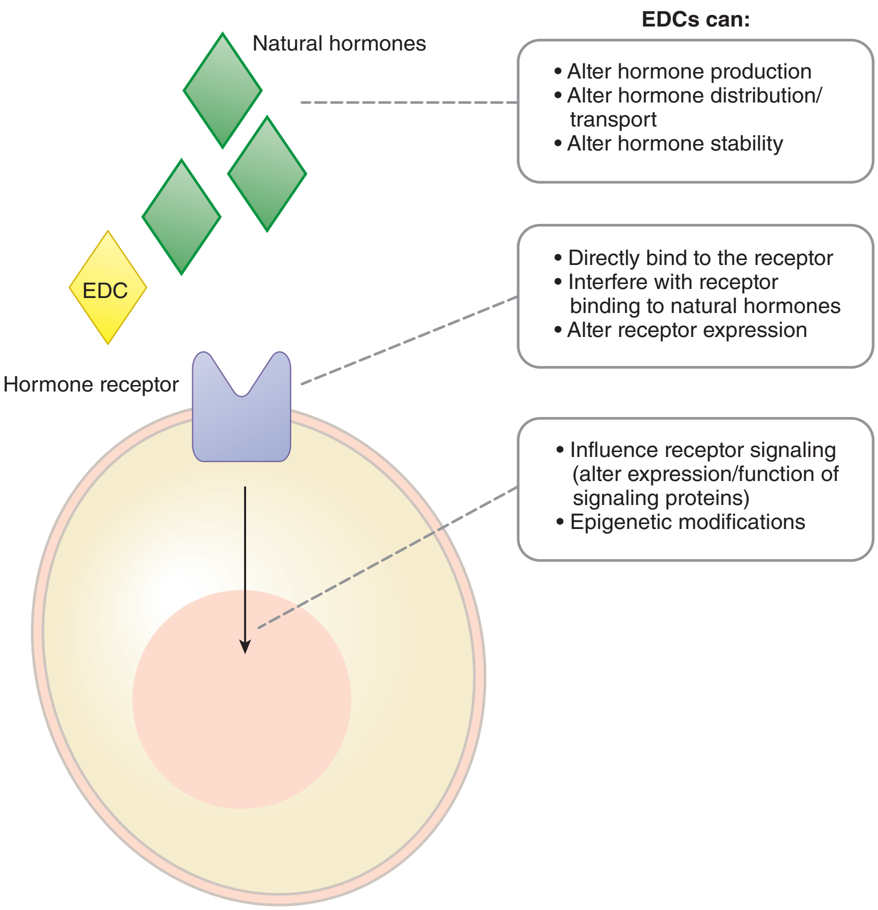
Natural hormones
EDCs can:
• Alter hormone production
• Alter hormone distribution/ transport
• Alter hormone stability
EDC
• Directly bind to the receptor
• Interfere with receptor binding to natural hormones
• Alter receptor expression
Hormone receptor
• Influence receptor signaling (alter expression/function of signaling proteins)
• Epigenetic modifications

Figure 76–11 *Mechanisms through which chemicals can disrupt endocrine function.* (Adapted from La Merrill et al., 2020.)

through consumption of contaminated food, where DDT or DDE has bioaccumulated. Bioaccumulation of DDT and its metabolites is especially problematic in fish and seafood, although dietary exposure can occur through most lipid-rich foods. In addition to the consumption of food where fat bioaccumulation occurs, leafy vegetables coated with air-deposited DDT and root vegetables grown in contaminated soil can serve as a source of food contamination. DDT exposure can also occur *in utero* and via breastfeeding. In the air, the half-life of DDT is approximately 2 days as it can be broken down by sunlight; however, DDT persists in soil with a half-life of 2 to 15 years. Even though widespread use of DDT ceased decades ago, DDE can still be found in biological samples from most humans worldwide (Koureas, 2019). In regions where DDT is still used, primarily for malaria control, inhalation of contaminated air (from DDT that did not adhere to a surface or that vaporized following application to a surface) serves as an additional source of exposure.

Chemistry and Mode of Action

DDT is a chlorinated ethane derivative and part of the organochlorine pesticide family. In technical grade DDT, p,p′-DDT is the predominant isomer, although it is a mixture of several isomers (o,p′-DDT and p,p′-DDE are the most abundant contaminants). DDT confers its pesticide activity by impairing the nervous system of insects through disruption of voltage-gated ion channels. Likewise, acute toxicity in humans results from prolonged Na^+ channel opening and delayed channel closing. In addition to neurotoxicity, there is evidence that DDT targets several other organ systems, including the endocrine system. The o,p′-DDT isomer, which makes up approximately 15% of DDT, acts as an agonist for both ERα and ERβ. The p,p′-DDE isomer, which makes up approximately 4% of technical grade DDT and is the primary metabolite of p,p′-DDT, acts as an AR antagonist. Additionally, p,p′-DDT interacts with the transmembrane domain of follicle-stimulating hormone receptor (FSHR), positively modulating of its downstream signaling (Munier et al., 2016).

ADME

Exposure to DDT, DDE, and dichlorodiphenyldichloroethane (DDD) primarily occurs through ingestion. The percentage of DDT and related isomers that is absorbed via the intestine is high, and the extent of absorption increases when DDT is dissolved in oils. Following absorption, DDT is initially distributed throughout all tissues in the body but ultimately accumulates in tissues proportional to their lipid content. DDE, the primary stable metabolite of DDT, is formed directly by dehydrochlorinase. Alternatively, DDE can be formed from a p,p′-DDD intermediate through reductive chlorination of DDT followed by dehydrogenase conversion of p,p′-DDD to DDE. DDE can persist in the body for decades. DDA [2,2-bis(p-chlorophenyl)acetic acid], an oxidized form of DDT and DDE, is the primary excreted metabolite and is eliminated through the urine; fecal elimination and breast milk serve as additional minor routes of excretion.

Health Effects

The EDC-related health effects of DDT have been determined based on a combination of epidemiological studies and causal studies in animal models (ATSDR, 2019). Using a systematic review approach of published epidemiological studies, there is consistent evidence that serum concentrations of DDT, DDE, and DDD correlate with an increased risk of abortions and preterm births in humans. While correlations with other reproductive effects have also been assessed, including fecundity, reproductive hormone levels in males and females, and early menopause, those data have been inconsistent.

Animal studies have provided more direct evidence of causal EDC activity that has supplemented the limitations of clinical studies. In rodent models, the health effects of p,p′-DDT, DDE, and DDD have mostly involved their antiadrenergic activity, whereas the health effects of o,p′-DDT are related to its estrogenic activity. DDT exposure in males led to a decrease in reproductive tissue weights, and in females, it led to increased uterine weight, decreased fertility, and decreased estradiol levels. Although mild effects on thyroid function have been observed in animal studies, there is less direct evidence for an involvement of DDT in thyroid function (T_3, T_4, and thyroid-stimulating hormone levels) in humans. The health effects of DDT exposure may be dependent on timing and could explain some of the inconsistencies in the epidemiological data. DDT exposure during development has been shown to impact health effects later in life, which is relevant today for older adults who were born prior to the ban of DDT. In this regard, there is evidence that DDT developmental exposure is associated with an increase in type 2 diabetes, obesity, and liver cancer (Russ and Howard, 2016; vonderEmbse et al., 2021).

Phthalates

Phthalates are a group of chemicals that are commonly used as plasticizers to increase flexibility of polyvinyl chloride (PVC) and in consumer industries for the production of personal care products, medical tubing, and pharmaceuticals.

Exposure

The prevalence of phthalates in plastics and personal care products and the increase in plastic waste make exposure unavoidable in daily life. Accordingly, exposure routes to phthalates predominately occur through ingestion, inhalation, and dermal absorption. Animal meats, cooking oils, and dairy products consistently reportedly have high levels of phthalates such as di-2-ethylhexyl phthalate (DEHP). Consumption of food wrapped in plastic packaging is also a source of ingestion-based exposure. Individuals who live in proximity to plastic manufacturers may be exposed through air, contaminated water, dust, and soil. Since phthalates are capable of crossing the placental barrier, exposures also occur at the embryonic and fetal developmental stages.

Chemistry and Mode of Action

Phthalates are esters of phthalic acid with the basic structure and predominant derivatives shown in Figure 76–12. Depending on the modifications of the two side chains, phthalates are grouped into two categories: long-chain, high-molecular-weight phthalates and short-chain, low-molecular-weight phthalates. The side chains can be linear, branched, or cyclic, but generally, structures with greater than a six-carbon backbone are considered high-molecular-weight phthalates; DEHP and diisononyl phthalate (DINP) predominate and are used as plasticizers. In contrast, the low-molecular-weight compounds (e.g., dimethyl phthalate [DMP], diethyl phthalate [DEP]) are more often used in the manufacturing of personal care products. Phthalates impose their EDC activity through multiple mechanisms, including modulating hormone levels, altering hormone receptor expression, direct binding to receptors, and epigenetic modifications of genes involved in hormone signaling pathways (Hlisníková, 2020). In rodent studies, the impact of phthalate exposure is not monotonic. Exposure at high concentrations of individual phthalates or in mixtures led to increased FSHR, gonadotropin-releasing hormone, and luteinizing hormone receptor (LHR), whereas lower levels of exposure resulted in decreased FSHR and LHR. Phthalates directly bind nuclear receptors (NR) by mimicking the steroid A ring of hormones. As a result, phthalates can influence AR, ER, and peroxisome proliferator-activated receptor (PPAR) signaling by either activating or inhibiting these receptors depending on the phthalate side chain, the size of the ligand-binding domain of the NR, and interactions between NRs and coactivators. Phthalates also affect cell-signaling pathways that converge with NRs, including the MAPK, nuclear factor-κB (NF-κB), and PI3K/Akt pathways, which can result in altered cell proliferation and apoptosis.

ADME

Absorption, bioavailability, and elimination of phthalates are dependent on the route of exposure and hydrophobicity of the specific compound. Metabolites eliminated in the urine are similar across exposure routes (Domínguez-Romero and Scheringer, 2019). Absorption after dermal exposure can range from 1% to 60%; absorption is higher by the oral route. Following oral and dermal exposure, phthalates undergo first-pass metabolism; diesters are hydrolyzed into monoester metabolites by lipases and esterases. Low-molecular-weight phthalates and monoesters are excreted in the urine, whereas high-molecular-weight phthalates undergo phase II conjugation prior to elimination. Approximately 60% of total phthalates are eliminated in the urine and feces after dermal exposure, whereas 80% are eliminated following oral exposure. In general, the elimination of total phthalates increases with decreasing hydrophobicity of the parent phthalate compound. Phthalates are rapidly metabolized, with the plasma half-life of most phthalates being less than 24 h. Therefore, acute exposure to phthalates is often determined by measuring metabolites in the urine.

Health Effects

Research in human and animal models has established the developing reproductive system as a particularly sensitive target of phthalates. In adults, phthalate exposure is associated with reduced reproductive ability (reduced semen quality in males, increased pregnancy loss in females) and increased risk of type 2 diabetes, obesity, and inflammation. In epidemiological studies of children, phthalate exposure is associated with decreased luteinizing hormone and testosterone, reduced anogenital distance, thyroid dysfunction, and precocious puberty (Hlisníková, 2020; Sedha et al., 2021).

Bisphenol A

Bisphenol A (BPA) is another prevalent synthetic compound used as a plasticizer in the manufacturing of polycarbonate plastics and epoxy resins.

Exposure

Bisphenol A is commonly used in the manufacturing of food and beverage packaging including water bottles, protective coatings in food cans, and bottle tops. The primary route of exposure to BPA is through ingestion by direct contact with packaging, or indirectly by consuming water and food contaminated by plastic leaching. The rate that BPA leaches into the environment increases with heat, interactions with liquids of nonneutral pH, harsh detergents, and physical disruption of BPA-containing plastics. BPA can cross the placenta and has been measured in the umbilical cord,

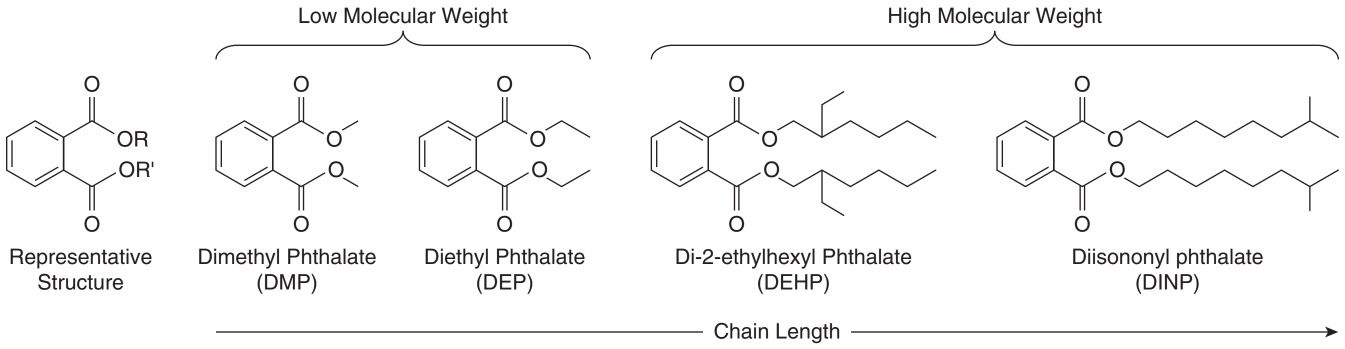

Figure 76–12 *Representative phthalate structures.*

amniotic fluid, and fetal plasma. BPA has also been measured in breast milk, which serves as an additional route of exposure in infants.

Chemistry and Mode of Action

Bisphenol A is generated from a reaction between phenols and acetone and is structurally composed of two phenols connected by an alkyl group. Because of its structural similarity to estrogen, BPA can bind to both ERα and ERβ, and has weak estrogenic activity (~1000-fold lower affinity than estradiol). BPA can activate ERα signaling but differentially interacts with the ERβ ligand-binding domain, preventing the conformational changes needed for productive signaling. As a result, BPA serves as a selective ER modulator and can act as both an agonist and antagonist. Most studies focus on the effects of high doses of BPA on ER transcriptional activity; however, at low concentrations, BPA can interfere with nongenomic ER pathways, including the formation of extranuclear complexes and Ca^{2+} release (Acconcia et al., 2015).

ADME

Following ingestion, BPA undergoes first-pass metabolism in the intestines and liver. BPA is primarily metabolized by the CYP2C subfamily and undergoes glucuronidation and, to a lesser extent, sulfation. BPA-monoglucuronide lacks estrogenic activity and is the primary metabolite found in bile and urine.

Health Effects

Bisphenol A has been identified in the urine of nearly all adults and children, as well as in the serum of pregnant women, breast milk, follicular and amniotic fluid, cord blood, and placental tissue. Even though it is difficult to form causal links with epidemiological studies, the evidence supports the hypothesis that BPA exposure is harmful to the human endocrine system (Abraham and Chakraborty, 2020). In regard to fertility and sexual function, higher urinary BPA levels were associated with lower serum E2, poorer oocyte yield, reduced maturation of oocytes, higher implantation failure, and a decrease in male sexual function. Males exposed to BPA have lower daily sperm production, sperm count, and motility. For women of childbearing age, higher BPA levels are associated with a shorter luteal phase. Urinary BPA correlates with other diseases involving endocrine disruption such as type 2 diabetes and thyroid dysfunction. Despite compelling associations, limitations to these studies include small sample sizes, confounding variables, and study designs that make it difficult to predict causations. Because of the inconclusive evidence in humans, regulation of BPA differs across countries, although bans of BPA in baby bottles have been enacted. Based on consumer demand, BPA has been phased out of food packaging with containers labeled as "BPA free," although replacement chemicals are often less studied.

In laboratory studies, mice exposed to BPA have impaired reproductive organ development, sperm development, and testosterone excretion (Adegoke et al., 2020). BPA exposure alters the function and structure of the brain via neurohormone receptors and the expression of the genes involved in thyroid hormone synthesis (Bansal and Zoeller, 2019; Kim and Park, 2019). Dams exposed to BPA gave birth to offspring with decreased insulin secretion. BPA has its greatest effect on health outcomes during critical stages of development such as embryonic development, gestational stages when BPA crosses the placental barrier, the neonatal period, childhood, and the peripubertal and pubertal periods (Eckstrum et al., 2018; Tuduri et al., 2018).

Chemicals That Target the Immune System

Immunotoxicants encompass a broad category of xenobiotics that interfere with or modulate the function of the immune system. In general, immunotoxicants act through one or more of the following mechanisms that target the immune system:

- Functional changes
- Structural changes
- Compositional changes
- Changes secondary to other target organ toxicities

Chemicals that induce functional changes modulate the ability of immune cells to perform their effector functions, such as antibody production, release of cytokines, processing and presentation of antigen, and cell proliferation or differentiation. Chemicals that lead to structural changes alter the expression of cellular receptors and their ligands or damage lymphoid organ architecture. Chemicals that induce compositional changes may lead to alterations in the proportion or number of immune cell populations and subsets. Since the immune system has significant crosstalk with other organ systems, chemicals such as EDCs that target other organs can indirectly impair immune system function. Immunotoxicants can interfere with immune homeostasis or appropriate immune responses, which can lead to immunosuppression or immune overactivation. Immunosuppressive chemicals increase the risk of infection and cancer, whereas immunostimulants can increase the risk for autoimmunity and allergic reactions/hypersensitivity, especially in genetically susceptible individuals.

The following section covers 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most potent immunotoxicant. TCDD mediates its toxicity through the aryl hydrocarbon receptor (AhR), and immunotoxicity of other persistent organic pollutants adsorbed to ambient particulate matter will also be briefly covered. Finally, emerging data on the immunotoxicity of per- and poly-fluoroalkyl substances (PFAS) are discussed.

TCDD

TCDD is a by-product or contaminant formed in the manufacturing of 2,4,5-trichlorophenol (TCP), a chemical once used as an herbicide and fungicide and that is also a combustion product in the burning of organic matter. Due to its resistance to breakdown, it is a persistent environmental pollutant. TCDD gained notoriety following three well-known exposure incidents:

- As a contaminant of Agent Orange, an herbicide that was used during the Vietnam War
- As the chemical accidentally released following a pressure tank incident in Seveso, Italy
- As the agent used in an assassination attempt of the Ukrainian presidential candidate, Viktor Yushchenko (Kerkvliet, 2012)

Exposure

TCDD, a lipophilic chemical, is resistant to metabolism and accumulates within the food chain. Today, greater than 90% of exposure is through the ingestion of contaminated food, with TCDD particularly soluble in fat-rich foods (e.g., dairy products, meat, and fish). Exposure also occurs occupationally from municipal, medical, and hazardous waste incineration and through natural combustion reactions.

Chemistry and Mode of Action

TCDD is part of a family of chemicals known as dioxins. There are greater than 400 structurally similar chemicals including polychlorinated dibenzo-para-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs). TCDD is the most potent of the congeners, based on its high affinity for the AhR and resistance to metabolism by CYPs, a profile that ensures a strong sustained AhR activation. Immunotoxicity of TCDD is mediated through AhR, which is expressed either constitutively or upon activation in most cells of the immune system. T cells are the primary target of AhR-mediated immunosuppression, with TCDD promoting the differentiation of newly activated $CD4^+$ T cells toward a regulatory T-cell phenotype (Ehrlich et al., 2017). TCDD also influences antiviral responses in $CD8^+$ T cells, induces tolerogenic dendritic cells, and inhibits antibody production in B cells (Franchini et al., 2019; Rothhammer and Quintana, 2019).

ADME

Following oral exposure, approximately 90% of TCDD is absorbed when dissolved in oil and approximately 50% to 60% is absorbed when administered in the diet. TCDD initially distributes through blood, liver,

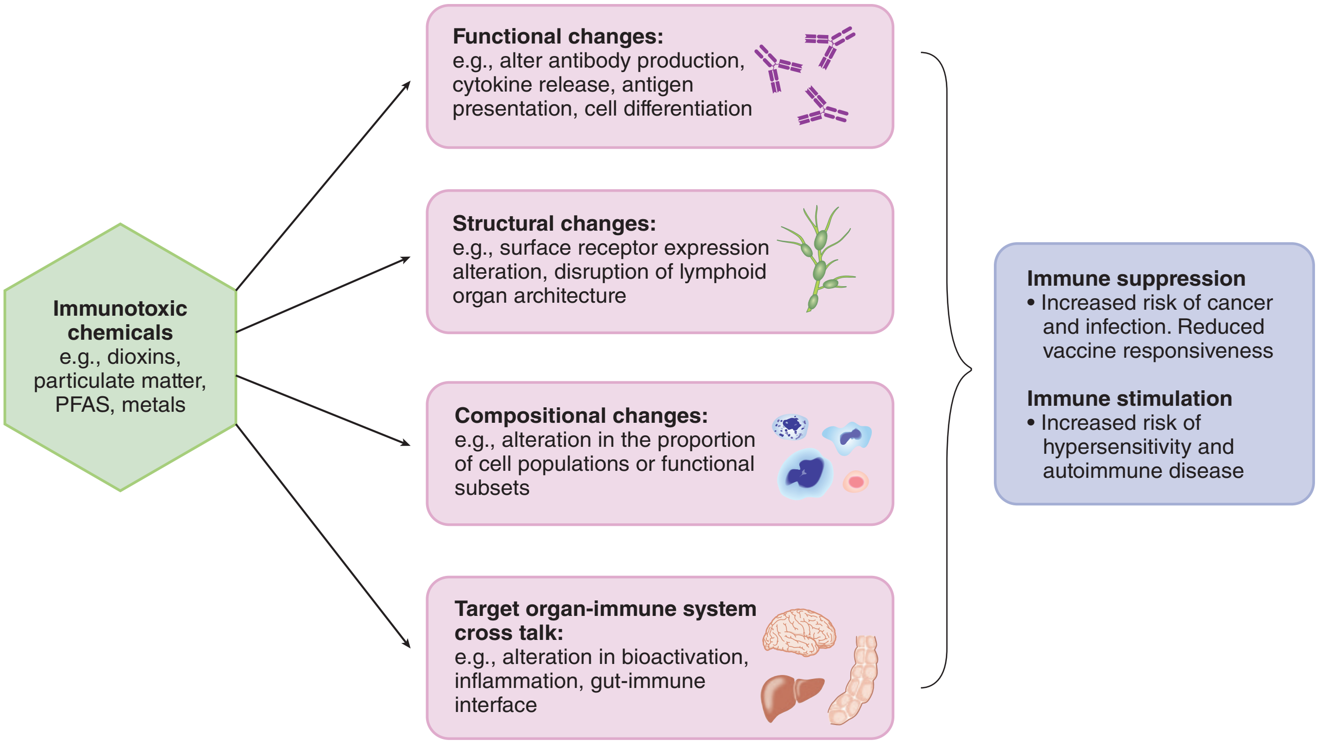
Immunotoxic chemicals
e.g., dioxins, particulate matter, PFAS, metals
Functional changes:
e.g., alter antibody production, cytokine release, antigen presentation, cell differentiation
Structural changes:
e.g., surface receptor expression alteration, disruption of lymphoid organ architecture
Compositional changes:
e.g., alteration in the proportion of cell populations or functional subsets
Target organ-immune system cross talk:
e.g., alteration in bioactivation, inflammation, gut-immune interface
Immune suppression
• Increased risk of cancer and infection. Reduced vaccine responsiveness
Immune stimulation
• Increased risk of hypersensitivity and autoimmune disease

Figure 76–13 *Mechanisms through which chemicals can cause immunotoxicity.*

muscles, skin, and adipose tissue, and ultimately bioaccumulates in the liver and adipose tissue. TCDD is generally resistant to metabolism, although there are differences in metabolism across species. In humans, the half-life of TCDD is 7 to 11 years, and elimination primarily occurs through the feces.

Health Effects

The toxic effects of TCDD are mostly garnered from occupational and accidental exposure incidents, with the most consistent findings being the development of chloracne and increased risk of certain cancers. Most consistently, a reduction in CD4$^+$ T helper cell subsets has been reported in several studies and confirmed in nonhuman primates. In animal models, TCDD exposure results in thymic involution or atrophy (Harris et al., 1973). Additionally, rodents become more susceptible to influenza infection, *Trichinella spiralis* infection, and systemic (but not respiratory) *Streptococcus pneumoniae* infection (Lawrence and Vorderstrasse, 2013). Notably, exposure to TCDD does not render animals more susceptible but even is protective in certain infection paradigms. Collectively, these studies highlight the multitude of (often contradictory) roles AhR plays in different cell types and pathways, which can be impacted by ligand dose, exposure route, tissue location of AhR activation, and timing/duration of AhR activation (Boule et al., 2018; Ehrlich et al., 2017).

AhR Ligands and Particulate Matter

Aryl hydrocarbon receptor signaling is now known to play a role in immune cell development, maintenance, and function, and in the absence of AhR or its ligands, immune homeostasis is interrupted. AhR has been shown to play a key role in T_H17 differentiation, innate lymphocyte development, and the production of the cytokine interleukin-22. The health effects of AhR-driven proinflammatory pathways is highlighted by studies with air pollution and ambient particular matter (PM) (Vogel, 2020).

Air pollution can trigger or exacerbate asthma, allergies, and chronic obstructive pulmonary disease and increase risk of cancer and autoimmune diseases. Indeed, air pollution is a major cause premature deaths worldwide, estimated by the World Health Organization at millions per year (World Health Organization, 2021).

The physiological consequences of PM depend on both physical properties (e.g., size, mass, shape) and properties of the chemicals absorbed to the particulates (e.g., polyaromatic hydrocarbons [PAHs], PCBs, metals). PAHs and dioxin-like PCB-bound PM can activate AhR in both *in vivo* and *ex vivo* assays and induce downstream signaling as measured by induction of CYPs 1A1, 1A2, and B1. Unlike immunosuppression associated with systemic TCDD exposure, AhR activation by PM is associated with an increase in ROS and oxidative stress, interactions with NF-κB and Nrf2 signaling pathways, and an increase in inflammation. PM2.5 alters dendritic cell differentiation toward a T_H17-promoting phenotype in an AhR-dependent manner (van Hooris et al., 2013). The proinflammatory outcomes of PM were also observed in the Seveso, Italy, cohort, where exposed individuals were twice as likely to develop chronic obstructive pulmonary disease (Baccarelli et al., 2004). The inflammatory consequences of AhR activation by PM are of particular interest with overlap between the severe acute respiratory distress syndrome coronavirus-2 (SARS-CoV-2) pandemic, wildfires, and climate change emergencies impacting air quality and environmental health outcomes.

PFAS

Per- and poly-fluoroalkyl substances (PFAS) are a class of anthropogenic chemicals commonly used in aqueous firefighting foams and in the manufacture of commercial surfactants (Figure 76–14). PFAS are often referred to as "forever chemicals" because of their long half-life in environmental and biological matrices.

Exposure

Due to their hydrophobic and oleophobic properties, PFAS are used in food packaging, cookware, furniture, and carpeting. Exposure occurs through consumer use of these products as well as through contaminated drinking water. PFAS contamination is highest near airports and chemical manufacturing plants. In addition to ingestion, PFAS exposure can occur through inhalation of indoor dust and, to a much lesser extent, through dermal exposure. PFAS do not readily degrade in the environment; therefore, these compounds have been accumulating since they were first introduced in the 1940s. While legacy PFAS such as perfluorooctane sulfonate (PFOS) have been voluntarily phased out of products in the U.S., these compounds are still used globally, and next-generation replacement

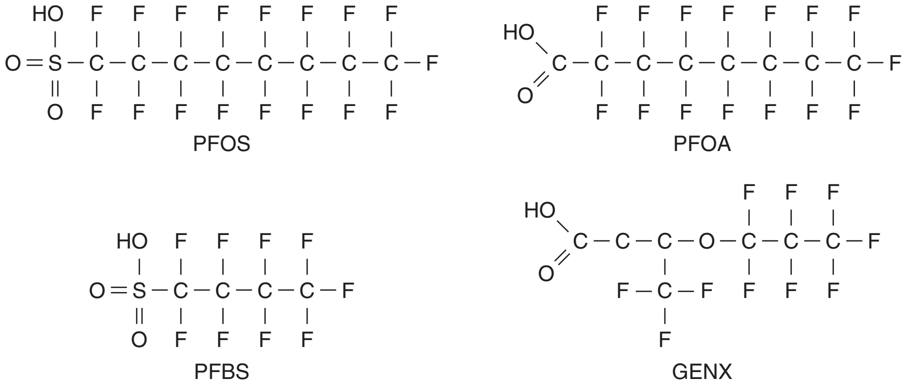

Figure 76–14 *Per- and poly-fluoroalkyl substances (PFAS).* PFAS are fully fluorinated carbon chains with a substituent as a functional head group. Their properties vary with chain length and head group. They have the general formula R-C_nF_{2n+1} (R, head group, e.g., carboxylate, sulfonate, amine, phosphate). Long-chain PFAS, n ≥ 6, have a greater tendency to bioaccumulate than PFAS with shorter chain lengths. There are also branched-chain PFAS, such as GenX, an industrial environmental toxin introduced as a replacement for PFOS. GenX is now found in drinking water drawn from the Cape Fear River around Wilmington, North Carolina (EPA, 2021; NC Policy Watch, 2021).

compounds with shorter chain lengths are continually developed. More than 4000 PFAS have been synthesized, and more than 100 have been measured in environmental samples.

Chemistry and Mode of Action

Per- and poly-fluoroalkyl substances contain at least one perfluoroalkyl moiety of the formula C_nF_{2n+1-} and a functional head group R. PFAS with fully fluorinated carbon chains are referred to as polyfluoroalkyl substances; those with incomplete replacement of the hydrogen atoms with fluorine are called perfluoralkyl compounds. The fluorinated carbon chain renders PFAS hydrophobic and oleophobic, although the specific chemical properties of PFAS are dependent on chain lengths and the functional head group. Perfluoroalkyl carboxylic acids and perfluoroalkane sulfonates with a chain length greater than 7 and 6, respectively, are considered long-chain PFAS and have a greater tendency to bioaccumulate. Perfluorooctanoic acid (PFOA) and PFOS are the most extensively manufactured and widely studied compounds. PFAS can interfere with endocrine function through disruption of lipid metabolism and steroid hormone biosynthesis, inducing ER stress and ROS, and directly interfering with PPAR (α and γ), Nrf2, and NF-κB signaling pathways (Corsini et al., 2012; Sant et al., 2021).

ADME

Following exposure by ingestion, greater than 90% of PFAS are absorbed and tend to partition to biological matrices with high protein content due to their hydrophobic nature. In the blood, most PFAS are bound to serum albumin. PFOA and PFOS preferentially partition to the liver; distribution of the next-generation PFAS, perfluorohexanesulfonic acid (PFHxS), favors the serum. PFAS are highly resistant to metabolism and bioaccumulate. The primary route of elimination is through the bile and urine, although menstruation and lactation serve as additional elimination routes. Serum concentrations tend to be higher in males than females, with elimination by menstruation partly explaining this sex difference. In humans, the half-lives of PFOA and PFOS are estimated to be 2 to 5 years.

Health Effects

Based on a combination of epidemiological and animal model studies, PFOS and PFOA are presumed to be an immune hazard to humans (National Toxicology Program, 2016). This conclusion is largely based on studies showing a negative correlation between PFAS levels and antigen-specific antibody responses. Several studies have shown that developmental exposures to PFAS are correlated with a reduction in antibody responses to childhood vaccines and with a decrease in antibodies to tetanus and diphtheria vaccines to below the clinically protective levels (Timmermann et al., 2022). Epidemiological studies are supported by murine studies showing that exposure to PFAS suppresses the T-cell–dependent antibody response, a robust *in vivo* assay of immunosuppression. There is also evidence in both human and mouse models that PFAS exposures can increase hypersensitivity responses, although the data are inconsistent and likely dependent on dose, timing, and duration of exposures (DeWitt et al., 2019). Understanding the mechanisms by which PFAS can induce its immunosuppressive and immunostimulant effects may help design epidemiological studies that can tease out these separate immunotoxic outcomes.

GI Microbiome and Environmental Toxicants

Exposure to environmental toxicants can lead to structural and functional changes in the gut microbiome. Consequences of gut microbiome toxicity include loss of bacterial diversity, changes in bacterial metabolites, and alterations in energy metabolism and balance (Tu, 2020). The loss of diversity due to toxicants can increase the susceptibility of invasion by pathogenic bacteria. Metabolites of the microbiome act as signaling molecules, and alterations of these pathways can have serious consequences on host physiology and homeostasis. The presence of specific bacterial species in the microbiome is critical for proper energy metabolism (see Chapter 6), and disturbances of these species are linked to obesity and malnutrition.

Dysbiosis, which can be generated by environmental chemicals, is linked to a broad range of diseases including inflammatory bowel disease, obesity, diabetes, cardiovascular disease, liver disease, colorectal cancer, and neurological disorders (Honda and Littman, 2016; Yuan et al., 2019). Pesticides can exert toxicity by disturbing bacterial communication through quorum sensing bacteria (Gao et al., 2018). Heavy metals can cause changes in the composition of the microbiome and alter the metabolic capabilities of the bacterial species (Li et al., 2019)

The gut microbiota can also mediate the toxicity of environmental chemicals (Claus et al., 2016). The gut flora express enzymes able to bioactivate or detoxify environmental compounds. Several key enzymes involved in bacteria-mediated metabolism of chemicals of concern include azoreductases, nitroreductases, β-glucuronidases, sulfatases, and β-lyases. These enzymes contribute to the metabolism of PAHs, nitrotoluenes, pesticides, PCBs, metals, azo dyes, melamine, and artificial sweeteners.

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Drug Facts for Your Personal Formulary: *Metal Chelators*

Drugs	Therapeutic Uses	Clinical Pharmacology and Tips
Heavy Metal Chelators		
$CaNa_2EDTA$ (calcium disodium ethylenediaminetetraacetic acid)	• Acute lead poisoning	• IV or IM administration • Not effective for chronic lead poisoning • Nephrotoxic • IV administration can increase intracranial pressure in patients with lead encephalopathy and cerebral edema • Zinc supplementation may be beneficial
Dimercaprol	• Acute arsenic, gold, and mercury poisoning • Acute lead poisoning (in combination with $CaNa_2EDTA$)	• Administered IM • Not effective for chronic intoxication • Increases toxicity of iron, cadmium, and selenium • Toxicity profile is worse than for succimer
Succimer	• Treatment of children with blood lead >45 μg/dL • Off label for adults with lead poisoning and for arsenic and mercury poisoning	• Orally bioavailable • Improved toxicity profile over dimercaprol • Reduced mobilization of lead to brain • May cause allergic reactions
Copper Chelators		
Penicillamine	• Treatment of copper intoxication due to Wilson disease • Chelates heavy metals, but more toxic, less potent, and less selective than other options	• Orally bioavailable • Allergenic • Nephrotoxic • Causes hematological toxicities • Causes a variety of other side effects
Trientine	• Treatment of Wilson disease in those intolerant of penicillamine	• Orally bioavailable • Less potent than penicillamine
Iron Chelators		
Deferoxamine	• Treatment of acute iron intoxication • Treatment of chronic iron overload due to transfusion	• IV, IM, or SC administration required • SC administration preferred for chronic iron overload • IV use for cardiovascular collapse or shock • IM administration for other acute iron intoxication cases
Deferasirox	• Treatment of chronic iron overload due to transfusion • Treatment of non–transfusion-dependent iron overload	• Orally bioavailable • Renal failure, hepatic failure, and GI hemorrhage are concerns • Not recommended over deferoxamine
Deferiprone	• Treatment of chronic iron overload due to transfusion	• Orally bioavailable • Causes agranulocytosis and neutropenia • Not recommended over deferoxamine

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Appendix

Design and Optimization of Dosage Regimens: Pharmacokinetic Data

Isabelle Ragueneau-Majlessi, Jingjing Yu, and Nina Isoherranen

TABULATED PHARMACOKINETIC PARAMETERS

- Bioavailability
- Urinary Excretion of Unchanged Drug
- Binding to Plasma Proteins
- Plasma Clearance
- Volume of Distribution
- Half-Life
- Time to Peak Concentration
- Peak Concentration

ALTERATIONS OF PARAMETERS IN THE INDIVIDUAL PATIENT

- Plasma-Protein Binding
- Clearance
- Volume of Distribution
- Half-Life
- Drug-Drug Interactions

This appendix provides a summary of basic pharmacokinetic information pertaining to small-molecule drugs that are in common clinical use and are delivered to the systemic circulation by parenteral or nonparenteral administration. Because of space limitations, this list cannot be exhaustive. Drugs designed exclusively for topical administration and not significantly absorbed into the bloodstream (e.g., ophthalmic and some dermal applications) are not included. A few other selection criteria have influenced the makeup of the list, but, in general, the authors have tried to include one or more representative drugs in each of the therapeutic areas in this text, based on distinct mechanism(s) of action. In some instances, drugs may be excluded because pharmacokinetics are not relevant to their therapeutic dosing regimen design. An obvious case is when drug efficacy is not apparently correlated with drug concentration in a reversible fashion (e.g., some cytotoxic anticancer drugs). In some instances, sufficient pharmacokinetic data are not available.

Often, the issue comes down to deciding which of the many drugs within a class should be selected. This is particularly problematic when the choices are largely therapeutically equivalent. Two criteria that have proven useful are prevalence of use and uniqueness of mechanism of action. For the present edition, we have consulted the top 200 selling drugs in 2020 for usage data. Drugs that fall into that list and meet the criteria above are generally selected. We have also consulted all new FDA drug approvals between 2016 and 2020. As mentioned above, a less used drug may be included if it has a different mechanism of action than the frequently used drugs, some additional actions that offer a unique therapeutic advantage, or a more acceptable side effect profile. Pharmacokinetic data for many older drugs not included in this appendix can be found in earlier editions of this book.

With rare exceptions (e.g., interferons), recombinant therapeutic proteins have been excluded from this compilation. In many cases, the therapeutic protein interacts with specific tissue or cellular targets with exquisite affinity; as a result, clinical efficacy seldom correlates with circulating drug concentration and pharmacokinetics are not considered critical in guiding dosing. For example, for a number of therapeutic antibodies, the antibody is administered at a fixed dose and at prolonged intervals that allow for its near-complete clearance (e.g., *infliximab*). There is also the constraint of space in the book; hence, the appendix focuses on small-molecule drugs.

A major objective of this appendix is to present pharmacokinetic data in a format that informs the clinician of the essential characteristics of drug disposition that form the basis of drug-dosage regimen design. Table AI-1 contains quantitative information about the absorption, distribution, and elimination of drugs; the effects of some disease states, age, pregnancy, and sex on these processes, where significant; and the correlation of efficacy and toxicity with drug concentrations in blood/plasma. The general principles that underlie the design of appropriate maintenance dose and dosing interval (and, where appropriate, the loading dose) for the average patient are described in Chapter 2. Their application using the data in Table AI-1 for individualization of dosage regimens is presented here.

To use the data that are presented, one must understand clearance concepts and their application to drug-dosage regimens. One also must know average values of clearance as well as some kinetic measures of drug absorption and distribution. The text below defines the eight basic parameters that are listed in the tabular material for each drug and key factors that influence these values in both healthy subjects and patients with renal or hepatic impairment. It obviously would be more straightforward if there were a consensus on a standard value for a given pharmacokinetic parameter; instead, literature estimates usually vary over a wide range, and a consensus set of pharmacokinetic values has been reached for only a limited number of drugs.

In Table AI-1, a single set of values for each parameter and its variability in a relevant population has been selected from the literature, based on the scientific judgment of the authors. Most data are in the form of a study population mean value ± 1 standard deviation (mean ± SD). However, some data are presented as mean and range of values (in parentheses) observed for the study population (i.e., the lowest and highest value reported). There are times when data are reported as a geometric mean with a 95% confidence interval or a coefficient of variation (in percentage). When sufficient data were available, a range of mean values obtained from different studies of similar design is presented: these ranges are in parentheses, sometimes below the primary study data. Occasionally, only a single mean value for the study population was available in the literature and is reported as such. Finally, some drugs can be administered intravenously in an unmodified form and orally as a prodrug. When relevant information about both the prodrug and the active molecule is needed, we have included both, using an abbreviation to indicate the species that was measured, followed by another abbreviation in parentheses to indicate the species that was dosed [e.g., *G* (V) indicates a parameter for *ganciclovir* after administration of the prodrug, *valganciclovir*].

A number of approved drugs are actually the active metabolite or stereoisomer of an earlier marketed drug. For example, *desloratadine* is the *O*-desmethyl metabolite of *loratadine*, and *esomeprazole* is the active *S*-enantiomer of *omeprazole*. Unless the parent drug and the active

Abbreviations

AUC: area under the plasma drug concentration-time curve
NDA: New Drug Application

metabolite offer distinct therapeutic advantages, only the more established or more commonly used drug is listed, and relevant information on its alternate active form is presented in the same table. This approach has permitted us to include more drugs in the Appendix, hopefully without undue confusion. The only exception is with *prednisone* and *prednisolone* which are both included as individual drugs and which undergo interconversion in the body.

Unless otherwise indicated in footnotes, data reported in the table are those determined in healthy adults. The direction of change for these values in particular disease states or due to physiological variables is noted below the average value. In previous editions, we have included disease states where studies have shown no change or clinically insignificant change for a particular pharmacokinetic measure. For the sake of space and consistency, we have eliminated the results of negative studies; hence, when a disease state is not listed, it implies no significant change due to the disease or that no information is available. One or more references are provided for each of the established drugs, typically an original journal publication, a review on its clinical pharmacokinetics, or a web-based drug database; the latter two secondary sources provide a broader range of papers for the interested reader. In some instances (e.g., many new drug approvals), we have relied on unpublished data provided by the drug sponsor in its New Drug Application (NDA) files or product labeling submitted to the U.S. Food and Drug Administration (FDA). This information can be accessed at *Drugs@FDA: FDA-Approved Drugs* (https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm). After searching by drug name, pharmacokinetic data are found most often in the *Clinical Pharmacology and Biopharmaceutics* section. Recent standardization in the presentation of data supporting the NDA in this section makes this an increasingly valuable resource.

Tabulated Pharmacokinetic Parameters

Each of the eight parameters presented in Table AI-1 has been discussed in detail in Chapter 2. The following discussion focuses on the format in which the values are presented.

Bioavailability

The *extent of oral bioavailability* (F) is expressed as a percentage of the administered dose. This value represents the percentage of the administered dose that is available to the systemic circulation–the fraction of the oral dose that reaches the arterial blood in an active or prodrug form, expressed as a percentage (0%–100%). T_{max} as a measure of the *rate of availability* is also presented in the table under peak time in hours after dosing. Values for multiple routes of administration are provided, when appropriate and available. In most cases, the tabulated value represents an absolute oral bioavailability that has been determined from a comparison of area under the plasma drug concentration–time curve (AUC) between the oral dose and an intravenous reference dose. For those drugs where intravenous administration is not feasible, an approximate estimate of oral bioavailability based on secondary information (e.g., urinary excretion of unchanged drug, especially when the nonrenal route of elimination is minimal) is presented, or the column is left blank (denoted by a long dash [–]). A dash also will appear when a drug is given by parenteral administration only.

A low oral bioavailability may result from a poorly formulated dosage form that fails to disintegrate or dissolve in the gastrointestinal fluid, degradative loss of drug in the gastrointestinal fluid, poor mucosal permeability including active efflux transport of drug back into the gut lumen, first–pass metabolism during transit through the intestinal epithelium, or first–pass hepatic metabolism or biliary excretion (see Chapter 2). In the case of drugs with extensive first-pass metabolism, hepatic disease may increase oral availability because hepatic metabolic capacity decreases and/or because vascular shunts develop around the liver.

Urinary Excretion of Unchanged Drug

The second parameter in Table AI-1 is the amount of drug eventually excreted unchanged in the urine, expressed as a percentage of the administered dose. Values represent the percentage expected in a healthy young adult (creatinine clearance (CL_{cr}) ≥100 mL/min). When possible, the value listed is that determined after bolus intravenous administration of the drug, for which bioavailability is 100%. If the drug is given orally, this parameter may be underestimated due to incomplete absorption of the dose; such approximated values are indicated with a footnote. The parameter obtained after intravenous dosing is of greater utility because it reflects the fractional contribution of renal clearance (CL_R) to total body clearance (CL) irrespective of bioavailability.

Renal disease is the primary factor that causes changes in this parameter. This is especially true when alternate pathways (nonrenal clerance CL_{NR}) of elimination are available; thus, as renal function decreases, a greater fraction of the dose is eliminated via other routes. Because renal function generally decreases as a function of age, the percentage of drug excreted unchanged also decreases with age when alternate pathways of elimination are available. In addition, for a number of weakly acidic and basic drugs with pK_a values within the normal range for urine pH, changes in urine pH will affect their urinary excretion.

Binding to Plasma Proteins

The tabulated value is the percentage of drug in plasma that is bound to plasma proteins at concentrations of the drug that are achieved clinically. In almost all cases, the values are from measurements performed *in vitro* (rather than from *ex vivo* measurements of binding to proteins in plasma obtained from patients to whom the drug had been administered). When a single mean value is presented, it signifies that there is no apparent change in percent bound over the range of plasma drug concentrations resulting from the usual clinical doses. In cases in which saturation of binding to plasma proteins is approached within the therapeutic range of plasma drug concentrations, a range of bound percentages is provided for concentrations at the lower and upper limits of the range. For some drugs, there is disagreement in the literature about the extent of plasma-protein binding; in those cases, the range of reported values is given.

Plasma-protein binding is affected primarily by disease states, notably hepatic and renal impairment, and inflammatory diseases that alter the concentration of albumin, α_1-acid glycoprotein, or other proteins in plasma that bind drugs. Uremia also changes the apparent binding affinity of albumin for some drugs. Disease-induced changes in plasma-protein binding can dramatically affect the volume of distribution (V), clearance (CL), and elimination $t_{1/2}$ of a drug. In regard to clinical relevance, it is important to assess the change in unbound drug concentration or AUC, particularly when only unbound drug can cross biological barriers and gain access to the site of action.

Plasma Clearance

Systemic clearance of total drug from plasma or blood is given in Table AI-1. Clearance varies as a function of body size and most frequently is presented in the table in units of mL/min/kg of body weight. Normalization to measures of body size other than weight may at times be more appropriate, such as normalization to body surface area in infants to better reflect the growth and development of the liver and kidneys. However, weight is easy to obtain, and its use often offsets any small loss in accuracy of clearance estimates, especially in adults. Exceptions to this rule are the anticancer drugs, for which dosage normalization to body surface area is conventionally used. When unit conversion was necessary, individual or mean body weight or body surface area (when appropriate) from the cited study were used, or if this was not available, a body mass of 70 kg or a body surface area of 1.73 m^2 for healthy adults was assumed.

For the few drugs that exhibit saturation kinetics following therapeutics doses, the Michaelis Menten constant, K_m, and the maximum velocity of drug elimination, V_{max}, are given if available. These values represent, respectively, the plasma concentration at which half of the maximal rate of elimination is reached in units of mass/volume and the maximal rate of elimination in units of mass/time/kg of body weight. K_m must be in the same units as the concentration of drug in plasma (C_p).

In almost all cases, clearances based on plasma (or serum) concentration data are presented in Table AI-1, because drug analysis most often is performed on plasma samples. The few exceptions where clearance from blood is presented are indicated by a footnote. Clearance estimates based on blood concentration may be useful when a drug concentrates in the blood cells.

To be accurate, clearances must be determined after intravenous drug administration. When only nonparenteral data are available, the ratio of *CL/F* is given; values offset by the fractional availability (*F*) are indicated in a footnote. When a drug, or its active isomer for racemic compounds, is known to be a substrate for a cytochrome P450 (CYP) or drug transporter, this information is provided in a footnote. This information is important for understanding pharmacokinetic variability due to genetic polymorphisms and for predicting metabolically based drug-drug interactions (see Appendix II).

Volume of Distribution

The volume of distribution at steady state (V_{ss}) is given in Table AI-1 and is expressed in units of L/kg or in units of L/m^2 for some anticancer drugs. Again, when unit conversion was necessary, individual or mean body weights or body surface area (when appropriate) from the cited study were used; if such data were not available, a body mass of 70 kg or a body surface area of 1.73 m^2 for healthy adults were assumed.

When estimates of V_{ss} were not available, values for V_{area} (also known as V_β or V_z) were provided; V_{area} represents the volume at equilibrated distribution during the terminal elimination phase. Unlike V_{ss}, V_{area} varies when drug elimination changes, even though there is no change in extravascular distribution. Because we may wish to know whether a particular disease state influences either the clearance or the tissue distribution of the drug, it is preferable to define volume in terms of V_{ss}, a parameter that is less likely to depend on changes in the rate of elimination. Occasionally, the condition under which the distribution volume was obtained was not specified in the primary reference; this is denoted by the absence of a subscript (shown as V).

As with clearance, V_{ss} usually is defined in the table in terms of concentration in plasma rather than blood. Further, if data were not obtained after intravenous administration of the drug, a footnote will make clear that the apparent volume estimate, V_{ss}/F or V_{area}/F, was reported.

Half-Life

Half-life ($t_{1/2}$) is the time required for the plasma concentration to decline by one-half *when elimination is first order*. It also governs the rate of approach to steady state and the degree of drug accumulation during multiple dosing or continuous infusion. For example, at a fixed dosing interval, the patient will be at 50% of steady state after one half-life, 75% of steady state after two half-lives, 93.75% of steady state after four half-lives, etc. Determination of $t_{1/2}$ is straightforward when drug elimination follows a monoexponential pattern (i.e., one-compartment model). However, for a number of drugs, plasma concentration follows a multiexponential pattern of decline over time. The mean value listed in Table AI-1 corresponds to an effective rate of elimination that covers the clearance of a major fraction of the absorbed dose from the body. In many cases, this $t_{1/2}$ refers to the rate of elimination in the terminal exponential phase. For a number of drugs, however, the $t_{1/2}$ of an earlier phase is presented, even though a prolonged $t_{1/2}$ may be observed later at very low plasma concentrations when very sensitive analytical techniques are used. If the latter component accounts for 10% or less of the AUC, predictions of drug accumulation in plasma during continuous or repetitive dosing will be in error by no more than 10% if this longer $t_{1/2}$ is ignored. The effective $t_{1/2}$ that predicts time to steady state for multicompartmental drug kinetics is given in Table AI-1.

Half-life usually is independent of body size because it is a function of the ratio of two parameters, volume of distribution and clearance, each of which is proportional to body size. It also should be noted that the $t_{1/2}$ is preferably obtained from intravenous studies, if feasible, because the $t_{1/2}$ of decline in plasma drug concentration after oral dosing can be influenced by prolonged absorption, such as when slow-release formulations are given. If the $t_{1/2}$ is derived from an oral dose, this will be indicated in a footnote of Table AI-1.

Time to Peak Concentration

Because clearance concepts are used most often in the design of multiple dosage regimens, the extent rather than the rate of availability is more critical to estimate the average steady-state concentration of drug in the body. In some circumstances, the degree of fluctuation in plasma drug concentration (i.e., peak (C_{max}) and trough (C_{min}) concentrations), which govern drug efficacy and side effects, can be greatly influenced by modulation of drug absorption rate through the use of sustained- or extended-release formulations. Controlled-release formulations often permit a reduction in dosing frequency from three or four times daily to once or twice daily. There also are drugs that are given on an acute basis (e.g., for the relief of breakthrough pain or to induce sleep), for which the rate of drug absorption is a critical determinant of onset of effect. Thus, information about the expected average time to achieve maximal plasma or blood concentration and the degree of interindividual variability in that parameter have been included in Table AI-1.

When more than one type of drug formulation is available commercially, we have provided absorption information for both the immediate- and extended-release formulations. Not surprisingly, the presence of food in the gastrointestinal tract can alter both the rate and extent of drug availability. We have indicated with footnotes when the consumption of food near the time of drug ingestion may have a significant effect on the drug bioavailability.

Peak Concentration

There is no general agreement about the best way to describe the relationship between the concentration of drug in plasma and its effect. Many different kinds of data are present in the literature, and use of a single-effect parameter or effective concentration is difficult. This is particularly true for antimicrobial agents because the effective concentration depends on the identity of the microorganism causing the infection. It also is important to recognize that concentration-effect relationships are most easily obtained at steady state or during the terminal log-linear phase of the concentration-time curve, when the drug concentration(s) at the site(s) of action is (are) expected to parallel those in plasma. Thus, when attempting to correlate a blood or plasma level to effect, the temporal aspect of distribution of drug to its site of action must be taken into account.

Despite these limitations, it is possible to define the minimum effective or toxic concentrations or the plasma concentration at which 50% of the maximum effect is observed (EC_{50}) for some of the drugs currently in clinical use. However, in reviewing the list of drugs approved within the past 10 years, it is rare to find a declaration of an *effective concentration range*, even in the manufacturer's product label. Thus, it is necessary to infer therapeutic concentrations from concentrations observed following *effective dosage* regimens. For a given dosage regimen, a time-averaged steady-state blood or plasma concentration (i.e., $\overline{C}_{ss}$ as estimated by dividing the mean AUC by the duration of the dosing interval) and the associated interindividual variability might be one appropriate parameter to report; however, such data often are not available. Also, $\overline{C}_{ss}$ does not take into account the onset and offset of effect during fluctuation of plasma drug concentration over a dosing interval. In some instances, drug efficacy may be more closely linked with peak concentration than with the average or trough concentration, and differences in peak concentration for specific populations sometimes are associated with increased incidence of drug toxicity.

For practical reasons, the most reported parameter, C_{max} (peak concentration), rather than effective or toxic concentrations, is presented in Table AI-1. This provides a more consistent body of information about drug exposure from which one can infer, if appropriate, efficacious or toxic blood levels. Although the value reported is the highest that would be encountered in a given dose interval, C_{max} can be related to the trough concentration (C_{min}) through appropriate mathematical predictions (see Chapter 2). Because peak levels will vary with dose, we have attempted to present concentrations observed with a customary dose regimen that is recognized to be effective in the majority of patients. When a higher or lower dose rate is used, the expected peak concentration can be adjusted by assuming dose proportionality, unless nonlinear kinetics are indicated. In some instances, only limited data pertaining to multiple dosing are available, so single-dose peak concentrations are presented. When specific information is available about an effective therapeutic range of concentrations, about concentrations at which toxicity occurs or the EC_{50} value for a specific effect they have been incorporated in a footnote.

It is important to recognize that significant differences in C_{max} will occur when comparing similar daily-dose regimens for an immediate-release and extended-release product. Indeed, the extended-release product sometimes is administered to reduce peak-trough fluctuations during the dosing interval and to minimize swings between potentially toxic or ineffective drug concentrations. Again, we report C_{max} for both immediate- and extended-release formulations, when available. In addition to parent drug concentrations, information on any active metabolite that circulates at a concentration that may contribute to the overall pharmacological effect have been included, particularly those active metabolites that accumulate with multiple dosing. Likewise, for chiral drugs whose stereoisomers differ in their pharmacological activity and clearance characteristics, information on concentrations of the individual enantiomers or the active enantiomer that contributes most to the drug's efficacy is presented.

Alterations of Parameters in the Individual Patient

Dose adjustments for an individual patient should be made according to the manufacturer's recommendation in the FDA-approved product label when available. This information generally is available when disease, age, or genetics have a significant impact on drug disposition. In some cases, a significant difference in drug disposition from the "average" healthy adult can be expected but may not require dose adjustment because of a sufficiently broad therapeutic index. In other cases, dose adjustment may be necessary, but no specific information is available.

Unless otherwise specified, the values in Table AI-1 represent mean values for populations of healthy adults; it may be necessary to modify them for calculation of dosage regimens for individual patients. The fraction available (F) and clearance (CL) would need to be adjusted to compute a maintenance dose necessary to achieve a desired average steady-state concentration. To calculate the loading dose, knowledge of the volume of distribution is needed. The estimated $t_{1/2}$ is used in deciding a dosing interval that provides an acceptable peak-trough fluctuation; note that this may be the apparent $t_{1/2}$ following dosing of a slowly absorbed formulation. The values reported in the table and the adjustments apply only to adults; exceptions are footnoted. Although the values at times may be applied to children who weigh more than approximately 30 kg, it is best to consult textbooks of pediatrics or other sources for definitive advice.

For each drug, changes in the parameters caused by certain disease states are noted within the eight segments of the table. In all cases, the qualitative direction of changes is noted, such as "↓ LD," which indicates a significant decrease in the parameter in a patient with chronic liver disease. The relevant literature and the product label should be consulted for more definitive, quantitative information for dosage adjustment recommendations.

Plasma-Protein Binding

Most acidic drugs that are extensively bound to plasma proteins are bound to albumin. Basic lipophilic drugs, such as *propranolol*, often bind to other plasma proteins (e.g., α_1-acid glycoprotein and lipoproteins). The degree of drug binding to proteins will differ in pathophysiological states that cause changes in plasma-protein concentrations. Significant pharmacokinetic effects from a change in plasma-protein binding will be denoted under clearance or volume of distribution.

Although pharmacokinetic parameters based on total drug or metabolite concentrations often are reported, it is important to recognize that in many cases it is the concentration of *unbound drug* that drives access to the site of action and the degree of pharmacological effect. Remarkable changes in the total plasma concentration may accompany disease-induced alteration in protein binding; however, the clinical outcome is not always affected because an increase in free fraction also will increase the apparent clearance of an orally administered drug and of a low-extraction drug dosed intravenously. Under such a scenario, the time-averaged unbound plasma concentration over a dosing interval at steady state will not change with reduced or elevated plasma-protein binding, despite a significant change in time-averaged total drug concentration. If so, no adjustment of daily maintenance dose is needed.

Clearance

For drugs that are partly or predominantly eliminated by renal excretion, plasma clearance changes with the renal function of an individual patient. This necessitates dosage adjustment that is dependent on the fraction of normal renal function remaining and the fraction of drug normally excreted unchanged in the urine. The latter quantity appears in the table; the former can be estimated as the ratio of the patient's estimated or measured creatinine clearance (CL_{cr}) to a normal value (100 mL/min/1.73 m^2). Creatinine clearance is often estimated from standardized serum creatinine concentration (C_{cr}) using the Modification of Diet in Renal Disease (MDRD) Study equation for CL_{cr} less than 60 mL/min/1.73 m^2 and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Except for certain oncolytic agents, the data presented in the table are normalized to weight. Thus, interindividual variability in the weight-normalized clearance reflects a variation in the intrinsic metabolic or transport clearance and not the size of the organ. Further, these differences can be attributed to variable expression/function of metabolic enzymes or transporters. However, it is important to recognize that liver mass and total enzyme/transporter content may not increase or decrease in proportion to weight in individuals with obesity or malnourishment. Alternative approaches such as normalization by body surface area or other measures of body mass may be more appropriate. For example, many of the drugs used to treat cancer are dosed according to body surface area. In the tabulation, if the literature reported dose per body surface area, we present the data in the same units. If the cited clearance data were not normalized, but the preponderance of the literature used body surface area, we followed the practice of using values of body surface area from the literature source or a standard of 1.73 m^2 for a healthy adult.

Volume of Distribution

Volume of distribution should be adjusted for the modifying factors indicated in Table AI-1 as well as for body size. Again, the data in the table most often are normalized to weight. Unlike clearance, volume of distribution in an individual is generally proportional to weight itself. Whether this applies to a specific drug depends on the actual sites of distribution of drug; no absolute rule applies.

Whether to adjust volume of distribution for changes in binding to plasma proteins cannot be decided in general; the decision depends critically on whether the factors that alter binding to plasma proteins also alter binding to tissue proteins. Qualitative changes in volume of distribution, when they occur, are indicated in the table.

Half-Life

Half-life may be estimated from the adjusted values of clearance (CL_{pt}) and volume of distribution (V_{pt}) for the patient: $t_{1/2} = 0.693 * V_{pt} / CL_{pt}$. Because $t_{1/2}$ has been the parameter most often measured and reported in the literature, qualitative changes for this parameter almost always are given in the table.

Drug-Drug Interactions

Appendix II, Table AII-1, and Table AII-2 provide information on drug-drug interactions that can lead to large changes in drug exposure, with special reference to drugs included in Table AI-1, the large table of pharmacokinetic data.

TABLE AI–1 ■ PHARMACOKINETIC DATA

Key: Unless otherwise indicated by a specific footnote, the data are presented for the study population as a mean value ± 1 standard deviation (SD), a mean and range (lowest-highest in parentheses) of values, a range of the lowest-highest values, or a single mean value. ACE, angiotensin-converting enzyme; Aged, aged; AIDS, acquired immunodeficiency syndrome; CHF, congestive heart failure; C_{max}, peak concentration; CYP, cytochrome P450; F or Fem, female; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IM, intramuscular; IV, intravenous; LD, chronic liver disease; M, male; MAO, monoamine oxidase; NAT, *N*-acetyltransferase; Neo, neonate; Obes, obesity; *PDR*58, *Physicians' Desk Reference,* 54th ed. Montvale, NJ, Medical Economics Co., 2000; *PDR*58, *Physicians' Desk Reference,* 58th ed. Montvale, NJ, Medical Economics Co., 2004; Pneu, pneumonia; PO, oral administration; Preg, pregnant; Prem, premature infants; Rac, racemic mixture of stereoisomers; RD, chronic renal disease; SC, subcutaneous; Smk, smoking; ST, sulfotransferase; T_{max}, peak time; UGT, UDP-glucuronosyl transferase. Other abbreviations are defined in the text section of this appendix.

Acetaminophen[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
88 ± 15	3 ± 1	<20	5.0 ± 1.4[b]	0.95 ± 0.12	2.7 ± 0.6	0.31–1.4	20 μg/mL[c]
			↓ LD, Aged		↑ LD		
			↑ Obes				

[a] Pharmacokinetic values reported are for doses <2 g; drug exhibits concentration-dependent kinetics above this dose. [b] Acetaminophen is eliminated predominantly via glucuronidaion and sulfation with a minor pathway via CYP2E1-mediated metabolism. [c] Mean concentration following a 20-mg/kg oral dose. Hepatic toxicity associated with levels >300 μg/mL at 4 h after an overdose.

References: Forrest JA, et al. Clinical pharmacokinetics of paracetamol. *Clin Pharmacokinet,* **1982**, *7*:93–107; van Rongen A, et al. Morbidly obese patients exhibit increased CYP2E1-mediated oxidation of acetaminophen. *Clin Pharmacokinet,* **2016**, *55*:833–847.

Acyclovir

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
15–30[a]	75 ± 10	15 ± 4	*CL* = 3.37 CL_{cr} + 0.41	0.69 ± 0.19	2.4 ± 0.7	1.5–2[b]	3.5–5.4 μM[b]
			↓ Neo	↑ Neo	↑ RD, Neo		

[a] Decreases with increasing dose. [b] Range of steady-state concentrations following a 400-mg dose given orally every 4 h to steady state.

Reference: Laskin OL. Clinical pharmacokinetics of acyclovir. *Clin Pharmacokinet,* **1983**, *8*:187–201.

Albendazole[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—[b]	<1	70	10.5–30.7[c]	—	8 (6–15)[d]	2–4[e]	0.50–1.8 μg/mL[e]
↑ Food							

[a] Oral albendazole undergoes rapid and essentially complete first-pass metabolism to albendazole sulfoxide (ALBSO), which is pharmacologically active. Pharmacokinetic data for ALBSO in male and female adults are reported. [b] The absolute bioavailability of ALBSO is not known but is increased by high-fat meals. [c] *CL/F* following twice-daily oral dosing to steady state. Chronic albendazole treatment appears to induce the metabolism of ALBSO. [d] $t_{1/2}$ reportedly shorter in children with neurocysticercosis compared with adults; may need to be dosed more frequently (three times a day) in children, rather than twice a day, as in adults. [e] Following a 7.5-mg/kg oral dose given twice daily for 8 days to adults.

References: Marques MP, et al. Enantioselective kinetic disposition of albendazole sulfoxide in patients with neurocysticercosis. *Chirality,* **1999**, *11*:218–223. *PDR*58, **2004**, p. 1422. Sanchez M, et al. Pharmacokinetic comparison of two albendazole dosage regimens in patients with neurocysticercosis. *Clin Neuropharmacol,* **1993**, *76*:77–82. Sotelo J, et al. Pharmacokinetic optimisation of the treatment of neurocysticercosis. *Clin Pharmacokinet,* **1998**, *34*:503–515.

Albuterol[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
PO, *R*: 30 ± 7	*R*: 46 ± 8	*Rac*: 7 ± 1	*R*: 10.3 ± 3.0	*R*: 2.00 ± 0.49	*R*: 2.00 ± 0.49	*R*: 1.5[b]	*R*: 3.6 (1.9–5.9) ng/mL[b]
PO, *S*: 71 ± 9	*S*: 55 ± 11		*S*: 6.5 ± 2.0	*S*: 1.77 ± 0.69	*S*: 2.85 ± 0.85	*S*: 2.0[b]	*S*: 11.4 (7.1–16.2) ng/mL[b]
IH, *R*: 25			↓ RD	↓ RD			
IH, *S*: 47							

[a] Data from healthy subjects for *R*- and *S*-enantiomers. No major sex differences. No kinetic differences in asthmatics. β-Adrenergic activity resides primarily with R-enantiomer. PO, oral; IH, inhalation. Oral dose undergoes extensive first-pass sulfation at the intestinal mucosa. [b] Median (range) following a single 4-mg oral dose of racemic albuterol.

References: Boulton DW, et al. Enantioselective disposition of albuterol in humans. *Clin Rev Allergy Immunol,* **1996**, *14*:115–138. Mohamed MH, et al. Effects of sex and race on albuterol pharmacokinetics. *Pharmacotherapy,* **1999**, *19*:157–161.

Alendronate[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
<0.7[b]	44.9 ± 9.3	78	1.11 (1.00–1.22)[c]	0.44 (0.34–0.55)[c]	~1.0[e]	IV: 2[f]	IV: ~275 ng/mL[f]
↓ Food			↓ RD[d]				PO: <5–8.4 ng/mL[f]

[a] Data from healthy postmenopausal female subjects. [b] Based on urinary recovery; reduced when taken <1 h before or up to 2 h after a meal. [c] *CL* and V_{ss} values represent mean and 90% confidence interval. [d] Mild to moderate renal impairment. [e] The $t_{1/2}$ for release from bone is ~11.9 years. [f] Following a single 10-mg IV infusion over 2 h and a 10-mg oral dose daily for >3 years.

References: Cocquyt V, et al. Pharmacokinetics of intravenous alendronate. *J Clin Pharmacol,* **1999**, *39*:385–393. Porras AG, et al. Pharmacokinetics of alendronate. *Clin Pharmacokinet,* **1999**, *36*:315–328.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Alfentanil

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
–	<1	92 ± 2	6.7 ± 2.4[a]	0.8 ± 0.3	1.6 ± 0.2	—	100–200 ng/mL[b]
↓ Food[c]		↓ LD	↓ Aged, LD	↓ LD	↑ Aged, LD		310–340 ng/mL[c]

[a]Metabolically cleared by CYP3A. [b]Provides adequate anesthesia for superficial surgery. [c]Provides adequate anesthesia for abdominal surgery.

Reference: Bodenham A, et al. Alfentanil infusions in patients requiring intensive care. *Clin Pharmacokinet,* **1988**, *75*:216–226.

Alfuzosin[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
50%[b]	11[d]	~90	6.4 ± 1.8[e]	3.2 ± 1.1	6.3 ± 0.9[g]	9[h]	16.6 ± 5.5 ng/mL[h]
↓ Food[c]			↓ LD[f]				

[a]Administered as racemate. [b]Value reported for 10-mg, extended-release formulation, once daily, given with a high-fat meal. [c]Oral bioavailability in the fasted state is one-half that of the fed state. [d]Value reported in product label; however, route of administration uncertain. [e]Alfuzosin is cleared primarily through CYP3A-dependent hepatic metabolism. *R/S* AUC ratio = 1.35. [f]Study in patients with moderate to severe liver impairment; *CL/F* reduced to one-third to one-quarter of control. [g]Apparent $t_{1/2}$ = 9 h for extended-release product; reflects absorption-limited kinetics. [h]Following a 10-mg dose of extended-release formulation given once a day for 5 days.

References: McKeage K, et al. Alfuzosin: a review of the therapeutic use of the prolonged-release formulation given once daily in the management of benign prostatic hyperplasia. *Drugs,* **2002**, *62*:135–167. Drugs@FDA. Uroxatral NDA and label. NDA approved on 6/12/03; label approved on 5/20/09. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2003/21-287_Uroxatral_BioPharmr_P1.pdf and http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/021287s013lbl.pdf. Accessed May 17, 2010.

Aliskiren

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
2.6[a]	7.5	49 (47–51)	2.1[b]	1.9	24–40[d]	2.5[e]	72 ± 67 ng/mL[e]
↓ Food[a]			↓ RD[c]				

[a]Absolute bioavailability of 75 mg in hard gelatin capsule reported. Oral AUC decreased 71% with high-fat meal. [b]Aliskiren is cleared by both renal excretion and CYP3A-dependent hepatic metabolism; however, the relative contribution of each pathway to total drug clearance is unclear. [c]Study in patients with mild to severe renal impairment; systemic exposure increased 1.0- to 2.5-fold but was independent of disease severity. [d]$t_{1/2}$ estimated from single oral dose data. [e]Following 150-mg oral dose given once daily to steady state in healthy adults.

References: Azizi M. Direct renin inhibition: clinical pharmacology. *J Mol Med,* **2008**, *86*:647–654. Azizi M, et al. Renin inhibition with aliskiren: where are we now, and where are we going? *J Hypertens,* **2006**, *24*:243–256. Vaidyanathan S, et al. Clinical pharmacokinetics and pharmacodynamics of aliskiren. *Clin Pharmacokinet*, **2008**, *27*:515–531.

Allopurinol[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
53 ± 13	12	—	9.9 ± 2.4[b]	0.87 ± 0.13	A: 1.2 ± 0.3	A: 1.7 ± 1.0[c]	A: 1.4 ± 0.5 μg/mL[c]
			↑ RD, Aged[b]		O: 24 ± 4.5	O: 4.1 ± 1.4[c]	O: 6.4 ± 0.8 μg/mL[c]

[a]Data from healthy male and female subjects. Allopurinol (A) is rapidly metabolized to the pharmacologically active oxypurinol (O). [b]Increased oxypurinol AUC during renal impairment and in the elderly. [c]Following a single 300-mg oral dose.

*References: PDR*58, **2000**, p. 1976. Turnheim K, et al. Pharmacokinetics and pharmacodynamics of allopurinol in elderly and young subjects. *Br J Clin Pharmacol,* **1999**, *48*:501–509.

Alosetron[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
57 (33–97)[a]	—	82	8.3 (6.5–10.8)[b]	0.91 (0.70–1.12)	1.4 (1.3-1.6)	1[d]	5.5 (4.8-6.4) ng/mL[d]
			↓ LD[c]				

[a]Absolute bioavailability of a 4-mg dose, as compared to IV infusion. [b]Alosetron is cleared primarily by CYP1A2-dependent hepatic metabolism. [c]Study in patients with moderate to severe liver impairment; oral AUC 1.6- to 14-fold higher than control. [d]Following a 1-mg oral dose, given twice a day to steady state.

References: Balfour JA, et al. Alosetron. *Drugs*, **2000**, *59*:511–518. Drugs@FDA. Lotronex NDA and label. NDA approved on 2/11/00; label approved on 4/1/08. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2000/21107a_Lotronex_clinphrmr_P3.pdf and http://www.accessdata.fda.gov/drugsatfda_docs/label/2008/021107s013lbl.pdf. Accessed May 17, 2010. Koch KM, et al. Sex and age differences in the pharmacokinetics of alosetron. *Br J Clin Pharmacol*, **2002**, *53*:238–242.

Alprazolam

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
88 ± 16	20	71 ± 3	0.74 ± 0.14[a]	0.72 ± 0.12	12 ± 2	1.5 (0.5–3.0)[c]	21 (15–32) ng/mL[c]
		↑ LD	↓ Obes, LD, Aged[b]		↑ Obes, LD, Aged[b]		

[a]Metabolically cleared by CYP3A and other CYP isozymes. [b]Data from male subjects only. [c]Mean (range) from 19 studies following a single 1-g oral dose given to adults.

Reference: Greenblatt DJ, et al. Clinical pharmacokinetics of alprazolam. Therapeutic implications. *Clin Pharmacokinet,* **1993**, *24*:453–471.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Amiodarone[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
46 ± 22	0	99.98 ± 0.01	1.9 ± 0.4[b]	66 ± 44	25 ± 12 days[c]	2–10[d]	1.5–2.4 μg/mL[d]

[a] Significant plasma concentrations of an active desethyl metabolite are observed (ratio of drug/metabolite ~1); $t_{1/2}$ for metabolite = 61 days. [b] Metabolically cleared by CYP3A. [c] Longer $t_{1/2}$ noted in patients (53 ± 24 days); all reported $t_{1/2}$s may be underestimated because of insufficient length of sampling. [d] Following a 400-mg/day oral dose to steady state in adult patients.

Reference: Gill J, et al. Amiodarone. An overview of its pharmacological properties, and review of its therapeutic use in cardiac arrhythmias. *Drugs,* **1992**, *43*:69–110.

Amlodipine[a]

74 ± 17	10	93 ± 1	5.9 ± 1.5[b]	16 ± 4	39 ± 8	5.4–8.0[c]	18.1 ± 7.1 ng/mL[c]
			↓ Aged, LD		↑ Aged, LD		

[a] Racemic mixture; in young, healthy subjects, there are no apparent differences between the kinetics of the more active *R*-enantiomer and *S*-enantiomer. [b] Amlodipine is mainly cleared by CYP3A4-dependent metabolism. [c] Following a 10-mg oral dose given once daily for 14 days to healthy male adults.

Reference: Meredith PA, et al. Clinical pharmacokinetics of amlodipine. *Clin Pharmacokinet,* **1992**, *22*:22–31.

Amoxicillin

93 ± 10[a]	86 ± 8	18	2.6 ± 0.4	0.21 ± 0.03	1.7 ± 0.3	1–2	IV: 46 ± 12 μg/mL[c]
			↑ Preg		↑ RD, Aged[b]		PO: 5 μg/mL[c]
			↓ RD, Aged[b]				

[a] Dose dependent; value shown is for a 375-mg dose; decreases to ~50% at 3000 mg. [b] No change if renal function is not decreased. [c] Following a single 500-mg IV bolus dose in healthy elderly adults or a single 500-mg oral dose in adults.

References: Andrew MA, et al. Amoxicillin pharmacokinetics in pregnant women: modeling and simulations of dosing strategies. *Clin Pharmacol Ther,* **2007**, *81*:547–556. Hoffler D. The pharmacokinetics of amoxicillin [in German]. *Adv Clin Pharmacol,* **1974**, *7*:28–30. Sjovall J, et al. Intra- and inter-individual variation in pharmacokinetics of intravenously infused amoxicillin and ampicillin to elderly volunteers. *Br J Clin Pharmacol,* **1986**, *27*:171–181.

Amphotericin B[a]

<5	2–5	>90	0.46 ± 0.20[b]	0.76 ± 0.52[c]	18 ± 7[d]	—	1.2 ± 0.33 μg/mL[e]

[a] Data for amphotericin B shown. [b] Data for eight children (ages 8 months to 14 years) yielded a linear regression, with *CL* decreasing with age: *CL* = −0.046 · age (years) + 0.86. Newborns show highly variable *CL* values. [c] Volume of central compartment. V_{ss} increases with dose from 3.4 L/kg for a 0.25-mg/kg dose to 8.9 L/kg for a 1.5-mg/kg dose. Also available as liposomal encapsulated formulations (ABELCET and AMBISOME). Amphotericin distribution and *CL* properties of these products are different from the nonencapsulated form; they have a terminal $t_{1/2}$ of 173 ± 78 and 110–153 h, respectively; however, an effective steady-state concentration can be achieved within 4 days. [d] $t_{1/2}$ for multiple dosing. In single-dose studies, a prolonged dose-dependent $t_{1/2}$ is seen. [e] Following a 0.5-mg/kg IV dose of amphotericin B given as a 1-h infusion, once daily for 3 days. Whole blood concentrations (free and liposome encapsulated) of 1.7 ± 0.8 μg/mL and 83 ± 35 μg/mL were reported following a 5-mg/kg/day IV dose (presumed 60- to 120-min infusion) of ABELCET and AMBISOME, respectively.

References: Gallis HA, et al. Amphotericin B: 30 years of clinical experience. *Rev Infect Dis,* **1990**, *12*:308–329. *PDR*54, **2000**, pp. 1090–1091, 1654.

Apixaban

~50	17–30	87	0.77–0.84[a,b]	0.38–0.42[b]	3.7–8.4[b,c]	3–4[d]	34–110 ng/mL[d]

[a] Cleared from blood primarily by CYP3A-dependent metabolism. [b] Calculated assuming 70-kg body weight; range of mean values for single IV doses of 0.5–5 mg. [c] Apparent $t_{1/2}$ after oral dose is ~12 h because of slow absorption. [d] Following a single 5-mg oral dose to 16 healthy adults.

References: Drugs@FDA. FDA-approved drug products: apixaban (Eliquis). Available at: http://www.accessdata.fda.gov/scripts/cder/daf/. Accessed April 23, 2022. Zheng SS, et al. Pharmacodynamics, pharmacokinetics and clinical efficacy of apixaban in the treatment of thrombosis. *Expert Opin Drug Metab Toxicol,* **2016,** *12*:575–580.

Aripiprazole[a]

87	<1	>99	0.83 ± 0.17[b,c]	4.9[c]	47 ± 10	3.0 ± 0.6[d]	242 ± 36 ng/mL[d]

[a] The major metabolite, dehydro-aripiprazole, has affinity for D_2 receptors similar to parent drug; found at 40% of parent drug concentration in plasma; $t_{1/2}$ is 94 h. No significant sex differences. [b] Eliminated primarily by CYP2D6- and CYP3A4-dependent metabolism. CYP2D6 poor metabolizers exhibit increased exposure (80%) to parent drug but reduced exposure (30%) to the active metabolite. [c] *CL/F* and *V/F* at steady state reported. [d] Following a 15-mg oral dose given once daily for 14 days.

References: DeLeon A, et al. Aripiprazole: A comprehensive review of its pharmacology, clinical efficacy, and tolerability. *Clin Ther,* **2004**, *26*:649–666. Mallikaarjun S, et al. Effects of hepatic or renal impairment on the pharmacokinetics of aripiprazole. *Clin Pharmacokinet,* **2008**, *47*:533–542. Mallikaarjun S, et al. Pharmacokinetics, tolerability, and safety of aripiprazole following multiple oral dosing in normal healthy volunteers. *J Clin Pharmacol,* **2004**, *44*:179–187. *PDR*58, **2004**, pp. 1034–1035.

Atazanavir[a]

—[b]	7	86	3.4 ± 1.0[c,d]	1.6–2.7[d]	7.9 ± 2.9	2.5[e]	5.4 ± 1.4 μg/mL[e]
↑ Food			↓ LD		↑ LD		

[a] Pharmacokinetic data reported for healthy adults. No significant sex or age differences. [b] Absolute bioavailability is not known, but food enhances the extent of absorption. [c] Undergoes extensive hepatic metabolism, primarily by CYP3A. Metabolic elimination affected by inhibitors and inducers of CYP3A. Coadministration with low-dose ritonavir increases systemic atazanavir exposure. [d] *CL/F* and *V/F* reported. [e] Following a 400-mg oral dose given with a light meal once daily to steady state.

References: Orrick JJ, et al. Atazanavir. *Ann Pharmacother,* **2004**, *38*:1664–1674. *PDR*58, **2004**, p. 1081.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Atenolol[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
58 ± 16	94 ± 8	<5	2.4 ± 0.3	1.3 ± 0.5[b]	6.1 ± 2.0[c]	3.3 ± 1.3[d]	0.28 ± 0.09 μg/mL[d]
			↓ Aged, RD		↑ RD, Aged		

[a]Atenolol is administered as a racemic mixture. No significant differences in the pharmacokinetics of the enantiomers. [b]V_{area} reported. [c]$t_{1/2}$ of *R*- and *S*-atenolol are similar. [d]Following a single 50-mg oral dose. [e]*CL/F* unchanged during pregnancy; however, renal clearance of atenolol is increased during pregnancy.

References: Boyd RA, et al. The pharmacokinetics of the enantiomers of atenolol. *Clin Pharmacol Ther,* **1989**, *45*:403–410. Mason WD, et al. Kinetics and absolute bioavailability of atenolol. *Clin Pharmacol Ther,* **1979**, *25*:408–415.

Atomoxetine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
EM: 63[b]	1%–2%	98.7 ± 0.3	EM: 6.2[b]	EM: 2.3[b]	EM: 5.3[b]	EM/PM: 2[c]	EM: 160 ng/mL[c]
PM: 94[b]			PM: 0.60[b]	PM: 1.1[b]	PM: 20[b]		PM: 915 ng/mL[c]
			EM: ↓ LD				

[a]Metabolized by CYP2D6 (polymorphic). Poor metabolizers (PM) exhibit a higher oral bioavailability, higher C_{max}, lower *CL*, and longer $t_{1/2}$ than extensive metabolizers (EM). No differences between adults and children >6 years of age. [b]*CL/F, V/F,* and $t_{1/2}$ measured at steady state. [c]Following a 20-mg oral dose given twice daily for 5 days.

References: Sauer JM, et al. Disposition and metabolic fate of atomoxetine hydrochloride: the role of CYP2D6 in human disposition and metabolism. *Drug Metab Dispos,* **2003**, *37*:98–107. Simpson D, et al. Atomoxetine: a review of its use in adults with attention deficit hyperactivity disorder. *Drugs,* **2004**, *64*:205–222.

Atorvastatin[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
12	<2	≥98	29[b]	~5.4	19.5 ± 9.6	Acid 1.8 h Lactone 3.4 h[d]	Acid 6.9 ng/mL Lactone 3.6 ng/mL[d]
			↓ LD[c], Aged		↑ LD, Aged		

[a]Data from healthy adult male and female subjects. No clinically significant sex differences. Atorvastatin undergoes extensive CYP3A-dependent first-pass metabolism. Metabolites are active and exhibit a longer $t_{1/2}$ (20–30 h) than parent drug. [b]Mean *CL/F* calculated from reported *AUC* data at steady state after a once-a-day 20-mg oral dose, assuming a 70-kg body weight. [c]AUC following oral administration increased; mild-to-moderate hepatic impairment. [d]Following a 20-mg single oral dose oral dose. Following once daily 20mg oral dosing for 14 days the C_{max} of atorvastatin equivalents that include pharmacologically active metabolites is 14.9 ± 1.8 ngEq/mL[d].

References: Gibson DM, et al. Effect of age and sex on pharmacokinetics of atorvastatin in humans. *J Clin. Pharmacol,* **1996**, *36*:242–246. Lennernas H, Clinical Pharmacokinetics of Atorvastatin, *Clin Pharmacokinet,* 2003, 42:1141-1160. Mazzu AL, Lasseter KC, Shamblen EC, Agarval V, Lettieri J, Sundaresen P. Itraconazole alter the pharmacokinetics of atorvastatin to a greater extent than either cerivastatin or pravastatin *Clin Pharmacol Ther* 2000, 68:391-400.

Atovaquone

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
23 ± 11[a]	<1	>99	1.26, 2.95, 2.84[b,c]	7.98[c]	84.9, 31.3, 35.2[c]	1.5–3[d]	4.25 ± 2.15 μg/mL[e]
↑ Food							

[a]Value reported when taken with food. [b]Cleared from blood primarily by biliary excretion; undergoes enterohepatic cycling, with eventual fecal elimination. [c]Population estimates of *CL/F*, *V/F* and $t_{1/2}$ in Black, Asian, and Malay patients, respectively, treated for malaria. [d]Longer T_{max} values also reported, possibly because of enterohepatic recycling. [e]Following a 250-mg oral dose (Malarone) given once a day for 4 days.

References: Hussein Z, et al. Population pharmacokinetics of atovaquone in patients with acute malaria caused by *Plasmodium falciparum*. *Clin Pharmacol Ther,* **1997**, *61*:518–530. Marra F, et al. Atovaquone-proguanil for prophylaxis and treatment of malaria. *Ann Pharmacother,* **2003**, *37*:1266–1275. Drugs@FDA. FDA-approved drug products: atovaquone (Mepron and Malarone). Available at: http://www.accessdata.fda.gov/scripts/cder/daf/. Accessed April 23, 2022.

Azathioprine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
60 ± 31[b]	<2	—	57 ± 31[c]	0.81 ± 0.65[c]	0.16 ± 0.07[c]	MP: 1–2[d]	MP: 20–90 ng/mL[d]

[a]Azathioprine is metabolized to mercaptopurine (MP), listed later in this table. [b]Determined as the bioavailability of MP; intact azathioprine is undetectable after oral administration because of extensive first-pass metabolism. Kinetic values are for IV azathioprine. [c]Data from kidney transplant patients. [d]MP concentration following a 135 ± 34-mg oral dose of azathioprine given daily to steady state in kidney transplant patients.

Reference: Lin SN, et al. Quantitation of plasma azathioprine and 6-mercaptopurine levels in renal transplant patients. *Transplantation,* **1980**, *29*:290–294.

Azithromycin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
34 ± 19	12	7–50[a]	9	31	40[b]	2–3[c]	0.4 μg/mL[c]
↓ Food (capsules)							
↑ Food (suspension)							

[a]Dose-dependent plasma binding. The bound fraction is 50% at 50 ng/mL and 12% at 500 ng/mL. [b]A longer terminal plasma $t_{1/2}$ of 68 ± 8 h, reflecting release from tissue stores, overestimates the multiple-dosing $t_{1/2}$. [c]Following a 250-mg/day oral dose to adult patients with an infection.

Reference: Lalak NJ, et al. Azithromycin clinical pharmacokinetics. *Clin Pharmacokinet,* **1993**, *25*:370–374.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Baclofen[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
>70[b]	69 ± 14	31 ± 11	2.72 ± 0.93[c]	0.81 ± 0.12[c]	3.75 ± 0.96	1.0 (0.5–4)[e]	160 ± 49 ng/mL[e]
			↓ RD[d]				

[a]Data from healthy adult male subjects. [b]Bioavailability estimate based on urine recovery of unchanged drug after oral dose. [c]*CL/F* and V_{area}/F reported for intestinal infusion of drug. [d]Limited data suggest *CL/F* reduced with renal impairment. [e]Following a single 10-mg oral dose.

References: Kochak GM, et al. The pharmacokinetics of baclofen derived from intestinal infusion. *Clin Pharmacol Ther,* **1985**, *38*:251–257. Wuis EW, et al. Plasma and urinary excretion kinetics of oral baclofen in healthy subjects. *Eur J Clin Pharmacol,* **1989**, *37*:181–184.

Buprenorphine[a]

SL: 51 ± 13 BC: 28 ± 9	Negligible	96	14.9 ± 5.2	4.8 ± 1.7	16.2 ± 20.1	SL:1.2 ± 0.1[c]	SL: 2.7 ± 0.3 ng/mL[c]
			↑ Child[b]	↑ Child[b]	↓ Child[b]	BC: 0.8 ± 0.2[c]	BC: 2.0 ± 0.6 ng/mL[c]

[a]Data from male and female subjects undergoing surgery. Buprenorphine is metabolized in the liver by CYP3A4 to an active metabolite, norbuprenorphine, and via conjugation. Majority of the dose is excreted into feces. [b]*CL*, 60 ± 19 mL/min/kg; V_{ss}, 3.2 L/kg; $t_{1/2}$, 1.03 ± 0.22 h; children 4–7 years of age. [c]Following 8-mg sublingual solution (SL) or 4-mg buccal (BC) dose.

References: Elkader A, Sproule B. Buprenorphine: clinical pharmacokinetics in the treatment of opioid dependence. *Clin Pharmacokinet,* **2005**, *44*:661–680. Olkkola KT, et al. Pharmacokinetics of intravenous buprenorphine in children. *Br J Clin Pharmacol,* **1989**, *28*:202–204.

Bupropion[a]

—	<1	>80	R: 43 (58)[b] S: 257 (72) Rac: 74 (61)	R: 40.7 (78)[b] S: 152 (83) Rac: 65.7 (80)	R: 11. 6 (49)[b] S: 7.2 (103) Rac: 10.8 (54) OH: 19.2 (21) Erythro: 21.6 (36) Threo: 30.8 (41)		Rac: 58 (52–63) ng/mL[c] OH: 464 (406–522) ng/mL[c] Erythro: 38 (35–42) ng/mL[c] Threo: 208 (181–236) ng/mL[c]
			↓ Aged, RD, LD		↑ Aged, LD		

[a]Bupropion is administered as a racemic mixture of *R* and *S* bupropion that can interconvert. Data from healthy adult male volunteers. Bupropion is metabolized by CYP2B6 to hydroxy-bupropion and by 11β-hydroxysteroid-dehydrogenase and aldo-ketoreductases to threo- and erythrohydrobupropion. All three metabolites accumulate in blood and are active. [b]*CL/F,* V_{ss}/F, and $t_{1/2}$ reported for oral dose. Percent coefficient of variation shown in parentheses. [c]Following oral dosing of 150-mg XL (sustained release) tablet to 42 healthy subjects for 7 days (steady state). 95% confidence interval shown in brackets.

References: Benowitz NL, et al. Influence of CYP2B6 genetic variants on plasma and urine concentrations of bupropion and metabolites at steady state. *Pharmacogenet Genomics,* **2013**, *23*:135–141. DeVane CL, et al. Disposition of bupropion in healthy volunteers and subjects with alcoholic liver disease. *J Clin Psychopharmacol,* **1990**, *10*:328–332. Masters AR, et al. Chiral plasma pharmacokinetics and urinary excretion of bupropion and metabolites in healthy volunteers. *J Pharmacol Exp Ther*, **2016**, *358*:230–238.

Buspirone[a]

3.9 ± 4.3	<0.1	>95	28.3 ± 10.3	5.3 ± 2.6	2.4 ± 1.1	0.71 ± 0.06[e]	1.66 ± 0.21 ng/mL[e]
↑ Food[b]			↓ LD,[c] RD[d]		↑ LD, RD		

[a]Data from healthy adult male subjects. No significant sex differences. Undergoes extensive CYP3A-dependent first-pass metabolism. The major metabolite (l-pyrimidinyl piperazine) is active in some behavioral tests in animals (one-fifth potency) and accumulates in blood to levels severalfold higher than buspirone. [b]Bioavailability increased ~84%; appears to be secondary to reduced first-pass metabolism. [c]*CL/F* reduced, hepatic cirrhosis. [d]*CL/F* reduced, mild renal impairment; unrelated to CL_{cr}. [e]Following a single 20-mg oral dose.

References: Barbhaiya RH, et al. Disposition kinetics of buspirone in patients with renal or hepatic impairment after administration of single and multiple doses. *Eur J Clin Pharmacol,* **1994**, *46*:41–47. Gammans RE, et al. Metabolism and disposition of buspirone. *Am J Med,* **1986**, *80*:41–51.

Calcitriol[a]

PO: ~61	<10%	99.9	0.43 ± 0.04	—	16.5 ± 3.1[b]	PO: 3–6[d]	IV: ~460 pg/mL[d]
IP: ~67					↑ Child[c]	IP: 2–3[d]	PO: ~90 pg/mL[d]
							IP: ~105 pg/mL[d]

[a]Data from young (15–22 years) patients on peritoneal dialysis. Metabolized by 23-, 24-, and 26-hydroxylases and also excreted into bile as its glucuronide. [b]Calcitriol $t_{1/2}$ is 5–8 h in healthy adult subjects. [c]Oral dose $t_{1/2}$ = 27 ± 12 h, children 2–16 years. [d]Following a single 60-ng/kg IV, intraperitoneal (IP) dialysate, or PO dose. Baseline plasma levels were <10 pg/mL.

References: Jones CL, et al. Comparisons between oral and intraperitoneal 1,25-dihydroxyvitamin D_3 therapy in children treated with peritoneal dialysis. *Clin Nephrol,* **1994**, *42*:44–49. *PDR54*, **2000**, p. 2650. Salusky IE, et al. Pharmacokinetics of calcitriol in continuous ambulatory and cycling peritoneal dialysis patients. *Am J Kidney Dis,* **1990**, *16*:126–132. Taylor CA, et al. Clinical pharmacokinetics during continuous ambulatory peritoneal dialysis. *Clin Pharmacokinet,* **1996**, *31*:293–308.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Canagliflozin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
65	<1[a]	98–99	2.74[b,c]	1.70[c]	6.9[d]	1.5 (1–5)[e]	1227 ± 481 ng/mL[e]

[a] Calculated as the ratio of dapagliflozin renal clearance/total IV clearance. [b] Cleared from blood primarily by UGT1A9 and 2B4-dependent metabolism. [c] Calculated assuming 70-kg body weight. [d] $t_{1/2}$ following IV dose reported; longer values (11–13 h) reported following oral administration. [e] Mean (range or ± SD) following a 100-mg oral dose given once a day for 7 days to patients with type 2 diabetes mellitus.

Reference: Drugs@FDA. FDA-approved drug products: canagliflozin (Invokamet). Available at: http://www.accessdata.fda.gov/scripts/cder/daf/. Accessed April 23, 2022. Scheen AJ. Pharmacokinetics, pharmacodynamics and clinical use of SGL2 inhibitors in patients with type 2 diabetes mellitus and chronic kidney disease. *Clin Pharmacokinet,* **2015**, *54*:691–708.

Candesartan[a]

42 (34–56)	52	99.8	0.37 (0.31–0.47)	0.13 (0.09–0.17)	9.7 (4.8–13)	4.0 ± 1.3	119 ± 43 ng/mL[c]
			↓ RD[b]		↑ RD[b]		

[a] Data from healthy adult male subjects. Candesartan cilexetil is rapidly and completely converted to candesartan through the action of gut wall esterases. Mean (range) for candesartan reported. No significant sex or age differences. [b] *CL/F* reduced in mild to severe renal disease. [c] Mean (SD) following a 16-mg oral dose (tablet), daily, for 7 days.

References: Hubner R, et al. Pharmacokinetics of candesartan after single and repeated doses of candesartan cilexetil in young and elderly healthy volunteers. *J Hum Hypertens*, **1997**, *11*(Suppl. 2):S19–S25. Stoukides CA, et al. Candesartan cilexetil: an angiotensin II receptor blocker. *Ann Pharmacother*, **1999**, *33*:1287–1298. van Lier JJ, et al. Absorption, metabolism and excretion of ^{14}C-candesartan and ^{14}C-candesartan cilexetil in healthy volunteers. *J Hum Hypertens*, **1997**, *11*(Suppl. 2):S27–S28.

Cannabidiol[a]

<10	—[b]	>94	PO: 265–455[c,d]	PO: 300–612[e]	PO: 56–61	PO: 2.5–5	PO:292 ± 88 ng/mL[d]
			IV:17.7 ± 3.4[f]	IV: 32.7 ± 8.6[f]	IV: 24 ± 6[f]		IV: 686 ± 239 ng/mL[f]
↑ Food			↓ LD		↑ LD, ↓ Food		

[a] Data from male and female healthy subjects. Cannabidiol exposure increases less than dose proportionally over the range of 5 to 25 mg/kg/day in patients. Cannabidiol is metabolized in the liver and the gut (primarily in the liver) by CYP2C19 and CYP3A4. It also undergoes glucuronidation by UGT1A7, UGT1A9, and UGT2B7 isoforms. After repeat dosing, the active metabolite of cannabidiol, 7-hydroxycannabidiol (equipotent with parent), has a 38% lower AUC than the parent drug. The 7-hydroxycannabidiol metabolite is metabolized to 7-carboxycannabidiol (pharmacologically inactive), which has an approximately 40-fold higher AUC than the parent drug. [b] After oral administration, cannabidiol is eliminated predominantly in feces, with metabolites found in urine. [c] *CL/F* reported. [d] After a 1500-mg single oral dose (approximately equal to the 20 mg/kg/day dosage). [e] V_{ss}/F reported. [f] Following 20 mg cannabidiol given IV to five male volunteers (infrequent to frequent cannabis smokers).

References: Crockett J, et al. A phase 1, randomized, pharmacokinetic trial of the effect of different meal compositions, whole milk, and alcohol on cannabidiol exposure and safety in healthy subjects. *Epilepsia*, **2020**, *61*:267–277. FDA. Product labeling: Epidiolex® (cannabidiol oral solution) Available at: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm. Accessed March 24, 2021. FDA. Cannabidiol NDA and label. NDA approved in 2018; label revised 10/2020. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/210365s008lbl.pdf. Accessed March 24, 2021. Ohlsson A, et al. Single-dose kinetics of deuterium-labelled cannabidiol in man after smoking and intravenous administration. *Biomed Environ Mass Spectrom*, **1986**, *13*:77–83. Taylor L, et al. A phase I, randomized, double-blind, placebo-controlled, single ascending dose, multiple dose, and food effect trial of the safety, tolerability and pharmacokinetics of highly purified cannabidiol in healthy subjects. *CNS Drugs,* **2018**, *32*:1053–1067. Taylor L, et al. A phase 1, open-label, parallel-group, single-dose trial of the pharmacokinetics and safety of cannabidiol (CBD) in subjects with mild to severe hepatic impairment. *J Clin Pharmacol*, **2019**, *59*:1110–1119.

Capecitabine[a]

—	3	<60	145 (34%) L/h/m^{2}[c,d]	270 L/m^{2} [c,d]	C: 1.3 (146%)[c]	C: 0.5 (0.5–1)[e]	C: 6.6 ± 6.0 μg/mL[e]
↓ Food[b]			↓ LD[e]		5-FU: 0.72 (16%)[c]	5-FU: 0.5 (0.5–2.1)[e]	5-FU: 0.47 ± 0.47 μg/mL[e]

[a] Data from male and female patients with cancer. Capecitabine (C) is a prodrug for 5-fluorouracil (5-FU; active), listed later in this table. It is well absorbed, and bioactivation is sequential in liver and tumor. [b] AUC for C and 5-FU decreased. [c] Geometric mean (coefficient of variation). [d] *CL/F* and V_{area}/F reported for oral dose. [e] Following 1255 mg/m^{2}.

References: Dooley M, et al. Capecitabine. *Drugs,* **1999**, *58*:69–76; discussion 77–78. Reigner B, et al. Effect of food on the pharmacokinetics of capecitabine and its metabolites following oral administration in cancer patients. *Clin Cancer Res,* **1998**, *4*:941–948.

Carbamazepine[a]

78 ± 24[b]	3	74 ± 6	0.73± 0.3[b]	1.1 ± 0.3[b]	20 ± 9[b]	—	11.2–11.7 (2–18) μg/mL[c]

[a] A metabolite, carbamazepine-10,11-epoxide, is equipotent in animal studies. Its formation is catalyzed primarily by CYP3A and secondarily by CYP2C8. [b] Data from 92 patients receiving carbamazepine oral therapy for treatment of epilepsy and given stable isotope-labeled carbamazepine intravenously. Carbamazepine induces its own metabolism; for a single dose, $CL/F = 0.36 \pm 0.07$ mL/min/kg and $t_{1/2} = 36 \pm 5$ h. [c] Mean steady-state C_{max} is similar following either dosing of immediate-release carbamazepine four times a day or extended-release carbamazepine once daily (800–1600 mg/day). Concentration following a daily 200-mg oral dose (immediate release) given to adult patients with epilepsy reported.

References: Garnett WR, et al. Pharmacokinetic evaluation of twice-daily extended release carbamazepine (CBZ) and four-time-daily immediate-release CBZ in patients with epilepsy. *Epilepsia,* **1998**, *39*:274–279. Marino SE, et al. Steady-state carbamazepine pharmacokinetics following oral and stable–labeled intravenous administration I epilepsy patients: effects of race and sex. *Clin Pharmacol Ther*, **2012**, *91*:483–488.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Carbidopa[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—[b]	5.3 ± 2.1	—	18 ± 7[c]	—	~2	2.1 ± 1.0	S: 165 ± 77 ng/mL[d]
							S-CR: 81 ± 28 ng/mL[d]

[a] Data from healthy adult subjects. Combined with levodopa for treatment of Parkinson disease. [b] Absolute bioavailability is unknown, but it is presumably low based on a high value for *CL/F*. Bioavailability of SINEMET CR (S-CR) is 55% of standard SINEMET (S). [c] *CL/F* reported for 2 tablets of SINEMET 25/100. [d] Following a single oral dose of 2 tablets of SINEMET 25/100 or 1 tablet of SINEMET CR 50/200.

Reference: Yeh KC, et al. Pharmacokinetics and bioavailability of Sinemet CR: a summary of human studies. *Neurology,* **1989**, *39*:25–38.

Carvedilol[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
25	<2	95[b]	8.7 ± 1.7	1.5 ± 0.3	2.2 ± 0.3[c]	1.3 ± 0.3[d]	105 ± 12 ng/mL[d]
S-(−): 15			↓ LD	↑ LD	↑, ↔ LD		
R-(+): 31							
↑ LD							

[a] Racemic mixture: *S*-(−)-enantiomer responsible for β_1 adrenergic–receptor blockade. *R*-(+)- and *S*-(−)-enantiomers have nearly equivalent α_1 receptor blocking activity. [b] *R*-(+)-enantiomer is more tightly bound than the *S*-(−)-antipode. [c] Longer $t_{1/2}$ of ~6 h has been measured at lower concentrations. [d] Following a 12.5-mg oral dose given twice a day for 2 weeks to healthy young adults.

References: Morgan T. Clinical pharmacokinetics and pharmacodynamics of carvedilol. *Clin Pharmacokinet,* **1994**, *26*:335–346. Morgan T, et al. Pharmacokinetics of carvedilol in older and younger patients. *J Hum Hypertens,* **1990**, *4*:709–715.

Caspofungin[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—[a]	~2	96.5	0.16 (0.14–0.18)	0.12[b]	9.6 ± 0.8[b]	—	8.7 (7.9–9.6) μg/mL[c]

[a] Caspofungin is available for IV administration only. [b] Initial distribution volume and $t_{1/2}$ reported. Exhibits biphasic elimination with a larger V_{area} (0.3–2.1 L/kg) and longer (>25 h) terminal $t_{1/2}$; the terminal phase accounts for a small fraction of the dose. [c] Following a 50-mg, 1-h IV infusion given once daily for 14 days.

References: Stone JA, et al. Single- and multiple-dose pharmacokinetics of caspofungin in healthy men. *Antimicrob Agents Chemother,* **2002**, *46*:739–745. Stone JA, et al. Disposition of caspofungin: role of distribution in determining pharmacokinetics in plasma. *Antimicrob Agents Chemother,* **2004**, *48*:815–823.

Cefazolin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
>90	80 ± 16	89 ± 2	0.95 ± 0.17	0.19 ± 0.06[a]	2.2 ± 0.02	IM: 1.7 ± 0.7[b]	IV: 237 ± 285 μg/mL[b]
		↓ RD, LD, Neo, Child	↓ RD ↑ Preg	↑ RD, Neo	↑ RD, Neo ↓ Preg, LD		IM: 42 ± 9.5 μg/mL[b]

[a] V_{area} reported. [b] Following a single 1-g IV (model-fitted C_{max}) or IM dose to healthy adults.

Reference: Scheld WM, et al. Moxalactam and cefazolin: comparative pharmacokinetics in normal subjects. *Antimicrob Agents Chemother,* **1981**, *79*:613–619.

Cefdinir

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Cap: 16–21[a]	13–23[b]	89[c]	11–15[d]	1.6–2.1[d]	1.4–1.5	Cap: 3 ± 0.7[e]	Cap: 2.9 ± 1.0 μg/mL[e]
Susp: 25[a]		↓ RD				Susp: 2 ± 0.4[e]	
↓ Iron							Susp: 3.9 ± 0.6 μg/mL[e]

[a] Bioavailability following ingestion of a capsule (Cap) or suspension (Susp) formulated dose. [b] Determined after a single oral dose. [c] Lower plasma protein binding (71%–74%) reported in patients undergoing dialysis. [d] *CL/F* and *V/F* reported. [e] Following ingestion of a single 600-mg capsule given to adults or a 14-mg/kg suspension dose given to children (6 months to 12 years). No accumulation after multiple dosing.

References: Guay DR. Pharmacodynamics and pharmacokinetics of cefdinir, an oral extended spectrum cephalosporin. *Pediatr Infect Dis J,* **2000**, *19*:S141–S146. *PDR*58, **2004**, p. 503. Tomino Y, et al. Pharmacokinetics of cefdinir and its transfer to dialysate in patients with chronic renal failure undergoing continuous ambulatory peritoneal dialysis. *Arzneimittelforschung,* **1998**, *48*:862–867.

Cefepime[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—	80	16–19	1.8 (1.7–2.5)[b]	0.26 (0.24–0.31)[d]	2.1 (1.3–2.4)[b]	—	65 ± 7 μg/mL[e]
			↓ RD[c]		↑ RD[c]		

[a] Data from healthy adult patients. Available only in parenteral form. [b] Median (range) of reported *CL* and $t_{1/2}$ values from 16 single-dose studies. [c] Mild renal impairment. [d] Median (range) of reported V_{ss} from 6 single-dose studies. [e] Following a 1-g IV dose.

References: Okamoto MP, et al. Cefepime clinical pharmacokinetics. *Clin Pharmacokinet,* **1993**, *25*:88–102. Rybak M. The pharmacokinetic profile of a new generation of parenteral cephalosporin. *Am J Med,* **1996**, *100*:39S–44S.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Ceftazidime

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—	84 ± 4	21 ± 6	$CL = 1.05\,CL_{cr} + 0.12$	0.23 ± 0.02	1.6 ± 0.1	IM: 0.7–1.3[a]	IV: 119–146 μg/mL[a]
IM: 91				↑ Aged	↑ RD, Prem, Neo, Aged		IM: 29–39 μg/mL[a]

[a] Range of mean data from different studies following a 1-g bolus IV or IM dose given to healthy adults.

Reference: Balant L, et al. Clinical pharmacokinetics of the third generation cephalosporins. *Clin Pharmacokinet,* **1985**, *10*:101–143.

Celecoxib[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—	<3	~97	6.60 ± 1.85[c]	6.12 ± 2.08[c]	11.2 ± 3.47	2.8 ± 1.0[f]	705 ± 268 ng/mL[f]
↑ Food[b]			↓ Aged, LD[d]				
			↑ RD[e]				

[a] Data from healthy subjects. [b] High-fat meal. Absolute bioavailability is unknown. [c] *CL/F* and *V/F* values reported. Cleared primarily by CYP2C9 (polymorphic). [d] *CL/F* reduced, mild or moderate hepatic impairment. [e] *CL/F* increased, moderate renal impairment, but unrelated to CL_{cr}. [f] Following a single 200-mg oral dose.

References: Goldenberg MM. Celecoxib, a selective cyclooxygenase-2 inhibitor for the treatment of rheumatoid arthritis and osteoarthritis. *Clin Ther,* **1999**, *21*:1497–1513; discussion 1427–1428. *PDR*54, **2000**, p. 2334.

Cephalexin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
90 ± 9	91 ± 18	14 ± 3	4.3 ± 1.1[a]	0.26 ± 0.03[a]	0.90 ± 0.18	1.4 ± 0.8[a]	28 ± 6.4 μg/mL[a]
			↓ RD		↑ RD		

[a] Following a single 500-mg oral dose given to healthy male adults.

Reference: Spyker DA, et al. Pharmacokinetics of cefaclor and cephalexin: Dosage nomograms for impaired renal function. *Antimicrob Agents Chemother,* **1978**, *14*:172–177.

Cetirizine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Rac: >70[b]	Rac: 70.9 ± 7.8	Rac: 89.2 ± 0.4	Rac: 0.74 ± 0.19[c]	Rac: 0.58 ± 0.16[c]	Rac: 9.42 ± 2.4	Rac: 0.9 ± 0.2[g]	Rac: 313 ± 45 ng/mL[g]
Levo: >68[b]	Levo: 68.1 ± 10.2	Levo: 92.0 ± 0.3	Levo: 0.62 ± 0.11[c]	Levo: 0.41 ± 0.10	Levo: 7.8 ± 1.6	Levo: 0.8 ± 0.5[g]	Levo: 270 ± 40 ng/mL[g]
			Rac: ↓ LD,[d] RD,[e] Aged		Rac: ↑ LD, RD, Aged		
			Levo: ↓ RD		Levo: ↑ RD		
			Rac/Levo: ↑ Child[f]		Rac/Levo: ↓ Child		

[a] Data from healthy male and female subjects receiving cetirizine (Rac) or the active R-enantiomer, levocetirizine (Levo). [b] Based on recovery of unchanged drug in urine. [c] *CL/F* and V_d/F reported for oral dose. [d] *CL/F* reduced, hepatocellular and cholestatic liver diseases. [e] *CL/F* reduced, moderate to severe renal impairment. [f] *CL/F* increased, ages 1–5 years. [g] Following a single 10-mg oral dose of Rac or 5 mg of Levo.

References: Baltes E, et al. Absorption and disposition of levocetirizine, the eutomer of cetirizine, administered alone or as cetirizine to healthy volunteers. *Fundam Clin Pharmacol,* **2001**, *15*:269–277. Benedetti MS, et al. Absorption, distribution, metabolism and excretion of [14C]levocetirizine, the R enantiomer of cetirizine, in healthy volunteers. *Eur J Clin Pharmacol*, **2001**, *57*:571–582. Horsmans Y, et al. Single-dose pharmacokinetics of cetirizine in patients with chronic liver disease. *J Clin Pharmacol,* **1993**, *33*:929–932. Matzke GR, et al. Pharmacokinetics of cetirizine in the elderly and patients with renal insufficiency. *Ann Allergy,* **1987**, *59*:25–30. *PDR*54, **2000**, p. 2404. Spicák V, et al. Pharmacokinetics and pharmacodynamics of cetirizine in infants and toddlers. *Clin Pharmacol Ther,* **1997**, *61*:325–330. Strolin Benedetti M, et al. Stereoselective renal tubular secretion of levocetirizine and dextrocetirizine, the two enantiomers of the H1-antihistamine cetirizine. *Fundam Clin Pharmacol,* **2008**, *22*:19–23.

Chloroquine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
~80	52–58[b]	*S*: 66.6 ± 3.3[c] *R*: 42.7 ± 2.1	3.7–13[b]	132–261[b]	10–24 days[b,d]		IV: 837 ± 248 ng/mL[e]
						IM: 0.25[e]	IM: 57–480 ng/mL[e]
						PO: 3.6 ± 2.0[e]	PO: 76 ± 14 ng/mL[e]

[a] Active metabolite, desethylchloroquine, accounts for 20% ± 3% of urinary excretion; $t_{1/2}$ = 15 ± 6 days. Racemic mixture; kinetic parameters for the two isomers are slightly different, *CL/F* = 136 mL/min and 237 mL/min and *V/F* = 3410 L and 4830 L for the *R*-isomer and *S*-isomer, respectively. [b] Range of mean values from different studies (IV administration). [c] Concentrates in red blood cells. Blood-to-plasma concentration ratio for racemate = 9. [d] A longer $t_{1/2}$ (41 ± 14 days) has been reported with extended blood sampling. [e] Following a single 300-mg IV dose (24-min infusion) of chloroquine HCl or a single 300-mg IM or oral dose of chloroquine phosphate given to healthy adults. Effective concentrations against *Plasmodium vivax* and *Plasmodium falciparum* are 15 ng/mL and 30 ng/mL, respectively. Diplopia and dizziness can occur >250 ng/mL.

References: Krishna S, et al. Pharmacokinetics of quinine, chloroquine and amodiaquine. Clinical implications. *Clin Pharmacokinet,* **1996**, *30*:263–299. White NJ. Clinical pharmacokinetics of antimalarial drugs. *Clin Pharmacokinet,* **1985**, *10*:187–215.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Chlorpromazine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
32 ± 19[b]	<1	95–98	8.6 ± 2.9[c]	21 ± 9[c]	30 ± 7[c]	1–4[d]	25–150 ng/mL[d]
			↓ Child				

[a]Active metabolites, 7-hydroxychlorpromazine ($t_{1/2}$ = 25 ± 15 h) and possibly chlorpromazine *N*-oxide, yield AUCs comparable to the parent drug (single doses). [b]After a single dose. Bioavailability may decrease to ~20% with repeated dosing. [c]*CL/F*, V_{area}, and terminal $t_{1/2}$ following IM administration. [d]Following a 100-mg oral dose given twice a day for 33 days to adult patients. Neurotoxicity (tremors and convulsions) occurs at concentrations of 750–1000 ng/mL.

Reference: Dahl SG, et al. Pharmacokinetics of chlorpromazine after single and chronic dosage. *Clin Pharmacol Ther,* **1977**, *21*:437–448.

Chlorthalidone

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
64 ± 10	65 ± 9[a]	75 ± 1	0.04 ± 0.01	0.14 ± 0.07	47 ± 22[b]	13.8 ± 6.3[c]	3.7 ± 0.9 μg/mL[c]
			↓ Aged		↑ Aged		

[a]Value is for 50- and 100-mg doses; renal *CL* is decreased at an oral dose of 200 mg, and there is a concomitant decrease in the percentage excreted unchanged. [b]Chlorthalidone is sequestered in erythrocytes. $t_{1/2}$ is longer if blood, rather than plasma, is analyzed. Parameters reported based on blood concentrations. [c]Following a single 50-mg oral dose (tablet) given to healthy male adults.

Reference: Williams RL, et al. Relative bioavailability of chlorthalidone in humans: adverse influence of polyethylene glycol. *J Pharm Sci,* **1982**, *71*:533–535.

Cidofovir[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
SC: 98 ± 10	70.1 ± 21.4[b]	<6	2.1 ± 0.6[b]	0.36 ± 0.13[b]	2.3 ± 0.5[b]	—	19.6 ± 7.2 μg/mL[d]
PO: <5			↓ RD[c]		↑ RD		

[a]Data from patients with HIV infection and positive for cytomegalovirus. Cidofovir is activated intracellularly by phosphokinases. For parenteral use. [b]Parameters reported for a dose given in the presence of probenecid. [c]*CL* reduced, mild renal impairment (cleared by high-flux hemodialysis). [d]Following a single 5-mg/kg IV infusion given over 1 h, with concomitant oral probenecid and active hydration.

References: Brody SR, et al. Pharmacokinetics of cidofovir in renal insufficiency and in continuous ambulatory peritoneal dialysis or high-flux hemodialysis. *Clin Pharmacol Ther,* **1999**, *65*:21–28. Cundy KC, et al. Clinical pharmacokinetics of cidofovir in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother,* **1995**, *39*:1247–1252. *PDR*54, **2000**, p. 1136. Wachsman M, et al. Pharmacokinetics, safety and bioavailability of HPMPC (cidofovir) in human immunodeficiency virus-infected subjects. *Antiviral Res,* **1996**, *29*:153–161.

Cinacalcet[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
~20	—[b]	93–97	~18	~17.6	34 ± 9	2–6	10.6 ± 2.8 ng/mL[c]
↑ Food			↓ LD		↑ LD		

[a]Cinacalcet is a chiral molecule; the *R*-enantiomer is more potent than the *S*-enantiomer and is thought to be responsible for the drug's pharmacological activity. Cinacalcet is metabolized primarily by CYP3A4, CYP2D6, and CYP1A2. [b]Unreported, but presumably negligible. [c]Following a single 75-mg oral dose.

References: FDA. Pharmacology and toxicology review of NDA. Application 21–688. U.S. FDA, CDER. Available at: http://www.fda.gov/drugs at fda_docs/nda/2004/21-688.pdf. Sensipar_Pharmr_Pl.pdf. Accessed July 7, 2010. Joy MS, et al. Calcimimetics and the treatment of primary and secondary hyperparathyroidism. *Ann Pharmacother,* **2004**, *38*: 1871–1880. Kumar GN, et al. Metabolism and disposition of calcimimetic agent cinacalcet HCl in humans and animal models. *Drug Metab Dispos,* **2004**, *32*:1491–1500.

Ciprofloxacin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
60 ± 12	50 ± 5	40	7.6 ± 0.8	2.2 ± 0.4[a]	3.3 ± 0.4	0.6 ± 0.2[b]	2.5 ± 1.1 μg/mL[b]
			↓ RD, Aged	↓ Aged	↑ RD		

[a]V_{area} reported. [b]Following a 500-mg oral dose given twice daily for ≥3 days to patients with chronic bronchitis or bronchiectasis.

References: Begg EJ, et al. The pharmacokinetics of oral fleroxacin and ciprofloxacin in plasma and sputum during acute and chronic dosing. *Br J Clin Pharmacol,* **2000**, *49*:32–38. Sorgel F, et al. Pharmacokinetic disposition of quinolones in human body fluids and tissues. *Clin Pharmacokinet,* **1989**, *16*(suppl):5–24.

Clarithromycin[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
55 ± 8[b]	36 ± 7[b]	42–50	7.3 ± 1.9[b]	2.6 ± 0.5	3.3 ± 0.5[b]	C: 2.8[c]	C: 2.4 μg/mL[c]
			↓ Aged, RD	↑ LD	↑ Aged, RD, LD	HC: 3[c]	HC: 0.7 μg/mL[c]

[a]Active metabolite, 14(*R*)-hydroxyclarithromycin. [b]At higher doses, metabolic *CL* saturates, resulting in increases in the percentage of urinary excretion and $t_{1/2}$ and a decrease in *CL*. [c]Mean data for clarithromycin (C) and 14-hydroxyclarithromycin (HC), following a 500-mg oral dose given twice daily to steady state in healthy adults.

References: Chu SY, et al. Absolute bioavailability of clarithromycin after oral administration in humans. *Antimicrob Agents Chemother*, **1992**, *36*:1147–1150. Fraschini F, et al. Clarithromycin clinical pharmacokinetics. *Clin Pharmacokinet,* **1993**, *25*:189–204.

Clindamycin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
~87[a]	13	93.6 ± 0.2	4.7 ± 1.3	1.1 ± 0.3[b]	2.9 ± 0.7	—	IV: 17.2 ± 3.5 μg/mL[c]
Topical: 2					↑ Prem		PO: 2.5 μg/mL[d]

[a]Clindamycin hydrochloride given orally. [b]V_{area} reported. [c]Following a 1200-mg IV dose (30-min infusion) of clindamycin phosphate (prodrug) given twice daily to steady state in healthy male adults. [d]Following a single 150-mg oral dose of clindamycin hydrochloride to adults.

*References: PDR*54, **2000**, p. 2421. Plaisance KI, et al. Pharmacokinetic evaluation of two dosage regimens of clindamycin phosphate. *Antimicrob Agents Chemother,* **1989**, *33*:618–620.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Clonazepam

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
IM: 93 ± 27	<1	86 ± 0.5	0.79 ± 0.12	2.6 ± 0.7	38 ± 9	IM: 3.1 ± 1.7[a]	IM: 11.0± 5.4 ng/mL[a]
PO: 90 ± 22						PO: 1.7 ± 0.9[a]	
							PO: 14.9 ± 3.9 ng/mL[a]

[a] Following a single 2-mg IM or PO dose given to healthy adults. Secondary plasma peaks have been observed after IV and IM administration

Reference: Crevoisier C, et al. Comparative single-dose pharmacokinetics of clonazepam following intravenous, intramuscular and oral administration to healthy volunteers. *Eur Neurol,* **2003**, *49*:173–177.

Clonidine

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
PO: 95	62 ± 11	20	3.1 ± 1.2[a]	2.1 ± 0.4	12 ± 7	PO: 2[b]	PO: 0.8 ng/mL[b]
TD: 60			↓ RD		↑ RD	TD: 72[b]	TD: 0.3–0.4 ng/mL[b]
			↑ Preg				

[a] Clonidine is cleared via renal route and CYP2D6-mediated hydroxylation. [b] Mean data following a 0.1-mg oral dose given twice a day to steady state or steady-state concentration (C_{ss}) following a 3.5-cm^2 transdermal (TD) patch administered to normotensive male adults. Concentrations of 0.2–2 ng/mL are associated with a reduction in blood pressure; >1 ng/mL will cause sedation and dry mouth.

Reference: Lowenthal DT, et al. Clinical pharmacokinetics of clonidine. *Clin Pharmacokinet,* **1988**, *14*:287–310.

Clopidogrel[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—[b]	—	Clo: 98	—	—	Clo: 4-6	Clo: 0.5–1	Clo[d]
					AM: 0.5[c]	AM: 1.0[d]	EM: 3.8 ± 2.5 ng/mL
							IM: 6.8 ± 3.6 ng/mL
							PM: 18 ± 14 ng/mL
							AM[d]
							EM: 39 ± 15 ng/mL
							IM: 26 ± 11 ng/mL
							PM: 24 ± 6 ng/mL

[a] Clopidogrel (Clo) is a prodrug that is converted to a minor unstable active metabolite (AM) via two sequential CYP dependent reactions. The majority of the dose gets converted rapidly to an inactive hydrolytic product by esterases. Although multiple CYP isoforms contribute to the formation of AM, blood levels of AM have been associated with the CYP2C19 genotype and phenotype status, with poor metabolizers (PM) exhibiting lower levels, on average, than extensive metabolizers (EM). Formation of AM accounts for ≤10% of the administered dose and may be dose dependent due to saturable metabolism. [b] The absolute bioavailability of Clo is unknown. There is one report of food greatly enhancing the systemic exposure of Clo following oral administration. [c] The reported value may represent disappearance of high levels of metabolite formed during first pass and not the terminal $t_{1/2}$, which should be formation limited. [d] Following a single 300-mg loading dose of Clo. IM, intermediate metabolizer.

References: Farid NA, et al. Metabolism and disposition of the thienopyridine antiplatelet drugs ticlopidine, clopidogrel, and prasugrel in humans. *J Clin Pharmacol,* **2009**, *50*: 126-42. Kim KA, et al. The effect of CYP2C19 polymorphism on the pharmacokinetics and pharmacodynamics of clopidogrel: a possible mechanism for clopidogrel resistance. *Clin Pharmacol Ther,* **2008**, *84*:236–242. Takahashi M, et al. Quantitative determination of clopidogrel active metabolite in human plasma by LC-MS/MS. *J Pharm Biomed Anal,* **2008**, *48*:1219–1224. Umemura K, et al. The common gene variants of CYP2C19 affect pharmacokinetics and pharmacodynamics in an active metabolite of clopidogrel in healthy subjects. *J Thromb Haemost,* **2008**, *6*:1439–1441.

Clorazepate[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
N: 91 ± 6[a]	N: <1	N: 97.5	N: 0.17 ± 0.02[b]	N: 1.24 ± 0.09[b]	N: 93 ± 11[b]	N: 0.9 ± 0.01[a,c]	N: 356 ± 27 ng/mL[a,c]
		↓ RD	↓ LD, Obes	↑ Obes	↑ Obes		
					↓ LD		

[a] Clorazepate is essentially a prodrug for nordiazepam (N, desmethyldiazepam). Bioavailability, T_{max}, and C_{max} values for N were derived after oral administration of clorazepate. [b] *CL*, V_{ss}, and $t_{1/2}$ values are for IV nordiazepam. [c] Data for N following a 20-mg oral dose of clorazepate.

References: Greenblatt DJ, et al. Desmethyldiazepam pharmacokinetics: studies following intravenous and oral desmethyldiazepam, oral clorazepate, and intravenous diazepam. *J Clin Pharmacol,* **1988**, *28*:853–859. Ochs HR, et al. Desmethyldiazepam kinetics after intravenous, intramuscular, and oral administration of clorazepate dipotassium. *Klin Wochenschr,* **1982**, *75*:175–180.

Clozapine

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
55 ± 12	<1	>95	6.1 ± 1.6[a]	5.4 ± 3.5	12 ± 4	1.9 ± 0.8[b]	546 ± 307 ng/mL[b]
			↓ Aged				
			↑ Smk				

[a] Clozapine is metabolically cleared, with a major role by CYP1A2 and more minor contributions by CYP3A4, CYP2C19, and CYP2D6 [b] Following titration up to a 150-mg oral dose (tablet) given twice daily for 7 days to adult chronic schizophrenics.

References: Choc MG, et al. Multiple-dose pharmacokinetics of clozapine in patients. *Pharm Res,* **1987**, *4*:402–405. Jann MW, et al. Pharmacokinetics and pharmacodynamics of clozapine. *Clin Pharmacokinet,* **1993**, *24*:161–176.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Codeine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
50 ± 7^{b}	Negligible	7	11 ± 2^{c}	2.6 ± 0.3^{c}	2.9 ± 0.7	C: 1.0 ± 0.5^{d}	C: 149 ± 60 ng/mL^{d}
						M: 1.0 ± 0.4^{d}	M: 3.8 ± 2.4 ng/mL^{d}

[a] Codeine is metabolized by CYP2D6 (polymorphic) to morphine. Analgesic effect is thought to be due largely to derived morphine. [b] Oral/IM bioavailability reported. [c] CL/F and V_{area}/F reported. [d] Data for codeine (C) and morphine (M) following a 60-mg oral codeine dose given three times daily for 7 doses to healthy male adults.

Reference: Quiding H, et al. Plasma concentrations of codeine and its metabolite, morphine, after single and repeated oral administration. *Eur J Clin Pharmacol,* **1986**, *30*:673–677.

Colchicine

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
37 ± 12^{a}	25–65	39 ± 5	1.8 ± 0.4^{b}	5.3 ± 1.2^{c}	58 ± 11	1.0 ± 0.6^{d}	6.5 ± 1 ng/mL^{d}
			↓ RD, LD				

[a] Decreased bioavailability after multiple doses has been reported. [b] Colchicine is a substrate of P-glycoprotein, which may contribute to its renal and biliary excretion and enterohepatic recirculation. Colchicine is also metabolized by CYP3A4. [c] Colchicine displays multicompartment kinetics with an initial central distribution volume of 0.26 L/kg. [d] Reported after administration of a single 1-mg tablet.

Reference: Ferron GM, et al. Oral absorption characteristics and pharmacokinetics of colchicine in healthy volunteers after single and multiple doses. *Eur J Clin Pharmacol,* **1996**, *36*:874–883.

Cyclophosphamide[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
74 ± 22	6.5 ± 4.3	13	1.3 ± 0.5	0.78 ± 0.57	7.5 ± 4.0	—	121 ± 21 μM^{b}
			↑ Child		↓ Child		
			↓ LD		↑ LD		

[a] Cyclophosphamide is activated to hydroxycyclophosphamide primarily by CYP2C9. The metabolite is further converted to the active alkylating species, phosphoramide mustard ($t_{1/2}$ = 9 h) and nornitrogen mustard (apparent $t_{1/2}$ = 3.3 h). Kinetic parameters are for cyclophosphamide. [b] Following a 600-mg/m^2 IV (bolus) dose given to breast cancer patients.

References: Grochow LB, et al. Clinical pharmacokinetics of cyclophosphamide. *Clin Pharmacokinet,* **1979**, *4*:380–394. Moore MJ, et al. Variability in the pharmacokinetics of cyclophosphamide, methotrexate and 5-fluorouracil in women receiving adjuvant treatment for breast cancer. *Cancer Chemother Pharmacol,* **1994**, *33*:472–476.

Cyclosporine

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
SI: 28 ± 18^{a,b}	<1	93 ± 2	5.7 (0.6–24)^{b,c}	4.5 (0.12–15.5)^{b}	10.7 (4.3–53)^{b}	NL: 1.5–2.0^{d}	NL: 1333 ± 469 ng/mL^{d}
			↓ LD, Aged	↓ Aged			
			↑ Child	↑ Child	↓ Child		SI: 1101 ± 570 ng/mL^{d}

[a] NEORAL (NL) exhibits a more uniform and slightly greater (125%–150%) relative oral bioavailability than the SANDIMMUNE (SI) formulation. [b] Pharmacokinetic parameters based on measurements in blood with a specific assay. Data from renal transplant patients shown. [c] Metabolized by CYP3A to three major metabolites, which are subsequently biotransformed to numerous secondary and tertiary metabolites. [d] Steady-state C_{max} following a 344 ± 122-mg/day oral dose (divided into two doses) of cyclosporine (NL, soft gelatin capsule) or a 14-mg/kg/day (range 6–22 mg/kg/day) oral dose of cyclosporine (SI) given to adult renal transplant patients in stable condition. Mean trough concentration after NL was 251 ± 116 ng/mL; therapeutic range (trough) is 150–400 ng/mL.

References: Fahr A. Cyclosporin clinical pharmacokinetics. *Clin Pharmacokinet,* **1993**, *24*:472–495. *PDR*54, **2000**, pp. 2034–2035. Pollak R, et al. Cyclosporine bioavailability of Neoral and Sandimmune in white and black *de novo* renal transplant recipients. Neoral Study Group. *Ther Drug Monit,* **1999**, *27*:661–663. Ptachcinski RJ, et al. Cyclosporine kinetics in renal transplantation. *Clin Pharmacol Ther,* **1985**, *38*:296–300.

Dabigatran[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
6 (3–7)^{b}	77	35	2.21 ± 0.29	0.98 ± 0.14	7.31 ± 0.74^{d}	2.7^{d}	159 ng/mL^{e}
			↓ RD^{c}		↑ RD^{c}		

[a] Dosed orally as dabigatran etexilate prodrug; conversion to dabigatran by carboxylesterases. [b] Absolute bioavailability of dabigatran after an oral dose of dabigatran etexilate. [c] The CL and $t_{1/2}$ of dabigatran are altered as a function of the severity of renal disease. [d] $t_{1/2}$ after an IV dose reported; much longer values (12–17 h) reported following multiple oral doses. [e] Dabigatran values following a 150-mg dose of the etexilate prodrug given twice a day to steady-state in surgical patients.

References: Blech S, et al. The metabolism and disposition of the oral direct thrombin inhibitor, dabigatran, in humans. *Drug Metab Disp,* **2008**, *36*:386–399. FDA. Drugs@FDA: FDA approved drug products. Dabigatran (Pradaxa). Available at: http://www.accessdata.fda.gov/scripts/cder/daf/. Accessed April 26, 2022. Stangier J. Clinical pharmacokinetics and pharmacodynamics of oral direct thrombin inhibitor dabigatran etexilate. *Clin Pharmacokinet,* **2008**, *47*:285–295.

Dapagliflozin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
78 (9)^{a}	3^{b}	91 ± 0.65	2.96 (23)^{c,d}	1.69 ± 0.45^{d}	12.2 ± 5.25	1 (0.5–2.0)^{d}	68 (32) ng/mL^{e}
			↓ RD^{f}				

[a] Mean (CV% reported); high-fat meal alters the absorption profile but not the AUC. [b] Calculated as the ratio of dapagliflozin renal clearance/total IV clearance. [c] Cleared from blood primarily by UGT1A9-dependent metabolism. [d] Mean (CV% or ± SD) calculated assuming 70-kg body weight. [e] Mean (range or CV%) reported for a 5-mg dose given once a day for 7 days. [f] Clearance declines as a function of the severity of renal disease by an unknown mechanism, as the fraction of dapagliflozin excreted unchanged is 3%.

References: FDA. Drugs@FDA: FDA approved drug products. Dapagliflozin (Farxiga). Available at: http://www.accessdata.fda.gov/scripts/cder/daf/. Accessed April 26, 2022. Scheen AJ, Pharmacokinetics, pharmacodynamics and clinical use of SGL2 inhibitors in patients with type 2 diabetes mellitus and chronic kidney disease. *Clin Pharmacokinet,* **2015**, *54*:691–708.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Dapsone

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
93 ± 8[a]	5–15[b]	73 ± 1	0.60 ± 0.17[c]	1.0 ± 0.1	22.4 ± 5.6	SD: 2.1 ± 0.8[d]	SD: 1.6 ± 0.4 μg/mL[d]
			↑ Neo				MD: 3.3 μg/mL[d]

[a]Decreased in severe leprosy by 70%–80%; estimates based on urinary recovery of radioactive dose. [b]Urine pH = 6–7. [c]Undergoes reversible metabolism to a monoacetyl metabolite; the reaction is catalyzed by NAT2 (polymorphic); also undergoes *N*-hydroxylation (CYP3A, CYP2C9). [d]Following a single 100-mg oral dose (SD) or a 100-mg oral dose given once daily to steady state (MD) in healthy adults.

References: Mirochnick M, et al. Pharmacokinetics of dapsone administered daily and weekly in human immunodeficiency virus-infected children. *Antimicrob Agents Chemother,* **1999**, *43*:2586–2591. Pieters FA, et al. The pharmacokinetics of dapsone after oral administration to healthy volunteers. *Br J Clin Pharmacol,* **1986**, *22*:491–494. Venkatesan K. Clinical pharmacokinetic considerations in the treatment of patients with leprosy. *Clin Pharmacokinet,* **1989**, *16*:365–386. Zuidema J, et al. Clinical pharmacokinetics of dapsone. *Clin Pharmacokinet,* **1986**, *11*:299–315.

Daptomycin

—[a]	47 ± 12	92	0.14 ± 0.01	0.096 ± 0.009	7.8 ± 1.0	—	99 ± 12 μg/mL[c]
			↑ RD[b]	↑ RD[b]	↑ RD[b]		

[a]Available for IV administration only. [b]Changes reported for patients with severe renal impairment. [c]C_{max} at the end of a 30-min IV infusion of a 6-mg/kg dose given once daily for 7 days. No significant accumulation with multiple dosing.

References: Dvorchik BH, et al. Daptomycin pharmacokinetics and safety following administration of escalating doses once daily to healthy subjects. *Antimicrob Agents Chemother,* **2003**, *47*:1318–1323. Product information: Cubicin™ (daptomycin for injection). Cubist Pharmaceuticals, Lexington, MA, **2004**.

Dextroamphetamine[a]

—[b]	Rac: 14.5[c]	Rac: 23–26	Dextro: 3.4–7.7[d]	Rac: 6.11 ± 0.22	Rac: 3.5–4.2[d]	Dextro:	Dextro: 61 ± 20 ng/mL[f]
			(Acidic urine)		(Acidic urine)	3.1 ± 1.1[f]	
			Dextro: 0.23–1.71[d]		Rac: 14–22[d]		
			(Alkaline urine)		(Alkaline urine)		
					Dextro: 6.8 ± 0.5[e]		
					(Uncontrolled urine pH)		

[a]Amphetamine is available as a racemate (Rac), dextro-isomer (Dextro), and a mixture of the two, in both immediate- and extended-release formulations. Pharmacokinetic data on both racemic and dextroamphetamine are presented. [b]Absolute bioavailability not reported; >55% based on urine recovery of unchanged drug under acidic urinary pH conditions. [c]Measured under uncontrolled urinary pH condition. Renal *CL* of amphetamine is dependent on urine pH. Acidification of urine results in increased urinary excretion, up to 55%. [d]*CL/F* and $t_{1/2}$ following oral dose to adults is reported. [e]$t_{1/2}$ in children reported. [f]Following a 20-mg immediate-release oral dose given once daily for >1 week. An extended-release formulation consisting of a mixture of dextroamphetamine and amphetamine salts (ADDERALL XR) exhibits a delayed T_{max} of ~7 h.

References: Busto U, et al. Clinical pharmacokinetics of non-opiate abused drugs. *Clin Pharmacokinet,* **1989**, *16*:1–26. Helligrel ET, et al. Steady-state pharmacokinetics and tolerability of modafinil administered alone or in combination with dextroamphetamine in healthy volunteers. *J Clin Pharmacol,* **2002**, *42*:450–460. McGough JJ, et al. Pharmacokinetics of SLI381 (ADDERALL XR), an extended-release formulation of Adderall. *J Am Acad Child Adolesc Psychiatry,* **2003**, *42*:684–691.

Diazepam[a]

PO: 100 ± 14	<1	98.7 ± 0.2	0.38 ± 0.06	1.1 ± 0.3	43 ± 13	PO: 1.3 ± 0.2[b]	IV: 400–500 ng/mL[d]
Rectal: 90		↓ RD, LD, Preg, Neo, Aged	↓ LD	↑ LD, Aged	↑ Aged, LD	Rectal: 1.5[c]	PO: 317 ± 27 ng/mL[b]
							Rectal: ~400 ng/mL[c]

[a]Active metabolites, desmethyldiazepam and oxazepam, formed by CYP2C19 (polymorphic) and CYP3A. [b]following a single 10-mg oral dose to healthy adults. [c]Following a 15-mg rectal dose given to healthy adults. A concentration of 300–400 ng/mL provides an anxiolytic effect, and >600 ng/mL provides control of seizures. [d]Range of data following a single 5- to 10-mg IV dose (15- to 30-sec bolus).

References: Friedman H, et al. Pharmacokinetics and pharmacodynamics of oral diazepam: effect of dose, plasma concentration, and time. *Clin Pharmacol Ther,* **1992**, *52*:139–150. Greenblatt DJ, et al. Diazepam disposition determinants. *Clin Pharmacol Ther,* **1980**, *27*:301–312. *PDR*54, **2000**, p. 1012.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Dicloxacillin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
50–85	60 ± 7	95.8 ± 0.2	1.6 ± 0.3[a]	0.086 ± 0.017	0.70 ± 0.07	0.5–1.6[b]	47–91 μg/mL[b]
		↓ RD, Aged, LD	↓ RD	↑ RD	↑ RD		

[a] Possible saturation of renal clearance at doses of 1–2 g. Active tubular secretion is mediated by OAT. [b] Estimated range of data following a single 2-g oral dose given to healthy (fasted) adults.

Reference: Nauta EH, Mattie H. Dicloxacillin and cloxacillin: pharmacokinetics in healthy and hemodialysis subjects. *Clin Pharmacol Ther*, **1976**, *20*:98–108.

Didanosine

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
38 ± 15	36 ± 9	<5	16 ± 7	1.0 ± 0.2	1.4 ± 0.3	B: 0.67 (0.33–1.33)[b]	B: 1.5–0.7 μg/mL[b]
↓ Food[a], Child						EC: 2.0 (1.0–5.0)[b]	EC: 0.93 ± 0.43 μg/mL[b]

[a] The magnitude of the food effect may depend on the product used, the type of meal consumed (light vs. high fat), and whether or not didanosine is coadministered with tenofovir, an inhibitor of didanosine metabolism. [b] Following a single 400-mg oral dose of didanosine formulated as a buffered tablet (B) or enteric-coated beads (EC), taken after a fast by patients with HIV infection.

References: Knupp CA, et al. Pharmacokinetics of didanosine in patients with acquired immunodeficiency syndrome or acquired immunodeficiency syndrome-related complex. *Clin Pharmacol Ther*, **1991**, *49*:523–535. Morse GD, et al. Single-dose pharmacokinetics of delavirdine mesylate and didanosine in patients with human immunodeficiency virus infection. *Antimicrob Agents Chemother*, **1997**, *41*:169–174.

Digoxin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Tablet 65.7 ± 2.0[a]	81 ± 2[b]	25 ± 5	2.8 ± 0.35	7 ± 1 L/kg	39 ± 13	1–3[c]	1.4 ± 0.7 ng/mL[c]
		↓ RD	↓ RD	↓ RD			
			↑ Neo, Child, Preg	↑ Child	↑ RD, Aged		

[a] LANOXIN tablets; digoxin solutions, elixirs, and capsules are absorbed more completely. [b] 19% of the dose is excreted into bile and into feces following IV administration. Intestinal and renal efflux transport of digoxin is mediated by P-glycoprotein and other digoxin-specific transporters. [c] Following an oral dose of 0.31 ± 0.19 mg/day in patients with CHF who exhibited no signs of digitalis toxicity.

References: Birkenfeld AL, et al. Genetic influences on the pharmacokinetics of orally and intravenously administered digoxin as exhibited by monozygotic twins. *Clin Pharmacol Ther,* **2009**, *86*:605–608. Hinderling PH, Hartmann D. Pharmacokinetics of digoxin and main metabolites/derivatives in healthy humans. *Ther Drug Monit,* **1991**, *13*:381–401. Mooradian AD. Digitalis. An update of clinical pharmacokinetics, therapeutic monitoring techniques and treatment recommendations. *Clin Pharmacokinet,* **1988**, *15*:165–179. Smith TW, et al. Digoxin intoxication: the relationship of clinical presentation to serum digoxin concentration. *J Clin Invest,* **1970**, *49*:2377–2386.

Diltiazem[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
38 ± 11	<4	78 ± 3	11.8 ± 2.2[b]	3.3 ± 1.2	4.4 ± 1.3[c]	4.0 ± 0.4[d]	151 ± 46 ng/mL[d]
			↓ RD	↓ RD			

[a] Active metabolites, desacetyldiltiazem ($t_{1/2}$ = 9 ± 2 h) and *N*-desmethyldiltiazem ($t_{1/2}$ = 7.5 ± 1 h). Formation of desmethyl metabolite (major pathway of *CL*) catalyzed primarily by CYP3A. [b] More than a 2-fold decrease with multiple dosing. [c] $t_{1/2}$ for oral dose is 5–6 h; does not change with multiple dosing. [d] Following a single 120-mg oral dose to healthy adults.

Reference: Echizen H, et al. Clinical pharmacokinetics of verapamil, nifedipine, and diltiazem. *Clin Pharmacokinet,* **1986**, *11*:425–449.

Docetaxel[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—	2.1 ± 0.2	94	22.6 ± 7.7 L/h/m^2	72 ± 24 L/m^2	13.6 ± 6.1	—	2.4 ± 0.9 μg/mL[c]
			↓ LD[b]				

[a] Data from male and female patients treated for cancer. Metabolized by CYP3A and excreted into bile. Parenteral administration. [b] Mild to moderate liver impairment. [c] Following an IV infusion of 85 mg/m^2 over 1.6 h.

References: Clarke SJ, et al. Clinical pharmacokinetics of docetaxel. *Clin Pharmacokinet,* **1999**, *36*:99–114. Extra JM, et al. Phase I and pharmacokinetic study of Taxotere (RP 56976; NSC 628503) given as a short intravenous infusion. *Cancer Res,* **1993**, *53*:1037–1042. *PDR*54, **2000**, p. 2578.

Donepezil[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—[b]	10.6 ± 2.7	92.6 ± 0.9[c]	2.90 ± 0.74[d,e]	14.0 ± 2.42[e]	59.7 ± 16.1[e]	3–4[g]	30.8 ± 4.2 ng/mL[g]
			↓ LD[f]	↑ Aged	↑ Aged		

[a] Data from young, healthy, male and female subjects. No significant sex differences. [b] Absolute bioavailability is unknown. [c] A fraction bound value of 96% also has been reported. [d] Metabolically cleared by CYP2D6, CYP3A4, and UGT. [e] *CL/F*, V_{ss}/F, and $t_{1/2}$ reported for oral dose. [f] *CL/F* reduced slightly (~20%), alcoholic cirrhosis. [g] Following a 5-mg oral dose given once daily to steady state.

References: Ohnishi A, et al. Comparison of the pharmacokinetics of E2020, a new compound for Alzheimer's disease, in healthy young and elderly subjects. *J Clin Pharmacol,* **1993**, *33*:1086–1091. *PDR*54, **2000**, p. 2323.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Dolutegravir

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—[a]	—	98.9	0.27 (45)[b,c]	0.33 (45)[c]	14.4 (19)[c]	2–3	4.15 (29) μg/mL[e]
↑ Food					↓ HIV-1[d]		

[a]The absolute oral bioavailability is not known. [b]Cleared from blood predominantly by UGT1A1-depedent metabolism. [c]*CL/F* and V_d/F calculated assuming 70-kg body weight; mean (CV%) reported for single oral dose to healthy volunteers. [d]$t_{1/2}$ of 11–12 h reported for patients with HIV-1 infection. [e]Mean plasma concentration (CV%) in HIV-1–infected patients receiving 50 mg dolutegravir, twice a day, to steady state.

References: FDA. Drugs@FDA: FDA approved drug products. Dolutegravir (Tivicay). Available at: http://www.accessdata.fda.gov/scripts/cder/daf/. Accessed April 26, 2022. Podany AT, et al. Comparative clinical pharmacokinetics and pharmacodynamics of HIV-1 integrase strand transfer inhibitors. *Clin Pharmacokinet*, **2017**, *56*:25–40.

Doxazosin[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Y: 65 ± 14	5	98	Y: 1.26 ± 0.27[b]	Y: 1.0 ± 0.1	20.5 ± 6.1[d,e]	3.9 ± 1.2[e]	67 ± 19 ng/mL[e]
E: 68 ± 16			E: 2.25 ± 1.42[b]	E: 1.7 ± 1.0	GITS: 19 ± 4[e]	GITS: 9 ± 5[d]	GITS: 28 ± 12 ng/mL[d]
GITS: F_{rel} = 59 ± 12			↓ LD[c]				

[a]Where indicated, data for young (Y) and elderly (E) normotensive adults are reported. Also reported are data for a gastrointestinal sustained-release device (GITS); oral bioavailability relative to standard formulation (F_{rel}). [b]Cleared primarily by CYP dependent metabolism. [c]Study in patients with mild to moderate liver impairment; AUC increased 43%. [d]Shorter $t_{1/2}$ following IV dosing reported; (Y) 10 ± 1 h, (E) 12 ± 5 h; attributed to inadequate duration of blood sampling. [e]Following an 8-mg dose of standard formulation or GITS, once daily, to steady state in young and elderly normotensive volunteers.

References: Chung M, et al. Clinical pharmacokinetics of doxazosin in a controlled-release gastrointestinal therapeutic system (GITS) formulation. *Br J Clin Pharmacol,* **1999**, *48*:678–687. Elliott HL, et al. Pharmacokinetic overview of doxazosin. *Am J Cardiol,* **1987**, *59*:78G–81G. Penenberg D, et al. The effects of hepatic impairment on the pharmacokinetics of doxazosin. *J Clin Pharmacol,* **2000**, *40*:67–73. Vincent J, et al. The pharmacokinetics of doxazosin in elderly normotensives. *Br J Clin Pharmacol,* **1986**, *21*:521–524.

Doxorubicin[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
5	<7	76	666 ± 339 mL/min/m^2	682 ± 433 L/m^2	26 ± 17[b]	—	High[c]
			↓ LD, Obes		↑ LD		D: ~950 ng/mL
			↑ Child				DL: 30–1008 ng/mL
							Low[c]
							D: 6.0 ± 3.2 ng/mL
							DL: 5.0 ± 3.5 ng/mL

[a]Active metabolites; $t_{1/2}$ for doxorubicinol is 29 ± 16 h. [b]Prolonged when plasma bilirubin concentration is elevated; undergoes biliary excretion. [c]Mean data for doxorubicin (D) and range of data for doxorubicinol (DL). High: a single 45- to 72-mg/m^2 high-dose 1-h IV infusion given to patients with small cell lung cancer. Low: continuous IV infusion at a rate of 3.9 ± 0.65 mg/m^2/day for 12.4 (2–50) weeks to patients with advanced cancer.

References: Ackland SP, et al. Pharmacokinetics and pharmacodynamics of long-term continuous-infusion doxorubicin. *Clin Pharmacol Ther,* **1989**, *45*:340–347. Piscitelli SC, et al. Pharmacokinetics and pharmacodynamics of doxorubicin in patients with small cell lung cancer. *Clin Pharmacol Ther,* **1993**, *53*:555–561.

Doxycycline

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
93	41 ± 19	88 ± 5	0.53 ± 0.18	0.75 ± 0.32	16 ± 6	Oral: 1–2[b]	IV: 2.8 μg/mL[b]
		↓ RD[a]	↓ Aged	↓ Aged			PO: 1.7–2 μg/mL[b]

[a]Decreases in plasma protein binding to 71% ± 3% in patients with uremia. [b]Mean data following a single 100-mg IV dose (1-h infusion) or range of mean data following a 100-mg oral dose given to adults.

Reference: Saivin S, et al. Clinical pharmacokinetics of doxycycline and minocycline. *Clin Pharmacokinet,* **1988**, *15*:355–366.

Duloxetine

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
42.8 (18.5–71.2)	—	>90	10.6 ± 2.4[b]	7.0 ± 1.3	9.3 (6.4–12)	4.5 (2.5–6)[e]	32.9 ng/mL[e]
↓ Smk[a]			↓ LD,[c] RD[d]		↑ LD[c]		

[a]Approximately 30% lower bioavailability based on population pharmacokinetic analysis; no dose adjustment recommended. [b]Cleared primarily by CYP1A2- and CYP2D6-dependent metabolism. [c]A 5-fold increase in oral AUC in patients with moderate liver impairment. [d]A 2-fold increase in oral AUC in patients with end-stage RD receiving intermittent dialysis. [e]Following a single 60-mg oral dose.

References: FDA. Drugs@FDA. Cymbalta label approved on 6/16/09. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/021427s030lbl.pdf. Accessed May 17, 2010. Lobo ED, et al. In vitro and in vivo evaluations of CYP1A2 interactions with duloxetine. *Clin Pharmacokinet*, **2008,** *47*:191–202. Lobo ED, et al. Population pharmacokinetics of orally administered duloxetine in patients: implications for dosing recommendation. *Clin Pharmacokinet*, **2009**, *48*:189–197.

Dutasteride

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
60 (40–94)	—	99	—[a,b]	—[b]	840[c]	1 (1–3)[d]	38 ± 13 ng/mL[d]

[a]Dutasteride is cleared primarily by CYP3A-dependent metabolism. [b]*CL/F* = 0.20–0.37 mL/min/kg, and *V/F* = 4.3–7.1 L/kg; calculated from steady-state (24 weeks) serum concentrations. [c]Terminal $t_{1/2}$ reported. [d]Following a 0.5-mg dose given once daily to steady state (24 weeks).

References: Clark RV, et al. Marked suppression of dihydrotestosterone in men with benign prostatic hyperplasia by dutasteride, a dual 5-alpha-reductase inhibitor. *J Clin Endocrinol Metab,* **2004**, *89*:2179–2178. Keam SJ, et al. Dutasteride: a review of its use in the management of prostate disorders. *Drugs*, **2008**, *68*:463–485.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Efavirenz[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—[b]	<1	99.5–99.75	3.1 ± 1.2[c,d]	—	SD: 52–76[d]	4.1 ± 1.7[e]	4.0 ± 1.7 μg/mL[e]
↑ Food					MD: 40–55[d]		

[a] Data from patients with HIV infection. No significant sex differences. [b] Absolute oral bioavailability is unknown. Oral AUC increased 50% with high-fat meal. [c] Metabolized primarily by CYP2B6 and to a lesser extent by CYP2A6 and through *N*-glucuronidation. [d] Single-dose (SD) data reported for *CL/F* and both SD and multiple-dose (MD) data for $t_{1/2}$. Efavirenz is a weak inducer of CYP3A4 and its own metabolism. [e] Following a 600-mg oral dose given daily to steady state.

References: Adkins JC, et al. Efavirenz. *Drugs*, **1998**, *56*:1055–1064. *PDR*54, **2000**, p. 981. Villani P, et al. Pharmacokinetics of efavirenz (EFV) alone and in combination therapy with nelfinavir (NFV) in HIV-1 infected patients. *Br J Clin Pharmacol*, **1999**, *48*:712–715.

Elbasvir/Grazoprevir[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Elbasvir: 32 ↓ Food	<1[b]	>99.9	6.2 (5.0–7.7) ↓ Aged[c]	9.7	24[d]	3 (3–6)[d,e]	121 (118, 123) ng/mL[f]
Grazoprevir: 27	<1[b]	>98.8	2.7 (2.0–3.7)	17.9	31[d]	2 (0.5–3)[d,e]	165 (161, 176) ng/mL[f]
↑ Food			↓ Aged[c], ↓ LD				

[a] Data from non–HCV-infected subjects unless otherwise specified. [b] The primary route of elimination of elbasvir and grazoprevir is through feces, with >90% of radiolabeled dose recovered in feces compared to <1% in urine following oral administration. Elbasvir and grazoprevir are partially eliminated by oxidative metabolism, primarily by CYP3A. No circulating metabolites of either elbasvir or grazoprevir were detected in human plasma. Elbasvir and grazoprevir are substrates of P-glycoprotein, but the role of intestinal P-glycoprotein in the absorption of elbasvir and grazoprevir appears to be minimal. Grazoprevir is a substrate of OATP1B1/3. [c] From population pharmacokinetic analysis. [d] In HCV-infected subjects. [e] Median (range) measured in HCV subjects. [f] Steady-state concentrations following administration of ZEPATIER (elbasvir 50 mg and grazoprevir 100 mg) once a day for 12 weeks in non-HCV subjects. Steady-state is reached within approximately 6 days; values are mean (90% confidence intervals) and estimated based on population pharmacokinetic modeling. Elbasvir pharmacokinetics are similar in healthy subjects and HCV-infected subjects, while grazoprevir oral exposures are approximately 2-fold greater in HCV-infected subjects when compared to healthy subjects. Elbasvir pharmacokinetics were approximately dose-proportional over the range of 5–100 mg once daily. Grazoprevir AUC increased more than dose proportionally over 10–800 mg once-a-day dosing in HCV-infected subjects.

Reference: Caro L, et al. Pharmacokinetics of elbasvir and grazoprevir in subjects with end-stage renal disease or severe renal impairment. *Eur J Clin Pharmacol*, **2019**, *75*:665–675. FDA. Drugs@FDA: FDA-approved drugs product labeling: Zepatier® (elbasvir and grazoprevir oral tablets). Available at: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm. Accessed April 1, 2021. FDA. Elbasvir and grazoprevir NDA and label. NDA approved in 2016; label revised 12/2019. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/208261s006lbl.pdf. Accessed April 1, 2021.

Eletriptan

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
~50	9 (7–12)[b]	85	5.6 (3.7–6.7)[b,c]	2.0 (1.4–2.4)[b]	4.1 (2.8–5.5)[b]	MF: 0.75–1.5[e]	57–115 ng/mL[f]
						M: 2.0–2.8[e]	
↑ Food[a]			↓ LD[d]		↑ LD[d]		

[a] Systemic exposure increased 20%–30% with high-fat meal. [b] Data from 50-μg/kg IV dose reported; lack of dose proportionality for oral AUC between 20- and 40- or 80-mg doses. [c] Cleared primarily by CYP3A-dependent metabolism. [d] Study in patients with mild to moderate hepatic impairment. [e] Following single 20- to 80-mg oral doses; MF: migraine-free period; M: during a migraine attack. [f] Range of mean values from different studies following a single 30-mg oral dose.

References: FDA. Drugs@FDA. Relpax label approved on 12/26/09. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2002/21016_relpax_lbl.pdf. Accessed May 17, 2010. McCormack PL, et al. Eletriptan: a review of its use in the acute treatment of migraine. *Drugs*, **2006**, *66*:1129–1149. Milton KA, et al. Pharmacokinetics, pharmacodynamics, and safety of the 5-HT(1B/1D) agonist eletriptan following intravenous and oral administration. *J Clin Pharmacol*, **2002**, *42*:528–539.

Enalapril[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
41 ± 15	88 ± 7[b]	50–60	4.9 ± 1.5[c]	1.7 ± 0.7[c]	11[d]	3.0 ± 1.6[e]	69 ± 37 ng/mL[e]
↓ LD	↓ LD		↓ RD, Aged, Neo		↑ RD, LD		
			↑ Child				

[a] Hydrolyzed by esterases to the active metabolite, enalaprilic acid (enalaprilat); except when noted, pharmacokinetic values and disease comparisons are for enalaprilat, following oral enalapril administration. [b] For IV enalaprilat. [c] *CL/F* and V_{ss}/F after multiple oral doses of enalapril. Values after single IV dose of enalaprilat are misleading because binding to ACE leads to a prolonged $t_{1/2}$, which does not represent a significant fraction of the *CL* upon multiple dosing. [d] Estimated from the approach to steady state during multiple dosing. [e] Mean values for enalaprilat following a 10-mg enalapril oral dose given daily for 8 days to healthy young adults. The EC_{50} for ACE inhibition is 5–20 ng/mL enalaprilat.

References: Lees KR, et al. Age and the pharmacokinetics and pharmacodynamics of chronic enalapril treatment. *Clin Pharmacol Ther*, **1987**, *41*:597–602. MacFadyen RJ, et al. Enalapril clinical pharmacokinetics and pharmacokinetic-pharmacodynamic relationships. An overview. *Clin Pharmacokinet*, **1993**, *25*:274–282.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Enoxaparin[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
SC: 92	—[b]	—	0.3 ± 0.1[c]	0.12 ± 0.04[c]	3.8 ± 1.3[d]	3[e]	ACLM: 145 ± 45 ng/mL[e]
			↓ RD		↑ RD		BCLM: 414 ± 87 ng/mL[e]

[a]Enoxaparin consists of low-molecular-weight heparin fragments of varying lengths. [b]43% is recovered in urine when administered as ^{99}Tc-labeled enoxaparin; 8%–20% anti–factor Xa activity. [c]*F, CL/F,* and V_{area}/F for SC dose measured by functional assay for anti–factor Xa activity. [d]Measured by functional assay of anti–factor Xa activity. Using anti–factor IIa activity or displacement binding assay gives a $t_{1/2}$ of ~1–2 h. [e]Following a single 40-mg SC dose to healthy adult subjects. High-affinity antithrombin III molecules: ACLM, above-critical-length molecules (anti–factor Xa and IIa activity); BCLM, below-critical-length molecules (anti–factor Xa activity).

References: Bendetowicz AV, et al. Pharmacokinetics and pharmacodynamics of a low molecular weight heparin (enoxaparin) after subcutaneous injection, comparison with unfractionated heparin—a three way cross over study in human volunteers. *Thromb Haemost,* **1994**, *71*:305–313. *PDR*54, **2000**, p. 2561.

Eplerenone[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—	7[b]	33–60[c]	2.4[d]	0.6–1.3[d]	4–6	1.8 ± 0.7[e]	1.0 ± 0.3 μg/mL[e]
			↓ LD				

[a]Eplerenone is converted (reversibly) to an inactive ring-open hydroxy acid. Both eplerenone (E) and the hydroxy acid (EA) circulate in plasma; concentrations of E are much higher than EA. Irreversible metabolism is catalyzed predominantly by CYP3A4. Data for E in healthy male and female volunteers reported; no significant sex differences. [b]Recovered as E and EA following an oral dose. [c]Protein binding is concentration dependent over the therapeutic range; lower at the highest concentration. [d]*CL/F* and V_{ss}/F reported. [e]Following a 50-mg oral dose given once daily for 7 days.

References: FDA. Clinical Pharmacology and Biopharmaceutics Review. Application 21-437/S-002. U.S. Food and Drug Administration Center for Drug Evaluation and Research. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2002/21-437_Inspra.cfm. Accessed July 9, 2010. Cook CS, et al. Pharmacokinetics and metabolism of [14C] eplerenone after oral administration to humans. *Drug Metab Dispos,* **2003**, *31*:1448–1455. Product information: Inspra™ (eplerenone tablets). Chicago, IL, Pfizer, **2004**.

Erlotinib

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
59 (55–66)	—[b]	93 (92–95)	1.0 ± 0.4[c,d]	1.2 ± 0.25[f]	13[g]	2–4[h]	1.1–1.7 μg/mL[h]
↑ Food[a]			↑ Smk[e]				

[a]Bioavailability increases to ~100% when taken with a meal; not recommended because food effect is highly variable. Likely to be low based on reported bioavailability and low recovery of unchanged drug (<2%) after an oral dose. [c]Erlotinib is cleared primarily by CYP3A- and CYP1A2-dependent metabolism. [d]Calculated from a 25-mg IV dose assuming a 70-kg body weight. [e]Systemic exposure reduced by half compared to nonsmokers. [f]Calculated from a 25-mg IV dose assuming a 70-kg body weight. [g]Median $t_{1/2}$ in a patient population receiving 150 mg orally once daily; a shorter $t_{1/2}$ of 36 h was reported for a single 25-mg IV dose. [h]Following 150-mg oral dose given once daily to steady state.

References: FDA. Drugs@FDA. Tarceva NDA and label; label approved on 4/27/09. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-743_Tarceva.cfm. Accessed May 17, 2010. Frohna P, et al. Evaluation of the absolute oral bioavailability and bioequivalence of erlotinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in a randomized, crossover study in healthy subjects. *J Clin Pharmacol,* **2006**, *46*:282–290. Lu JF, et al. Clinical pharmacokinetics of erlotinib in patients with solid tumors and exposure-safety relationship in patients with non-small cell lung cancer. *Clin Pharmacol Ther,* **2006**, *80*:136–134.

Erythromycin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
35 ± 25[a]	12 ± 7	84 ± 3[c]	9.1 ± 4.1[d]	0.78 ± 0.44	1.6 ± 0.7	B: 2.1–3.9[e]	B: 0.9–3.5 μg/mL[e]
↓ Preg[b]				↑ RD	↑ LD	S: 2–3[e]	S: 0.5–1.4 μg/mL[e]

[a]Value for enteric-coated erythromycin base. [b]Decreased concentrations in pregnancy possibly due to decreased bioavailability (or increased *CL*). [c]Erythromycin base. [d]Erythromycin is a CYP3A substrate; *N*-demethylation. It also is transported by P-glycoprotein, which may contribute to biliary excretion of parent drug and metabolites. [e]Range of mean values from studies following a 250-mg oral enteric-coated free base in a capsule (B) given four times daily for 5–13 doses or a 250-mg film-coated tablet or capsule of erythromycin stearate (S) given four times daily for 5–12 doses.

Reference: Periti P, et al. Clinical pharmacokinetic properties of the macrolide antibiotics. Effects of age and various pathophysiological states (part I). *Clin Pharmacokinet,* **1989**, *16*:193–214.

Escitalopram[a], Citalopram

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—	Es: 8	Es: 56	Es: 8.8 ± 3.2[b,c]	Es: 15.4 ± 2.4[c]	Es: 22 ± 6[b]	—	Es: 21 ± 4 ng/mL[d]
Rac: 80 ± 13	Rac: 10.5 ± 1.4	Rac: 80	Rac: 4.3 ± 1.2[b]	Rac: 12.3 ± 2.3	Rac: 33 ± 4[b]	Rac/Es: 4–5[d]	Rac: 50 ± 9 ng/mL[d]
			↓ Aged, LD[d]		↑ Aged, LD,[d] RD[e]		

[a]Escitalopram is the active *S*-enantiomer of racemic citalopram. Pharmacokinetic data after dosing of escitalopram (Es) and citalopram racemate (Rac) are reported. No significant sex differences. Citalopram is metabolized by CYP2C19 (polymorphic) and CYP3A4 to desmethylcitalopram. [b]Data from CYP2C19 extensive metabolizers. CYP2C19 poor metabolizers exhibit a lower (~44%) *CL/F* and longer $t_{1/2}$ than extensive metabolizers. [c]*CL/F* and *V/F* for Es reported. [d]Following a single 40-mg (Rac) or 20-mg (Es) oral dose.

References: Gutierrez MM, et al. An evaluation of the potential for pharmacokinetic interaction between escitalopram and the cytochrome P450 3A4 inhibitor ritonavir. *Clin Ther,* **2003**, *25*:1200–1210. Joffe P, et al. Single-dose pharmacokinetics of citalopram in patients with moderate renal insufficiency or hepatic cirrhosis compared with healthy subjects. *Eur J Clin Pharmacol,* **1998**, *54*:237–242. *PDR*58, **2004**, pp. 1292, 1302–1303. Sidhu J, et al. Steady-state pharmacokinetics of the enantiomers of citalopram and its metabolites in humans. *Chirality,* **1997**, *9*:686–692. Sindrup SH, et al. Pharmacokinetics of citalopram in relation to the sparteine and the mephenytoin oxidation polymorphisms. *Ther Drug Monit,* **1993**, *15*:11–17.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Esomeprazole[a], Omeprazole

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Es: 89 (81–98)[b]	Es/Rac: <1	Es/Rac: 95–97	Es: 4.1 (3.3–5.0)[c,d]	Es: 0.25 (0.23–0.27)	Es: 0.9 (0.7–1.0)[d]	Es: 1.5 (1.3–1.7)[f]	Es: 4.5 (3.8–5.7) μM[f]
					Rac: 0.7 ± 0.5		Rac, EM: 0.68 ± 0.43 μM[g]
Rac: 53 ± 29[b]			Rac: 7.5 ± 2.7[c]	Rac: 0.34 ± 0.09		Rac, EM: ~1[g]	Rac, PM: 3.5 ± 1.4 μM[g]
			↓ LD[e]		↑ LD[e]	Rac, PM: ~3–4[g]	

[a] Esomeprazole is the *S*-enantiomer of omeprazole. Both esomeprazole (Es) and racemic omeprazole (Rac) are available. Data for both formulations are reported. [b] Bioavailability determined after multiple dosing. Lower Es values 64% (54%–75%) reported for single dose. [c] The metabolic *CL* of the Es is slower than that of the *R*-enantiomer. Both Es and Rac are metabolized by CYP2C19 (polymorphic) and CYP3A4. *CL* of Es and Rac is decreased and $t_{1/2}$ increased in CYP2C19 poor metabolizers. [d] Following a single 40-mg IV dose. *CL* of Es decreases and $t_{1/2}$ of Es increases with multiple dosing. [e] Reduced *CL* and increased $t_{1/2}$ in patients with severe (Child-Pugh class C) hepatic impairment. [f] Following a 40-mg oral dose of Es given once daily for 5 days to healthy subjects of unspecified CYP2C19 phenotype. [g] Following a 20-mg oral dose of Rac given twice daily for 4 days to healthy subjects phenotyped as CYP2C19 extensive metabolizers (EM) and poor metabolizers (PM).

References: Andersson T, et al. Pharmacokinetic studies with esomeprazole, the (S)-isomer of omeprazole. *Clin Pharmacokinet*, **2001**, *40*:411–426. Chang M, et al. Interphenotype differences in disposition and effect on gastrin levels of omeprazole—suitability of omeprazole as a probe for CYP2C19. *Br J Clin Pharmacol*, **1995**, *39*:511–518.

Eszopiclone,[a] Zopiclone

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—	Es: <10	Es: 52-59	—[b]	—[c]	Es: 7.2 ± 1.3	Es: 1 (0.4–2.1)	Es: 39.8 ± 8.6 ng/mL[e]
					Es: ↑ LD[d]		

[a] Eszopiclone is the (*S*)-isomer of zopiclone. Pharmacokinetic data after dosing of eszopiclone (Es) is shown. Es is metabolized extensively by CYP3A4 and CYP2E1. [b] *CL/F* following a 15-mg oral dose of zopiclone is 2.7 mL/min/kg for Es and 4.4 mL/min/kg for racemic zopiclone. [c] *V/F* following a 15-mg oral dose of racemic zopiclone is 1.4 L/kg for eszopiclone and 2 L/kg for racemic zopiclone. [d] In patients with severe hepatic impairment. [e] Following 3 mg Es given once daily to steady state.

References: FDA. Drugs@FDA. Lunesta label approved on 04/06/09. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/021476s012lbl.pdf. Accessed July 9, 2010. Najib J. Eszopiclone, a nonbenzodiazepine sedative-hypnotic agent for the treatment of transient and chronic insomnia. *Clin Ther,* **2006**, *28*:491–516.

Ethambutol

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
77 ± 8	79 ± 3	6–30	8.6 ± 0.8	1.6 ± 0.2	3.1 ± 0.4	2–4[a]	2–5 μg/mL[a]
					↑ RD		

[a] Following a single 800-mg oral dose to healthy subjects. Concentrations >10 μg/mL can adversely affect vision. No accumulation with once-a-day dosing in patients with normal renal function.

Reference: Holdiness MR. Clinical pharmacokinetics of the antituberculosis drugs. *Clin Pharmacokinet,* **1984**, *9*:511–544.

Etoposide

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
52 ± 17[a]	35 ± 5	96 ± 0.4[b]	0.68 ± 0.23[c]	0.36 ± 0.15	8.1 ± 4.3	1.3	NT: 2.7 μg/mL[d] T: 4.7 μg/mL[d]
			↓ RD		↑ RD		

[a] Decreases at oral doses >200 mg. [b] Decreases with hyperbilirubinemia. [c] Metabolized by CYP3A; also a substrate for P-glycoprotein. [c] Mean C_{max} for patients without (NT) and with (T) serious hematological toxicity following a 75- to 200-mg/m² dose given as a 72-h continuous IV infusion.

References: Clark PI, Slevin ML. The clinical pharmacology of etoposide and teniposide. *Clin Pharmacokinet*, **1987**, *12*:223–252. McLeod HL, Evans WE. Clinical pharmacokinetics and pharmacodynamics of epipodophyllotoxins. *Cancer Surv*, **1993**, *17*:253–268.

Exenatide[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
SC: ~100	—	—	8.1[b]	0.1[c]	1.5 (0.9–2.0)	2 (1–3)[c]	821 ± 500 pg/mL[d]
			↓ RD		↑ RD		

[a] Exenatide is a synthetic peptide cleared primarily by the kidney through filtration, reabsorption, and proteolytic degradation. [b] *CL/F* after SC injection reported. [c] V_{area}/F after SC injection reported. [d] Following a 10-μg SC injection.

Reference: Linnebjerg H, et al. Effects of renal impairment on the pharmacokinetics of exenatide. *Br J Clin Pharmacol*, **2007**, *64*:317–327.

Ezetimibe[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—	~2	>90[b]	6.6[c]	1.5[c]	28–30[d]	1[e]	122 ng/mL[e]
			↓ Aged, RD, LD				

[a] Ezetimibe is extensively metabolized to a glucuronide, which is more active than ezetimibe in inhibiting cholesterol absorption. Clinical effects are related to the total plasma concentration of ezetimibe and ezetimibe–glucuronide, with ezetimibe concentrations being only 10% of the total. [b] For ezetimibe and ezetimibe–glucuronide. [c] *CL/F* and a volume for the central compartment (V_c/F) for total (unconjugated and glucuronide conjugate) ezetimibe reported. [d] Ezetimibe undergoes significant enterohepatic recycling, leading to multiple secondary peaks. An effective $t_{1/2}$ is estimated. [e] Total (unconjugated and glucuronide conjugate) ezetimibe following a 10-mg oral dose given once daily for 10 days.

References: Mauro VF, et al. Ezetimibe for management of hypercholesterolemia. *Ann Pharmacother,* **2003**, *37*:839–848. Patrick JE, et al. Disposition of the selective cholesterol absorption inhibitor ezetimibe in healthy male subjects. *Drug Metab Dispos,* **2002**, *30*:430–437. *PDR*58, **2004**, pp. 3085–3086.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Famotidine

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
37 (20–66)	65–80	20	4.3–6.9[a]	1.1–1.4	2.5–4.0	2.3 (1–4)	76–104 ng/mL[c]
			↓ Aged, RD, Neo[b]		↑ RD		

[a]Cleared primarily by the kidney. Renal clearance after IV administration was ~4.3 mL/min/kg. [b]The pharmacokinetics of IV famotidine were similar in children >1 year of age and adults. [c]Following a single 40-mg oral dose.

References: Krishna DR, et al. Newer H_2-receptor antagonists. Clinical pharmacokinetics and drug interaction potential. *Clin Pharmacokinet,* **1988**, *15*:205–215. Maples HD, et al. Famotidine disposition in children and adolescents with chronic renal insufficiency. *J Clin Pharmacol,* **2003**, *43*:7–14. Wenning LA, et al. Pharmacokinetics of famotidine in infants. *Clin Pharmacokinet,* **2005**, *44*:395–406.

Felodipine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
15 ± 8	<1	99.6 ± 02	12 ± 5[b]	10 ± 3	14 ± 4	IR: 0.9 ± 0.4[d]	IR: 34 ± 26 nM[d]
↑ Food			↓ Aged, LD	↓ LD	↑ Aged	ER: 3.7 ± 0.9[d]	ER: 9.1 ± 7.3 nM[d]

[a]Racemic mixture; *S*-(–)-enantiomer is an active Ca^{+2} channel blocker; different enantiomer pharmacokinetics result in *S*-(–)-enantiomer concentrations about 2-fold higher than those of *R*-(+)-isomer. [b]Undergoes significant CYP3A-dependent first-pass metabolism in the intestine and liver. [c]May be age related rather than CHF related. [d]Following a 10-mg oral immediate-release (IR) or extended-release (ER) tablet given twice daily to steady state in healthy subjects. EC_{50} for diastolic pressure decrease is 8 ± 5 nM in patients with hypertension.

Reference: Dunselman PH, et al. Felodipine clinical pharmacokinetics. *Clin Pharmacokinet,* **1991**, *21*:418–430.

Fenofibrate[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—[b]	0.1–10[c]	>99	0.45[d]	0.89[d]	20–27	IR: 6–8[e]	IR: 8.6 ± 0.9 μg/mL[e]
↑ Food			↓ RD		↑ RD	Mic: 4–6[f]	Mic: 10.8 ± 0.6 μg/mL[f]

[a]Fenofibrate is a prodrug that is hydrolyzed by esterases to fenofibric acid, the pharmacologically active compound. All values reported are for fenofibric acid. [b]Absolute bioavailability is not known. Recovery of radiolabeled dose in urine as fenofibric acid, and its glucuronide is 60%. Immediate-release (IR) tablet and micronized (Mic) capsule are bioequivalent. Bioavailability is increased when taken with a standard meal. [c]Recovery following oral dose. [d]*CL/F* and *V/F* reported. [e]Following a 300-mg IR fenofibrate tablet given once daily to steady state. [f]Following a 200-mg Mic capsule given once daily to steady state.

References: Balfour JA, et al. Fenofibrate. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in dyslipidaemia. *Drugs,* **1990**, *40*:260–290. Miller DB, et al. Clinical pharmacokinetics of fibric acid derivatives (fibrates). *Clin Pharmacokinet,* **1998**, *34*:155–162.

Fentanyl

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
TM: ~50	8	84 ± 2	13 ± 2[a]	4.0 ± 0.4	3.7 ± 0.4	TD: 35 ± 15[b]	TD: 1.4 ± 0.5 ng/mL[b]
			↓ Aged		↑ Aged, Prem	TM: 0.4 (0.3–6)[b]	TM: 0.8 ± 0.3 ng/mL[b]
			↑ Neo				

[a]Metabolically cleared primarily by CYP3A to norfentanyl and hydroxy metabolites. [b]Following a 5-mg transdermal (TD) dose administered at 50 μg/h through a DURAGESIC system or a single 400-μg ransmucosal (TM) dose. Postoperative and intraoperative analgesia occurs at plasma concentrations of 1 ng/mL and 3 ng/mL, respectively. Respiratory depression occurs at >0.7 ng/mL.

References: Olkkola KT, et al. Clinical pharmacokinetics and pharmacodynamics of opioid analgesics in infants and children. *Clin Pharmacokinet,* **1995**, *28*:385–404. *PDR*54, **2000**, pp. 405, 1445.

Fexofenadine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—[b]	12[c]	60–70	9.4 ± 4.2[d,e]	—	14 ± 6[e]	1.3 ± 0.6[g]	286 ± 143 ng/mL[g]
					↑ RD[f]		

[a]Data from healthy adult male subjects. [b]Absolute bioavailability is unknown. [c]Urine recovery of unchanged drug following an oral dose. [d]Negligible metabolism with 85% of a dose recovered in feces unchanged; a substrate for hepatic and intestinal uptake and efflux transporters. [e]*CL/F* and $t_{1/2}$ reported for oral dose. [f]Mild renal impairment. [g]Following a 60-mg oral dose twice a day to steady state.

References: Markham A, et al. Fexofenadine. *Drugs,* **1998**, *55*:269–274; discussion 275–276. Robbins OK, et al. Dose proportionality and comparison of single and multiple dose pharmacokinetics of fexofenadine (MDL 16455) and its enantiomers in healthy male volunteers. *Biopharm Drug Dispos,* **1998**, *19*:455–463.

Finasteride

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
63 ± 21	<1	90	2.3 ± 0.8	1.1 ± 0.2	7.9 ± 2.5	1–2[a]	37 (27–49) ng/mL[a]

[a]Following a single 5-mg oral dose given to healthy adults. Drug accumulates 2-fold with once-daily dosing.

Reference: Sudduth SL, et al. Finasteride: The first 5α-reductase inhibitor. *Pharmacotherapy,* **1993**, *13*:309–325; discussion 325–329.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Flecainide[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
70 ± 11	43 ± 3	61 ± 10	5.6 ± 1.3[b]	4.9 ± 0.4[c]	11 ± 3[b]	~3 (1–6)[d]	458 ± 100 ng/mL[d]
			↓ RD, LD,	↑ LD	↑ RD, LD,		
			↑ Child		↓ Child		

[a] Racemic mixture; enantiomers exert similar electrophysiological effects. [b] Metabolized by CYP2D6 (polymorphic); except for a shortened elimination $t_{1/2}$ and nonlinear kinetics in extensive metabolizers, CYP2D6 phenotype had no significant influence on flecainide pharmacokinetics or pharmacodynamics. [c] V_{area} reported. [d] Following a 100-mg oral dose given twice daily for 5 days in healthy adults. Similar levels for CYP2D6 extensive and poor metabolizers.

Reference: Funck-Brentano C, et al. Variable disposition kinetics and electrocardiographic effects of flecainide during repeated dosing in humans: contribution of genetic factors, dose-dependent clearance, and interaction with amiodarone. *Clin Pharmacol Ther,* **1994**, *55*:256–269.

Fluconazole

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
>90	75 ± 9	11 ± 1	0.27 ± 0.07	0.60 ± 0.11	32 ± 5	1.7–4.3[a]	10.6 ± 0.4 μg/mL[a]
			↓ RD, Prem	↑ Prem, Neo	↑ LD, RD, Prem		
					↓ Child		

[a] Following a 200-mg oral dose given twice a day for 4 days to healthy adults.

References: Debruyne D, et al. Clinical pharmacokinetics of fluconazole. *Clin Pharmacokinet,* **1993**, *24*:10–27. Varhe A, et al. Effect of fluconazole dose on the extent of fluconazole-triazolam interaction. *Br J Clin Pharmacol,* **1996**, *42*:465–470.

Fludarabine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—	24 ± 3	—	3.7 ± 1.5	2.4 ± 0.6	10–30	—	0.57 μg/mL[b]
			↓ RD				

[a] Data from adult male and female cancer patients following IV administration. Fludarabine is rapidly dephosphorylated to 2-fluoro-arabinoside-A (F-ara-A), transported into cells, and phosphorylated to the active triphosphate metabolite. Pharmacokinetics of F-ara-A are reported. [b] Following a single 25-mg/m² IV dose of fludarabine (30-min infusion); no accumulation after five daily doses.

References: Hersh MR, et al. Pharmacokinetic study of fludarabine phosphate (NSC 312887). *Cancer Chemother Pharmacol,* **1986**, *17*:277–280. *PDR*54, **2000**, p. 764. Plunkett W, et al. Fludarabine: pharmacokinetics, mechanisms of action, and rationales for combination therapies. *Semin Oncol,* **1993**, *20*:2–12.

5-Fluorouracil (5-FU)

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
28 (0–80)[a]	<10	8–12	16 ± 7	0.25 ± 0.12	11 ± 4 min[b]	—	11.2 μM[c]

[a] Higher *F* with rapid absorption and lower *F* with slower absorption, due to a saturable first-pass effect. [b] A much longer (~20 h) terminal $t_{1/2}$ has been reported, representing a slow redistribution of drug from tissues. [c] Steady-state concentration following a continuous IV infusion of 300–500 mg/m²/day to cancer patients.

Reference: Diasio RB, et al. Clinical pharmacology of 5-fluorouracil. *Clin Pharmacokinet,* **1989**, *16*:215–237.

Fluoxetine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—[a]	<2.5	94	9.6 ± 6.9[b,c]	35 ± 21[d]	53 ± 41[e]	F: 6–8[f]	F: 200–531 ng/mL[f]
			↓ LD		↑ LD		NF: 103–465 ng/mL[f]

[a] Active metabolite, norfluoxetine; $t_{1/2}$ of norfluoxetine is 6.4 ± 2.5 days (12 ± 2 days in cirrhosis). Absolute bioavailability is unknown, but ≥80% of the dose is absorbed. [b] Reduced *CL* with repetitive dosing (~2.6 mL/min/kg) and with increasing dose between 40 and 80 mg. [c] *CL/F* reported; fluoxetine is a CYP2D6 and CYP2C19 substrate and inhibitor, but steady-state elimination is not dependent on these enzymes because of autoinhibition. [d] V_{area}/F reported. [e] Longer $t_{1/2}$ with repetitive dosing and with increasing doses. [f] Range of data for fluoxetine (F) and norfluoxetine (NF) following a 60-mg oral dose given once daily for 1 week. NF continues to accumulate for several weeks.

Reference: Altamura AC, et al. Clinical pharmacokinetics of fluoxetine. *Clin Pharmacokinet,* **1994**, *26*:201–214.

Fluphenazine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
PO: 2.7 (1.7–4.5)[b]	Negligible	—	10 ± 7[c]	11 ± 10	IV: 12 ± 4[d]	IR: 2.8 ± 2.1[d]	IR: 2.3 ± 2.1 ng/mL[e]
SC or IM: 3.4 (2.5–5.0)[b]					IR: 14.4 ± 7.8[d]	DN: 24–48[e]	DN: 1.3 ng/mL[e]
					SR: 20.3 ± 7.9[d]	EN: 48–72[e]	EN: 1.1 ng/mL[e]

[a] Data from healthy male and female volunteers. [b] Available in immediate-release (IR) oral and IM formulations and depot SC or IM injections as the enanthate (EN) or decanoate (DN) esters. Geometric mean (90% confidence interval). [c] Fluphenazine is extensively metabolized. [d] Reported $t_{1/2}$ for a single IV dose and apparent $t_{1/2}$ following oral administration of IR and slow-release (SR) formulations. Longer apparent $t_{1/2}$s with oral dosing reflect an absorption-limited elimination. [e] Following a single 12-mg oral dose (IR) or 5-mg IM injections of DN and EN.

References: Jann MW, et al. Clinical pharmacokinetics of the depot antipsychotics. *Clin Pharmacokinet,* **1985**, *10*:315–333. Koytchev R, et al. Absolute bioavailability of oral immediate and slow release fluphenazine in healthy volunteers. *Eur J Clin Pharmacol,* **1996**, *51*:183–187.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Foscarnet							
9 ± 2	95 ± 5	14–17	1.6 ± 0.2	0.35	5.7 ± 0.2	1.4 ± 0.6[b]	86 ± 36 µM[b]
			↓ RD[a]		↑ RD[a]		

[a]In patients with moderate to severe renal impairment. [b]Following an 8-mg/kg oral dose given once daily for 8 days to HIV-seropositive patients.

References: Aweeka FT, et al. Effect of renal disease and hemodialysis on foscarnet pharmacokinetics and dosing recommendations. *J Acquir Immune Defic Syndr Hum Retrovirol,* **1999**, *20*:350–357. Noormohamed FH, et al. Pharmacokinetics and absolute bioavailability of oral foscarnet in human immunodeficiency virus-seropositive patients. *Antimicrob Agents Chemother,* **1998**, *42*:293–297.

Furosemide[a]							
71 ± 35 (43–73)	71 ± 10 (50–80)	98.6 ± 0.4 (96–99)	1.66 ± 0.58 (1.5–3.0)	0.13 ± 0.06 (0.09–0.17)	1.3 ± 0.8 (0.5–2.0)	1.4 ± 0.8[c]	1.7 ± 0.9 µg/mL[c]
		↓ RD, LD	↓ RD,[b] Neo, Prem	↑ Neo, Prem, LD	↑ RD,[b] Prem, Neo, LD		

[a]Data from healthy adult male subjects. No significant sex differences described. Range of mean values from multiple studies shown in parentheses. [b]The renal clearance of furosemide is mediated by renal OATs and MRPs. *CL/F* reduced with declining renal function. [c]Following a single 40-mg oral dose (tablet). Ototoxicity occurs at concentrations >25 µg/mL.

References: Andreasen F, et al. The pharmacokinetics of frusemide are influenced by age. *Br J Clin Pharmacol,* **1983**, *16*:391–397. Ponto LL, et al. Furosemide (frusemide). A pharmacokinetic/pharmacodynamic review (part I). *Clin Pharmacokinet,* **1990**, *18*:381–408. Waller ES, et al. Disposition and absolute bioavailability of furosemide in healthy males. *J Pharm Sci,* **1982**, *71*:1105–1108.

Gabapentin							
60[a]	64–68	<3	1.6 ± 0.3	0.80 ± 0.09	6.5 ± 1.0	2–3[b]	4 µg/mL[b]
			↓ Aged, RD		↑ RD		

[a]Decreases with increasing dose. Value for 300- to 600-mg dose reported. [b]Following an 800-mg oral dose given three times daily to steady state in healthy adults. Efficacious at concentrations >2 µg/mL.

References: Bialer M. Comparative pharmacokinetics of the newer antiepileptic drugs. *Clin Pharmacokinet,* **1993**, *24*:441–452. McLean MJ. Gabapentin. In: Wyllie E, ed. *The Treatment of Epilepsy: Principles and Practice,* 2nd ed. Williams & Wilkins, Baltimore, **1997**, pp. 884–898.

Galantamine[a]							
100 (91–110)	20 (18–22)	18	5.7 (5.0–6.3)[b]	2.6 (2.4–2.9)	5.7 (5.2–6.3)	2.6 ± 1.0[d]	96 ± 29 ng/mL[d]
			↓ RD,[c] LD[c]				

[a]Primarily metabolized by CYP2D6, CYP3A4, and glucuronidation. [b]CYP2D6 poor metabolizers show a lower *CL,* but dose adjustment is not required. [c]In patients with mild to moderate hepatic or renal insufficiency. [d] Following a 12-mg oral dose given twice daily for 7 days in healthy, elderly adults.

References: Bickel U, et al. Pharmacokinetics of galanthamine in humans and corresponding cholinesterase inhibition. *Clin Pharmacol Ther,* **1991**, *50*:420–428. Huang F, et al. Pharmacokinetic and safety assessments of galantamine and risperidone after the two drugs are administered alone and together. *J Clin Pharmacol,* **2002**, *42*:1341–1351. Scott LJ, et al. Galantamine: a review of its use in Alzheimer's disease. *Drugs,* **2000**, *60*:1095–1122.

Ganciclovir							
3–5	91 ± 5	1–2	3.4 ± 0.5	1.1 ± 0.2	3.7 ± 0.6	PO: 3.0 ± 0.6[a]	IV: 6.6 ± 1.8 µg/mL[a]
↑ Food			↓ RD		↑ RD		PO: 1.2 ± 0.4 µg/mL[a]

[a]Following a single 6-mg/kg IV dose (1-h infusion) or a 1000-mg oral dose given with food three times a day to steady state.

References: Aweeka FT, et al. Foscarnet and ganciclovir pharmacokinetics during concomitant or alternating maintenance therapy for AIDS-related cytomegalovirus retinitis. *Clin Pharmacol Ther,* **1995**, *57*:403–412. *PDR*54, **2000**, p. 2624.

Gemcitabine[a]							
—	<10	Negligible	37.8 ± 19.4[b]	1.4 ± 1.3[c]	0.63 ± 0.48[c]	—	26.9 ± 9 µM[d]
			↓ Aged		↑ Aged		

[a]Data from patients with leukemia. Rapidly metabolized intracellularly to active di- and triphosphate products; IV administration. [b]Weight-normalized *CL* is ~25% lower in women compared to men. [c]V_d and $t_{1/2}$ are reported to increase with long duration of IV infusion. [d]Steady-state concentration during a 10-mg/m^2/min infusion for 120–640 min.

References: Grunewald R, et al. Gemcitabine in leukemia: a phase I clinical, plasma, and cellular pharmacology study. *J Clin Oncol,* **1992**, *10*:406–413. *PDR*54, **2000**, p. 1586.

Gemfibrozil							
98 ± 1	<1	97	1.7 ± 0.4[a]	0.14 ± 0.03	1.1 ± 0.2	1–2[a]	15–25 µg/mL[a]

[a]Gemfibrozil is mainly cleared by UGT2B7-dependant glucuronidation. Both gemfibrozil and gemfibrozil glucuronide inhibit CYP2C8 and OATP1B1 and OATP1B3. [b]Following a 600-mg oral dose given twice daily to steady state.

Reference: Todd PA, et al. Gemfibrozil. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in dyslipidaemia. *Drugs,* **1988**, *36*:314–339.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Gentamicin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
IM: ~100	>90	<10	0.62 ± 0.16	0.31 ± 0.10	2–3[b]	IV: 1[b]	IV: 4.9 ± 0.5 μg/mL[c]
			↓ RD[a]	↑ Neo		IM: 0.3–0.75[b]	IM: 5.0 ± 0.4 μg/mL[c]

[a]Gentamicin clearance declines in proportion to a decline in CL_{CR}. [b]Gentamicin has a very long terminal $t_{1/2}$ of 53 ± 25 h (slow release from tissues), which accounts for urinary excretion for up to 3 weeks after a dose. Majority of the dose is eliminated during the distribution phase. [c]Following a single 100-mg IV infusion (1 h) or IM injection given to healthy adults.

References: Schentag JJ, et al. Gentamicin disposition and tissue accumulation on multiple dosing. *J Pharmacokinet Biopharm,* **1977**, *6*:559–577. Regamey C, et al. Comparative pharmacokinetics of tobramycin and gentamicin. *Clin Pharmacol Ther,* **1973**, *14*:396–403.

Glimepiride[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
~100	<0.5	>99.5	0.62 ± 0.26	0.18	3.4 ± 2.0	2–3[c]	359 ± 98 ng/mL[c]
			↑ RD[b]	↑ RD[b]			

[a]Data from healthy male subjects. No significant sex differences. Glimepiride is metabolized by CYP2C9 to an active (approximately one-third potency) metabolite, Ml. [b]CL/F and V_d/F increased and $t_{1/2}$ unchanged, moderate to severe renal impairment; presumably mediated through an increase in plasma-free fraction. Ml AUC also increased. [c]Following a single 3-mg oral dose.

References: Badian M, et al. Determination of the absolute bioavailability of glimepiride (HOE 490), a new sulphonylurea. *Int J Clin Pharmacol Ther Toxicol,* **1992**, *30*:481–482. *PDR*54, **2000**, pp. 1346–1349. Rosenkranz B, et al. Pharmacokinetics and safety of glimepiride at clinically effective doses in diabetic patients with renal impairment. *Diabetologia,* **1996**, *39*:1617–1624.

Glipizide

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
95	<5	98.4	0.52 ± 0.18[a]	0.17 ± 0.02[a]	3.4 ± 0.7	2.1 ± 0.9[b]	465 ± 139 ng/mL[b]

[a]CL/F and V_{ss}/F reported. [b]Following a single 5-mg oral dose (immediate-release tablet) given to healthy young adults. An extended-release formulation exhibits a delayed T_{max} of 6–12 h.

Reference: Kobayashi KA, et al. Glipizide pharmacokinetics in young and elderly volunteers. *Clin Pharm,* **1988**, *7*:224–228.

Glyburide

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
G: 90–100[a]	Negligible	99.8	1.3 ± 0.5	0.20 ± 0.11	4 ± 1[b]	G: ~1.5[c]	G: 106 ng/mL[c]
M: 64–90[a]		↓ Aged	↓ LD		↑ LD	M: 2-4[c]	M: 104 ng/mL[c]

[a]Data for GLYNASE PRESTAB micronized tablet (G) and MICRONASE tablet (M). [b]$t_{1/2}$ for micronized formulation reported. $t_{1/2}$ for nonmicronized formulation is 6–10 h, reflecting absorption rate limitation. A long terminal $t_{1/2}$ (15 h), reflecting redistribution from tissues, has been reported. Longer $t_{1/2}$ in patients with type 2 diabetes mellitus also reported. [c]Following a 3-mg oral GLYNASE tablet taken with breakfast or a 5-mg oral MICRONASE tablet given to healthy adult subjects.

References: Jonsson A, et al. Slow elimination of glyburide in NIDDM subjects. *Diabetes Care,* **1994**, *17*:142–145. *PDR54,* **2000**, p. 2457.

Haloperidol[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
60 ± 18	1	92 ± 2	11.8 ± 2.9[b]	18 ± 7	18 ± 5[b]	IM: 0.6 ± 0.1[c]	IM: 22 ± 18 ng/mL[c]
		↑ LD	↑ Child, Smk		↓ Child	PO: 1.7 ± 3.2[c]	PO: 9.2 ± 4.4 ng/mL[c]
			↓ Aged				

[a]Undergoes reversible metabolism to a less active reduced haloperidol. [b]Represents net CL of parent drug; reduced haloperidol CL = 10 ± 5 mL·min^{-1}·kg^{-1} and $t_{1/2}$ = 67 ± 51 h. Slow conversion from reduced haloperidol to parent compound probably responsible for prolonged terminal $t_{1/2}$ (70 h) for haloperidol observed with 7-day sampling. [c]Following a single 20-mg oral or 10-mg IM dose. Effective concentrations are 4–20 ng/mL.

Reference: Froemming JS, et al. Pharmacokinetics of haloperidol. *Clin Pharmacokinet*, **1989**, *17*:396–423.

Hydrochlorothiazide

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
71 ± 15	>95	58 ± 17	4.9 ± 1.1[a]	0.83 ± 0.31[b]	2.5 ± 0.2[c]	SD: 1.9 ± 0.5[d]	SD: 75 ± 17 ng/mL[d]
			↓ RD, Aged	↓ Aged	↑ RD, Aged	MD: 2[d]	MD: 91 ± 0.2 ng/mL[d]

[a]Renal CL reported, which should approximate total plasma CL. [b]V_{area} calculated from individual values of renal CL, terminal $t_{1/2}$, and fraction of drug excreted unchanged; 70-kg body weight assumed. [c]Longer terminal $t_{1/2}$ of 8 ± 2.8 h has been reported with a corresponding increase in V_{area} to 2.8 L/kg. [d]Following a single (SD) or multiple (MD) 12.5-mg oral dose of hydrochlorothiazide; MD given once daily for 5 days to healthy adults.

References: Beermann B, et al. Pharmacokinetics of hydrochlorothiazide in man. *Eur J Clin Pharmacol,* **1977**, *12*:297–303. Jordo L, et al. Bioavailability and disposition of metoprolol and hydrochlorothiazide combined in one tablet and of separate doses of hydrochlorothiazide. *Br J Clin Pharmacol,* **1979**, *7*:563–567. O'Grady P, et al. Fosinopril/hydrochlorothiazide: single dose and steady-state pharmacokinetics and pharmacodynamics. *Br J Clin Pharmacol,* **1999**, *48*:375–381.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Hydrocodone[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—	EM: 10.2 ± 1.8	—	EM: 11.1 ± 3.57[b]	—	EM: 4.24 ± 0.99[b]	EM: 0.72 ± 0.46[c]	EM: 30 ± 9.4 ng/mL[c]
	PM: 18.1 ± 4.5		PM: 6.54 ± 1.25[b]		PM: 6.16 ± 1.97[b]	PM: 0.93 ± 0.59[c]	PM: 27 ± 5.9 ng/mL[c]

[a] Data from healthy male and female subjects. The metabolism of hydrocodone to hydromorphone (more active) is catalyzed by CYP2D6. Subjects were phenotyped as extensive metabolizers (EM) and poor metabolizers (PM). [b] *CL/F* and $t_{1/2}$ reported for oral dose. [c] Following a 10-mg oral dose (syrup). Maximal hydromorphone concentrations are higher in EM than in PM (5.2 vs. 1.0 ng/mL). Hydromorphone is metabolized extensively to principal metabolite 3-glucuronie, which accumulates to much higher (27-fold) levels that hydromorphone. Hydromorphone half-life is 2.4 ± 0.6 h, *CL* 14.6 ± 7.6 mL/min/kg, and V_{area} 2.90 ± 1.31 L/kg.

References: Hagen N, et al. Steady-state pharmacokinetics of hydromorphone and hydromorphone-3-glucuronide in cancer patients after immediate and controlled-release hydromorphone. *J Clin Pharmacol,* **1995**, *35*:37–44. Otton SV, et al. CYP2D6 phenotype determines the metabolic conversion of hydrocodone to hydromorphone. *Clin Pharmacol Ther,* **1993**, *54*:463–472. Parab PV, et al. Pharmacokinetics of hydromorphone after intravenous, peroral and rectal administration to human subjects. *Biopharm Drug Dispos,* **1988**, *9*:187–199.

Hydroxychloroquine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
79 ± 12	27	45 ± 3	11.9 ± 5.4[b]	525 ± 158	1056 (624–1512)	3.2 (2–4.5)	46 ng/mL (34–79 ng/mL)[c]

[a] Hydroxychloroquine is marketed as a racemic mixture of *R*- and *S*-hydroxychloroquine. Data for the racemic mixture are reported. [b] Plasma clearance is reported. Hydroxychloroquine accumulates in red blood cells with an average blood-to-plasma ratio of 7.2. Blood clearance of hydroxychloroquine is 1.3 mL/min/kg. [c] Following oral administration of a single 155-mg tablet.

References: Tett SE, et al. A dose-ranging study of the pharmacokinetics of hydroxychloroquine following intravenous administration to healthy volunteers. *Br J Clin Pharmacol,* **1988**, *26*:303–313. Tett SE, et al. Bioavailability of hydroxychloroquine tablets in healthy volunteers. *Br J Clin Pharmacol,* **1989**, *27*:771–779.

Hydroxyurea[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
108 ± 18	35.8 ± 14.2	Negligible	72 ± 17 mL/min/m^2[b]	19.7 ± 4.6 L/m^2	3.4 ± 0.7	IV: 0.5[c]	IV: 1007 ± 371 μM[c]
(79–108)			(36.2–72.3)		(2.8–4.5)	PO: 1.2 ± 1.2[c]	PO: 794 ± 241 μM[c]

[a] Data from male and female patients treated for solid tumors. A range of mean values from multiple studies is shown in parentheses. [b] Nonrenal elimination of hydroxyurea is thought to exhibit saturable kinetics through a 10- to 80-mg/kg dose range. [c] Following a single 2-g, 30-min IV infusion or oral dose.

References: Gwilt PR, et al. Pharmacokinetics and pharmacodynamics of hydroxyurea. *Clin Pharmacokinet,* **1998**, *34*:347–358. Rodriguez GI, et al. A bioavailability and pharmacokinetic study of oral and intravenous hydroxyurea. *Blood,* **1998**, *91*:1533–1541.

Hydroxyzine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—	—	—	A: 9.8 ± 3.3[b]	A: 16 ± 3[b]	A: 20 ± 4[b]	A: 2.1 ± 0.4[d]	A: 72 ± 11 ng/mL[d]
			C: 32 ± 11[b]	C: 19 ± 9[b]	C: 7.1 ± 2.3[b,c]	C: 2.0 ± 0.9[d]	C: 47 ± 17 ng/mL[d]
				↑ Aged	↑ Aged, LD		

[a] Hydroxyzine is metabolized to an active metabolite, cetirizine. Plasma concentrations of cetirizine exceed those of the parent drug; its $t_{1/2}$ is similar to that of hydroxyzine when formed from parent drug. Hydroxyzine data for adults (A) and children (C) are reported. [b] *CL/F*, V_d/F, and $t_{1/2}$ after oral dose reported. [c] $t_{1/2}$ increases with increasing age (1–15 years of age). [d] Following a single 0.7-mg/kg oral dose given to healthy adults and children.

References: Paton DM, et al. Clinical pharmacokinetics of H_1-receptor antagonists (the antihistamines). *Clin Pharmacokinet,* **1985**, *10*:477–497. Simons FE, et al. Pharmacokinetics and antipruritic effects of hydroxyzine in children with atopic dermatitis. *J Pediatr,* **1984**, *104*:123–127. Simons FE, et al. The pharmacokinetics and antihistaminic of the HI receptor antagonist hydroxyzine. *J Allergy Clin Immunol,* **1984**, *73*(pt 1):69–75. Simons FE, et al. The pharmacokinetics and pharmacodynamics of hydroxyzine in patients with primary biliary cirrhosis. *J Clin Pharmacol,* **1989**, *29*:809–815. Simons KJ, et al. Pharmacokinetic and pharmacodynamic studies of the H_1-receptor antagonist hydroxyzine in the elderly. *Clin Pharmacol Ther,* **1989**, *45*:9–14.

Ibandronate

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
0.63	54 ± 13	85	1.8 ± 0.1[a]	5.8 ± 1.5	37 ± 5	1	11 ± 4 ng/mL[c]
			↓ RD[b]				

[a] Cleared primarily by the kidney. [b] Exposure increases 50%–100% in patients with moderate and severe renal impairment. [c] Following a single 50-mg oral dose.

References: Barrett J, et al. Ibandronate: a clinical pharmacological and pharmacokinetic update. *J Clin Pharmacol,* **2004**, *44*:951–965. Bergner R, et al. Renal safety and pharmacokinetics of ibandronate in multiple myeloma patients with or without impaired renal function. *J Clin Pharmacol,* **2007**, *47*:942–950.

Ibuprofen[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
>80	<1	>99[b]	0.75 ± 0.20[b,c]	0.15 ± 0.02[c]	2 ± 0.5[b]	1.6 ± 0.3[d]	61.1 ± 5.5 μg/mL[d]
					↑ LD		

[a] Racemic mixture. Kinetic parameters for the active *S*-(+)-enantiomer do not differ from those for the inactive *R*-(–)-enantiomer when administered separately; 63 ± 6% of the *R*-(–)-enantiomer undergoes inversion to the active isomer. [b] Unbound percent of *S*-(+)-ibuprofen (0.77% ± 0.20%) is significantly greater than that of *R*-(–)-ibuprofen (0.45% ± 0.06%). Binding of each enantiomer is concentration dependent and is influenced by the presence of the optical antipode, leading to nonlinear elimination kinetics. [c] *CL/F* and V_{ss}/F reported. [d] Following a single 800-mg dose of racemate. A level of 10 μg/mL provides antipyresis in febrile children.

References: Lee EJ, et al. Stereoselective disposition of ibuprofen enantiomers in man. *Br J Clin Pharmacol,* **1985**, *19*: 669–674. Lockwood GF, et al. Pharmacokinetics of ibuprofen in man. I. Free and total area/dose relationships. *Clin Pharmacol Ther,* **1983**, *34*:97–103.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Ifosfamide[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
92	Low: 12–18	Negligible	Low: 63 mL/min/m^2[b]	Low: —	Low: 5.6	Oral: 0.5–1.0[c]	IV: 203 (168–232) μM[c] Oral: 200 (163–245) μM[c]
	High: 53.1± 9.6		High: 6.6 ± 1.9 mL/min/m^2	High: 12.5 ± 3.6 L/m^2	High: 15.2 ± 3.6		
				↑ Aged	↑ Aged		

[a]Data from male and female patients treated for advanced cancers. Administered IV with mesna to avoid hemorrhagic cystitis. Exhibits dose-dependent kinetics, with apparent saturation of hepatic metabolism. Parameters reported for a 1.5-g/m^2 (low) or 5-g/m^2 (high) IV dose and 1.5-g/m^2 oral dose. [b]*CL* reported to increase with daily dosing. [c]Geometric mean (range) following a single 1.5-g/m^2 IV infusion (30–60 min).

References: Allen LM, Creaven PJ. Pharmacokinetics of ifosfamide. *Clin Pharmacol Ther*, **1975**, *17*:492–498. Kurowski V, et al. Metabolism and pharmacokinetics of oral and intravenous ifosfamide. *J Cancer Res Clin Oncol*, **1991**, *117*:S148–S153. Lind MJ, et al. The effect of age on the pharmacokinetics of ifosfamide. *Br J Clin Pharmacol*, **1990**, *30*:140–143. *PDR*54, **2000**, pp. 866–867.

Imatinib

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
98 (87–111)	5	95	3.3 ± 1.2[a]	6.2 ± 2.2	22 ± 4	3.3 ± 1.1[b]	2.6 ± 0.8 μg/mL[b]

[a]Imatinib is metabolically cleared primarily by CYP3A4. [b]Following a 400-mg oral dose given once daily to steady state.

References: Peng B, et al. Absolute bioavailability of imatinib (Glivec) orally versus intravenous infusion. *J Clin Pharmacol,* **2004**, *44*:158–162. Peng B, et al. Pharmacokinetics and pharmacodynamics of imatinib in a phase I trial with chronic myeloid leukemia patients. *J Clin Oncol,* **2004**, *22*:935–942. Product information: Gleevec (imatinib mesylate). Basel, Switzerland, Novartis, **2004**.

Imipenem/Cilastatin[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Imipenem	69 ± 15	<20	2.9 ± 0.3	0.23 ± 0.05	0.9 ± 0.1	IM: 1–2[b]	IV: 60–70 μg/mL[b]
—	↓ Neo		↑ Child	↑ Neo, Child, Prem			IM: 8.2–12 μg/mL[b]
			↓ RD				
Cilastatin	70 ± 3	~35	3.0 ± 0.3	0.20 ± 0.03	0.8 ± 0.1		
—	↓ Neo		↑ Child				
			↓ Neo, RD, Prem	↑ Prem	↑ Neo, Prem		

[a]Formulated as a 1:1 (mg/mg) mixture for parenteral administration; cilastatin inhibits the metabolism of imipenem by the kidney, increasing concentrations of imipenem in the urine; cilastatin does not change imipenem plasma concentrations appreciably. [b]Plasma C_{max} of imipenem following a single 1-g IV infusion over 30 min or 750-mg IM injection.

Reference: Buckley MM, et al. Imipenem/cilastatin. A reappraisal of its antibacterial activity, pharmacokinetic properties and therapeutic efficacy. *Drugs,* **1992**, *44*:408–444.

Indomethacin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
~100	15 ± 8	90	1.4 ± 0.2[a]	0.29 ± 0.04	2.4 ± 0.2[a]	~1.3[b]	~2.4 μg/mL[b]
			↓ Prem, Neo, Aged		↑ Neo, Prem, Aged		

[a]Undergoes significant enterohepatic recycling (~50% after an IV dose). [b]Following a single 50-mg oral dose given after a standard breakfast. Effective at concentrations of 0.3–3 μg/mL and toxic at >5 μg/mL.

Reference: Oberbauer R, et al. Pharmacokinetics of indomethacin in the elderly. *Clin Pharmacokinet,* **1993**, *24*:428–434.

Interferon Alfa[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
I-SC: 90	—[b]	—	I: 2.8 ± 0.6[c]	I: 0.40 ± 0.19[c]	I: 0.67[d]	I: 7.3[e]	I: 1.7 (1.2–2.3) ng/mL[e]
			PI$_{12kD}$: 0.17	PI$_{12kD}$: 0.44–1.04	PI$_{12kD}$: 37 (22–60)	PI$_{12kD}$: 22[f]	
			PI$_{40kD}$: 0.014–0.024	PI$_{40kD}$: 0.11–0.17	PI$_{40kD}$: 65	PI$_{40kD}$: 80[g]	PI$_{12kD}$: 0.91 ± 0.33 ng/mL[f]
							PI$_{40kD}$: 26 ± 8.8 ng/mL[g]

[a]Values for recombinant interferon alfa-2a (I) and its 40-kDa pegylated form (PI$_{40kD}$) and the 12-kDa pegylated form of interferon alfa-2b (PI$_{12kD}$) are reported. [b]I undergoes renal filtration, tubular reabsorption, and proteolytic degradation within tubular epithelial cells. Renal elimination of PI forms is much less significant than that of I, although not negligible. [c]*CL* values in four patients with leukemia were more than halved (1.1 ± 0.3 mL/min/kg), while V_{ss} increased more than 20-fold (9.5 ± 3.5 L/kg) and terminal $t_{1/2}$ changed only minimally (7.3 ± 2.4 h). [d]A terminal $t_{1/2}$ of 5.1 ± 1.6 h accounts for 23% of the *CL* of I. [e]Following a single 36 × 10^6 units SC dose of I. [f]Following 4 weeks of multiple SC dosing of 1 μg of PI$_{12kD}$. [g]Following 48 weekly SC doses of 180 μg/kg of PI$_{40kD}$.

References: Glue P, et al. Pegylated interferon-2b: pharmacokinetics, pharmacodynamics, safety, and preliminary efficacy data. Hepatitis C Intervention Therapy Group. *Clin Pharmacol Ther,* **2000**, *68*:556–567. Harris JM, et al. Pegylation: a novel process for modifying pharmacokinetics. *Clin Pharmacokinet,* **2001**, *40*:539–551. *PDR*54, **2000**, p. 2654. Wills RJ. Clinical pharmacokinetics of interferons. *Clin Pharmacokinet,* **1990**, *19*:390–399.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Interferon Beta

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
SC: 51 ± 17	—[a]	—	13 ± 5[a]	2.9 ± 1.8	4.3 ± 2.3	SC: 1–8[b]	IV: 1491 ± 659 IU/mL
							[b] SC: 40 ± 20 IU/mL[b]

[a] Undergoes renal filtration, tubular reabsorption, and renal catabolism, but hepatic uptake and catabolism are thought to dominate systemic *CL*. [b] Concentration at 5 min following a single 90×10^6 IU IV dose or following a single 90×10^6 IU SC dose of recombinant interferon beta-1b.

Reference: Chiang J, et al. Pharmacokinetics of recombinant human interferon-β_{ser} in healthy volunteers and its effect on serum neopterin. *Pharm Res,* **1993**, *10*:567–572.

Irbesartan[a]

60–80	2.2 ± 0.9	90	2.12 ± 0.54[b]	0.72 ± 0.20	13 ± 6.2	1.2 (0.7–2)[d]	1.3 ± 0.4 μg/mL[d]
			↓ Aged[c]				

[a] Data from healthy male subjects. No significant sex differences. [b] Metabolically cleared by UGT and CYP2C9. [c] *CL/F* reduced; no dose adjustment required. [d] Following a single 50-mg oral dose (capsule).

References: Gillis JC, et al. Irbesartan. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in the management of hypertension. *Drugs,* **1997**, *54*:885–902. *PDR*54, **2000**, p. 818. Vachharajani NN, et al. Oral bioavailability and disposition characteristics of irbesartan, an angiotensin antagonist, in healthy volunteers. *J Clin Pharmacol,* **1998**, *38*:702–707.

Irinotecan[a]

—	I: 16.7 ± 1.0	I: 30–68	I: 14.8 ± 4 L/h/m^2	I: 150 ± 49 L/m^2	I: 10.8 ± 0.5	I: 0.5[b]	I: 1.7 ± 0.8 μg/mL[b]
		SN-38: 95			SN-38: 10.4 ± 3.1	SN-38: ≤1[b]	SN-38: 26 ± 12 ng/mL[b]

[a] Data from male and female patients with malignant solid tumors. No significant sex differences. Irinotecan (I) is metabolized to an active metabolite, SN-38 (100-fold more potent but with lower blood levels). [b] Following a 125-mg/m^2 IV infusion over 30 min.

References: Chabot GG, et al. Population pharmacokinetics and pharmacodynamics of irinotecan (CPT-11) and active metabolite SN-38 during phase I trials. *Ann Oncol,* **1995**, *6*:141–151. *PDR*54, **2000**, pp. 2412–2413.

Isoniazid[a]

—[b]	RA: 7 ± 2[c]	~0	RA: 7.4 ± 2.0[d]	0.67 ± 0.15[d]	RA: 1.1 ± 0.1	RA: 1.1 ± 0.5[f]	RA: 5.4 ± 2.0 μg/mL[f]
↓ Food	SA: 29 ± 5[c]		SA: 3.7 ± 1.1[d]		SA: 3.1 ± 1.1	SA: 1.1 ± 0.6[f]	SA: 7.1 ± 1.9 μg/mL[f]
			↓ RD[e]		↑ LD, Neo, RD		

[a] Metabolized by NAT2 (polymorphic). Data for slow acetylators (SA) and rapid acetylators (RA) reported. [b] It is usually stated that isoniazid is completely absorbed; however, good estimates of possible loss due to first-pass metabolism are not available. Absorption is decreased by food and antacids. [c] Recovery after oral administration; assay includes unchanged drug and acid-labile hydrazones. Higher percentages have been noted after IV administration, suggesting significant first-pass metabolism. [d] *CL/F* and V_{ss}/F reported. [e] Decrease in CL_{NR}/F as well as CL_R. [f] Following a single 400-mg oral dose to healthy RAs and SAs.

Reference: Kim YG, et al. Decreased acetylation of isoniazid in chronic renal failure. *Clin Pharmacol Ther,* **1993**, *54*:612–620.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Isosorbide Dinitrate[a]							
PO: 22 ± 14[b]	<1	28 ± 12	46 (38–59)[c]	3.1 (2.2–8.6)	0.7 (0.6–2.0)[c]	IR[d]	IR[d]
↑ LD							
SL: 45 ± 16[b]			↓ LD			ISDN: 0.3 (0.2–0.5)	ISDN: 42 (59–166) nM
PC: 33 ± 17[b]							
						IS-2-MN: 0.6 (0.2–1.6)	IS-2-MN: 207 (197–335) nM
						IS-5-MN: 0.7 (0.3–1.9)	IS-5-MN: 900 (790–1080) nM
						SR[d]	SR[d]
						ISDN: ~0	ISDN: ~0
						IS-2-MN: 2.8 (2.7–3.7)	IS-2-MN: 28 (23–33) nM
						IS-5-MN: 5.1 (4.2–6.6)	IS-5-MN: 175 (154–267) nM

[a] Isosorbide dinitrate (ISDN) is metabolized to the 2- and 5-mononitrates (IS-2-MN and IS-5-MN). Both metabolites and the parent compound are thought to be active. Data for the dinitrate are reported except where indicated. [b] Bioavailability calculations from single dose. SL, sublingual; PC, percutaneous. [c] *CL* may be decreased and $t_{1/2}$ prolonged after chronic dosing. [d] Mean (range) for ISDN and IS-2-MN and IS-5-MN following a single 20-mg oral immediate-release (IR) and sustained-release (SR) dose.

References: Abshagen U, et al. Pharmacokinetics and metabolism of isosorbide-dinitrate after intravenous and oral administration. *Eur J Clin Pharmacol*, **1985**, *27*:637–644. Fung HL. Pharmacokinetics and pharmacodynamics of organic nitrates. *Am J Cardiol*, **1987**, *60*:4H–9H.

Isosorbide 5-Mononitrate (Isosorbide Nitrate)[a]							
93 ± 13	<5	0	1.80 ± 0.24	0.73 ± 0.09	4.9 ± 0.8	1–1.5[b]	314–2093 nM[b]

[a] Active metabolite of isosorbide dinitrate. [b] Following a 20-mg oral dose given by asymmetric dosing (0 and 7 h) for 4 days.

Reference: Abshagen UW. Pharmacokinetics of isosorbide mononitrate. *Am J Cardiol*, **1992**, *70*:61G–66G.

Isotretinoin[a]							
40[b]	Negligible	>99	5.5 (0.9–11.1)[c]	5 (1–32)[c]	17 (5–167)[d]	I: 4.5 ± 3.4[e]	I: 208 ± 92 ng/mL[e]
↑ Food						4-oxo: 6.8 ± 6.5[e]	4-oxo: 473 ± 171 ng/mL[e]

[a] Isotretinoin (I) is eliminated through metabolic oxidations catalyzed by multiple CYPs (2C8, 2C9, 3A4, and 2B6). The 4-oxo-isotretinoin metabolite (4-oxo) is active and found at higher concentrations than parent drug at steady state. [b] Bioavailability when taken with food is reported. [c] *CL/F* and *V/F* reported. [d] 4-Oxo has an apparent mean $t_{1/2}$ of 29 ± 6 h. [e] Values for I and 4-oxo following a 30-mg oral dose given once daily to steady state.

References: Larsen FG, et al. Pharmacokinetics and therapeutic efficacy of retinoids in skin diseases. *Clin Pharmacokinet*, **1992**, *23*:42–61. Nulman I, et al. Steady-state pharmacokinetics of isotretinoin and its 4-oxo metabolite: implications for fetal safety. *J Clin Pharmacol*, **1998**, *38*:926–930. Wiegand UW, et al. Pharmacokinetics of oral isotretinoin. *J Am Acad Dermatol*, **1998**, *39*:S8–S12.

Itraconazole[a]							
55	<1	99.8	5.1[c]	10.7[d]	21 ± 6[e]	3–5[f]	649 ± 289 ng/mL[f]
↑ Food[b]							

[a] Metabolized predominantly by CYP3A4 to an active metabolite, hydroxyitraconazole, and other sequential metabolites. [b] Relative to oral dosing with food. [c] Blood CL is 9.4 mL/min/kg. *CL* is concentration dependent; the value given is nonsaturable range. $K_m = 330 \pm 200$ ng/mL, $V_{max} = 2.2 \pm 0.8$ pg · mL^{-1} · min^{-1} · kg^{-1}. Apparent *CL/F* at steady state reported to be 5.4 mL · min^{-1} · kg^{-1}. [d] V_{area} reported. Follows multicompartment kinetics. Does not appear to be concentration dependent. [e] $t_{1/2}$ for the nonsaturable concentration range. $t_{1/2}$ at steady state reported to be 64 h. [f] Following a 200-mg oral dose given daily for 4 days to adults.

References: Heykants J, et al. The pharmacokinetics of itraconazole in animals and man. An overview. In: Fromtling RA, ed. *Recent Trends in the Discovery, Development and Evaluation of Antifungal Agents.* Prous Science Publisher, Barcelona, **1987**, pp. 223–249. Jalava KM, et al. Itraconazole greatly increases plasma concentrations and effects of felodipine. *Clin Pharmacol Ther,* **1997**, *61*:410–415.

Ivermectin[a]							
—	<1	93.1 ± 0.2	2.06 ± 0.81[b,c]	9.91 ± 2.67[c]	56.5 ± 7.5[c]	4.7 ± 0.5[d]	38.2 ± 5.8 ng/mL[d]

[a] Data from male and female patients treated for onchocerciasis. [b] Metabolized by hepatic enzymes and excreted into bile. [c] *CL/F*, V_{area}/F, and $t_{1/2}$ reported for oral dose. Terminal $t_{1/2}$ reported. [d] Following a single 150-μg/kg oral dose (tablet).

References: Okonkwo PO, et al. Protein binding and ivermectin estimations in patients with onchocerciasis. *Clin Pharmacol Ther,* **1993**, *53*:426–430. *PDR54*, **2000**, p. 1886.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Labetalol[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
30–65[b]	—	50	23 ± 5.3	9.9 ± 1.5	8.3–1.4[c]	1.5–2[d]	274 ± 99 ng/mL[d]
↑ LD			↑ Preg				

[a]The clinical formulation of labetalol consists of equal proportions of four optical isomers. Labetalol is mainly cleared by glucuronidation [b]Bioavailability correlates with age and increases with age. Bioavailability appears to increase with multiple dosing. [c]Labetalol displays multicompartment distribution kinetics with an initial $t_{1/2}$ of 1.4 h. [d]Following a 200-mg dose of labetalol every 12 h for five doses.

References: Lalonde RL, et al. Labetalol pharmacokinetics and pharmacodynamics: evidence of stereoselective disposition. *Clin Pharmacol Ther,* **1990**, *48*:509–519. McNeil JJ, Louis WJ. Clinical pharmacokinetics of labetalol. *Clin Pharmacokinet,* **1984**, *9*:157–167.

Lamivudine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
86 ± 17	49–85	<36	4.95 ± 0.75	1.30 ± 0.36	9.11 ± 5.09	0.5–1.5[d]	1.0 (0.86–1.2) μg/mL[d]
			↓ RD[b]		↑ RD[b]		
			↑ Child[c]		↓ Child[c]		

[a]Data from healthy male subjects. No significant sex differences. [b]*CL/F* decreased, moderate and severe renal impairment. [c]Weight-normalized *CL/F* increased, children <12 years of age. [d]Following a single 100-mg oral dose (tablet).

References: Heald AE, et al. Pharmacokinetics of lamivudine in human immunodeficiency virus-infected patients with renal dysfunction. *Antimicrob. Agents Chemother*, **1996**, *40*:1514–1519. Johnson MA, et al. Clinical pharmacokinetics of lamivudine. *Clin Pharmacokinet*, **1999**, *36*:41–66. *PDR*54, **2000**, p. 1172. Yuen GJ, et al. Pharmacokinetics, absolute bioavailability, and absorption characteristics of lamivudine. *J Clin Pharmacol*, **1995**, *35*:1174–1180.

Lamotrigine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
97.6 ± 4.8	10	56	0.38–0.61[b,c,d]	0.87–1.2	24–35[c]	2.2 ± 1.2[g]	2.5 ± 0.4 μg/mL[g]
			↓ LD,[e] RD[f]		↑ LD,[e] RD[f]		

[a]Data from healthy adults and patients with epilepsy. Range of mean values from multiple studies reported. [b]Lamotrigine is eliminated primarily by glucuronidation. The parent-metabolite pair may undergo enterohepatic recycling. [c]*CL/F* increases slightly with multiple-dose therapy. [c]*CL/F* increased and $t_{1/2}$ decreased in patients receiving enzyme-inducing anticonvulsant drugs. [e]*CL/F* reduced, moderate to severe hepatic impairment. [f]*CL/F* reduced, severe RD. [g]Following a single 200-mg oral dose to healthy adults.

References: Chen C, et al. Pharmacokinetics of lamotrigine in children in the absence of other antiepileptic drugs. *Pharmacotherapy,* **1999**, *19*:437–441. Garnett WR. Lamotrigine: pharmacokinetics. *J Child Neurol,* **1997**, *12*(suppl 1):S10–S15. *PDR*54, **2000**, p. 1209. Wootton R, et al. Comparison of the pharmacokinetics of lamotrigine in patients with chronic renal failure and healthy volunteers. *Br J Clin Pharmacol,* **1997**, *43*:23–27.

Leflunomide[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—[b]	Negligible	99.4	0.012[c]	0.18 (0.09–0.44)[c]	377 (336–432)[d]	6–12[e]	35 μg/mL[e]
		↓ RD	↑ RD	↑ RD			

[a]Leflunomide is a prodrug that is converted almost completely (~95%) to an active metabolite, A77–1726 [2-cyano-3-hydroxy-*N*-(4-trifluoromethylphenyl)-crotonamide]. All pharmacokinetic data reported are for the active metabolite. [b]Absolute bioavailability is not known; parent drug/metabolite are well absorbed. [c]Apparent *CL/F* and *V/F* in healthy volunteers reported. Both parameters are a function of the bioavailability of leflunomide and the extent of its conversion to A77–1726. [d]In patients with rheumatoid arthritis (RA). [e]Following a 20-mg oral dose given once daily to steady state in patients with RA.

Reference: Rozman B. Clinical pharmacokinetics of leflunomide. *Clin Pharmacokinet,* **2002**, *41*:421–430.

Levetiracetam[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
~100	66	<10	0.96	0.5–0.7	7 ± 1	0.5–1.0[e]	~10 μg/mL[e]
			↓ RD,[b] Aged, LD[c]		↑ RD,[b] Aged		
			↑ Child[d]				

[a]Data from healthy adults and patients with epilepsy. No significant sex differences. [b]*CL/F* reduced, mild renal impairment (cleared by hemodialysis). [c]*CL/F* reduced, severe hepatic impairment. [d]*CL/F* increased, 6–12 years of age. [e]Following a single 500-mg dose given to healthy adults.

*Reference: PDR*55, **2001**, pp. 3206–3207.

Levodopa[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
41 ± 16	<1	—	23 ± 4	1.7 ± 0.4	1.4 ± 0.4		
↑ Aged			↓ Aged	↓ Aged			
86 ± 19[b]			9 ± 1[b]	0.9 ± 0.2[b]	1.5 ± 0.3[b]	Y: 1.4 ± 0.7[c]	Y: 1.7 ± 0.8 μg/mL[c]
			↓ Aged	↓ Aged		E: 1.4 ± 0.7[c]	E: 1.9 ± 0.6 μg/mL[c]

[a]Naturally occurring precursor to dopamine. [b]Values obtained with concomitant carbidopa (inhibitor of dopa decarboxylase). [c]Following a single 125-mg oral dose of levodopa given with carbidopa (100 mg 1 h before and 50 mg 6 h after levodopa) in young (Y) and elderly (E) subjects.

Reference: Robertson DR, et al. The effect of age on the pharmacokinetics of levodopa administered alone and in the presence of carbidopa. *Br J Clin Pharmacol*, **1989**, *28*:61–69.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Levofloxacin[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
99 ± 10	61–87	24–38	2.52 ± 0.45	1.36 ± 0.21	7 ± 1	1.6 ± 0.8[c]	4.5 ± 0.9 µg/mL[c]
			↓ RD[b]		↑ RD[b]		

[a]Data from healthy adult male subjects. Sex and age differences related to renal function. [b]*CL/F* reduced, mild to severe renal impairment (not cleared by hemodialysis). [c]Following a single 500-mg oral dose. No significant accumulation with once-daily dosing.

References: Chien SC, et al. Pharmacokinetic profile of levofloxacin following once-daily 500-milligram oral or intravenous doses. *Antimicrob Agents Chemother,* **1997**, *41*:2256–2260. Fish DN, et al. The clinical pharmacokinetics of levofloxacin. *Clin Pharmacokinet*, **1997**, *32*:101–119. *PDR*54, **2000**, p. 2157.

Lidocaine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
37 ± 9	2 ± 1	69 ± 2	13.5 ± 3.1	2.3 ± 0.6	2.6 ± 4	0.78 ± 0.4	9.8 ± 3.3 µmol/L[b]
			↓ LD		↑ LD		

[a]Lidocaine is cleared mainly by CYP1A2 and partially by CYP3A4-dependent metabolism. Lidocaine has an active metabolite, monoethylglycylxylidide (MEGX), which has 33%–83% of the antiarrhythmic activity of lidocaine, and it can also cause convulsions. [b]Following a 750-mg single oral dose given twice daily to steady state in cancer patients.

References: O'Byrne S, et al. Plasma protein binding of lidocaine and warfarin in insulin-dependent and non-insulin dependent diabetes mellitus. *Clin Pharmacokinet,* **1993**, *24*:183–186. Olkkola KT et al. The effect of erythromycin and fluvoxamine on the pharmacokinetics of intravenous lidocaine. *Anesth Analg,* **2005**, *100*:1352–1356. Perucca E, Richens A. Reduction of oral bioavailability of lignocaine by induction of first pass metabolism in epileptic patients. *Br J Clin Pharmacol,* **1979**, *8*:21–31.

Linezolid

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
100	35	31	2.1 ± 0.8	0.57–0.71	5.2 ± 1.7	PO: 1.4 ± 0.5[a]	PO: 16 ± 4 µg/mL[a]
			↑ Child		↓ Child		IV: 15 ± 3 µg/mL[b]

[a]Following a 600-mg oral dose given twice daily to steady state. [b]Following a 30-min IV infusion of a 600-mg dose given twice daily to steady state in patients with gram-positive infection.

References: MacGowan AP. Pharmacokinetic and pharmacodynamic profile of linezolid in healthy volunteers and patients with Gram-positive infections. *J Antimicrob Chemother,* **2003**, *51*(suppl 2):ii17–ii25. Stalker DJ, et al. Clinical pharmacokinetics of linezolid, a novel oxazolidinone antibacterial. *Clin Pharmacokinet,* **2003**, *42*:1129–1140.

Lisinopril

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
25 ± 20	88–100	0	4.2 ± 2.2[a]	2.4 ± 1.4[a]	12[b]	~7[c]	50 (6.4–343) ng/mL[c]
			↓ RD, Aged		↑ RD, Aged		

[a]*CL/F* and V_{area}/F reported. [b]Effective $t_{1/2}$ to predict steady-state accumulation upon multiple dosing; a terminal $t_{1/2}$ of 30 h reported. [c]Following a 2.5- to 40-mg oral dose given daily to steady state in elderly patients with hypertension and varying degrees of renal function. EC_{90} for ACE inhibition is 27 ± 10 ng/mL.

Reference: Thomson AH, et al. Lisinopril population pharmacokinetics in elderly and renal disease patients with hypertension. *Br J Clin Pharmacol,* **1989**, *27*:57–65.

Lithium

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
100[a]	95 ± 15	0	0.35 ± 0.11[b]	0.66 ± 0.16	22 ± 8[c]	IR: 0.5–3[d]	IR: 1–2 mM[d]
			↓ RD, Aged	↓ Obes	↑ RD, Aged	SR: 2–6[d]	SR: 0.7–1.2 mM[d]
			↑ Preg		↓ Obes		

[a]Values as low as 80% reported for some prolonged-release preparations. [b]Renal *CL* of Li^+ parallels that of Na^+. The ratio of Li^+ and creatinine *CL* is ~0.2 ± 0.03. [c]The distribution $t_{1/2}$ is 5.6 ± 0.5 h; this influences drug concentrations for at least 12 h. [d]Following a single 0.7-mmol/kg oral dose of immediate-release (IR) lithium carbonate and sustained-release (SR) tablets.

Reference: Ward ME, et al. Clinical pharmacokinetics of lithium. *J Clin Pharmacol*, **1994**, *34*:280–285.

Lopinavir[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—[b]	<3	98–99	1.2[c]	0.6[c]	5.3 ± 2.5	4.4 ± 2.4[d]	9.8 ± 3.7 µg/mL[d]
↑ Food							

[a]Currently formulated in combination with ritonavir (KALETRA). Ritonavir inhibits the CYP3A-dependent metabolism of lopinavir, enhancing its bioavailability, increasing plasma concentrations (50- to 100-fold), and extending its $t_{1/2}$. Pharmacokinetic data from male and female patients with HIV are reported. [b]Absolute bioavailability is not known; the relative bioavailability increases with a high-fat meal. [c]*CL/F* and V_{area}/F reported; calculated from steady-state AUC data. [d]Following a 400/100-mg lopinavir/ritonavir oral dose given twice daily in combination with stavudine and lamivudine to steady state.

References: Boffito M, et al. Lopinavir protein binding in vivo through the 12-hour dosing interval. *Ther Drug Monit*, **2004**, *26*:35–39. Corbett AH, et al. Kaletra (lopinavir/ritonavir). *Ann Pharmacother*, **2002**, *36*:1193–1203. Eron JJ, et al. Once-daily versus twice-daily lopinavir/ritonavir in antiretroviral-naive HIV-positive patients: a 48-week randomized clinical trial. *J Infect Dis*, **2004**, *189*:265–272. King JR, et al. Pharmacokinetic enhancement of protease inhibitor therapy. *Clin Pharmacokinet*, **2004**, *43*:291–310.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Loratadine[a]							
L: —[b]	L: Negligible	L: 97	L: 142 ± 57[d]	L: 120 ± 80[d]	L: 8 ± 6	L (L): 2.0 ± 2.0[e]	L (L): 3.4 ± 3.4 ng/mL[e]
			↓ LD		↑ LD	DL (L): 2.6 ± 2.9[e]	DL (L):4.1 ± 2.6 ng/mL[e]
DL: —[b]	DL: —	DL: 82–87[c]	DL: 14–18[d]	DL: 26[d]	DL: 21–24	DL (DL): 3.2 ± 1.8[f]	DL(DL): 4.0 ± 2.1 ng/mL[f]
			↓ RD, LD			HDL (DL): 4.8 ± 1.9[f]	HDL (DL): 2.0 ± 0.6 ng/mL[f]

[a]Loratadine (L) is converted to a major active metabolite, desloratadine (DL). Almost all patients achieve higher plasma concentrations of DL than of L. DL (CLARINEX) is approved for similar clinical indications as L. DL is eliminated by metabolism. DL is eliminated by metabolism to an active metabolite, 3-hydroxydesloratidine (HDL). Approximately 7%–20% of patients are slow metabolizers of DL; frequency varies with ethnicity. [b]Bioavailability of L and DL is not known; L is probably low due to extensive first-pass metabolism. [c]Plasma protein binding of HDL is 85%–89%. [d]*CL/F* and V_{area}/F reported. For DL, oral *CL/F* calculated from AUC data following a single 5- to 20-mg oral dose given to healthy adults. [e]Mean for L and DL following a 10-mg oral L dose (CLARITIN-D 24 HOUR) given once daily for 7 days to healthy adults. [f]Mean for DL and HDL following a 5-mg oral DL dose (CLARINEX) given once daily for 10 days to healthy adults.

References: Affrime M, et al. A pharmacokinetic profile of desloratadine in healthy adults, including elderly. *Clin Pharmacokinet*, **2002**, *41*(suppl):13–19. Gupta S, et al. Desloratadine demonstrates dose proportionality in healthy adults after single doses. *Clin Pharmacokinet*, **2002**, *41*(suppl):l–6. Haria M, et al. Loratadine. A reappraisal of its pharmacological properties and therapeutic use in allergic disorders. *Drugs*, **1994**, *48*:617–637. Kosoglou T, et al. Pharmacokinetics of loratadine and pseudoephedrine following single and multiple doses of once- versus twice-daily combination tablet formulations in healthy adult males. *Clin Ther*, **1997**, *19*:1002–1012. *PDR*58, **2004**, p. 3044.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Lorazepam							
93 ± 10	<1	91 ± 2	0.61± 0.01[a]	1.3 ± 0.2[b]	14 ± 5	IM: 1.2[c]	IV: ~75 ng/mL[c]
		↓ LD, RD		↑ LD, RD	↑ LD, Neo, RD	PO: 1.2–2.6[c]	IM: ~30 ng/mL[c] PO: ~28 ng/mL[c]

[a]Cleared primarily by glucuronidation by UGT2B7 and UGT2B15. [b]V_{area} reported. [c]Following a single 2-mg IV bolus dose, IM dose, or oral dose given to healthy adults.

References: Chung J-Y, et al. Pharmacokinetic and pharmacodynamic interaction of lorazepam and valproic acid in relation to *UGT2B7* genetic polymorphism in healthy subjects. *Clin Pharmacol Ther*, **2007**, *83*:595-600. Greenblatt DJ. Clinical pharmacokinetics of oxazepam and lorazepam. *Clin Pharmacokinet*, **1981**, *6*:89–105.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Losartan[a]							
L: 35.8 ± 15.5	L: 12 ± 2.8	L: 98.7	L: 8.1 ± 1.8	L: 0.45 ± 0.24	L: 2.5 ± 1.0	L: 1.0 ± 0.5[d]	L: 296 ± 217 ng/mL[d]
		LA: 99.8	↓ RD[b], LD[c]		LA: 5.4 ± 2.3	LA: 4.1 ± 1.6[d]	LA: 249 ± 74 ng/mL[d]

[a]Data from healthy male subjects. Losartan (L) is metabolized primarily by CYP2C9 to an active 5-carboxylic acid metabolite (LA). [b]*CL/F* for L but not LA decreased in severe renal impairment (L/LA not removed by hemodialysis). No dose adjustment required. [c]*CL/F* for L reduced in mild to moderate hepatic impairment. LA AUC also increased. [d]Following a single 50-mg oral dose (tablet). Higher plasma levels of L (but not LA) in female subjects than in male subjects.

References: Lo MW, et al. Pharmacokinetics of losartan, an angiotensin II receptor antagonist, and its active metabolite EXP3174 in humans. *Clin Pharmacol Ther*, **1995**, *58*:641–649. *PDR*54, **2000**, pp. 1809–1812.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Lovastatin[a]							
≤5	10	>95	4.3–18.3[b]	—	1–4	AI: 2.0 ± 0.9[c]	AI: 41 ± 6 ng-Eq/mL[c]
↑ Food			↓ RD			TI: 3.1 ± 2.9[c]	TI: 50 ± 8 ng-Eq/mL[c]

[a]Lovastatin is an inactive lactone that is metabolized to the corresponding active β-hydroxy acid. Pharmacokinetic values are based on the sum of 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase inhibition activity by the β-hydroxy acid and other less potent metabolites. [b]The lactone (in equilibrium with β-hydroxy acid metabolite) is metabolized by CYP3A. [c]Following an 80-mg oral dose given once daily for 17 days. Peak levels represent total active inhibitors (AI) and total inhibitors (TI) of HMG-CoA reductase.

References: Corsini A, et al. New insights into the pharmacodynamic and pharmacokinetic properties of statins. *Pharmacol Ther*, **1999**, *84*:413–428. Desager JP, et al. Clinical pharmacokinetics of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors. *Clin Pharmacokinet,* **1996**, *31*:348–371. McKenney JM. Lovastatin: a new cholesterol-lowering agent. *Clin Pharm,* **1988**, *7*:21–36.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Mefloquine[a]							
—[b]	<1	98.2	0.43 ± 0.14[c]	19 ± 6[c]	20 ± 4 days	SD: 7–19.6[d]	SD: 800–1020 ng/mL[d]
			↑ Preg		↓ Preg	MD: 12 ± 8[d]	MD: 420 ± 141 ng/mL[d]

[a]Racemic mixture; no information on relative kinetics of the enantiomers. [b]Absolute bioavailability is not known; reported values of >85% represent comparison of oral tablet to solution. [c]*CL/F* and V_{ss}/F reported. [d]Range of mean values from different studies following a single 1000-mg oral dose (SD) and mean following a 250-mg oral dose given once weekly for 4 weeks (MD).

Reference: Karbwang J, et al. Clinical pharmacokinetics of mefloquine. *Clin Pharmacokinet*, **1990**, *19*:264–279.

(*Continued*)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Meperidine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
52 ± 3	~5 (1–25)^{b}	58 ± 9^{c}	17 ± 5	4.4 ± 0.9	3.2 ± 0.8^{d}	IM: <1^{e}	IV: 0.67 μg/mL^{e}
↑ LD		↓ Aged, RD	↓LD, RD, Prem, Neo	↑ Aged, Prem	↑ LD, Prem, Neo, Aged, RD		IM: ~0.7 μg/mL^{e}

[a] Meperidine undergoes CYP dependent *N*-demethylation to normeperidine. The metabolite is not an analgesic but is a potent central nervous system–excitatory agent and is associated with adverse side effects of meperidine. [b]Meperidine is a weak base (pK_a = 8.6) and is excreted to a greater extent in the urine at low urinary pH and to a lesser extent at high urinary pH. [c]Correlates with the concentration of α_1-acid glycoprotein. [d]A longer $t_{1/2}$ (7 h) also is observed. [e]Following a continuous 24-mg/h IV infusion or 100-mg IM injection every 4 h to steady state. Postoperative analgesia occurs at 0.4–0.7 μg/mL.

Reference: Edwards DJ, et al. Clinical pharmacokinetics of pethidine: 1982. *Clin Pharmacokinet,* **1982**, *7*:421–433.

Mercaptopurine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
12 ± 7^{b}	22 ± 12	19	11 ± 4^{c}	0.56 ± 0.38	0.90 ± 0.37		IV: 6.9 μM^{d}
						PO (–): 2.4 ± 0.4^{d}	PO (–): 0.74 ± 0.28 μM^{d}
						PO (+): 2.8 ± 0.4^{d}	PO (+): 3.7 ± 0.6 μM^{d}

[a] Inactive prodrug is metabolized intracellularly to 6-thioinosinate. Pharmacokinetic values for mercaptopurine are reported. [b]Increases to 60% when first-pass metabolism is inhibited by allopurinol (100 mg three times daily). [c]Metabolically cleared by xanthine oxidase and thiopurine methyltransferase (polymorphic). Despite inhibition of intrinsic *CL* by allopurinol, hepatic metabolism is limited by blood flow, and *CL* is thus little changed by allopurinol. [d]Following an IV infusion of 50 mg/m^2/h to steady state in children with refractory cancers or a single oral dose of 75 mg/m^2 with (+) or without (–) allopurinol pretreatment.

References: Lennard L. The clinical pharmacology of 6-mercaptopurine. *Eur J Clin Pharmacol*, **1992**, *43*:329–339. *PDR*54, **2000**, p. 1255.

Metformin[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
52 ± 5 (40–55)	99.9 ± 0.5 (79–100)	Negligible	7.62 ± 0.30^{b} (6.3–10.1)	1.12 ± 0.08 (0.9–3.94)	1.74 ± 0.20 (1.5–4.5)	1.9 ± 0.4^{d} (1.5-3.5)^{d}	1.6 ± 0.2 μg/mL^{d}
			↓ RD^{c}, Aged ↑ Preg		↑ RD^{c}, Aged		(1.0–3.1 μg/mL)^{d}

[a] Data from healthy male and female subjects. No significant sex differences. Shown in parentheses are mean values from different studies. [b]Clearance of metformin is mediated in part by OCTs in the kidney. [c]*CL/F* reduced, mild to severe renal impairment. [d]Following a single 0.5-g oral dose (tablet) and range for a 0.5- to 1.5-g oral dose.

References: Harrower AD. Pharmacokinetics of oral antihyperglycaemic agents in patients with renal insufficiency. *Clin Pharmacokinet*, **1996**, *37*:111–119. Pentikainen PJ, et al. Pharmacokinetics of metformin after intravenous and oral administration to man. *Eur J Clin Pharmacol*, **1979**, *16*:195–202. *PDR*54, **2000**, pp. 831–835. Scheen AJ. Clinical pharmacokinetics of metformin. *Clin Pharmacokinet*, **1996**, *30*:359–371.

Methadone[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
92 ± 21	24 ± 10^{b}	89 ± 2.9^{c}	1.7 ± 0.9^{b}	3.6 ± 1.2^{d}	27 ± 12^{e}	~3^{f}	IV: 450–550 ng/mL^{f}
			↓ Child		↓ Child		PO: 69–980 ng/mL^{f}
			↑ Preg				

[a] Data for racemic mixture. Opioid activity resides with the *R*-enantiomer. *In vivo* disposition is stereoselective. *N*-demethylation is mediated by CYP3A4 and CYP2B6. [b]Inversely correlated with urine pH. [c]*d*-Methadone slightly higher percent bound. [d]V_{area} reported. Directly correlated with urine pH. [e]Directly correlated with urine pH. [f]Following a single 10-mg IV bolus dose in patients with chronic pain or a 0.12- to 1.9-mg/kg oral dose once daily for at least 2 months in subjects with opioid dependency. Levels >100 ng/mL prevent withdrawal symptoms; EC_{50} for pain relief and sedation in cancer patients is 350 ± 180 ng/mL.

References: Dyer KR, et al. Steady-state pharmacokinetics and pharmacodynamics in methadone maintenance patients: Comparison of those who do and do not experience withdrawal and concentration-effect relationships. *Clin Pharmacol Ther*, **1999**, *65*:685–694. Inturrisi CE, et al. Pharmacokinetics and pharmacodynamics of methadone in patients with chronic pain. *Clin Pharmacol Ther*, **1987**, *41*:392–401.

Methotrexate[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
70 ± 27^{b,c}	81 ± 9	46 ± 11	2.1 ± 0.8^{d}	0.55 ± 0.19	7.2 ± 2.1^{e}	SC: 0.9 ± 0.2^{f}	SC: 1.1 ± 0.2 μM^{f}
			↓ RD				IV: 37–99 μM^{f}

[a] Plasma concentrations of the 7-hydroxy metabolite approach those of the parent drug. Metabolite may have both therapeutic and toxic effects. [b]Bioavailability is dose dependent and may be as low as 20% when doses are >80 mg/m^2. [c]IM bioavailability is only slightly higher. [d]Methotrexate clearance is mediated by several transporters including OATs. [e]Exhibits triexponential elimination kinetics. A shorter $t_{1/2}$ (2 h) is seen initially, and a longer (52 h) terminal $t_{1/2}$ has been observed with increased assay sensitivity. [f]Following a 15-mg SC dose given once weekly to steady state in adult patients with inflammatory bowel disease. Initial steady-state concentrations in young (1.5–22 years of age) leukemia patients receiving a 500-mg/m^2 loading dose given over 1 h followed by an infusion of 196 mg/m^2/h for 5 h.

References: Egan LJ, et al. Systemic and intestinal pharmacokinetics of methotrexate in patients with inflammatory bowel disease. *Clin Pharmacol Ther*, **1999**, *65*:29–39. Tracy TS, et al. Methotrexate disposition following concomitant administration of ketoprofen, piroxicam and flurbiprofen in patients with rheumatoid arthritis. *Br J Clin Pharmacol*, **1994**, *37*:453–456. Wall AM, et al. Individualized methotrexate dosing in children with relapsed acute lymphoblastic leukemia. *Leukemia*, 2000, 14:221–225.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Methylphenidate[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
(+): 22 ± 8	(+): 1.3 ± 0.5	(+/−): 15–16	(+): 6.7 ± 2.0[b]	(+): 2.7 ± 1.1	(+): 6.0 ± 1.7[c]	(+): 2.4 ± 0.8[c,d]	(+): 18 ± 4.3 ng/mL[d]
(−): 5 ± 3	(−): 0.6 ± 0.3		(−): 12 ± 4.7[b]	(−): 1.8 ± 0.9	(−): 3.6 ± 1.1	(−): 2.1 ± 0.6[d]	(−): 3.0 ± 0.9 ng/mL[d]

[a] Methylphenidate is available as a racemate and the active (+)-dextro-enantiomer, dexmethylphenidate. Methylphenidate and dexmethylphenidate are extensively metabolized, primarily through ester hydrolysis to ritalinic acid. Data for individual enantiomers following racemate administration to healthy adult male subjects. No significant sex differences. [b] The (+)-enantiomer exhibits dose-dependent kinetics at high doses of racemate, with an ~50% reduction in *CL/F* between a 10- and 40-mg dose. [c] When dexmethylphenidate is given alone, its $t_{1/2}$ is 2.2 h, and T_{max} is 1–1.5 h. [d] Following a single 40-mg oral dose (immediate release). Longer T_{max} (3–5 h) and lower C_{max} reported for sustained-released oral formulation.

References: Aoyama T, et al. Nonlinear kinetics of threo-methylphenidate enantiomers in a patient with narcolepsy and in healthy volunteers. *Eur J Clin Pharmacol*, **1993**, *44*:79–84. Keating GM, et al. Dexmethylphenidate. *Drugs,* **2002**, *62*:1899–1904; discussion 1905–1908. Kimko HC, et al. Pharmacokinetics and clinical effectiveness of methylphenidate. *Clin Pharmacokinet,* **1999**, *37*:457–470. *PDR*58, **2004**, pp. 2265, 2297–2298. Srinivas NR, et al. Enantioselective pharmacokinetics of dl-threo-methyl-phenidate in humans. *Pharm Res*, **1993**, *10*:14–21.

Methylprednisolone

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
82 ± 13[a]	4.9 ± 2.3	78 ± 3	6.2 ± 0.9	1.2 ± 0.2	2.3 ± 0.5		IV: 225 ± 44 ng/mL[b]
						PO: 1.64 ± 0.64[c]	PO: 178 ± 44 ng/mL[c]
		↓ LD	↓ Obes	↓ Obes, Fem	↑ Obes		
			↑ Fem		↓ Fem		

[a] May be decreased to 50%–60% with high doses. [b] Mean at 1 h following a 28-mg IV infusion over 20 min given twice daily for 6 ± 4 days during the perioperative period following kidney transplantation. [c] Mean data following a 24-mg oral dose given twice daily for 3 days in healthy adult male subjects.

References: Lew KH, et al. Sex-based effects on methylprednisolone pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther*, **1993**, *54*:402–414. Rohatagi S, et al. Pharmacokinetics of methylprednisolone and prednisolone after single and multiple oral administration. *J Clin Pharmacol*, **1997**, *37*:916–925. Tornatore KM, et al. Methylprednisolone and cortisol metabolism during the early post-renal transplant period. *Clin Transplant*, **1995**, *9*:427–432.

Metoclopramide

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
76 ± 38	20 ± 9	40 ± 4	6.2 ± 1.3	3.4 ± 1.3	5.0 ± 1.4	A: ≤1[a]	A: 80 ng/mL[a]
			↓ RD, LD		↑ RD, LD	I: 2.5 ± 0.7[a]	I: 18 ± 6.2 ng/mL[a]
			↓ Neo	↓ Neo			

[a] Following a single 20-mg oral dose given to healthy adults (A) or an oral (nasogastric) dose of 0.10–0.15 mg/kg given four times daily to steady state to premature infants (I), 1–7 weeks of age (26–36 weeks postconceptional).

References: Kearns GL, et al. Pharmacokinetics of metoclopramide in neonates. *J Clin Pharmacol*, **1998**, *38*:122–128. Lauritsen K, et al. Clinical pharmacokinetics of drugs used in the treatment of gastrointestinal diseases (part I). *Clin Pharmacokinet,* **1990**, *19*:11–31. Rotmensch HH, et al. Comparative central nervous system effects and pharmacokinetics of neu-metoclopramide and metoclopramide in healthy volunteers. *J Clin Pharmacol*, **1997**, *37*:222–228.

Metoprolol[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
38 ± 14[b]	10 ± 3[b]	11 ± 1	15 ± 3[b]	4.2 ± 0.7	3.2 ± 0.2[b]	EM: ~2[c]	EM: 99 ± 53 ng/mL[c]
↑ LD			↑ Preg		↑ LD, Neo	PM: ~3[c]	PM: 262 ± 29 ng/mL[c]
↓ Preg			↑ Fem	↑ Fem			

[a] Data for racemic mixture reported. Metabolism of less active *R*-(+)-enantiomer (*CL/F* = 28 mL/min/kg; V_{area}/F = 7.6 L/kg; $t_{1/2}$ = 2.7 h) is slightly faster than that of more active *S*-(−)-enantiomer (*CL/F* = 20 mL/min/kg; V_{area}/F = 5.5 L/kg; $t_{1/2}$ = 3 h). [b] Metabolically cleared by CYP2D6 (polymorphic). Compared to extensive metabolizers (EM), individuals who are poor metabolizers (PM) have a lower *CL/F* and a longer $t_{1/2}$ (7.6 ± 1.5 vs. 2.8 ± 1.2 h) and excrete more unchanged drug in urine (15% ± 7% vs. 3.2% ± 3%) due to reduced hepatic metabolism. [c] C_{3h} following a single 100-mg oral dose in CYP2D6 EM and PM patients with hypertension. Plasma concentrations of the more active *S*-enantiomer are ~35% higher than the *R*-antipode in CYP2D6 EM. No stereochemical difference was observed in PM subjects. EC_{50} for decreased heart rate during peak submaximal exercise testing was 16 ± 7 ng/mL; EC_{50} for decreased systolic blood pressure during exercise testing was 25 ± 18 ng/mL.

References: Dayer P, et al. Interindividual variation of beta-adrenoceptor blocking drugs, plasma concentration and effect: influence of genetic status on behaviour of atenolol, bopindolol and metoprolol. *Eur J Clin Pharmacol*, **1985**, *28*:149–153. Lennard MS, et al. Oxidation phenotype—a major determinant of metoprolol metabolism and response. *N Engl J Med*, **1982**, *307*:1558–1560. McGourty JC, et al. Metoprolol metabolism and debrisoquine oxidation polymorphism—population and family studies. *Br J Clin Pharmacol*, **1985**, *20*:555–566.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Metronidazole[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
99 ± 8[b]	10 ± 2	11 ± 3	1.3 ± 0.3	0.74 ± 0.10	8.5 ± 2.9		IV: 27 (11–41) μg/mL[c]
			↓ LD, Neo		↑ Neo, LD		
						PO: 2.8[c]	PO: 19.8 μg/mL[c]
						VA: 11 ± 2[c]	VA: 1.9 ± 0.2 μg/mL[c]

[a]Active hydroxylated metabolite accumulates in renal failure. [b]Bioavailability is 67%–82% for rectal suppositories and 53% ± 16% for intravaginal gel. [c]Following a single 100-mg dose of vaginal (VA) cream, a 100-mg IV infusion over 20 min three times daily to steady state, or a 100-mg oral dose three times daily to steady state.

Reference: Lau AM, et al. Clinical pharmacokinetics of metronidazole and other nitroimidazole anti-infectives. *Clin Pharmacokinet*, **1992**, *23*:328–364.

Micafungin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—	<1	99	0.14 ± 0.03[a]	0.20 ± 0.03	14.6 ± 3.0	—	8.8 ± 1.8 μg/mL[b]

[a]Undergoes arylsulfatase-dependent metabolism and biliary excretion. [b]Following a 100-mg IV infusion administered over 1 hour.

Reference: Hebert MF, et al. Pharmacokinetics of micafungin in healthy volunteers, volunteers with moderate liver disease and volunteers with renal dysfunction. *J Clin Pharmacol,* **2005**, *45*:1145–1152.

Midazolam

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
44 ± 17[a]	<1%	98	6.6 ± 1.8[b]	1.1 ± 0.6	1.9 ± 0.6		IV: 113 ± 16 ng/mL[d]
↑ LD		↓ Aged, RD	↑ RD[c]	↑ Obes	↑ Aged, Obes, LD	PO: 0.67 ± 0.45[d]	PO: 78 ± 27 ng/mL[d]
			↓ LD, Neo	↓ Neo			

[a]Undergoes extensive first-pass metabolism by intestinal and hepatic CYP3A. Bioavailability appears to be dose dependent; 35%–67% at 15-mg, 28%–36% at 7.5-mg, and 12%–47% at 2-mg oral dose, possibly due to saturable first-pass intestinal metabolism. [b]Metabolically cleared by CYP3A. [c]Increased *CL* due to increased plasma free fraction; unbound *CL* is unchanged. [d]Following a single 5-mg IV bolus or 10-mg oral dose.

References: Garzone PD, et al. Pharmacokinetics of the newer benzodiazepines. *Clin Pharmacokinet*, **1989**, *76*:337–364. Thummel KE, et al. Oral first-pass elimination of midazolam involves both gastrointestinal and hepatic CYP3A-mediated metabolism. *Clin Pharmacol Ther*, **1996**, *59*:491–502.

Midostaurin[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
>90 ↑ Food[f]	—[b]	>99.8	0.91 (32.96%)[c]	1.36 (31%)[c]	19 (39%)[c]	1 (1–3)[d] ↑ Food	1170 (26%) ng/mL[c,e,f]

[a]Data from female and male healthy subjects. [b]No unchanged drug was found in the urine after oral administration. Midostaurin is primarily metabolized by CYP3A4 to CGP52421 (hydroxyl metabolite) and CGP62221 (*O*-demthylated metabolite), both active metabolites. CGP62221 and CGP52421 account for 28% ± 2.7% and 38% ± 6.6% (mean ± SD), respectively, of the total circulating radioactivity. [c]Mean (CV%) reported in healthy subjects. [d]Median (range) reported in healthy subjects. [e]Following 50-mg single dose orally to healthy subjects. Midostaurin exhibits time-dependent pharmacokinetics with an increase in trough concentrations during the first week of dosing followed by a decline to a steady state after ~28 days. [f]Midostaurin AUC increased 1.2-fold when coadministered with a standard meal and 1.6-fold when coadministered with a high-fat meal compared to a fasted state. Midostaurin maximum concentrations (C_{max}) were reduced by 20% with a standard meal and by 27% with a high-fat meal compared to a fasted state.

References: FDA. Drugs@FDA: FDA-approved drugs. Product labeling: Rydapt® (midostaurin oral capsules). Available at: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm. Accessed April 2, 2021. FDA. Midostaurin NDA and label. NDA approved in 2017; label revised 11/2020. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/207997s006lbl.pdf. Accessed April 2, 2021.

Minocycline

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
95–100	11 ± 2	76	1.0 ± 0.3[a]	1.3 ± 0.2[b]	16 ± 2		IV: 3.5 μg/mL[c]
						PO: 2–4[c]	PO: 2.3–3.5 μg/mL[c]

[a]Cleared primarily by oxidative metabolism in the liver. [b]V_{area} reported. [c]Following a single 200-mg IV infusion (1 h) or range of values following a 100-mg oral dose given twice a day to steady state.

Reference: Saivin S, et al. Clinical pharmacokinetics of doxycycline and minocycline. *Clin Pharmacokinet*, **1988**, *15*:355–366.

Mirtazapine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
50 ± 10	—	85	9.12 ± 1.14[b]	4.5 ± 1.7	16.3 ± 4.6[b,e]	1.5 ± 0.7[f]	41.8 ± 7.7 ng/mL[f]
			↓ LD,[c] RD[d]		↑ LD,[c] RD[d]		

[a]Data from healthy adult subjects. Metabolized by CYP2D6 and CYP1A2 (8-hydroxy) and CYP3A (*N*-desmethyl, *N*-oxide). [b]Women of all ages exhibit a lower *CL/F* and longer $t_{1/2}$ than men. [c]*CL/F* reduced, hepatic impairment. [d]*CL/F* reduced, moderate to severe renal impairment. [e]The $t_{1/2}$ of the (–)-enantiomer is approximately twice as long as the (+)-antipode; approximately 3-fold higher blood concentrations (+ vs. –) are achieved. [f]Following a 15-mg oral dose given once daily to steady state.

References: Fawcett J, et al. Review of the results from clinical studies on the efficacy, safety and tolerability of mirtazapine for the treatment of patients with major depression. *J Affect Disord*, **1998**, *51*:267–285. *PDR54*, **2000**, p. 2109.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Montelukast[a]							
62	<0.2	>99	0.70 ± 0.17[b]	0.15 ± 0.02	4.9 ± 0.6	3.0 ± 1.0[d]	542 ± 173 ng/mL[d]
			↓ LD[c]		↑ LD[c]		

[a]Data from healthy adult subjects. No significant sex differences. [b]Montelukast is metabolized by CYP3A4 and CYP2C9. [c]*CL/F* is reduced by 41%, mild to moderate hepatic impairment with cirrhosis. [d]Following a single 10-mg oral dose.

*References: PDR*54, **2000**, p. 1882. Zhao JJ, et al. Pharmacokinetics and bioavailability of montelukast sodium (MK-0476) in healthy young and elderly volunteers. *Biopharm Drug Dispos,* **1997**, *18*:769–777.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Morphine[a]							
PO: 24 ± 12	4 ± 5	35 ± 2	24 ± 10	3.3 ± 0.9	1.9 ± 0.5	IM: 0.2–0.3[b]	IV: 200–400 ng/mL[b]
IM: ~100		↓ LD	↓RD, Prem	↓ RD	↑ Neo, Prem	PO-IR: 0.5–1.5[b]	IM: ~70 ng/mL[b]
						PO-SR: 3–8[b]	PO-IR: 10 ng/mL[b]
							PO-SR: 7.4 ng/mL[b]

[a]Mainly cleared by UGT2B7-dependent glucuronidation to morphine 3-glucuronide and to an active metabolite, morphine-6-glucuronide; the latter urinary excretion = 14% ± 7% and $t_{1/2}$ = 4.0 ± 1.5 h. Steady-state ratio of active metabolite to parent drug after oral dosing = 4.9 ± 3.8. In renal failure, $t_{1/2}$ increases to 50 ± 37 h, resulting in significant accumulation of active glucuronide metabolite. [b]Following a single 10-mg IV dose (bolus with 5-min blood sample), a 10-mg/70-kg IM, a 10-mg/70-kg immediate-release oral (PO-IR) dose, or a 50-mg sustained-release oral dose (PO-SR). Minimum analgesic concentration is 15 ng/mL.

References: Berkowitz BA. The relationship of pharmacokinetics to pharmacological activity: morphine, methadone and naloxone. *Clin Pharmacokinet*, **1976**, *1*:219–230. Glare PA, et al. Clinical pharmacokinetics of morphine. *Ther Drug Monit*, **1991**, *13*:1–23.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Moxifloxacin[a]							
86 ± 1	21.9 ± 3.6	39.4 ± 2.4	2.27 ± 0.24[b]	2.05 ± 1.15	15.4 ± 1.2	2.0 (0.5–6.0)[c]	2.5 ± 1.3 μg/mL[c]

[a]Data from healthy adult male subjects. [b]Moxifloxacin is metabolically cleared by ST and UGT. [c]Following a single oral 400-mg dose.

Reference: Stass H, et al. Pharmacokinetics and elimination of moxifloxacin after oral and intravenous administration in man. *J Antimicrob Chemother*, **1999**, *43*(suppl B):83–90.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Mycophenolate[a]							
MM: ~0	MPA: <1	MPA: 97.5	MM: 120–163	MPA: 3.6–4[c]	MM: <0.033	MPA: 1.1–2.2[d]	MPA: 8–19 μg/mL[d]
MPA: 94		↓ RD[b]	MPA: 2.5 ± 0.4[c]		MPA: 16.6 ± 5.8		
			↓ RD[b]				

[a]Data from healthy adult male and female subjects and organ transplant patients. No significant sex differences. Mycophenolate mofetil (MM) is rapidly converted to the active mycophenolic acid (MPA) after IV and oral doses. Kinetic parameters refer to MM and MPA after a dose of MM. MPA is metabolized by UGT to MPA-glucuronide (MPAG). MPA undergoes enterohepatic recycling; MPAG is excreted into bile and presumably is hydrolyzed by gut flora and reabsorbed as MPA. [b]Accumulation of MPA and MPAG and increased unbound MPA; severe renal impairment. [c]*CL/F* and V_{area}/F reported for MPA. [d]Range of mean MPA C_{max} and T_{max} from different studies following a 1- to 1.75-g oral dose given twice daily to steady state in renal transplant patients.

References: Bullingham R, et al. Effects of food and antacid on the pharmacokinetics of single doses of mycophenolate mofetil in rheumatoid arthritis patients. *Br J Clin Pharmacol,* **1996**, *47*:513–516. Bullingham RE, et al. Clinical pharmacokinetics of mycophenolate mofetil. *Clin Pharmacokinet,* **1998**, *34*:429–455. Kriesche HUM, et al. MPA protein binding in uremic plasma: prediction of free fraction. *Clin Pharmacol Ther,* **1999**, *65*:184. *PDR*54, **2000**, pp. 2617–2618.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Naltrexone[a]							
20 ± 5	2	21	18.3 ± 1.4	16.1 ± 5.2	10.3 ± 3.3[c]	1[d]	15–64 ng/mL[d]
			↓ LD[b]				

[a]Naltrexone has an active metabolite, 6β-naltrexol, that circulates at greater concentrations than naltrexone and has a 10-fold higher AUC than naltrexone after oral administration of naltrexone. [b]The oral AUC of naltrexone was significantly increased in patients with liver impairment, whereas the AUC of 6β-naltrexol was not changed. [c]A $t_{1/2}$ of 2.7 h after IV administration also reported. [d]Following a single 100-mg oral dose.

References: Bertolotti M, et al. Effect of liver cirrhosis on the systemic availability of naltrexone in humans. *J Hepatol,* **1997**, *27*:505–511. Bullingham RES, et al. Clinical pharmacokinetics of narcotic agonist-antagonist drugs. *Clin Pharmacokinet,* **1983**, *8*:332–343.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Naproxen

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
99[a]	5–6	99.7 ± 0.1[b]	0.13 ± 0.02[d,e]	0.16 ± 0.02[e]	14 ± 1	T-IR: 2–4[f]	T-IR: 37 μg/mL[f]
		↑ RD, Aged,[c] LD	↓ RD	↑ RD, Child	↑ Aged[c]	T-CR: 5[f] S: 2.2 ± 2.1[f]	T-CR: 94 μg/mL[f] S: 55 ± 14 μg/mL[f]

[a] Estimated bioavailability. [b] Saturable plasma protein binding yields apparent nonlinear elimination kinetics. [c] No change in total *CL*, but a significant (50%) decrease in *CL* of unbound drug; it is thus suggested that dosing rate be decreased. A second study in elderly patients found a decreased *CL* and increased $t_{1/2}$ with no change in percent bound. [d] Metabolically cleared by CYP2C9 (polymorphic) and CYP1A2. [e] *CL/F* and V_{area}/F reported. [f] Following a single 250-mg dose of suspension (S) given orally to pediatric patients or a 250-mg immediate-release tablet (T-IR) or a 500-mg controlled-release tablet (T-CR) given to adults.

Reference: Wells TG, et al. Comparison of the pharmacokinetics of naproxen tablets and suspension in children. *J Clin Pharmacol,* **1994**, *34*:30–33.

Nevirapine[a]

93 ± 9	<3	60	SD: 0.23–0.77[b] MD: 0.89[b]	SD: 1.2 ± 0.09 MD: 1.2	SD: 45[b] MD: 25–35[b]	2–4[d]	SD: 2 ± 0.4 μg/mL[d] MD: 4.5 ± 1.9 μg/mL[d]
			↑ Child[c]				

[a] Data from healthy adult and HIV-infected subjects. No significant sex differences. Metabolized by CYP3A. [b] Range of *CL/F* and *V/F* reported. Nevirapine appears to autoinduce its own metabolism. *CL/F* increases and $t_{1/2}$ decreases from a single dose (SD) to multiple doses (MD). [c] Patients <8 years. [d] Following a single 200-mg oral dose (SD), and 200 mg, twice a day, to steady state (MD).

References: Cheeseman SH, et al. Pharmacokinetics of nevirapine: initial single-rising-dose study in humans. *Antimicrob Agents Chemother*, **1993**, *37*:178–182. Luzuriaga, K, et al. Pharmacokinetics, safety, and activity of nevirapine in human immunodeficiency virus type 1-infected children. *J Infect Dis*, **1996**, *174*:713–721. *PDR*54, **2000**, p. 2721. Zhou XJ, et al. Population pharmacokinetics of nevirapine, zidovudine, and didanosine in human immunodeficiency virus-infected patients. The National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group Protocol 241 Investigators. *Antimicrob Agents Chemother*, **1999**, *43*:121–128.

Nifedipine

50 ± 13	~0	96 ± 1	7.0 ± 1.8[a]	0.78 ± 0.22	1.8 ± 0.4[b]	IR: 0.5 ± 0.2[c]	IR: 79 ± 44 ng/mL[c]
↑ LD, Aged		↓ LD, RD	↓ LD, Aged	↑ LD, RD, Aged	↑ LD, RD, Aged	ER: ~6[c]	ER: 35–49 ng/mL[c]

[a] Metabolically cleared by CYP3A; undergoes significant first-pass metabolism. [b] Longer apparent $t_{1/2}$ after oral administration because of absorption limitation, particularly for extended-release (ER) formulations. [c] Mean following a single 10-mg immediate-release (IR) capsule given to healthy male adults or a range of steady-state concentrations following a 60-mg ER tablet given daily to healthy male adults. Levels of 47 ± 20 ng/mL were reported to decrease diastolic pressure in hypertensive patients.

References: Glasser SP, et al. The efficacy and safety of once-daily nifedipine: the coat-core formulation compared with the gastrointestinal therapeutic system formulation in patients with mild-to-moderate diastolic hypertension. Nifedipine Study Group. *Clin Ther,* **1995**, *17*:12–29. Renwick AG, et al. The pharmacokinetics of oral nifedipine—a population study. *Br J Clin Pharmacol,* **1988**, *25*:701–708. Soons PA, et al. Intraindividual variability in nifedipine pharmacokinetics and effects in healthy subjects. *J Clin Pharmacol,* **1992**, *32*:324–331.

Nitrofurantoin

87 ± 13	47 ± 13	62 ± 4	9.9 ± 0.9	0.58 ± 0.12	1.0 ± 0.2	2.3 ± 1.4[a]	428 ± 146 ng/mL[a]
			↑ Alkaline urine				

[a] Following a single 50-mg oral dose (tablet) given to fasted healthy adults. No changes when taken with a meal.

Reference: Hoener B, et al. Nitrofurantoin disposition. *Clin Pharmacol Ther,* **1981**, *29*:808–816.

Nitroglycerin[a]

PO: <1	<1	—	195 ± 86[c]	3.3 ± 1.2[c,d]	2.3 ± 0.6 min	SL: 0.09 ± 0.03[e]	IV: 3.4 ± 1.7 ng/mL[e]
SL: 38 ± 26[b]						Top: 3–4[e]	SL: 1.9 ± 1.6 ng/mL[e]
Top: 72 ± 20						TD: 2[e]	

[a] Dinitrate metabolites have weak activity compared to nitroglycerin (<10%), but because of a prolonged $t_{1/2}$ (~40 min), they may accumulate during administration of sustained-release preparations to yield concentrations in plasma 10- to 20-fold greater than parent drug. [b] Following sublingual (SL) dose rinsed out of mouth after 8 min. Rinse contained 31% ± 19% of the dose. [c] Following a 40- to 100-min IV infusion. [d] V_{area} reported. [e] Steady-state concentration following a 20–54 μg/min IV infusion over 40–100 min or a 0.4-mg SL dose. Levels of 1.2–11 ng/mL associated with a 25% drop in capillary wedge pressure in patients with CHF. T_{max} for topical (Top) and transdermal (TD) preparations also reported.

References: Noonan PK, et al. Incomplete and delayed bioavailability of sublingual nitroglycerin. *Am J Cardiol,* **1985**, *55*:184–187. *PDR*54, **2000**, p. 1474. Thadani U, et al. Relationship of pharmacokinetic and pharmacodynamic properties of the organic nitrates. *Clin Pharmacokinet,* **1988**, *15*:32–43.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Olanzapine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
~60[b]	7.3	93	6.2 ± 2.9[c,d]	16.4 ± 5.1[d]	33.1 ± 10.3	6.1 ± 1.9[e]	12.9 ± 7.5 ng/mL[e]
					↑ Aged		

[a] Data from male and female schizophrenic patients. [b] Bioavailability estimated from parent metabolite recovery data. [c] Metabolized primarily by UGT, CYP1A2, and flavin-containing monooxygenase. [d] Summary of *CL/F* and V_{area}/F for 491 subjects receiving an oral dose. *CL/F* segregates by sex (F/M) and smoking status (nonsmoker [NS]/smoker [S]): M, S > F, S > M, NS > F, NS. [e] Following a single 9.5 ± 4-mg oral dose to healthy male subjects; $C_{max,ss}$ ~20 ng/mL following a 10-mg oral dose given once daily.

References: Callaghan JT, et al. Olanzapine. Pharmacokinetic and pharmacodynamic profile. *Clin Pharmacokinet,* **1999**, *37*:177–193. Kassahun K, et al. Disposition and biotransformation of the antipsychotic agent olanzapine in humans. *Drug Metab Dispos,* **1997**, *25*:81–93. *PDR*54, **2000**, p. 1649.

Olmesartan[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
26	35–50	99	0.31 ± 0.05	0.36 ± 0.18	13.7 ± 5.6	1.5 (1–2.5)[b]	1083 ± 283 ng/mL[b]

[a] Olmesartan is administered as a prodrug, olmesartan medoxomil. Pharmacokinetic data for olmesartan are reported. [b] Following 40 mg/day olmesartan medoxomil for 10 days.

References: FDA. Drugs@FDA. Benicar label approved on 07/13/05. Available at: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm. Accessed May 17, 2010. Rohatgi S, et al. Pharmacokinetics of amlodipine and olmesartan after administration of amlodipine besylate and olmesartan medoxomil in separate dosage forms and as a fixed-dose combination. *J Clin Pharmacol,* **2008**, *48*:1309–1322.

Oxaliplatin[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—[b]	—[c]	90[d]	49 (41–64)[e]	1.5 (1.1–2.1)	0.32 (0.27–0.46)[f]	—	Ox: 0.33 (0.28–0.38) μg Pt/mL
							PtDC: 0.008 (0.004–0.014) μg Pt/mL[g]

[a] Oxaliplatin is an organoplatinum complex; Pt is coordinated with diaminocyclohexane (DACH) and an oxalate ligand as a leaving group. Oxaliplatin (Ox) undergoes nonenzymatic biotransformation to reactive derivatives, notably Pt(DACH)Cl_2 (PtDC). Antitumor activity and toxicity are thought to relate to the concentration of Ox and PtDC in plasma ultrafiltrate (i.e., unbound concentration). [b] For IV administration only. [c] Approximately 54% of the platinum eliminated is recovered in urine. [d] Binding to plasma proteins is irreversible. [e] *CL* of total platinum is much lower; ~2–4 mL/min/kg. [f] The elimination of platinum species in plasma follows a triexponential pattern. The quoted $t_{1/2}$ reflects the $t_{1/2}$ of the first phase, which is the clinically relevant phase. The $t_{1/2}$ for the slower two phases are 17 and 391 h. [g] Steady-state plasma ultrafiltrate concentration of Ox and PtDC after an 85-mg/m² IV infusion over 2 h during cycles 1 and 2.

*References: PDR*58, **2004**, pp. 3024–3025. Shord SS, et al. Oxaliplatin biotransformation and pharmacokinetics: a pilot study to determine the possible relationship to neurotoxicity. *Anticancer Res,* **2002**, *22*:2301–2309.

Oxcarbazepine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—	O: <1	—	O: 67.4[b]	—	O: ~2	HC: 2–4[e]	HC: 8.5 ± 2.0 μg/mL[e]
	HC: 27	HC: 45	HC: ↓ RD,[c] Aged		HC: 8–15		
			HC: ↑ Child[d]		HC: ↑ RD, Aged		

[a] Data from healthy adult male subjects. No significant sex differences. Oxcarbazepine (O) undergoes extensive first-pass metabolism to an active metabolite, 10-hydroxycarbamazepine (HC). Reduction by cytosolic enzymes is stereoselective (80% *S*-enantiomer, 20% *R*-enantiomer), but both show similar pharmacological activity. [b] *CL/F* for O reported. HC eliminated by glucuronidation. [c] AUC for HC increased, moderate to severe renal impairment. [d] AUC for HC decreased, children <6 years of age. [e] Following a 300-mg oral oxcarbazepine dose given twice daily for 12 days.

References: Battino D, et al. Clinical pharmacokinetics of antiepileptic drugs in paediatric patients. Part II. Phenytoin, carbamazepine, sulthiame, lamotrigine, vigabatrin, oxcarbazepine and felbamate. *Clin Pharmacokinet,* **1995**, *29*:341–369. Lloyd P, et al. Clinical pharmacology and pharmacokinetics of oxcarbazepine. *Epilepsia,* **1994**, *35*(suppl 3): S10–S13. Rouan MC, et al. The effect of renal impairment on the pharmacokinetics of oxcarbazepine and its metabolites. *Eur J Clin Pharmacol,* **1994**, *47*:161–167. van Heiningen PN, et al. The influence of age on the pharmacokinetics of the antiepileptic agent oxcarbazepine. *Clin Pharmacol Ther,* **1991**, *50*:410–419.

Oxybutynin[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
1.6–10.9	<1	—	8.1 ± 2.3[b]	1.3 ± 0.4[b]	IV: 1.9 ± 0.35[b,c]	IR: 5.0 ± 4.2[d]	IR: 12.4 ± 4.1 ng/mL[d]
						XL: 5.2 ± 3.7[d]	XL: 4.2 ± 1.6 ng/mL[d]

[a] Data from healthy female subjects. No significant sex differences. Racemic mixture; anticholinergic activity resides predominantly with *R*-enantiomer; no stereoselectivity exhibited for antispasmodic activity. Oxybutynin undergoes extensive first-pass metabolism to *N*-desethyloxybutynin (DEO), an active, anticholinergic metabolite. Metabolized primarily by intestinal and hepatic CYP3A. Racemic oxybutynin kinetic parameters reported. [b] Data reported for a 1-mg IV dose, assuming a 70-kg body weight. A larger volume (2.8 L/kg) and longer $t_{1/2}$ (5.3 h) reported for a 5-mg IV dose. [c] Exhibits a longer apparent $t_{1/2}$ following oral dosing due to absorption rate-limited kinetics: immediate-release (IR) $t_{1/2}$ = 9 ± 2 h; extended-release (XL) $t_{1/2}$ = 14 ± 3 h. The apparent $t_{1/2}$ for DEO was 4.0 ± 1.4 h and 8.3 ± 2.5 h for the IR and XL formulations, respectively. [d] Following a dose of 5-mg IR given three times daily or 15-mg XL given once daily for 4 days. Peak DEO levels at steady state were 45 and 23 ng/mL for IR and XL, respectively.

References: Gupta SK, et al. Pharmacokinetics of an oral once-a-day controlled-release oxybutynin formulation compared with immediate-release oxybutynin. *J Clin Pharmacol,* **1999**, *39*:289–296. *PDR*54, **2000**, p. 507.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Oxycodone[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
CR: 60–87[b]	—[c]	45	12.4 (9.2–15.4)	2.0 (1.1–2.9)	2.6 (2.1–3.1)[d]	CR: 3.2 ± 2.2[e]	CR: 15.1 ± 4.7 ng/mL[e]
IR: 42 ± 7[b]						IR: 1.6 ± 0.8[e]	IR: 15.5 ± 4.5 ng/mL[e]

[a] Oxycodone is metabolized primarily by CYP3A4/5, with a minor contribution from CYP2D6. Oxymorphone is an active metabolite produced by CYP2D6-mediated *O*-dealkylation. The circulating concentrations of oxymorphone are too low to contribute significantly to the opioid effects of oxycodone. Data from healthy male and female subjects reported. [b] Values reported for OXYCONTIN (oxycodone controlled release [CR]) and immediate-release (IR) tablets. [c] Up to 19% excreted unchanged after an oral dose. [d] The apparent $t_{1/2}$ for the CR oral formulation is ~5 h; this most likely reflects absorption-limited terminal elimination kinetics. [e] Following 10 mg of OXYCONTIN (CR) given twice daily to steady state or a 5-mg IR tablet given every 6 h to steady state.

References: Benziger DP, et al. Differential effects of food on the bioavailability of controlled-release oxycodone tablets and immediate-release oxycodone solution. *J Pharm Sci,* **1996**, *85*:407–410. *PDR*58, **2004**, pp. 2854–2855. Takala A, et al. Pharmacokinetic comparison of intravenous and intranasal administration of oxycodone. *Acta Anaesthesiol Scand,* **1997**, *47*:309–312.

Paclitaxel

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Low	5 ± 2	88–98[a]	5.5 ± 3.5[b]	2.01 ± 1.2	31 ± 1[c]	—	0.85 ± 0.21 µM[d]

[a] Binding of drug to dialysis filtration devices may lead to overestimation of protein binding fraction (88% suggested). [b] Metabolized by CYP2C8 and CYP3A, and substrate for P-glycoprotein. [c] Average accumulation $t_{1/2}$; longer terminal $t_{1/2}$s up to 50 h are reported. [d] Steady-state concentration during a 250-mg/m^2 IV infusion given over 24 h to adult cancer patients.

Reference: Sonnichsen DS, et al. Clinical pharmacokinetics of paclitaxel. *Clin Pharmacokinet,* **1994**, *27*:256–269.

Paliperidone[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
28 (oral ER)[b,c]	59 (51–67) (oral ER)	74	3.70 ± 1.04[d]	9.1[d] (oral ER)	28.4 ± 5.1[d] (oral ER)	22 (2.0–24)[d] (oral ER)	10.7 ± 3.3 ng/mL[d] (oral ER)
		↓ LD	↑ LD[e]		25–49 days (IM PP)[f]	13 days (IM PP)	

[a] Paliperidone, otherwise known as the 9-hydroxy active metabolite of risperidone, is marketed as an oral extended-release (ER) tablet (INVEGA) or in the form of its water-insoluble palmitate ester as a once-monthly long-acting IM injection (INVEGA SUSTENNA). Paliperidone is a racemate; its enantiomers have similar pharmacological profiles. The (+) and (–)-enantiomers of paliperidone interconvert, reaching an AUC (+) to (–) ratio of ~1.6 at steady state. [b] High-fat/high-caloric meal increased C_{max} and AUC by 60% and 54%, respectively. [c] No data on the absolute bioavailability of IM paliperidone palmitate (IM PP). The initiation regimen for INVEGA SUSTENNA (234 mg/156 mg in the deltoid muscle on day 1/day 8) produces paliperidone concentrations matching the range observed with 6–12 mg oral ER paliperidone. [d] At steady-state during once-daily doses of 3 mg, assuming an average body weight of 73 kg. V_{area} is estimated from *CL/F* and $t_{1/2}$. [e] Patients with moderate hepatic impairment showed a modest increase in clearance and plasma free fraction with no significant change in unbound AUC. [f] The apparent long terminal $t_{1/2}$ of paliperidone following IM depot injection reflects the slow dissolution of paliperidone palmitate and release of active paliperidone.

References: Boom S, et al. Single- and multiple-dose pharmacokinetics and dose proportionality of the psychotropic agent paliperidone extended release. *J Clin Pharmacol*, **2009**, *49*:1318–1330. FDA. Drugs@FDA. Invega label approved on 4/27/07; Invega Sustenna label approved on 7/31/09. Available at: http://www.accessdata.fda.gov/Scripts/cder/DrugsatFDA/. Accessed on January 1, 2010.

Pantoprazole[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
77 (67–89)	—[b]	98	2.8 ± 0.9	0.17 ± 0.04	1.1 ± 0.4	2.6 ± 0.9	2.5 ± 0.7 µg/mL[c]
			↓ LD		↑ LD		

[a] Pantoprazole is available as a racemic mixture of (+) and (–) isomers. Pantoprazole is cleared primarily by CYP2C19 (polymorphic)-dependent metabolism. Poor metabolizers (PM) exhibit profound differences in *CL* (lower) and $t_{1/2}$ (higher) compared to extensive metabolizers (EM). In CYP2C19 EM, no significant differences in the pharmacokinetics of (+) and (–) pantoprazole were observed, whereas in CYP2C19 PM, the *CL* of (–) pantoprazole was significantly greater than that of (+) pantoprazole. [b] No unchanged drug recovered in urine. [c] Following a single 40-mg oral dose.

References: FDA. Drugs@FDA. Protonix label approved on 11/12/09. Available at: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm. Accessed December 26, 2009. Huber R, et al. Pharmacokinetics of lansoprazole in man. *Int J Clin Pharmacol Ther,* **1996**, *34*:185–194. Pue MA, et al. Pharmacokinetics of pantoprazole following single intravenous and oral administration to healthy male subjects. *Eur J Clin Pharmacol,* **1993**, *44*:575–578. Tanaka M, et al. Stereoselective pharmacokinetics of pantoprazole, a proton pump inhibitor, in extensive and poor metabolizers of S-mephenytoin. *Clin Pharmacol Ther*, **2001**, *69*:108–113.

Paroxetine

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Dose dependent[a]	<2	95	8.6 ± 3.2[a,b]	17 ± 10[c]	17 ± 3[d]	5.2 ± 0.5[e]	EM: ~130 nM[e]
			↓ LD, Aged		↑ LD, Aged		PM: ~220 nM[e]

[a] Metabolized by CYP2D6 (polymorphic); undergoes time- and dose-dependent autoinhibition of metabolic *CL* in extensive metabolizers (EM). [b] *CL/F* reported for multiple dosing in EM. Single-dose data are significantly higher. In CYP2D6 poor metabolizers (PM), *CL/F* = 5.0 ± 2.1 mL/min/kg for multiple dosing. [c] V_{area}/F reported. [d] Data reported for multiple doses in EM. In PM, $t_{1/2}$ = 41 ± 8 h. [e] Estimated mean C_{max} following a 30-mg oral dose given once daily for 14 days to adults phenotyped as CYP2D6 EM and PM. There is a significant disproportional accumulation of drug in blood when going from single to multiple dosing due to autoinactivation of CYP2D6.

*References: PDR*54, **2000**, p. 3028. Sindrup SH, et al. The relationship between paroxetine and the sparteine oxidation polymorphism. *Clin Pharmacol Ther,* **1992**, *51*:278–287.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Phenobarbital							
100 ± 11	24 ± 5[a]	51 ± 3	0.062 ± 0.013	0.54 ± 0.03	99 ± 18	2–4[b]	13.1 ± 4.5 μg/mL[b]
		↓ Neo	↑ Preg, Child, Neo	↑ Neo	↑ LD, Aged ↓ Child		

[a] Phenobarbital is a weak acid (pK_a = 7.3); urinary excretion is increased at an alkaline pH; it also is reduced with decreased urine flow. [b] Mean steady-state concentration following a 90-mg oral dose given daily for 12 weeks to patients with epilepsy. Levels of 10–25 μg/mL provide control of tonic-clonic seizures, and levels of at least 15 μg/mL provide control of febrile convulsions in children. Levels >40 μg/mL can cause toxicity; 65–117 μg/mL produce stage III anesthesia—comatose but reflexes present; 100–134 μg/mL produce stage IV anesthesia—no deep tendon reflexes.

References: Bourgeois BFD. Phenobarbital and primidone. In: Wyllie E, ed. *The Treatment of Epilepsy: Principles and Practice,* 2nd ed. Williams & Wilkins, Philadelphia, **1997**, pp. 845–855. Browne TR, et al. Studies with stable isotopes II: phenobarbital pharmacokinetics during monotherapy. *J Clin Pharmacol,* **1985**, *25*:51–58.

Phenytoin[a]							
90 ± 3	2 ± 8	89 ± 23	V_{max} = 5.9 ± 1.2 mg · kg^{-1} · day^{-1}	0.64 ± 0.04[d]	6-24[e]	3–12[f]	0–5 μg/mL (27%)[f]
		↓ RD, Neo, LD, Preg	↓ Aged	↑ Neo, RD	↑ Prem[c]		5–10 μg/mL (30%)[f]
			↑ Child		↓ RD[c]		10–20 μg/mL (29%)[f]
			K_m = 5.7 ± 2.9 mg/L[b]				20–30 μg/mL (10%)[f]
			↓ Child				>30 μg/mL (6%)[f]
			↑ RD[c]				
			↓ Prem[c]				

[a] Metabolized predominantly by CYP2C9 (polymorphic) and also by CYP2C19 (polymorphic); exhibits saturable kinetics with therapeutic doses. [b] Significantly decreased in the Japanese population. [c] Comparison of *CL*s and $t_{1/2}$s with similar doses in normal subjects and patients; nonlinear kinetics not considered. [d] V_{area} reported. [e] Apparent $t_{1/2}$ is dependent on plasma concentration. [f] Population frequency of total phenytoin concentrations following a 300-mg oral dose (capsule) given daily to steady state. Total levels >10 μg/mL associated with suppression of tonic-clonic seizures. Nystagmus can occur at levels >20 μg/mL and ataxia at levels >30 μg/mL.

References: Eldon, M.A., Loewen, G.R., *et al.* Pharmacokinetics and tolerance of fosphenytoin and phenytoin administered intravenously to healthy subjects. *Can. J. Neurol. Sci.,* **1993**, *20*(suppl 4):S180. Levine, M., and Chang, T. Therapeutic drag monitoring of phenytoin. Rationale and current status, *Clin. Pharmacokinet.,* **1990**, *79*:341–358. Tozer, T.N., and Winter, M.E. Phenytoin. In, *Applied Pharmacokinetics: Principles of Therapeutic Drug Monitoring,* 3rd ed. (Evans, W.E., Schentag, J.J., and Jusko, W.J., eds.) Applied Therapeutics, Vancouver, WA, **1992,** pp. 25-1–25-44. are missing

Pioglitazone[a]							
—	Negligible	>99	1.2 ± 1.7[b]	0.63 ± 0.41[b]	11 ± 6[c]	P: 3.5 (1–4)[d]	P: 1.6 ± 0.2 μg/mL[d]
						M-III: 11 (2–48)[d]	M-III: 0.4 ± 0.2 μg/mL[d]
						M-IV: 11 (4–16)[d]	M-IV: 1.4 ± 0.5 μg/mL[d]

[a] Data from healthy male and female subjects and patients with type 2 diabetes. Pioglitazone (P) is metabolized extensively by CYP2C8, CYP3A4, and other CYP isozymes. Two major metabolites (M-III and M-IV) accumulate in blood and contribute to the pharmacological effect. [b] *CL/F* and V_{area}/F reported. *CL/F* is lower in women than in men. [c] Steady-state $t_{1/2}$ of M-III and M-IV is 29 and 27 h, respectively. [d] Following a 45-mg oral dose given once daily for 10 days.

References: Budde K, et al. The pharmacokinetics of pioglitazone in patients with impaired renal function. *Br J Clin Pharmacol,* **2003**, *55*:368–374. *PDR*58, **2004**, p. 3186.

Posaconazole							
—[a]	—	98	11.7 ± 6.4[b]	11.9[b]	21.6 ± 8.4	4 (3–12)	324 ± 161 ng/mL[c]

[a] Approximately 66% of an oral posaconazole dose is excreted unchanged in feces. It is unclear whether this represents significant biliary excretion or unabsorbed drug. [b] *CL/F* and V_d/F reported. [c] Following a single 400-mg dose of an oral suspension.

References: Courtney R, et al. Posaconazole pharmacokinetics, safety, and tolerability in subjects with varying degrees of chronic renal disease. *J Clin Pharmacol,* **2005**, *45*:185–192. Dodds Ashley ES, et al. Pharmacokinetics of posaconazole administered orally or by nasogastric tube in healthy volunteers. *Antimicrob Agents Chemother,* **2009**, *53*:2960–2964.

Pramipexole[a]							
>90[b]	~90	15	8.2 ± 1.4[b]	7.3 ± 1.7[b]	11.6 ± 2.57	1–2	M: 1.6 ± 0.23 ng/mL[e]
			↓ Aged, RD,[c] PD[d]		↑ Aged, RD		F: 2.1 ± 0.25 ng/mL[e]

[a] Data from healthy adult male and female subjects. No significant sex differences. [b] Bioavailability estimated from urinary recovery of unchanged drug. *CL/F* and V/F_{area} reported. [c] *CL/F* reduced, moderate to severe renal impairment. [d] Parkinson disease (PD); *CL/F* reduced with declining renal function. [e] Following a 0.5-mg oral dose given three times daily for 4 days to male (M) and female (F) adults.

References: Lam YW. Clinical pharmacology of dopamine agonists. *Pharmacotherapy,* **2000**, *20*:17S–25S. *PDR*54, **2000**, p. 2468. Wright CE, et al. Steady-state pharmacokinetic properties of pramipexole in healthy volunteers. *J Clin Pharmacol,* **1997**, *37*:520–525.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Pramlintide[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
30%–40%[b]	—	~60[c]	Low: 14.9 ± 3.9[d]	0.43	IV: 0.4–0.75	0.32–0.35[e]	Low: 21 ± 3 pmol/L[e]
			High: 14.5 ± 4.0[d]	0.71	SC: 0.5–0.83		High: 77 ± 22 pmol/L[e]

[a]Pramlintide is a synthetic peptide analogue of amylin for the treatment of both type 1 and type 2 diabetes. It is metabolized in the kidneys to at least one primary active metabolite: des-lys(1)pramlintide (2-37 pramlintide) with a $t_{1/2}$ similar to that of the parent drug. [b]SC administration with greater variability in response when the injection is into the arm compared to into the abdomen or thigh. [c]Not extensively bound to blood cells or albumin. [d]Based on IV infusion of a low dose of 30 μg for type 1 diabetes and a high dose of 100 μg for type 2 diabetes. [e]Following a low SC dose of 30 μg and a high SC dose of 100 μg.

References: Colburn WA, et al. Pharmacokinetics and pharmacodynamics of AC137 (25,28,29 triproamylin, human) after intravenous bolus and infusion doses in patients with insulin-dependent diabetes. *J Clin Pharmacol*, **1996**, *36*:13–24. FDA. Drugs@FDA. Symlin label approved on 9/25/07. Available at: http://www.accessdata.fda.gov/Scripts/cder/DrugsatFDA/. Accessed August 1, 2009. Kolterman OG, et al. Effect of 14 days' subcutaneous administration of the human amylin analogue, pramlintide (AC137), on an intravenous insulin challenge and response to a standard liquid meal in patients with IDDM. *Diabetologia*, **1996**, *39*:492–499.

Pravastatin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
18 ± 8	47 ± 7	43–48	13.5 ± 2.4	0.46 ± 0.04	0.8 ± 0.2[b]	1–1.4[c]	28–38 ng/mL[c]
			↓ LD		↔ Aged, RD[a]		
			↔ Aged, RD[a]				

[a]Although renal *CL* decreases with reduced renal function, no significant changes in *CL/F* or $t_{1/2}$ are seen following oral dosing as a result of the low and highly variable bioavailability. [b]A longer $t_{1/2}$ = 1.8 ± 0.8 h reported for oral dosing; probably rate limited by absorption. [c]Range of mean values from different studies following a single 20-mg oral dose.

References: Corsini A, et al. New insights into the pharmacodynamic and pharmacokinetic properties of statins. *Pharmacol Ther*, **1999**, *84*:413–428. Desager JP, et al. Clinical pharmacokinetics of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors. *Clin Pharmacokinet*, **1996**, *31*:348–371. Quion JA, et al. Clinical pharmacokinetics of pravastatin. *Clin Pharmacokinet*, **1994**, *27*:94–103.

Praziquantel[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—[b]	Negligible	80–85	5 mg/kg: 467[c]	50 mg/kg: 9.55 ± 2.86	5 mg/kg: 0.8–1.5[c]	1.5–1.8[e]	0.8–6.3 μg/mL[e]
			40–60 mg/kg: 57–222[c]		40–60 mg/kg: 1.7-3.0[c]		
			↓ LD[d]		↑ LD		

[a]Data from male and female patients with schistosomiasis. [b]Absolute bioavailability is not known. Praziquantel is well absorbed (80%) but undergoes significant first-pass metabolism (hydroxylation), the extent of which appears to be dose dependent. [c]*CL/F* and V_{ss}/F reported; *CL/F* and $t_{1/2}$ are dose dependent. [d]*CL/F* reduced, moderate to severe hepatic impairment. [e]Range of mean values from different studies following a single 40- to 60-mg/kg oral dose.

References: Edwards G, et al. Clinical pharmacokinetics of anthelmintic drugs. *Clin Pharmacokinet*, **1988**, *15*:67–93. el Guiniady MA, et al. Clinical and pharmacokinetic study of praziquantel in Egyptian schistosomiasis patients with and without liver cell failure. *Am J Trop Med Hyg*, **1994**, *51*:809–818. Jung H, et al. Clinical pharmacokinetics of praziquantel. *Proc West Pharmacol Soc*, **1991**, *34*:335–340. Sotelo J, et al. Pharmacokinetic optimisation of the treatment of neurocysticercosis. *Clin Pharmacokinet*, **1998**, *34*:503–515. Watt G, et al. Praziquantel pharmacokinetics and side effects in *Schistosoma japonicum*-infected patients with liver disease. *J Infect Dis*, **1988**, *157*:530–535.

Prednisolone

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
82 ± 13	26 ± 9[a]	90–95 (<200 ng/mL)[b]	1.0 ± 0.16[c]	0.42 ± 0.11[e]	2.2 ± 0.5	1.5 ± 0.5[f]	458 ± 150 ng/mL[f]
		~70 (>1 μg/mL)					
	↑ Aged	↓ Aged, LD	↓ Aged,[d] LD[d]	↓ Aged, Obes[d]	↑ Aged[d]		

[a]Prednisolone and prednisone are interconvertible; an additional 3% ± 2% is excreted as prednisone. [b]Extent of binding to plasma proteins is dependent on concentration over range encountered. [c]Total *CL* increases as protein binding is saturated. *CL* of unbound drug increases slightly but significantly with increasing dose. [d]Changes are for unbound drug. [e]*V* increases with dose due to saturable protein binding. [f]Following a 30-mg oral dose given twice daily for 3 days to healthy adult male subjects. The ratio of prednisolone/prednisone is dose dependent and can vary from 3–26 over a prednisolone concentration range of 50–800 ng/mL.

References: Frey BM, et al. Clinical pharmacokinetics of prednisone and prednisolone. *Clin Pharmacokinet*, **1990**, *19*:126–146. Rohatagi S, et al. Pharmacokinetics of methylprednisolone and prednisolone after single and multiple oral administration. *J Clin Pharmacol*, **1997**, *37*:916–925.

Prednisone

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
80 ± 11[a]	3 ± 2[b]	75 ± 2[c]	3.6 ± 0.8[d]	0.97 ± 0.11[d]	3.6 ± 0.4[d]	P: 2.1–3.1[e]	P: 62–81 ng/mL[e]
						PL: 1.2–2.6[e]	PL: 198–239 ng/mL[e]

[a]Measured relative to equivalent IV dose of prednisolone (PL). [b]An additional 15% ± 5% excreted as PL. [c]In contrast to PL, there is no dependence on concentration. [d]Kinetic values for prednisone (P) often are reported in terms of values for PL, its active metabolite. However, the values cited here pertain to P. [e]Range of mean data for P and PL following a single 10-mg oral dose given as different proprietary formulations to healthy adults.

References: Gustavson LE, et al. The macromolecular binding of prednisone in plasma of healthy volunteers including pregnant women and oral contraceptive users. *J Pharmacokinet Biopharm*, **1985**, *13*:561–569. Pickup ME. Clinical pharmacokinetics of prednisone and prednisolone. *Clin Pharmacokinet*, **1979**, *4*:111–128. Sullivan TJ, et al. Comparative bioavailability: Eight commercial prednisone tablets. *J Pharmacokinet Biopharm*, **1976**, *4*:157–172.

(*Continued*)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Pregabalin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
≥90[a]	90–99	0	0.96–1.2[b,c]	0.5	5–6.5	1[a]	8.5 μg/mL[e]
			↓ RD[d]				

[a] Bioavailability does not vary with dose up to 600 mg. T_{max} is delayed from 1 h to 3 h and C_{max} decreased by 25%–30% when pregabalin is given with food. No change in AUC or extent of absorption is noted. [b] Pregabalin undergoes minimal metabolism; an *N*-methylated metabolite has been identified in urine and accounts for 0.9% of oral dose. [c] Mean renal clearance in healthy young subjects ranges from 67 to 81 mL/min and assuming 70-kg body weight. Pregabalin pharmacokinetics is dose independent and predictable from single to multiple dosing. [d] Pregabalin clearance is proportional CL_{cr}; hence, dosage can be adjusted in accordance to CL_{cr} in renal dysfunction. Plasma pregabalin decreased by ~50% after 4 h of hemodialysis. [e] Steady-state concentration in healthy subjects receiving 200 mg pregabalin every 8 h.

References: Bialer M, et al. Progress report on new antiepileptic drugs: a summary of the fifth Eilat conference (EILAT V). *Epilepsy Res*, **2001**, *43*:11–58. Brodie MJ, et al. Pregabalin drug interaction studies: lack of effect on the pharmacokinetics of carbamazepine, phenytoin, lamotrigine, and valproate in patients with partial epilepsy. *Epilepsia*, **2005**, *46*:1407–1413. *Physicians' Desk Reference,* 63rd ed. Physicians' Desk Reference Inc., Montvale, NJ, **2008**, pp. 2527–2534.

Procainamide[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
83 ± 16	67 ± 8	16 ± 5	$CL = 2.7\,CL_{cr} + 1.7$	1.9 ± 0.3	3.0 ± 0.6	M: 3.6[d]	M: 2.2 μg/mL[d]
	↓ LD		+ 3.2 (fast)[b] or + 1.1 (slow)[b]	↓ Obes	↑ RD[c]	F: 3.8[d]	F: 2.9 μg/mL[d]
			↑ Child		↓ Child, Neo		

[a] Active metabolite, *N*-acetylprocainamide (NAPA); $CL = 3.1 \pm 0.4$ mL/min/kg, $V = 1.4 \pm 0.2$ L/kg, and $t_{1/2} = 6.0 \pm 0.2$ h. [b] *CL* calculated using units of mL/min/kg for CL_{cr}. *CL* depends on NAT2 acetylation phenotype. Use a mean value of 2.2 if phenotype unknown. [c] $t_{1/2}$ for procainamide and NAPA increased in patients with RD. [d] Least square mean values following 1000-mg oral dose given twice daily to steady state in male (M) and female (F) adults. Mean peak NAPA concentrations were 2.0 and 2.2 μg/mL for male and female adults, respectively; $T_{max} = 4.1$ and 4.2 h, respectively.

References: Benet LZ, et al. Die renale Elimination von procainamide: pharmacokinetik bei niereninsuffizienz. In: Braun J, et al., eds. *Die Behandlung von Herzrhythmusstorungen bei Nierenkranken.* Karger, Basel, **1984**, pp. 96–111. Koup JR, et al. Effect of age, sex, and race on steady state procainamide pharmacokinetics after administration of Procanbid sustained-release tablets. *Ther Drug Monit,* **1998**, *20*:73–77.

Propofol[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—[b]	—	98.3–98.8[c]	27 ± 5	1.7 ± 0.7[f]	3.5 ± 1.2[f]	—	SS: 3.5 ± 0.06 μg/mL[g]
			↑ Child,[d]	↑ Child[d]			
			↓ Aged[e]	↓ Aged[e]			
							E: 1.1 ± 0.4 μg/mL[g]

[a] Data from patients undergoing elective surgery and healthy volunteers. Propofol is extensively metabolized by UGTs. [b] For IV administration only. [c] Fraction bound in whole blood. Concentration dependent; 98.8% at 0.5 μg/mL and 98.3 at 32 μg/mL. [d] *CL* and central volume increased in children 1–3 years of age. [e] *CL* and central volume decreased in elderly patients. [f] V_{area} is much larger than V_{ss}. A much longer terminal $t_{1/2}$ was reported following prolonged IV infusion. Concentration producing anesthesia after infusion to steady state (SS) and at emergence (E) from anesthesia.

References: Mazoit JX, et al. Binding of propofol to blood components: Implications for pharmacokinetics and for pharmacodynamics. *Br J Clin Pharmacol,* **1999**, *47*:35–42. Murat I, et al. Pharmacokinetics of propofol after a single dose in children aged 1–3 years with minor burns. Comparison of three data analysis approaches. *Anesthesiology,* **1996**, *84*:526–532. Servin F, et al. Pharmacokinetics of propofol infusions in patients with cirrhosis. *Br J Anaesth,* **1990**, *65*:177–183.

Propranolol[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
26 ± 10	<0.5	87 ± 6[b]	16 ± 5[c,d]	4.3 ± 0.6[c]	3.9 ± 0.4[c]	P: 1.5[e]	P: 49 ± 8 ng/mL[e]
↑ LD		↑ Preg, Obes	↑ LD	↑ Obes, Fem	↓ LD	HP: 1.0[e]	HP: 37 ± 9 ng/mL[e]
		↓ LD	↓ LD, Obes, Fem				

[a] Racemic mixture. For *S*-(–)-enantiomer (100-fold more active) compared to *R*-(+)-enantiomer, *CL* is 19% lower and V_{area} is 15% lower because of a higher degree of protein binding (18% lower free fraction); hence, there is no difference in $t_{1/2}$. Active metabolite, 4-hydroxypropranolol (HP). [b] Drug is bound primarily to α_1-acid glycoprotein, which is elevated in a number of inflammatory conditions. [c] Based on blood measurements; blood-to-plasma concentration ratio = 0.89 ± 0.03. [d] CYP2D6 catalyzes the formation of HP; CYP1A2 is responsible for most of the *N*-desisopropyl metabolite; UGT catalyzes the major conjugation pathway of elimination. [e] Following a single 80-mg oral dose given to healthy adults. Plasma accumulation factor was 3.6-fold after 80 mg was given four times daily to steady state. A concentration of 20 ng/mL gave a 50% decrease in exercise-induced cardioacceleration. Antianginal effects manifest at 15–90 ng/mL. A concentration up to 1000 ng/mL may be required for control of ventricular arrhythmias. P, propranolol.

References: Colangelo PM, et al. Age and propranolol stereoselective disposition in humans. *Clin Pharmacol Ther,* **1992**, *57*:489–494. Walle T, et al. 4-Hydroxypropranolol and its glucuronide after single and long-term doses of propranolol. *Clin Pharmacol Ther,* **1980**, *27*:22–31.

Pseudoephedrine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
~100	43–96[b]	—	7.33[b,c]	2.64-3.51[c]	4.3-8[b,c]	IR: 1.4–2[d]	IR: 177–360 ng/mL[d]
						CR: 3.8–6.1[d]	CR: 265–314 ng/mL[d]

[a] Data from healthy adult male and female subjects. [b] At a high urinary pH (>7.0), pseudoephedrine is extensively reabsorbed; $t_{1/2}$ increases, and *CL* decreases. [c] *CL/F, V/F,* and $t_{1/2}$ reported for oral dose. [d] Range of mean values from different studies following a single 60-mg immediate-release tablet or syrup (IR) or 120-mg controlled-release capsule (CR) oral dose.

Reference: Kanfer I, et al. Pharmacokinetics of oral decongestants. *Pharmacotherapy,* **1993**, *13*:116S–128S.

(*Continued*)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Pyrazinamide[a]							
—[b]	4–14[c]	10	1.1 (0.2–2.3)[d]	0.57 (0.13–1.04)[d]	6 (2–23)	1–2[e]	35 (19–103) μg/mL[e]
			↑ Child		↓ Child		

[a]Pyrazinamide is hydrolyzed in the liver to an active metabolite, 2-pyrazinoic acid. Reported peak 2-pyrazinoic acid concentrations range from 0.1- to 1-fold that of the parent drug. Pyrazinamide data reported are for male and female adults with tuberculosis. [b]Absolute bioavailability is not known, but the drug is well absorbed based on recovery of parent drug and metabolites (70%). [c]Recovery unchanged following an oral dose; the recovery of pyrazinoic acid is 37% ± 5%. [d]*CL/F* and V_{area}/F reported. [e]Following a 15- to 53-mg/kg daily oral dose to steady state.

References: Bareggi SR, et al. Clinical pharmacokinetics and metabolism of pyrazinamide in healthy volunteers. *Arzneimittelforschung*, **1987**, *37*:849–854. Lacroix C, et al. Pharmacokinetics of pyrazinamide and its metabolites in healthy subjects. *Eur J Clin Pharmacol*, **1989**, *36*:395–400. *PDR*58, **2004**, p. 766. Zhu M, et al. Population pharmacokinetic modeling of pyrazinamide in children and adults with tuberculosis. *Pharmacotherapy*, **2002**, *22*:686–695.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Quetiapine[a]							
9	<1%	83	19[b]	10 ± 4	6	1–1.8	278 ng/mL[c]
↑ Food			↓ Aged, LD				

[a]No significant sex differences. [b]Extensively metabolized through multiple pathways, including sulfoxidation, *N*- and *O*-dealkylation catalyzed by CYP3A4. Two minor active metabolites. [c]Following a 250-mg oral dose given daily for 23 days in patients with schizophrenia.

References: Goren JL, et al. Quetiapine, an atypical antipsychotic. *Pharmacotherapy*, **1998**, *18*:1183–1194. *PDR*54, **2000**, p. 563.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Quinapril[a]							
QT (Q): 52 ± 15[b]	Q (Q): 3.1 ± 1.2[c]	Q/QT: 97	QT (QT): 0.98 ± 0.22[d]	QT (QT): 0.19 ± 0.04[d]	Q (Q): 0.8-0.9[c]	Q (Q): 1.4 ± 0.8[e]	Q (Q): 207 ± 89 ng/mL[e]
	QT (QT): 96[d]				QT (QT): 2.1-2.9[d]	QT (Q): 2.3 ± 0.9[e]	QT (Q): 923 ± 277 ng/mL[e]
			↓ RD		↑ RD		

[a]Hydrolyzed to its active metabolite, quinaprilat. Pharmacokinetic data for quinapril (Q) and quinaprilat (QT) following oral Q and IV QT administration are presented. [b]Absolute bioavailability based on plasma QT concentrations. [c]Data for Q following a 2.5- to 80-mg oral Q dose. [d]Data for QT following a 2.5-mg IV QT dose. The $t_{1/2}$ of QT after dosing Q is similar. [e]Following a single 40-mg oral Q dose. No accumulation of QT with multiple dosing.

References: Breslin E, et al. A pharmacodynamic and pharmacokinetic comparison of intravenous quinaprilat and oral quinapril. *J Clin Pharmacol*, **1996**, *36*:414–421. Olson SC, et al. The clinical pharmacokinetics of quinapril. *Angiology*, **1989**, *40*:351–359. *PDR*58, **2004**, p. 2516.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Quinine[a]							
76 ± 11	N-A: 12–20	N-A: ~85–90[b]	N-A: 1.9 ± 0.5	N-A: 1.8 ± 0.4	N-A: 11 ± 2	PO: 3.5–8.4[d]	Adults
	M-A: 33 ± 18	M-A: 93–95[b]	M-A: 0.9–1.4	M-A: 1.0–1.7	M-A: 11–18		IV: 11 ± 2 μg/mL[d]
		↓ Neo	M-C: 0.4–1.4	M-C: 1.2–1.7	M-C: 12–16		PO: 7.3–9.4 μg/mL[d]
			↑ Smk	↓ Preg[c]	↓ Preg,[c] Smk		Children
			↓ Aged				IV: 8.7–9.4 μg/mL[d]
					↑ LD, Aged		PO: 7.3 ± 1.1 μg/mL[d]

[a]Data from normal adults (N-A) and range of means from different studies of adults (M-A) or children (M-C) with malaria reported. [b]Correlates with serum α_1-acid glycoprotein levels. Binding is increased in severe malaria. [c]From patients with malaria. [d]Following a single 10-mg/kg dose given as a 0.5- to 4-h IV infusion or orally (PO) to children or adults with malaria. A level >0.2 μg/mL for unbound drug is targeted for treatment of falciparum malaria. Oculotoxicity and hearing loss/tinnitus associated with unbound concentrations >2 μg/mL.

References: Edwards G, et al. Clinical pharmacokinetics in the treatment of tropical diseases. Some applications and limitations. *Clin Pharmacokinet*, **1994**, *27*:150–165. Krishna S, et al. Pharmacokinetics of quinine, chloroquine and amodiaquine. Clinical implications. *Clin Pharmacokinet*, **1996**, *30*:263–299.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Raloxifene[a]							
2[b]	<0.2	>95	735 ± 338[c]	2348 ± 1220[c]	28 (11–273)	6[d]	0.5 ± 0.3 ng/mL[d]
			↓ LD				

[a]Data from postmenopausal women. Undergoes extensive first-pass metabolism (UGT catalyzed) and enterohepatic recycling. [b]Approximately 60% absorption from the gastrointestinal tract; not significantly affected by food. [c]*CL/F* and *V/F* reported for an oral dose. [d]Following a single 1-mg/kg oral dose.

References: Hochner-Celnikier D. Pharmacokinetics of raloxifene and its clinical application. *Eur J Obstet Gynecol Reprod Biol*, **1999**, *85*:23–29. *PDR*54, **2000**, p. 1583.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Raltegravir[a]							
≥31.8 ± 9.4[b]	8.8 ± 4.7	83	16.1 (11.4, 22.6)[c]	—	α: 0.92 ± 0.21[d]	1.0[e]	4.5 (2.0, 10.2) μM[e]
					β: 12.5 ± 4.6[d]		

[a] Raltegravir undergoes *O*-glucuronidation mediated largely by UGT1A1 and to a lesser extent by UGT1A3 and UGT1A9. Raltegravir AUC is only modestly elevated in individuals with UGT1A1*28/*28 genotype compared to *1/*1 genotype. [b] The absolute oral bioavailability of raltegravir has not been determined. This minimum extent of oral absorption is based upon recovery of radioactivity in urine following oral administration of ^{14}C-labeled raltegravir in healthy human subjects. [c] Geometric mean (95% confidence interval) of pharmacokinetic parameters following a single 400-mg oral dose. Apparent oral clearance (*CL/F*) is listed. [d] Plasma concentration time course of raltegravir exhibits multiphasic washout kinetics. Initial (α) and terminal (β) $t_{1/2}$s are reported because the early phase accounts for a large portion of the AUC from time 0 to ∞. [e] Median for T_{max} and geometric mean (95% confidence interval) for C_{max} following a 400-mg twice-daily monotherapy regimen for 10 days in treatment-naive patients with HIV-1 infection.

References: FDA. Drugs@FDA. Isentress label approved on 7/8/09. Available at: http://www.accessdata.fda.gov/Scripts/cder/DrugsatFDA/. Accessed August 22, 2009. Kassahun K, et al. Metabolism and disposition in humans of raltegravir (MK-0518), an anti-AIDS drug targeting the human immunodeficiency virus 1 integrase enzyme. *Drug Metab Dispos*, **2007**, *35*:1657–1663. Wenning LA, et al. Lack of a significant drug interaction between raltegravir and tenofovir. *Antimicrob Agents Chemother*, **2008**, *52*:3253–3258. Wenning LA, et al. Pharmacokinetics of raltegravir in individuals with UGT1A1 polymorphisms. *Clin Pharmacol Ther*, **2009**, *85*:623–627.

Ramelteon[a]							
1.8[b]	<0.1%	82	883 ± 857[c]		P: 1.3 ± 0.5[e]	P: 1.6 ± 0.5	P: 6.9 ± 7.8 ng/mL[e,f]
			↓ Aged, LD[d]		M: 2.3 ± 0.5[e]		M: 110 ± 29 ng/mL[e,f]
					↑ Aged		

[a] Ramelteon undergoes primary oxidative metabolism followed by glucuronidation as secondary metabolism. CYP1A2 is the major enzyme involved in oxidative metabolism; CYP3A and CYP2C9 also are involved as minor enzymes. Remarkable elevation in C_{max} and AUC were observed with the concurrent administration of the strong CYP1A2 inhibitor fluvoxamine. The M-II metabolite contributes to the hypnotic effects of ramelteon. M-II has 1/5th to 1/10th the affinity of ramelteon as an agonist for the melatonin receptors (MT-1 and MT-2); however, it circulates at 20- to 100-fold higher concentrations relative to ramelteon. [b] Poor systemic availability of ramelteon is due to extensive first-pass metabolism. C_{max} and AUC are elevated by a high-fat meal; T_{max} is slightly delayed. [c] Intersubject variability is notably large. [d] Four- and 10-fold elevation in AUC in mild and moderate hepatic impairment. [e] P = parent drug; M = M-II metabolite. [f] C_{max} following a single 16-mg oral dose of ramelteon in young adult subjects. There is no measurable accumulation of parent drug or active metabolite because of their short elimination $t_{1/2}$.

References: FDA. Drugs@FDA. Rozerem label approved on 10/20/08. Available at: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm. Accessed August 23, 2009. Greenblatt DJ, et al. Age and sex effects on the pharmacokinetics and pharmacodynamics of ramelteon, a hypnotic agent acting via melatonin receptors MT 1 and MT2. *J Clin Pharmacol*, **2006**, *47*:485–496. McGechan A, et al. Ramelteon. *CNS Drugs*, **2005**, *19*:1057–1065.

Ramipril[a]							
R (R): 28[b]	R (R): <2[c]	R: 73 ± 2	R (R): 23[d]	—	R (R): 5 ± 2	R (R): 1.2 ± 0.3[g]	R (R): 43.3 ± 10.2 ng/mL[g]
RT (R): 48[b]	RT (R): 13 ± 6[c]	RT: 56 ± 2	RT: —[e]		RT (R): 9–18[f] ↑ RD	RT (R): 3.0 ± 0.7[g]	RT (R): 24.1 ± 5.6 ng/mL[g]

[a] Hydrolyzed to its active metabolite, ramiprilat (RT). Pharmacokinetic data for ramipril (R) and RT following oral and IV R administration are presented. [b] Based on plasma AUC of R and RT after IV and oral R administration. [c] Following an oral dose of R. [d] *CL/F* of R calculated from reported AUC data. [e] No data available; mean renal *CL* of RT is ~1.1 mL/min/kg. [f] $t_{1/2}$ for the elimination phase reported. A longer terminal $t_{1/2}$ of ~120 h most likely corresponds to the release of drug from ACE; contributes to the duration of effect but does not contribute to systemic drug accumulation. [g] Following a single 10-mg oral dose.

References: Eckert HG, et al. Pharmacokinetics and biotransformation of 2-[N-[(S)-l-ethoxycarbonyl-3-phenylpropyl]-L-alanyl]-(lS,3S,5S)-2-azabicyclo [3.3.0]octane-3-carboxylic acid (Hoe 498) in rat, dog and man. *Arzneimittelforschung,* **1984**, *34*:1435–1447. Meisel S, et al. Clinical pharmacokinetics of ramipril. *Clin Pharmacokinet,* **1994**, *26*:7–15. *PDR*58, **2004**, p. 2142. Song JC, et al. Clinical pharmacokinetics and selective pharmacodynamics of new angiotensin converting enzyme inhibitors: An update. *Clin Pharmacokinet,* **2002**, *41*:207–224. Thuillez C, et al. Pharmacokinetics, converting enzyme inhibition and peripheral arterial hemodynamics of ramipril in healthy volunteers. *Am J Cardiol,* **1987**, 59:38D–44D.

Ranitidine							
52 ± 11	69 ± 6	15 ± 3	10.4 ± 1.1	1.3 ± 0.4	2.1 ± 0.2	2.1 ± 0.31[a]	462 ± 54 ng/mL[a]
↑ LD	↓ RD		↓ RD, Aged		↑ RD, LD, Aged		

[a] Following a single 150-mg oral dose given to healthy adults. IC_{50} for inhibition of gastric acid secretion is 100 ng/mL.

Reference: Gladziwa U, et al. Pharmacokinetics and pharmacodynamics of H_2-receptor antagonists in patients with renal insufficiency. *Clin Pharmacokinet,* **1993**, *24*:319–332.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Regorafenib[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—[b]	—	99.5	0.54[c]	—	R: 28 (14–28)[d]	4	R: 3.9 (35–44) μg/mL[e]
↑ Food					M2: 20–30[d] M5: 50–60[d]		M2: 3.2 μg/mL[e] M5: 4.0 μg/mL[e]

[a] Regorafenib is cleared by multiple processes, including biliary excretion and CYP3A- and UGT1A9-dependent metabolism; two active metabolites (M2 and M5) are found in plasma at levels comparable to parent drug. Regorafenib undergoes enterohepatic cycling. [b] The absolute bioavailability is not known; administration with a low-fat meal recommended. [c] *CL/F* calculated following a single 160-mg oral dose, assuming 70-kg body weight. [d] Mean (range) $t_{1/2}$ of regorafenib (R), M2 and M5 following a single 160-mg oral dose. [e] Mean (range) concentration of regorafenib (R) and mean M2 and M5 concentrations following a 160-mg oral dose, given once a day for 21 days to patients with cancer.

References: FDA. Drugs@FDA: FDA approved drug products. Regorafenib (Stivarga). Available at: http://www.accessdata.fda.gov/scripts/cder/daf/. Accessed April 26, 2022. Shirley M, Keating G. Regorafenib: a review of its use in patients with advanced gastrointestinal stromal tumours. *Drugs,* **2015,** *75*:1009–1017.

Remifentanil[a]

—[b]	Negligible	92	40–60	0.3–0.4	0.13–0.33	—	~20 ng/mL[d]
			↓ Aged[c]	↓ Aged[c]			

[a] Data from healthy adult male subjects and patients undergoing elective surgery. Undergoes rapid inactivation by esterase-mediated hydrolysis; resulting carboxy metabolite has low activity. [b] For IV administration only. [c] *CL* and *V* decreased slightly in the elderly. [d] Mean CL_{min} following a 5-μg/kg IV dose (1-min infusion). Cp_{50} for skin incision is 2 ng/mL (determined in the presence of nitrous oxide).

References: Egan TD, et al. Remifentanil pharmacokinetics in obese versus lean patients. *Anesthesiology*, **1998**, *89*:562–573. Glass PS, et al. A review of the pharmacokinetics and pharmacodynamics of remifentanil. *Anesth Analg,* **1999**, *89*:S7–S14.

Repaglinide[a]

56 ± 7	0.3–2.6	97.4	9.3 ± 6.8[b]	0.52 ± 0.17	0.8 ± 0.2	0.25–0.75[e]	47 ± 24 ng/mL[e]
			↓ RD[c], LD[d]		↑ LD		

[a] Data from healthy adult male subjects. [b] Undergoes extensive oxidative and conjugative metabolism. CYP2C8 and CYP3A4 to a minor degree have been implicated in the metabolism of repaglinide. Repaglinide is also a substrate of OATP1B1, which is believed to contribute to the hepatic uptake of repaglinide. [c] *CL/F* reduced, severe renal impairment. [d] *CL/F* reduced, moderate to severe LD. [e] Following a single 4-mg oral dose (tablet).

References: Hatorp V, et al. Single-dose pharmacokinetics of repaglinide in subjects with chronic liver disease. *J Clin Pharmacol,* **2000**, *40*:142–152. Hatorp V, et al. Unavailability of repaglinide, a novel antidiabetic agent, administered orally in tablet or solution form or intravenously in healthy male volunteers. *Int J Clin Pharmacol Ther,* **1998**, *36*:636–641. Marbury TC, et al. Pharmacokinetics of repaglinide in subjects with renal impairment. *Clin Pharmacol Ther,* **2000**, *67*:7–15. van Heiningen PN, et al. Absorption, metabolism and excretion of a single oral dose of ^{14}C-repaglinide during repaglinide multiple dosing. *Eur J Clin Pharmacol,* **1999**, *55*:521–525.

Ribavirin[a]

45 ± 5	35 ± 8	0[b]	5.0 ± 1.0[c]	9.3 ± 1.5	28 ± 7[c]	RT: 3 ± 1.8[d]	R: 11.1 ± 1.2 μM[d]
							RT: 15.1 ± 12.8 μM[d]

[a] Values reported for studies conducted in asymptomatic HIV-positive men. [b] At steady state, red blood cell–to–plasma concentration ratio is ~60. [c] Following multiple oral dosing, *CL/F* decreases by >50%, and a long terminal $t_{1/2}$ of 150 ± 50 h is observed. [d] Following a 1200-mg oral ribavirin capsule (R) given daily for 7 days to adult subjects seropositive for HIV or a 600-mg oral REBETRON (RT) dose given twice daily to steady state to adults with hepatitis C infection.

References: Morse GD, et al. Single-dose pharmacokinetics of delavirdine mesylate and didanosine in patients with human immunodeficiency virus infection. *Antimicrob Agents Chemother,* **1997**, *47*:169–174. *PDR*54, **2000**, p. 2836. Roberts RB, et al. Ribavirin pharmacodynamics in high-risk patients for acquired immunodeficiency syndrome. *Clin Pharmacol Ther,* **1987**, *42*:365–373.

Ribociclib[a]

—	12[b]	70	6.1 (66%)[c,d]	15.6[e]	32.0 (63%)[c]	1–4	1720 (45%) ng/mL[c,f]
			↓ LD				

[a] Data from patients with advanced cancer unless otherwise specified. [b] Following 600-mg single oral dose administration in healthy subjects. Ribociclib was the major circulating drug-derived entity in plasma (44%). The major circulating metabolites included metabolite M13 (CCI284, *N*-hydroxylation), M4 (LEQ803, *N*-demethylation), and M1 (secondary glucuronide), each representing an estimated 9%, 9%, and 8% of total radioactivity, and 22%, 20%, and 18% of ribociclib exposure, respectively. Clinical activity (pharmacological and safety) of ribociclib was due primarily to parent drug, with negligible contribution from circulating metabolites. Ribociclib is extensively metabolized in the liver mainly via CYP3A. [c] Mean (CV%) in cancer patients. [d] *CL/F* reported. [e] V_{ss}/F reported. [f] Steady-state concentrations following 600 mg once a day for 21 days of a 28-day cycle; steady-state was generally achieved after 8 days. Ribociclib exhibited more than dose proportional increases in exposure (C_{max} and AUC) across the dose range of 50–1200 mg following both single dose and repeated doses.

References: FDA. Product labeling: Kisquli® (ribociclib oral capsules). Drugs@FDA: FDA-approved drugs. Available at: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm. Accessed April 26, 2022. FDA. Ribociclib NDA and label. NDA approved in 2017; label revised 07/2020. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/209092s005lbl.pdf Accessed April 2, 2021

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Rifampin[a]							
—[b]	7 ± 3	60–90	3.5 ± 1.6[c]	0.97 ± 0.36	3.5 ± 0.8[c]	1–3[e]	6.5 ± 3.5 μg/mL[e]
	↑ Neo		↑ Neo, ↓ RD[d]	↑ Neo	↑ LD, RD[d]		

[a]Active desacetyl metabolite. [b]Absolute bioavailability is not known, although some studies indicate complete absorption. Such reports presumably refer to rifampin plus its desacetyl metabolite because considerable first-pass metabolism is expected. [c]$t_{1/2}$ is shorter (1.7 ± 0.5) and *CL/F* is higher after repeated administration. Rifampin is a strong enzyme (CYP3A and others) inducer and appears to autoinduce its own metabolism. [d]Not observed with 300-mg doses but pronounced differences with 900-mg doses. $t_{1/2}$ is longer with high single doses. [e]Following a 600-mg dose given once daily for 15–18 days to patients with tuberculosis.

Reference: Israili ZH, et al. Pharmacokinetics of antituberculosis drugs in patients. *J Clin Pharmacol,* **1987**, *27*:78–83.

Riluzole							
64 (30–100)	<1	98	5.5 ± 0.9[b]	3.4 ± 0.6	14 ± 6	0.8 ± 0.5[c]	173 ± 72 ng/mL[c]
↓ Food[a]			↓ LD				

[a]High-fat meal. [b]Eliminated primarily by CYP1A2-dependent metabolism; metabolites are inactive. Involvement of CYP1A2 may contribute to ethnic (lower *CL/F* in Japanese) and sex (lower *CL* in women) differences and inductive effects of smoking (higher *CL* in smokers). [c]Following a 50-mg oral dose given twice daily to steady state.

References: Bruno R, et al. Population pharmacokinetics of riluzole in patients with amyotrophic lateral sclerosis. *Clin Pharmacol Ther,* **1997**, *62*:518–526. Le Liboux A, et al. Single- and multiple-dose pharmacokinetics of riluzole in white subjects. *J Clin Pharmacol,* **1997**, *37*:820–827. *PDR*58, **2004**, p. 769. Wokke J. Riluzole. *Lancet,* **1996**, *348*:795–799.

Risperidone[a]							
PO: 66 ± 28[b]	3 ± 2[b]	89[c]	5.4 ± 1.4[b]	1.1 ± 0.2	3.2 ± 0.8[a,b]	R: ~l[e]	R: 10 ng/mL[e]
IM: 103 ± 13			↓ RD[c], Aged[d]		↑ RD[c], Aged[d]		TA: 45 ng/mL[e]

[a]The active metabolite, 9-hydroxyrisperidone, is the predominant circulating species in extensive metabolizers and is equipotent to parent drug. 9-Hydroxyrisperidone has a $t_{1/2}$ of 20 ± 3 h. In extensive metabolizers, 35% ± 7% of an IV dose is excreted as this metabolite; its elimination is primarily renal and therefore correlates with renal function. Formation of 9-hydroxyrisperidone is catalyzed by CYP2D6. [b]Parameters reported for extensive metabolizers. In poor metabolizers, *F* is higher; ~20% of an IV dose is excreted unchanged, 10% as the 9-hydroxy metabolite; *CL* is slightly <1 mL/min/kg, and $t_{1/2}$ is similar to that of the active metabolite, ~20 h. [c]77% for 9-hydroxyrisperidone. [d]Changes in elderly subjects due to decreased renal function affecting the elimination of the active metabolite. [e]Mean steady-state C_{min} for risperidone (R) and total active (TA) drug, risperidone + 9-OH-risperidone, following a 3-mg oral dose given twice daily to patients with chronic schizophrenia. No difference in total active drug levels between CYP2D6 extensive and poor metabolizers.

References: Cohen LJ. Risperidone. *Pharmacotherapy,* **1994**, *14*:253–265. Heykants J, et al. The pharmacokinetics of risperidone in humans: a summary. *J Clin Psychiatry,* **1994**, *55*(suppl):13–17.

Ritonavir							
—[a]	3.5 ± 1.8	98–99	SD: 1.2 ± 0.4[b,c]	0.41 ± 0.25[c]	3–5[c]	2–4[e]	11 ± 4 μg/mL[e]
↑ Food			MD: 2.1 ± 0.8[c]		↑ LD[d]		
			↓ Child, LD[d]				

[a]Absolute bioavailability unknown (>60% absorbed); food elicits a 15% increase in oral AUC for capsule formulation. [b]Ritonavir is extensively metabolized primarily by CYP3A4. It also appears to induce its own *CL* with single-dose (SD) to multiple-dose (MD) administration. [c]*CL/F*, V_{area}/F, and $t_{1/2}$ reported for oral dose. [d]*CL/F* reduced slightly and $t_{1/2}$ increased slightly, moderate liver impairment. [e]Following a 600-mg oral dose given twice daily to steady state.

References: Hsu A, et al. Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. *Clin Pharmacokinet,* **1998**, *35*:275–291. *PDR*54, **2000**, p. 465.

Rivaroxaban							
80–100[a]	~40	92–95	2.33[b] ↓ RD	0.62	5–9 ↑ Aged	2.5 (1–4)[c]	138 (77–251) ng/mL[c]

[a]The oral bioavailability is lower (~60%) at doses >10 mg; food increases the fraction absorbed at those higher doses. [b]Rivaroxaban is cleared from blood by both renal and multiple biotransformation routes, including CYP3A-dependent metabolism. [c]Mean (range) following a single 10-mg oral dose in the fasted stated.

References: FDA. Drugs@FDA: FDA-approved drug products. Rivaroxaban (Xarelto). Available at: http://www.accessdata.fda.gov/scripts/cder/daf/. Accessed April 26, 2022. Mueck W, et al. Clinical pharmacokinetics and pharmacodynamics profile of rivaroxaban. *Clin Pharmacokinet,* **2014,** *53*:1–16.

Rivastigmine							
72 (22–119)[a]	Negligible	40	13 ± 4[b,c]	1.5 ± 0.6[c]	1.4 ± 0.4[c,d]	1.2 ± 1.0[e]	26 ± 10 ng/mL[e]
↑ Food, Dose							

[a]Following a 6-mg oral dose. Bioavailability increases with dose; following a 3-mg dose, the median bioavailability is 36%. [b]Rivastigmine is metabolized by cholinesterase. No apparent sex differences. [c]IV dose of 2 mg. [d]The pharmacodynamic $t_{1/2}$ is ~10 h due to tight binding to acetylcholinesterase. [e]Following oral administration of a 6-mg capsule. C_{max} increases more than proportionally at doses >3 mg.

References: Hossain M, et al. Estimation of the absolute bioavailability of rivastigmine in patients with mild to moderate dementia of the Alzheimer's type. *Clin Pharmacokinet,* **2002**, *41*:225–234. Williams BR, et al. A review of rivastigmine: a reversible cholinesterase inhibitor. *Clin Ther,* **2003**, 25:1634–1653.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Rizatriptan[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
47	F: 28 ± 9[b]	14	F: 12.3 ± 1.4[b]	F: 1.5 ± 0.2	F: 2.2	SD: 0.9 ± 0.4[e]	SD: 20 ± 4.9 ng/mL[e]
	M: 29[b]		M: 18.9 ± 2.8[b]	M: 2.2 ± 0.4	M: 2.4	MD: 4.8 ± 0.7[e]	MD: 37 ± 13 ng/mL[e]
			↓ LD[c], RD[d]				

[a]Data from healthy adult male (M) and female (F) subjects. Oxidative deamination catalyzed by MAO-A is the primary route of elimination. *N*-desmethyl rizatriptan (DMR) is a minor metabolite (~14%) that is active and accumulates in blood. [b]Evidence of minor dose-dependent metabolic *CL* and urinary excretion. [c]*CL/F* reduced, moderate hepatic impairment. [d]*CL/F* reduced, severe renal impairment. [e]Following a 10-mg single (SD) and multiple (MD) oral dose (10 mg every 2 h × 3 doses × 4 days). DMR C_{max} is 8.5 and 26.2 ng/mL with SD and MD, respectively.

References: Goldberg MR, et al. Rizatriptan, a novel $5\text{-HT}_{1B/1D}$ agonist for migraine: single-and multiple-dose tolerability and pharmacokinetics in healthy subjects. *J Clin Pharmacol,* **2000**, *40*:74–83. Lee Y, et al. Pharmacokinetics and tolerability of intravenous rizatriptan in healthy females. *Biopharm Drug Dispos,* **1998**, *19*:577–581. *PDR*54, **2000**, p. 1912. Vyas KP, et al. Disposition and pharmacokinetics of the antimigraine drug, rizatriptan, in humans. *Drug Metab Dispos,* **2000**, *28*:89–95.

Ropinirole[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
55	<10	~40	11.2 ± 5.0[b]	7.5 ± 2.4[b]	6[b]	1.0 (0.5–6.0)[d]	7.4 (2.4–13) ng/mL[d]
			↓ Aged[c]				

[a]Data from male and female patients with Parkinson disease. Metabolized primarily by CYP1A2 to inactive *N*-deisopropyl and hydroxy metabolites. [b]*CL/F*, V_d/F, and $t_{1/2}$ reported for oral dose. [c]*CL/F* reduced but dose titrated to desired effect. [d]Following a 2-mg oral dose given three times daily to steady state. Food increases the T_{max} and decreases the C_{max}.

References: Bloomer JC, et al. In vitro identification of the P450 enzymes responsible for the metabolism of ropinirole. *Drug Metab Dispos,* **1997**, *25*:840–844. *PDR*54, **2000**, p. 3037. Taylor AC, et al. Lack of a pharmacokinetic interaction at steady state between ropinirole and L-dopa in patients with Parkinson's disease. *Pharmacotherapy,* **1999**, *79*:150–156.

Rosuvastatin[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
20 (17–23)	30 ± 7	88	10.5 ± 4.7[b]	1.7 ± 0.5	20 ± 6	3 (1–6)[d]	4.6 ± 2.1 ng/mL[d]
			↓ RD[c]				

[a]Data from healthy men reported; no significant sex or age differences. [b]Eliminated primarily by biliary excretion; also appears to be actively transported into the liver by an organic anion transport protein (polymorphic OATP1B1, among others). [c]Reduced *CL/F* in patients with severe renal impairment. [d]Following a 10-mg oral dose taken once daily for 10 days.

References: Martin PD, et al. Absolute oral bioavailability of rosuvastatin in healthy white adult male volunteers. *Clin Ther,* **2003**, *25*:2553–2563. Martin PD, et al. Pharmacodynamic effects and pharmacokinetics of a new HMG-CoA reductase inhibitor, rosuvastatin, after morning or evening administration in healthy volunteers. *Br J Clin Pharmacol,* **2002**, *54*:472–477. Product labeling: Crestor® tablets (rosuvastatin calcium). Astra-Zeneca Pharmaceuticals LP, Wilmington, DE, **2003**. Schneck DW, et al. The effect of gemfibrozil on the pharmacokinetics of rosuvastatin. *Clin Pharmacol Ther,* **2004**, *75*:455–463.

Rucaparib[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
36 (30–45)[b]	44.9[c]	70	3.6–18.9[d]	1.6–3.7[e]	25.9[c]	1.9[c]	1940 (54%) ng/mL[f]
↑ Food			↓ LD				

[a]Data from patients with cancer. [b]Mean (range) absolute bioavailability of rucaparib immediate-release tablet. [c]Following 600-mg single dose oral administration. [d]*CL/F* measured at steady state after 600 mg twice-a-day dosing. [e]Following a single IV dose of 12–40 mg. [f]Mean (CV%) measured in cancer patients. Based on population pharmacokinetic analyses, steady-state concentrations following rucaparib 600 mg twice a day did not differ significantly across CYP1A2 or CYP2D6 genotype subgroups.

References: FDA. Product labeling: Rubraca® (rucaparib oral tablets). Drugs@FDA: FDA-approved drugs. Available at: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm. Accessed April 26, 2022. FDA. Rucaparib NDA and label. NDA approved in 2016; label revised 10/2020. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/209115s008lbl.pdf Accessed April 2, 2021. Grechko N, et al. Pharmacokinetics and safety of rucaparib in patients with advanced solid tumors and hepatic impairment. *Cancer Chemother Pharmacol,* **2021**, *88*:259–270. Liao ML, et al. Evaluation of absorption, distribution, metabolism, and excretion of [14 C]-rucaparib, a poly(ADP-ribose) polymerase inhibitor, in patients with advanced solid tumors. *Invest New Drugs*, **2020**, *38*:765–775.

Selegiline[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Negligible[b]	Negligible	94[c]	~1500[b]	1.9	1.91 ± 1.0[e]	S: 0.7 ± 0.4[f]	S: 1.1 ± 0.4 ng/mL[f]
			160[d]			DS: ~1 h	DS: ~15 ng/mL[f]

[a]MAO-B active metabolite: *l*-(–)-desmethylselegiline. [b]Extensive first-pass metabolism; estimate of *CL/F* reported. [c]Blood-to-plasma concentration ratio = 1.3–2.2 for parent drug and ~0.55 for *N*-desmethyl metabolite. [d]*CL/F* for *N*-desmethylselegiline, assuming quantitative conversion of parent to this metabolite. [e]For parent and *N*-desmethyl metabolite. $t_{1/2}$s for methamphetamine (major plasma species) and amphetamine are 21 and 18 h, respectively. [f]Mean data for selegiline (S) and its active metabolite, *N*-desmethylselegiline (DS), following a single 10-mg oral dose given to adults.

Reference: Heinonen EH, et al. Pharmacokinetic aspects of *l*-deprenyl (selegiline) and its metabolites. *Clin Pharmacol Ther*, **1994**, *56*:742–749.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Sertraline

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—[a]	<1	98–99	38 ± 14[b]	—	23	M: 6.9 ± 1.0[c]	M: 118 ± 22 ng/mL[c]
			↓ Aged, LD		↑ Aged, LD	F: 6.7 ± 1.8[c]	F: 166 ± 65 ng/mL[c]

[a]Absolute bioavailability is not known (>44% absorbed); undergoes extensive first-pass metabolism to essentially inactive metabolites; catalyzed by multiple CYP isoforms. [b]*CL/F* reported. [c]Following a dose titration up to 200 mg given once daily for 30 days to healthy male (M) and female (F) adults.

References: van Harten J. Clinical pharmacokinetics of selective serotonin reuptake inhibitors. *Clin Pharmacokinet*, **1993**, *24*:203–220. Warrington SJ. Clinical implications of the pharmacology of sertraline. *Int Clin Psychopharmacol*, **1994**, *6*(suppl 2):11–21.

Sildenafil[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
38	0	96	6.0 ± 1.1	1.2 ± 0.3	2.4 ± 1.0	1.2 ± 0.3[d]	212 ± 59 ng/mL[d]
			↓ LD[b], RD[c], Aged				

[a]Data from healthy male subjects. Sildenafil is metabolized primarily by CYP3A and secondarily by CYP2C9. Piperazine *N*-desmethyl metabolite is active (~50% parent) and accumulates in plasma (~40% parent). [b]*CL/F* reduced, mild to moderate hepatic impairment. [c]*CL/F* reduced, severe renal impairment. Increased unbound concentrations. [d]Following a single 50-mg oral (solution) dose.

References: *PDR*54, **2000**, p. 2382. Walker DK, et al. Pharmacokinetics and metabolism of sildenafil in mouse, rat, rabbit, dog and man. *Xenobiotica*, **1999**, *29*:297–310.

Simvastatin[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
≤5	Negligible	94	7.6[b]	—	2–3	AI: 1.4 ± 1.0[c]	AI: 46 ± 20 ngEq/mL[c]
						TI: 1.4 ± 1.0[c]	TI: 56 ± 25 ngEq/mL[c]

[a]Simvastatin is a lactone prodrug that is hydrolyzed to the active corresponding β-hydroxy acid. Values reported are for the disposition of the acid. [b]The β-hydroxy acid can be reconverted back to the lactone; irreversible oxidative metabolites are generated by CYP3A. [c]Data for active inhibitors (AI, ring-opened molecule) and total inhibitors (TI) following a 40-mg oral dose given once daily for 17 days to healthy adults.

References: Corsini A, et al. New insights into the pharmacodynamic and pharmacokinetic properties of statins. *Pharmacol Ther*, **1999**, *84*:413–428. Desager JP, et al. Clinical pharmacokinetics of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors. *Clin Pharmacokinet*, **1996**, *31*:348–371. Mauro VF. Clinical pharmacokinetics and practical applications of simvastatin. *Clin Pharmacokinet*, **1993**, *24*:195–202.

Sirolimus[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
~15[b]	—	40[c]	3.47 ± 1.58[d]	12 ± 4.6[d]	62.3 ± 16.2[d]	SD: 0.81 ± 0.17[e]	SD: 67 ± 23 ng/mL[e]
↑ Food[b]						MD: 1.4 ± 1.2[e]	MD: 94–210 ng/mL[e]

[a]Data from male and female renal transplant patients. All subjects were on a stable cyclosporine regimen. Sirolimus is metabolized primarily by CYP3A and is a substrate for P-glycoprotein. Several sirolimus metabolites are pharmacologically active. [b]Cyclosporine coadministration increases sirolimus bioavailability. *F* increased by high-fat meal. [c]Concentrates in blood cells; blood-to-plasma concentration ratio ~38 ± 13. [d]Blood *CL/F*, V_{ss}/F, and $t_{1/2}$ reported for oral dose. [e]Following a single 15-mg oral dose (SD) in healthy subjects and 4- to 6.5-mg/m² oral dose (with cyclosporine) given twice daily to steady state (MD) in renal transplant patients.

References: Kelly PA, et al. Conversion from liquid to solid rapamycin formulations in stable renal allograft transplant recipients. *Biopharm Drug Dispos*, **1999**, *20*:249–253. Zimmerman JJ, et al. Pharmacokinetics of sirolimus in stable renal transplant patients after multiple oral dose administration. *J Clin Pharmacol*, **1997**, *37*:405–415. Zimmerman JJ, et al. The effect of a high-fat meal on the oral bioavailability of the immunosuppressant sirolimus (rapamycin). *J Clin Pharmacol*, **1999**, *39*:1155–1161.

Sitagliptin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
87 ± 5.2	73.1 ± 15.9	38	4.42[a]	—	13.9 ± 2.0	1.5 ± 1.3	1046 ± 286 nM[c]
			↓ RD[b]				

[a]Cleared primarily by the kidney. Renal clearance is ~350 mL/min, which indicates active tubular secretion, possibly mediated by human organic anion transporter-3 (OAT3) and P-glycoprotein (ABCB1). [b]Apparent oral clearance increased by a respective 2.3-, 3.8- and 4.5-fold in patients with moderate (CL_{cr} = 30–50 mL/min) and severe (<30 mL/min) renal insufficiency and in patients with end-stage RD requiring hemodialysis. [c]Following a single 100-mg oral dose. Plasma AUC increased by ~14% following daily doses of 100 mg at steady state compared to the first dose.

References: Bergman A, et al. Absolute bioavailability of sitagliptin, an oral dipeptidyl peptidase-4 inhibitor, in healthy volunteers. *Biopharm Drug Dispos*, **2007**, *28*:315–22. Bergman AJ, et al. Effect of renal insufficiency on the pharmacokinetics of sitagliptin, a dipeptidyl peptidase-4 inhibitor. *Diabetes Care*, **2007**, *30*:1862–1864. FDA. Drugs@FDA. Januvia label approved on 7/22/08. Available at: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm. Accessed December 26, 2009. Migoya EM, et al. Effect of moderate hepatic insufficiency on the pharmacokinetics of sitagliptin. *Can J Clin Pharmacol*, **2009**, *16*:e165–e170. Vincent SH, et al. Metabolism and excretion of the dipeptidyl peptidase 4 inhibitor [^{14}C]sitagliptin in humans. *Drug Metab Dispos*, **2007**, *35*:533–538.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Sofosbuvir[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—[b]	—[c]	~82[d]	—	—	0.68 (0.53–1.00)[f]	1.00 (0.50–1.50)[f]	1356 (63.0) ng/mL
			↓ RD[e]				

[a]Sofosbuvir is sequentially metabolized to a pharmacologically active nucleoside analogue triphosphate; the active metabolite is undetected in plasma. The first hydrolytic step appears to be rapid (much of it may occur during first pass), yielding the intermediate metabolite, GS566500, which can either be fully activated by kinases to GS-461203 or inactivated by phosphatase activity to GS-331007. [b]The absolute oral bioavailability of sofosbuvir in humans is not known. Administration with a high-fat meal increases systemic sofosbuvir exposure by 67%–91%, although this is not thought to be clinically meaningful. [c]3.5% of the oral dose is recovered as unchanged sofosbuvir in urine; the true fraction excreted unchanged may be much higher, depending on the absolute bioavailability. [d]Also reported to be 62%–65% based on *in vitro* ultrafiltration experiments. [f]Systemic exposure (AUC) of sofosbuvir and GS-566500 increased 171% and 244%, respectively, in patients with severe renal impairment; smaller increases seen with less severe disease. [e]Mean (25th and 75th quartiles or CV%) reported following administration of 400-mg oral sofosbuvir, given once a day for 27 days.

Reference: FDA. Drugs@FDA: FDA-approved drug products. Sofosbuvir (Sovaldi). Available at: http://www.accessdata.fda.gov/scripts/cder/daf/. Accessed April 26, 2022. Kirby BJ, et al. Pharmacokinetic, pharmacodynamics and drug interaction profile of the hepatitis C virus NS5B polymerase inhibitor sofosbuvir. *Clin Pharmacokinet,* **2015**, 54:677–690.

Solifenacin[a]

90	3–6	98[b]	9.39 ± 2.68	671 ± 118	52.4 ± 13.9	4.2 ± 1.8[c]	40.6 ± 8.5 ng/mL[d]
			↓ LD, RD[c]		↑ LD, RD[c]		

[a]Solifenacin is extensively metabolized by CYP3A. The 4*R*-hydroxy-solifenacin metabolite is pharmacologically active but not likely to contribute to the therapeutic efficacy of solifenacin because of low circulating levels. [b]Primarily bound to α_1-acid glycoprotein. [c]Dosage reduction is advised in patients with severe renal impairment (CL_{cr} <30 mL/min), in whom a 2-fold reduction in clearance and prolongation in $t_{1/2}$ are expected. [d]At steady state following 21 days of dosing with 10 mg once daily.

References: FDA. Drugs@FDA. VESIcare label approved on 11/18/08. Available at: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm. Accessed December 27, 2009. Kuipers M, et al. Open-label study of the safety and pharmacokinetics of solifenacin in subjects with hepatic impairment. *J Pharmacol Sci*, **2006**, *102*:405–412. Kuipers ME, et al. Solifenacin demonstrates high absolute bioavailability in healthy men. *Drugs*, **2004**, *5*:73–81. Smulders RA, et al. Pharmacokinetics and safety of solifenacin succinate in healthy young men. *J Clin Pharmacol*, **2004,** *44*:1023–1033. Smulders RA, et al. Pharmacokinetics, safety, and tolerability of solifenacin in patients with renal insufficiency. *J Pharmacol Sci*, **2007**, *103*:67–74.

Sorafenib

—[a]	—	99.5	1.31[b,c]	3.19[c]	25.6 (20)[c]	3	7.7 (65.3) μg/mL[d]

[a]The absolute bioavailability is not known, and a significant fraction of the oral dose is recovered in feces as unchanged drug, suggesting either poor absorption or significant biliary excretion. [b]Sorafenib is cleared by CYP3A4- and UGT1A9-dependent metabolism. [c]CL/F, V_β/F, and $t_{1/2}$ calculated following a single 400-mg oral dose assuming 70-kg body weight. [d]Mean (CV%) following a 400-mg oral dose given twice daily to steady state in cancer patients.

References: FDA. Drugs@FDA: FDA-approved drug products. Sorafenib (Nexavar). Available at: http://www.accessdata.fda.gov/scripts/cder/daf/. Accessed April 26, 2022. van Erp NP, et al. Clinical pharmacokinetics of tyrosine kinase inhibitors. *Cancer Treat Rev,* **2009,** *35*:692–706.

Sotalol[a]

60–100	70 ± 15	Negligible	2.20 ± 0.67	1.21 ± 0.17	7.18 ± 1.30	3.1 ± 0.6	1.0 ± 0.5 μg/mL[b]
			↓ RD		↑ RD		

[a]Sotalol is available as a racemate. The enantiomers contribute equally to sotalol's antiarrhythmic action; hence, pharmacokinetic parameters for the total enantiomeric mixture is reported herein. β-Adrenoreceptor blockade resides solely with *S*-(–)-isomer. [b]Following 80-mg, twice-a-day dosing to steady state.

References: Berglund G, et al. Pharmacokinetics of sotalol after chronic administration to patients with renal insufficiency. *Eur J Clin Pharmacol*, **1980**, *18*:321–326. Kimura M, et al. Pharmacokinetics and pharmacodynamics of (+)-sotalol in healthy male volunteers. *Br J Clin Pharmacol*, **1996**, *42*:583–588. Poirier JM, et al. The pharmacokinetics of d-sotalol and d,l-sotalol in healthy volunteers. *Eur J Clin Pharmacol,* **1990**, *38*:579–582.

Spironolactone[a]

—[b]	<1[c]	>90[d]	93[e]	10[e]	S: 1.3 ± 0.3[f]	S: 1.0[f]	S: 185 ± 51 ng/mL[f]
↑ Food					C: 11.2 ± 2.3[f]	C: 2.9 ± 0.6[f]	C: 231 ± 49 ng/mL[f]
					TS: 2.8 ± 0.4[f]	TS: 1.8 ± 0.5[f]	TS: 571 ± 74 ng/mL[f]
					HTS: 10.1 ± 2.3[f]	HTS: 3.1 ± 0.9[f]	HTS: 202 ± 54 ng/mL[f]
					↑ LD[g]		

[a]Spironolactone (S) is extensively metabolized; it has three known active metabolites: canrenone (C), 7α-thiomethylspironolactone (TS), and 6β-hydroxy-7α-thiomethylspironolactone (HTS). [b]Absolute bioavailability is not known; old values reported in the literature were based on nonspecific assays for C; likely to exhibit first-pass metabolism. AUC of parent drug and metabolites increased when S taken with food. [c]Measured after an oral dose. [d]Binding of S and its active metabolites. [e]CL/F and V_{area}/F; calculated from reported AUC and $t_{1/2}$ data. [f]Following a single 200-mg oral dose of S. C accumulates 2.5-fold with multiple S dosing. [g]$t_{1/2}$ of parent drug and metabolites increased in patients with cirrhosis.

References: Ho PC, et al. Pharmacokinetics of canrenone and metabolites after base hydrolysis following single and multiple dose oral administration of spironolactone. *Eur J Clin Pharmacol*, **1984**, *27*:441–446. Overdiek HW, et al. Influence of food on the bioavailability of spironolactone. *Clin Pharmacol Ther*, **1986**, *40*:531–536. Overdiek HW, et al. New insights into the pharmacokinetics of spironolactone. *Clin Pharmacol Ther*, **1985**, *38*:469–474. *PDR*54, **2000**, p. 2883. Sungaila I, et al. Spironolactone pharmacokinetics and pharmacodynamics in patients with cirrhotic ascites. *Gastroenterology*, **1992**, *102*:1680–1685.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Sulfamethoxazole							
~100	14 ± 2	53 ± 5	0.31 ± 0.07[a,b]	0.26 ± 0.04[a]	10.1 ± 2.6[a]	4[b]	37.1 μg/mL[b]
		↓ RD		↑ RD	↑ RD		

[a]Studies include concurrent administration of trimethoprim and variation in urinary pH; these factors had no marked effect on the *CL* of sulfamethoxazole. Metabolically cleared primarily by N_4-acetylation. [b]Following a single 1000-mg oral dose given to healthy adults.

References: Hutabarat RM, et al. Disposition of drugs in cystic fibrosis. I. Sulfamethoxazole and trimethoprim. *Clin Pharmacol Ther*, **1991**, *49*:402–409. Welling PO, et al. Pharmacokinetics of trimethoprim and sulfamethoxazole in normal subjects and in patients with renal failure. *J Infect Dis*, **1973**, *128*(suppl):556–566.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Sumatriptan							
PO: 14 ± 5	22 ± 4	14–21	22 ± 5.4	2.0 ± 0.34	1.0 ± 0.3[a]	SC: 0.2 (0.1–0.3)[b]	SC: 72 (55–108) ng/mL[b]
SC: 97 ± 16						PO: ~1.5[b]	PO: 54 (27–137) ng/mL[b]

[a]An apparent $t_{1/2}$ of ~2 h reported for SC and oral doses. [b]Following a single 6-mg SC or 100-mg oral dose given to healthy young adults.

References: Scott AK. Sumatriptan clinical pharmacokinetics. *Clin Pharmacokinet*, **1994**, *27*:337–344. Scott AK, et al. Sumatriptan and cerebral perfusion in healthy volunteers. *Br J Clin Pharmacol,* **1992**, *33*:401–404.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Tacrolimus							
25 ± 10[a,b]	<1	75–99[c]	0.90 ± 0.29[a]	0.91 ± 0.29[a,d]	12 ± 5[a]	1.4 ± 0.5[e]	31.2 ± 10.1 ng/mL[e]
↓ Food				↑ LD	↑ LD		

[a]Drug disposition parameters calculated from blood concentrations. Data from liver transplant patients reported. Metabolized by CYP3A; also a substrate for P-glycoprotein. [b]A similar bioavailability (F = 21% ± 19%) reported for kidney transplant patients; F = 16% ± 7% for normal subjects. Low oral bioavailability likely due to incomplete intestinal availability. [c]Different values for plasma protein binding reported. Concentrates in blood cells; blood-to-plasma concentration ratio = 35 (12–67). [d]Slightly higher V_{ss} and $t_{1/2}$ reported for kidney transplant patients. Because of the very high and variable blood-to-plasma concentration ratio, markedly different V_{ss} values are reported for parameters based on plasma concentrations. [e]Following a single 7-mg oral dose given to healthy adults. Consensus target C_{min} at steady state is 5–20 ng/mL.

References: Bekersky I, et al. Dose linearity after oral administration of tacrolimus 1-mg capsules at doses of 3, 7, and 10 mg. *Clin Ther*, **1999**, *27*:2058–2064. Jusko WJ, et al. Pharmacokinetics of tacrolimus in liver transplant patients. *Clin Pharmacol Ther*, **1995**, *57*:281–290. *PDR*54, **2000**, pp. 1098–1099.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Tadalafil							
—	—	94	0.59[a,b]	0.89[b]	17.5	2[d]	378 ng/mL[d]
			↓ RD[c]				

[a]Eliminated primarily by CYP3A4-dependent metabolism. [b]*CL/F* and *V/F* reported. [c]AUC increased in patients with mild or moderate (2-fold) and severe (4-fold) renal insufficiency. [d]Following a single 20-mg oral dose.

References: Curran M, et al. Tadalafil. *Drugs*, **2003**, *63*:2203–2212; discussion 2213–2214. Product labeling: Cialis® (tadalafil tablets). Lilly Icos, Bothell, WA, **2004**.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Tafenoquine[a]							
— ↑ Food[b]	—[c]	>99.5	0.71[d]	22.9[e]	360 (336–456)	12–15	200 (20%) ng/mL[f]

[a]Data from female and male heathy subjects. [b]Tafenoquine was administered as an investigational capsule formulation with a high-calorie, high-fat meal. [c]After single oral dose administration of tafenoquine, the only circulating component is unchanged tafenoquine, with no major systemic metabolites observed in blood or plasma. Over a 6-day collection period, renal elimination of unchanged tafenoquine was negligible. [d]CL/F reported. [e]The apparent volume of distribution after oral dose (V/F) reported. [f]Mean (CV%) measured in healthy subjects following 300-mg single oral dose to healthy subjects.

References: FDA. Product labeling: Krintafel (tafenoquine oral tablets). Drugs@FDA: FDA-approved drugs. Available at: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm. Accessed April 26, 2022. FDA. Tefenoquine NDA and label. NDA approved in 2018; label revised 11/2020. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/210795s001lbl.pdf. Accessed April 2, 2021.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Tamoxifen[a]							
—	<1	>98	1.4[b,c]	50–60[b]	4–11 days[d]	5 (3–7)[e]	120 (67–183) ng/mL[e]

[a]Has active metabolites; 4-hydroxytamoxifen and 4-hydroxy-*N*-desmethyltamoxifen (endoxifen) are minor metabolites that exhibit affinity for the estrogen receptor that is greater than that of parent *trans*-tamoxifen. The $t_{1/2}$ of all metabolites are rate limited by tamoxifen elimination. [b]*CL/F* and V_{area}/F reported. [c]The major pathway of elimination, *N*-demethylation, is catalyzed by CYP3A. Polymorphic CYP2D6 catalyzes the 4-hydroxylation step key to metabolite activity. [d]$t_{1/2}$ consistent with accumulation and approach to steady state. Significantly longer terminal $t_{1/2}$s are observed. [e]Average C_{ss} following a 10-mg oral dose given twice daily to steady state.

References: Lønning PE, et al. Pharmacological and clinical profile of anastrozole. *Breast Cancer Res Treat*, **1998**, *49*(suppl 1):S53–S57. *PDR*54, **2000**, p. 557.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
Tamsulosin[a]							
100	12.7 ± 3.0	99 ± 1	0.62 ± 0.31[b]	0.20 ± 0.06	6.8 ± 3.5[d]	5.3 ± 0.7[e]	16 ± 5 ng/mL[e]
↓ Food		↑ RD	↑ RD[c], Aged		↑ RD, Aged		

[a]Data from healthy male subjects. [b]Metabolized primarily by CYP3A and CYP2D6. [c]*CL/F* reduced, moderate renal impairment. Unbound AUC relatively unchanged. [d]Apparent $t_{1/2}$ after oral dose in patients is ~14–15 h, reflecting controlled release from modified-release granules. [e]Following a single 0.4-mg modified-release oral dose in healthy subjects.

References: Matsushima H, et al. Plasma protein binding of tamsulosin hydrochloride in renal disease: role of α_1-acid glycoprotein and possibility of binding interactions. *Eur J Clin Pharmacol*, **1999**, *55*:437–443. van Hoogdalem EJ, et al. Disposition of the selective α_{1A} adrenoceptor antagonist tamsulosin in humans: comparison with data from interspecies scaling. *J Pharm Sci*, **1997**, *86*:1156–1161. Wolzt M, et al. Pharmacokinetics of tamsulosin in subjects with normal and varying degrees of impaired renal function: an open-label single-dose and multiple-dose study. *Eur J Clin Pharmacol*, **1998**, *4*:367–373.

(*Continued*)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Tecovirimat[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
—	—[c]	77–82	12.1 ± 7.9[d]	19.3 ± 12.0[e]	24 ± 14	4–6[f]	2106 (33%) ng/mL[g,h]
↑ Food[b]							

[a]Data from female and male adult healthy subjects. [b]Administration of tecovirimat with a moderate-fat meal increased tecovirimat AUC by 49% compared to the fasted state. [c]Tecovirimat is extensively metabolized, predominantly by hydrolysis of the amide bond and glucuronidation. After a single oral dose administration of $[^{14}C]$-tecovirimat in mass balance study, 73% of the dose was excreted in urine, predominantly as metabolites. [d]*CL/F* at steady state reported following 600-mg oral daily dose for 14 days. [e]The apparent volume of distribution during the terminal phase (V_{area}/F) reported, after oral administration of 600-mg daily dose. [f]Value reflects administration of drug with food. [g]Mean (CV%) measured in healthy subjects. [h]Steady-state concentrations at the recommended dosage of 600 mg twice a day for 14 days in adults; steady-state AUC is achieved by day 6.

References: Chinsangaram J, et al. Safety and pharmacokinetics of the anti-orthopoxvirus compound ST-246 following a single daily oral dose for 14 days in human volunteers. *Antimicrob Agents Chemother,* **2012**, *56*:4900–49055. FDA. Product labeling: Tpoxx (tecovirimat oral capsules). Drugs@FDA: FDA-approved drugs. Available at: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm. Accessed April 26, 2022. FDA. Tecovirimat NDA and label. NDA approved in 2018; label revised 07/2018. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/208627s000lbl.pdf. Accessed April 2, 2021.

Telithromycin

57 (41–112)	23 (19–27)	70	14 (12–16)[a]	3.0 (2.1–4.5)	12 (7–23)	1.0 (0.5–3.0)[c]	2.23 μg/mL[c]
			↓ RD[b]				

[a]Approximately 35% of the dose is metabolized by CYP3A4. [b]*CL/F* reduced in patients with severe renal impairment. [c]Following an 800-mg oral dose given once daily for 7 days.

References: Ferret C, et al. Pharmacokinetics and absolute oral bioavailability of an 800-mg oral dose of telithromycin in healthy young and elderly volunteers. *Chemotherapy,* **2002**, *48*:217–223. Namour F, et al. Pharmacokinetics of the new ketolide telithromycin (HMR 3647) administered in ascending single and multiple doses. *Antimicrob Agents Chemother*, **2001**, *45*:170–175. Zhanel GG, et al. The ketolides: a critical review. *Drugs,* **2002**, *62*:1771–1804.

Temsirolimus[a]

—	4.6[b]	—[c]	3.8 ± 0.6[d]	3.3 ± 0.5[d]	12.8 ± 1.1	—	595 ± 102 ng/mL[e]

[a]Temsirolimus, a water-soluble ester analogue of sirolimus or rapamycin, is available for IV use. Following IV administration, temsirolimus is converted to sirolimus; blood AUC of sirolimus is 3-fold higher than that of temsirolimus at the recommended dose of 25 mg for the treatment of advanced renal cell carcinoma. Both temsirolimus and sirolimus inhibit mTOR kinase activity and undergo oxidative metabolism mediated by CYP3A. [b]Recovery of radioactivity after a single IV dose of $[^{14}C]$-temsirolimus. [c]Both temsirolimus and sirolimus partition extensively into blood cells; a major fraction of temsirolimus and sirolimus in plasma is bound to plasma proteins. [d]Based on *CL* of 16.1 ± 2.5 L/h and V_{ss} of 232 ± 36 L at a dose of 25 mg, assuming an average body weight of 70 kg. All pharmacokinetic assessments are based on whole blood concentration. [e]Following the first dose of a 25-mg/wk regimen.

References: Atkins MB, et al. Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. *J Clin Oncol*, **2004**, *22*:909–918. FDA. Drugs@FDA. Torisel label approved on 5/30/07. Available at: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm. Accessed December 31, 2010.

Tenofovir[a]

25[b]	82 ± 13	<1	2.6 ± 0.9[c]	0.6 ± 0.1[c]	8.1 ± 1.8[c,d]	2.3[e]	326 ng/mL[e]
↑ Food			↓ RD		↑ RD		

[a]Tenofovir is formulated as an ester prodrug, VIREAD (tenofovir disoproxil fumarate), for oral administration. [b]Bioavailability under fasted state reported; increased to 39% with high-fat meal. [c]Data reported for steady-state 3-mg/kg IV dose given once a day for 2 weeks to HIV-1–infected male and female adults. Slightly higher *CL* with single IV dose. [d]Longer apparent plasma $t_{1/2}$ (17 h) reported for steady-state oral dosing; this may reflect a longer duration of blood sampling; also, phosphorylated "active" metabolite exhibits a longer intracellular $t_{1/2}$ (60 h). [e]Following a 300-mg oral dose given once a day with a meal to steady state.

References: Barditch-Crovo P, et al. Phase I/II trial of the pharmacokinetics, safety, and antiretroviral activity of tenofovir disoproxil fumarate in human immunodeficiency virus-infected adults. *Antimicrob Agents Chemother*, **2001**, *45*:2733–2739. Deeks SG, et al. Safety, pharmacokinetics, and antiretroviral activity of intravenous 9-[2-(R)-(Phosphonomethoxy) propyl]adenine, a novel anti-human immunodeficiency virus (HIV) therapy, in HIV-infected adults. *Antimicrob Agents Chemother*, **1998**, *42*:2380–2384. Kearney BP, et al. Tenofovir disoproxil fumarate: Clinical pharmacology and pharmacokinetics. *Clin Pharmacokinet*, **2004**, *43*:595–612.

Terazosin

82	11–14	90–94	1.1–1.2[a]	1.1	9–12	1.7[b]	16 ng/mL[b]

[a]Plasma *CL* reportedly reduced in patients with hypertension. [b]Following a 1-mg oral dose (tablet) given to healthy volunteers.

References: Senders RC. Pharmacokinetics of terazosin. *Am J Med*, **1986**, *80*:20–24. Sennello LT, et al. Effect of age on the pharmacokinetics of orally and intravenously administered terazosin. *Clin Ther*, **1988**, *10*:600–607.

Tetracycline

77	58 ± 8	65 ± 3	1.67 ± 0.24	1.5 ± 0.1[a]	10.6 ± 1.5	Oral: 4	IV: 16.4 ± 1.2 μg/mL[b]
							Oral: 2.3 ± 0.2 μg/mL[b]

[a]V_{area} reported. [b]Following a single 10-mg/kg IV dose or a single 250-mg oral dose (taken after a fast and with wafer).

References: Garty M, Hurwitz A. Effect of cimetidine and antacids on gastrointestinal absorption of tetracycline. *Clin Pharmacol Ther*, **1980**, *28*:203–207. Raghuram TC, Krishnaswamy K. Pharmacokinetics of tetracycline in nutritional edema. *Chemotherapy*, **1982**, *28*:428–433.

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TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Thalidomide[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—[b]	<1	—	2.2 ± 0.4[c]	1.1 ± 0.3[c]	6.2 ± 2.6[c]	3.2 ± 1.4[d]	2.0 ± 0.6 μg/mL[d]
						↑ HD, Food	↑ HD

[a]Data from healthy male subjects. Similar data reported for asymptomatic patients with HIV. No age or sex differences. Thalidomide undergoes spontaneous hydrolysis in blood to multiple metabolites. [b]Absolute bioavailability is not known. Altered absorption rate and extent, Hansen disease (HD). [c]CL/F, V_{area}/F, and $t_{1/2}$ reported for oral dose. [d]Following a single 200-mg oral dose.

References: Noormohamed FH, et al. Pharmacokinetics and hemodynamic effects of single oral doses of thalidomide in asymptomatic human immunodeficiency virus-infected subjects. *AIDS Res Hum Retrovir,* **1999**, *15*:1047–1052. *PDR*54, **2000**, p. 912. Teo SK, et al. Single-dose oral pharmacokinetics of three formulations of thalidomide in healthy male volunteers. *J Clin Pharmacol,* **1999**, *39*:1162–1168.

Tolterodine[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
EM: 26 ± 18	EM: Negligible	T: 96.3	EM: 9.6 ± 2.8	EM: 1.7 ± 0.4	EM: 2.3 ± 0.3	EM: 1.2 ± 0.5[c]	EM: 5.2 ± 5.7 ng/mL[c]
PM: 91 ± 40	PM: <2.5	5-HM: 64	PM: 2.0 ± 0.3	PM: 1.5 ± 0.4	PM: 9.2 ± 1.2	PM: 1.9 ± 1.0[c]	PM: 38 ± 15 ng/mL[c]
EM: ↑ Food			↓ LD[b]		↑ LD		

[a]Data from healthy adult male subjects. No significant sex differences. Tolterodine (T) is metabolized primarily by CYP2D6 to an active (100% potency) metabolite, 5-hydroxymethyl tolterodine (5-HM), in extensive metabolizers (EM); $t_{1/2}$ 5-HM = 2.9 ± 0.4 h. Also metabolized by CYP3A to an *N*-desalkyl product, particularly in poor metabolizers (PM). [b]CL/F reduced and AUC $\text{5-HM}_{unbound}$ increased, hepatic cirrhosis. [c]Following a 4-mg oral dose given twice daily for 8 days. C_{max} of 5-HM was 5 ± 3 ng/mL for EM.

References: Brynne N, et al. Influence of CYP2D6 polymorphism on the pharmacokinetics and pharmacodynamic of tolterodine. *Clin Pharmacol Ther,* **1998**, *63*:529–539. Hills CJ, et al. Tolterodine. *Drugs,* **1998**, *55*:813–820. *PDR*54, **2000**, p. 2439.

Topiramate[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
>70[b]	70–97	13–17	0.31–0.51[c]	0.6–0.8[c]	19–23[c]	1.7 ± 0.6[f]	
			↑ Child[d]		↑ RD		5.5 ± 0.6 μg/mL[f]
			↓ RD[e]				

[a]Data from healthy adult male and female subjects and patients with partial epilepsy. [b]Estimate of bioavailability based on urine recovery of unchanged drug. [c]CL/F, V_{area}/F, and $t_{1/2}$ reported for oral dose. Patients receiving concomitant therapy with enzyme-inducing anticonvulsant drugs exhibit increased CL/F and decreased $t_{1/2}$. [d]CL/F increased, <4 years of age (substantially), and 4–17 years of age. [e]CL/F reduced, moderate to severe renal impairment (drug cleared by hemodialysis). [f]Following a 400-mg oral dose given twice daily to steady state in patients with epilepsy.

References: Glauser TA, et al. Topiramate pharmacokinetics in infants. *Epilepsia,* **1999**, *40*:788–791. *PDR*54, **2000**, p. 2209. Rosenfeld WE. Topiramate: a review of preclinical, pharmacokinetic, and clinical data. *Clin Ther,* **1997**, *19*:1294–1308. Sachdeo RC, et al. Steady-state pharmacokinetics of topiramate and carbamazepine in patients with epilepsy during monotherapy and concomitant therapy. *Epilepsia,* **1996**, *37*:774–780.

Tramadol[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
70–75	10–30[b]	20	8 (6–12)	2.7 (2.3–3.9)	5.5 (4.5–7.5)	T: 2.3 ± 1.4[c]	T: 592 ± 178 ng/mL[c]
			↓ LD, RD		↑ LD, RD	M1: 2.4 ± 1.1[c]	M1: 110 ± 32 ng/mL[c]

[a]Tramadol (T) is available as a racemic mixture. At steady state, the plasma concentration of (+) (1*R*,2*R*)-tramadol is ~30% higher than that of (–) (1*S*,2*S*)-tramadol. Both isomers contribute to analgesia. Data reported are for total (+ and –) T. T is metabolized by CYP2D6 to an active *O*-desmethyl metabolite (M1); there are other CYP-catalyzed metabolites. [b]Recovery following an oral dose was reported. [c]Following a 100-mg immediate-release tablet given every 6 h for 7 days.

References: Klotz U. Tramadol—the impact of its pharmacokinetic and pharmacodynamic properties on the clinical management of pain. *Arzneimittelforschung,* **2003**, *53*:681–687. *PDR*58, **2004**, p. 2494.

Trazodone[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
81 ± 6	<1	93	2.1 ± 0.1	1.0 ± 0.1[d]	5.9 ± 0.4	2.0 ± 1.5[e]	1.5 ± 0.2 μg/mL[e]
			↓ Aged[b], Obes[c]	↑ Aged, Obes	↑ Aged, Obes		

[a]Active metabolite, *m*-chlorophenylpiperazine, is a tryptaminergic agonist; formation catalyzed by CYP3A. [b]Significant for male subjects only. [c]No difference when *CL* is normalized to ideal body weight. [d]V_{area} reported. [e]Following a single 100-mg oral dose (capsule) given with a standard breakfast to healthy adults.

References: Greenblatt DJ, et al. Trazodone kinetics: effect of age, sex, and obesity. *Clin Pharmacol Ther,* **1987**, *42*:193–200. Nilsen OG, et al. Single dose pharmacokinetics of trazodone in healthy subjects. *Pharmacol Toxicol,* **1992**, *71*:150–153.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Trimethoprim

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
>63	63 ± 10	37 ± 5	1.9 ± 0.3[a]	1.6 ± 0.2[a]	10 ± 2[a]	2[b]	1.2 μg/mL[b]
			↓ RD		↑ RD		
			↑ Child	↑ Neo, Child	↓ Child		

[a] Studies included concurrent administration of sulfamethoxazole and variation in urinary pH; these factors had no marked effect on the *CL*, V_{area}, and $t_{1/2}$ of trimethoprim. [b] Following a single 160-mg oral dose given to healthy adults.

References: Hutabarat RM, et al. Disposition of drugs in cystic fibrosis. I. Sulfamethoxazole and trimethoprim. *Clin Pharmacol Ther,* **1991**, *49*:402–409. Welling PO, et al. Pharmacokinetics of trimethoprim and sulfamethoxazole in normal subjects and in patients with renal failure. *J Infect Dis,* **1973**, *128*(suppl):556–566.

Valacyclovir[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
V: very low	V: <1	V: 13.5–17.9	V: —	—	V: —	V: 1.5	V: ≤0.56 μg/mL[e]
A: 54 (42–73)[b]	A: 44 ± 10[c]	A: 22–33			A: 2.5 ± 0.3	A: 1.9 ± 0.6[e]	A: 4.8 ± 1.5 μg/mL[e]
			A: ↓ RD[d]		A: ↑ RD		

[a] Data from healthy male and female adults. Valacyclovir is an L-valine prodrug of acyclovir. Extensive first-pass conversion by intestinal (gut wall and luminal) and hepatic enzymes. Parameters refer to acyclovir (A) and valacyclovir (V) following V administration. See "Acyclovir" for its systemic disposition parameters. [b] Bioavailability of A based on AUC of A following IV A and a 1-g oral dose of V. [c] Urinary recovery of A is dose dependent (76% and 44% following 100-mg and 1000-mg oral doses of V, and 87% following IV A). [d] *CL/F* reduced, end-stage RD (drug cleared by hemodialysis). [e] Following a single 1-g oral dose of V.

References: Perry CM, et al. Valaciclovir. A review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in herpesvirus infections. *Drugs,* **1996**, *52*:754–772. Soul-Lawton J, et al. Absolute bioavailability and metabolic disposition of valaciclovir, the L-valyl ester of acyclovir, following oral administration to humans. *Antimicrob Agents Chemother,* **1995**, *39*:2759–2764. Weller S, et al. Pharmacokinetics of the acyclovir pro-drug valaciclovir after escalating single- and multiple-dose administration to normal volunteers. *Clin Pharmacol Ther,* **1993**, *54*:595–605.

Valganciclovir[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
G (V): 61 ± 9[b]	—	—	—	—	V (V): 0.5 ± 0.2	V (V): 0.5 ± 0.3[d]	V (V): 0.20 ± 0.07 μg/mL[d]
↑ Food					G (V): 3.7 ± 0.6	G (V): l–3[e]	G (V): 5.6 ± 1.5 μg/mL[e]
					↑ RD[c]		

[a] Valganciclovir (V) is an ester prodrug for ganciclovir (G). It is rapidly hydrolyzed with a plasma $t_{1/2}$ = 0.5 h. G and V data following oral V dosing to male and female patients with viral infections are reported. See "Ganciclovir" for its systemic disposition parameters. [b] Increased and more predictable bioavailability of G when V is taken with a high-fat meal. [c] The apparent $t_{1/2}$ of G is increased in patients with renal impairment. [d] Following a single 360-mg oral dose of V taken without food. [e] Following a 900-mg oral dose of V taken once daily with food to steady state.

References: Cocohoba JM, et al. Valganciclovir: an advance in cytomegalovirus therapeutics. *Ann Pharmacother,* **2002**, *36*:1075–1079. Jung D, et al. Single-dose pharmacokinetics of valganciclovir in HIV- and CMV-seropositive subjects. *J Clin Pharmacol,* **1999**, *39*:800–804. *PDR*58, **2004**, pp. 2895, 2971.

Valproic Acid[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
100 ± 10[b]	1.8 ± 2.4	93 ± l[c]	0.11 ± 0.02[d,e]	0.22 ± 0.07	14 ± 3[d,e]	L–4[f]	34 ± 8 μg/mL[f]
		↓ RD, LD, Preg, Aged, Neo	↑ Child	↑ LD, Neo	↑ LD, Neo		
					↓ Child		

[a] Valproic acid is available either as the free acid or stable coordination compound comprised of sodium valproate and valproic acid (divalproex sodium). [b] Systemic availability of valproate ion is the same after molar equivalent oral doses of free acid and divalproex sodium. [c] Dose dependent; value shown for daily doses of 250 and 500 mg. At 1 g daily, percent bound = 90% ± 2%. [d] Data for multiple dosing (500 mg daily) reported. Single-dose value: 0.14 ± 0.04 mL/min/kg; $t_{1/2}$ = 9.8 ± 2.6 h. Total *CL* is the same at 100 mg daily, although *CL* of free drug increases with multiple dosing. Valproate is eliminated mainly by glucuronidation. [e] Increased *CL* and decreased $t_{1/2}$ from enzyme induction following concomitant administration of other antiepileptic drugs. [f] Average concentration following a 250-mg oral dose (capsule, DEPAKENE) given twice daily for 15 days to healthy male adults. A therapeutic range of 50–150 μg/mL is reported. T_{max} is 3–8 h for divalproex tablets and 7–14 h for extended-release divalproex tablets.

References: Dean JC. Valproate. In: Wyllie E, ed. *The Treatment of Epilepsy,* 2nd ed. Williams & Wilkins, Baltimore, **1997**, pp. 824–832. Pollack GM, et al. Accumulation and washout kinetics of valproic acid and its active metabolites. *J Clin Pharmacol,* **1986**, *26*:668–676. Zaccara G, et al. Clinical pharmacokinetics of valproic acid—1988. *Clin Pharmacokinet,* **1988**, *15*:367–389.

Valsartan[a]

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
23 ± 7	29.0 ± 5.8	95	0.49 ± 0.09[b]	0.23 ± 0.09	9.4 ± 3.8	2 (1.5–3)[d]	1.6 ± 0.6 μg/mL[d]
↓ Food			↓ Aged, LD[c]		↑ Aged		

[a] Data from healthy adult male subjects. No significant sex differences. [b] Valsartan is cleared primarily by biliary excretion. [c] *CL/F* reduced, mild to moderate hepatic impairment and biliary obstruction. [d] Following a single 80-mg oral dose (capsule).

References: Brookman LJ, et al. Pharmacokinetics of valsartan in patients with liver disease. *Clin Pharmacol Ther,* **1997**, *62*:272–278. Flesch G, et al. Absolute bioavailability and pharmacokinetics of valsartan, an angiotensin II receptor antagonist, in man. *Eur J Clin Pharmacol,* **1997**, *52*:115–120. Muller P, et al. Pharmacokinetics and pharmacodynamic effects of the angiotensin II antagonist valsartan at steady state in healthy, normotensive subjects. *Eur J Clin Pharmacol,* **1997**, *52*:441–449. *PDR*54, **2000**, p. 2015.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Vancomycin

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—[a]	79 ± 11	30 ± 11	$CL = 0.79CL_{cr} + 0.22$	0.39 ± 0.06	5.6 ± 1.8	—	18.5 (15–25) μg/mL[b]
			↓ RD, Aged, Neo	↓ Obes	↑ RD, Aged ↓ Obes		

[a]Very poorly absorbed after oral administration but used by this route to treat *Clostridium difficile* and staphylococcal enterocolitis. [b]Following a dose of 1000 mg IV (1-h infusion) given twice daily or 7.5 mg/kg IV (1-h infusion) given four times daily to adult patients with staphylococcal or streptococcal infections. Levels of 37–152 μg/mL have been associated with ototoxicity.

Reference: Leader WG, et al. Pharmacokinetic optimization of vancomycin therapy. *Clin Pharmacokinet,* **1995**, *28*:327–342.

Vardenafil[a]

15 (8–25)	2–6	93–95 (parent and M1)	56	3.0[b]	4–5 (parent and M1)	0.7 (0.25–3)[c]	19.3 ± 1.7 ng/mL[c]

[a]Vardenafil is primarily metabolized by CYP3A with minor involvement of CYP2C9. The major oxidative metabolite (M1), a product of *N*-desmethylation at the piperazine ring, is a less potent PDE5 inhibitor and circulates at a level 28% that of the parent drug. It contributes only 7% of the *in vivo* activity of vardenafil. [b]V_{ss} of 208 L, assuming 70-kg body weight. [c]Following a single 20-mg dose. There is no change in pharmacokinetics between single and multiple dosing.

References: FDA. Drugs@FDA. Levitra label approved on 3/19/08. Available at: http://www.access-data.fda.gov/scripts/cder/drugsatfda/index.cfm. Accessed December 31, 2009. Gupta M, et al. The clinical pharmacokinetics of phosphodiesterase-5 inhibitors for erectile dysfunction. *J Clin Pharmacol*, **2005**, *45*:987–1003.

Varenicline

≥87%[a]	86.2 ± 6.2	≤20	2.27 ± 0.34[b,c]	6.2[c]	31.5 ± 7.7[c]	2.0 (1.0–4.0)[d]	10.2 ± 1.0 ng/mL[d]

[a]87.1% ± 5.5% of radioactivity is excreted in urine after an oral dose of [^{14}C]-varenicline. Minimal first-pass metabolism is expected. [b]Varenicline is cleared mostly by renal excretion with minimal metabolism. Organic cation transporter 2 (OCT2) is involved in renal tubular secretion, as evidenced by inhibition of varenicline renal clearance by cimetidine, a known OCT2 inhibitor. [c]*CL/F* and V_{area}/F estimated from steady-state AUC and $t_{1/2}$ during 1-mg twice-daily dosing of varenicline in healthy adult smokers and assuming an average body weight of 70 kg. [d]After the first dose of a multiple-dosing regimen. An accumulation factor of 2.85 ± 0.73 was reported.

References: Faessel HM, et al. Multiple-dose pharmacokinetics of the selective nicotinic receptor partial agonist, varenicline, in healthy smokers. *J Clin Pharmacol*, **2006**, *46*:1439–1448. FDA. Drugs@FDA. Chantix label approved on 7/1/09. Available at: http://www.access-data.fda.gov/scripts/cder/drugsatfda/index.cfm. Accessed December 31, 2009. Obach RS, et al. Metabolism and disposition of varenicline, a selective α4β2 acetylcholine receptor partial agonist, in vivo and in vitro. *Drug Metab Dispos*, **2006**, *34*:121–130.

Venetoclax[a]

—	<0.1[c]	>99.9	3.9 ± 2.6[d]	3.7–4.6[e]	26	5–8[f]	2.1 ± 1.1 μg/mL[g]
↑ Food[b]							

[a]Data from patients with relapsed or refractory chronic lymphocytic leukemia or non-Hodgkin lymphoma. [b]Administration with a low-fat meal increased venetoclax exposure by approximately 3.4-fold and administration with a high-fat meal increased venetoclax exposure by 5.1- to 5.3-fold compared to fasting conditions. [c]Venetoclax is extensively metabolized, predominantly by CYP3A4/5, to the major circulating metabolite M27, which is also mainly metabolized by CYP3A4/5. After a single oral dose of radiolabeled [^{14}C]-venetoclax 200 mg to healthy subjects, >99.9% of the dose was recovered in feces (21% as unchanged drug) and <0.1% in urine within 9 days. The major metabolite identified in plasma, M27, is considered inactive, and its AUC represented 80% of the parent AUC. [d]*CL/F* measured at steady state following 400 mg orally once a day with low-fat meal. [e]The apparent volume of distribution (V_{ss}/F) reported. [f]Following multiple oral administration under fed conditions. [g]Steady-state concentrations following administration of 400 mg orally once a day with a low-fat meal. Venetoclax steady-state AUC increased dose proportionally over the dose range of 150–800 mg (0.25–1.33 times the maximum approved recommended dosage).

References: Deng R, et al. Bayesian population model of the pharmacokinetics of venetoclax in combination with rituximab in patients with relapsed/refractory chronic lymphocytic leukemia: results from the phase III MURANO Study. *Clin Pharmacokinet*, **2019**, *58*:1621–1634. FDA. Product Labeling: Venclexta® (venetoclax oral tablets). Drugs@FDA: FDA-approved drugs. Available at: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm. Accessed April 26, 2022. FDA. Venetoclax NDA and label. NDA approved in 2016; label revised 11/2020. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/208573s023lbl.pdf. Accessed April 2, 2021. Salem A, et al. Pharmacokinetics of venetoclax, a novel BCL-2 inhibitor, in patients with relapsed or refractory chronic lymphocytic leukemia or non-Hodgkin lymphoma. *J Clin Pharmacol,* **2017**, *57*:484–492.

Venlafaxine,[a] Desvenlafaxine[b]

10–45	V: 4.6 ± 3.0	V: 27 ± 2	22 ± 10[d]	7.5 ± 3.7[d]	4.9 ± 2.4	V: 2.0 ± 0.4	V: 167 ± 55 ng/mL[e]
	ODV: 29 ± 7[c]	ODV: 30 ± 12[c]			10.3 ± 4.3[c]	ODV: 2.8 ± 0.8[c]	ODV: 397 ± 81 ng/mL[e]
			↓ LD, RD		↑ LD, RD		

[a]Venlafaxine (V) is available as a racemic mixture; antidepressant activity resides with the *l*-(−)-enantiomer and its equipotent *O*-desmethyl metabolite (formation catalyzed by CYP2D6—polymorphic). Parameters for the derived *O*-desmethylvenlafaxine (ODV) are included. [b]*O*-Desmethyl metabolite is marketed as desvenlafaxine in an extended-release formulation as a successor to V. It has a higher oral bioavailability (80%), with a T_{max} of 7.5 h. Desvenlafaxine has a much lower *CL* (3.5 mL/min/kg) and a smaller V_{ss} (3.4 L/kg). Its reported $t_{1/2}$ matches that observed for metabolite derived from V. [c]Values for ODV after V dosing. [d]*CL/F* and V_{ss}/F reported. [e]Mean data for V and ODV, following a 75-mg oral dose (immediate-release tablet) given three times daily for 3 days to healthy adults. T_{max} for an extended-release formulation is 5.5 (V) and 9 (DV) h.

References: Klamerus KJ, et al. Introduction of a composite parameter to the pharmacokinetics of venlafaxine and its active *O*-desmethyl metabolite. *J Clin Pharmacol,* **1992**, *32*:716–724. *PDR*54, **2000**, p. 3237.

(Continued)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Verapamil[a,b]							
Oral: 22 ± 8	<3	90 ± 2	15 ± 6[c,d]	5.0 ± 2.1	4.0 ± 1.5[c]	IR: 1.1[e]	IR: 272 ng/mL[e]
SL: 35 ± 13		↑ LD	↓ LD	↑ LD	↑ LD	XR: 5.6–7.7[e]	XR: 118–165 ng/mL[e]
↑ LD							

[a]Racemic mixture; (–)-enantiomer is more active. Bioavailability of (+)-verapamil is 2.5-fold greater than that for (–)-verapamil because of a lower *CL* (10 ± 2 vs. 18 ± 3 mL/min/kg). Relative concentration of the enantiomers changes as a function of route of administration. [b]Active metabolite, norverapamil, is a vasodilator but has no direct effect on heart rate or PR interval. At steady state (oral dosing), AUC is equivalent to that of parent drug ($t_{1/2}$ = 9 ± 3 h). [c]Multiple dosing causes a greater than 2-fold decrease in *CL/F* and prolongation of $t_{1/2}$ in some studies, but no change of $t_{1/2}$ in others. [d]Verapamil is a substrate for CYP3A4, CYP2C9, and other CYPs and for the efflux transporter P-glycoprotein. [e]Mean data following a 120-mg oral conventional tablet (IR) given twice daily or range of data following a 240-mg oral extended-release (XR) dose given once daily, both for 7–10 days to healthy adults. EC_{50} for prolongation of PR interval after an oral dose of racemate is 120 ± 20 ng/mL; the value for IV administration is 40 ± 25 ng/mL. After oral administration, racemate concentrations >100 ng/mL cause >25% reduction in heart rate in atrial fibrillation, >10% prolongation of PR interval, and >50% increase in duration of exercise in angina patients. A level of 120 ± 40 ng/mL (after IV administration) was found to terminate reentrant supraventricular tachycardias.

Reference: McTavish D, et al. Verapamil. An updated review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in hypertension. *Drugs,* **1989**, *38*:19–76.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Vincristine[a]							
—	10–20	Low	4.92 ± 3.01 $L/h/m^2$	96.9 ± 55.7 L/m^2[c]	22.6 ± 16.7[c]	—	~250–425 nM[d]
			↓ LD[b]		↑ LD[b]		

[a]Data from adult male and female cancer patients. Metabolized by CYP3A and excreted unchanged into bile (substrate for P-glycoprotein). [b]*CL* reduced, cholestatic liver disease. [c]$t_{1/2}$ and V_{area} for terminal phase. Longer $t_{1/2}$ (~85 ± 69 h) also reported. [d]Following a 2-mg IV bolus dose.

References: Gelmon KA, et al. Phase I study of liposomal vincristine. *J Clin Oncol,* **1999**, *17*:697–705. Rahmani R, et al. Pharmacokinetics and metabolism of vinca alkaloids. *Cancer Surv,* **1993**, *17*:269–281. Sethi VS, et al. Pharmacokinetics of vincristine sulfate in adult cancer patients. *Cancer Res,* **1981**, *41*:3551–3555. Sethi VS, et al. Pharmacokinetics of vincristine sulfate in children. *Cancer Chemother Pharmacol,* **1981**, *6*:111–115. van den Berg HW, et al. The pharmacokinetics of vincristine in man: Reduced drug clearance associated with raised serum alkaline phosphatase and dose-limited elimination. *Cancer Chemother Pharmacol*, **1982**, *8*:215–219.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Vinorelbine							
27 ± 12[a]	11	87 (80–91)	21 ± 7	76 ± 41[b]	42 ± 21[b]	1.5 ± 1.0[c]	114 ± 43 ng/mL[c]
			↓ LD				1130 ± 636 ng/mL[d]

[a]For liquid-filled gelatin capsules. [b]Elimination kinetics of vinorelbine follow a three-compartment model with extensive tissue distribution. Values for the terminal elimination phase are reported. [c]Following a single 100-mg/m² oral dose (gel capsule). [d]Following a single 30-mg/g IV infusion over 15 min.

Reference: Leveque D, et al. Clinical pharmacokinetics of vinorelbine. *Clin Pharmacokinet,* **1996**, *37*:184–197.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Voriconazole							
96	<2	58	3.8[a,b]	1.6[b]	6.7[b]	PO: 1.1[d]	PO: 2356 ng/mL[d]
↓ Food			↓ LD[c]				IV: 3621 ng/mL[e]

[a]Metabolized mainly to an inactive *N*-oxide by CYP2C19 (major), CYP3A4, and CYP2C9. [b]Elimination is dose and time dependent. Pharmacokinetic parameters determined at steady state are reported. Mean *CL* was reduced (64%), V_{ss} reduced (32%), and $t_{1/2}$ increased (16%) with 12 days of twice-daily 3-mg/kg IV administration. Also, *CL* decreased 41% when dose was increased from 200 to 300 mg twice daily. [c]*CL* reduced in patients with mild to moderate hepatic insufficiency. [d]Following a 3-mg/kg oral dose given twice daily for 12 days. [e]Following a 3-mg/kg IV infusion over 1 h given twice daily for 12 days.

References: Boucher HW, et al. Newer systemic antifungal agents: pharmacokinetics, safety and efficacy. *Drugs,* **2004**, *64*:1997–2020. Purkins L, et al. The pharmacokinetics and safety of intravenous voriconazole—a novel wide-spectrum antifungal agent. *Br J Clin Pharmacol,* **2003**, *56*(suppl):2–9. Purkins L, et al. Voriconazole, a novel wide-spectrum triazole: oral pharmacokinetics and safety. *Br J Clin Pharmacol,* **2003**, *56*(suppl):10–16.

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK Time (h)	PEAK CONCENTRATION
Warfarin[a]							
93 ± 8	<2	99 ± 1[b]	0.045 ± 0.024[c,d,e]	0.14 ± 0.06[b,d]	37 ± 15[f]	<4[g]	*R*: 0.9 ± 0.4 μg/mL[g]
		↓ RD					*S*: 0.5 ± 0.2 μg/mL[g]

[a]Values are for racemic warfarin; the *S*-(–)-enantiomer is 3- to 5-fold more potent than the R-(+)-enantiomer. [b]No difference between enantiomers in plasma protein binding or V_{area}. [c]*CL* of the *R*-enantiomer is ~70% of that of the antipode (0.043 vs. 0.059 mL · min⁻¹ · kg⁻¹). [d]Conditions leading to decreased binding (e.g., uremia) presumably increase *CL* and *V*. [e]The *S*-enantiomer is metabolically cleared by CYP2C9 (polymorphic). [f]$t_{1/2}$ of the *R*-enantiomer is longer than that of the *S*-enantiomer (43 ± 14 vs. 32 ± 12 h). [g]Mean steady-state, 12-h postdose concentrations of warfarin enantiomers following a daily oral dose of 6.1 ± 2.3 mg of racemic warfarin given to patients with stabilized (1–5 months) anticoagulant therapy.

Reference: Chan E, et al. Disposition of warfarin enantiomers and metabolites in patients during multiple dosing with rac-warfarin. *Br J Clin Pharmacol,* **1994**, *37*:563–569.

(*Continued*)

TABLE AI–1 ■ PHARMACOKINETIC DATA (*CONTINUED*)

Zidovudine

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
63 ± 10	18 ± 5	<25	26 ± 6[a]	1.4 ± 0.4	1.1 ± 0.2	0.5–1[c]	IV: 2.6 μg/mL[c]
↑ Neo			↓ RD[b], Neo, LD	↓ RD[b], LD	↑ Neo, LD		PO: 1.6 μg/mL[c]

[a]Formation of 5-*O*-glucuronide is the major route of elimination (68%). [b]A change in *CL/F* and V_{area}/F reported. [c]Following a 5-mg/kg IV or oral dose given every 4 h to steady state.

References: Blum MR, et al. Pharmacokinetics and bioavailability of zidovudine in humans. *Am J Med,* **1988**, *85*:189–194. Morse GD, et al. Comparative pharmacokinetics of antiviral nucleoside analogues. *Clin Pharmacokinet,* **1993**, *24*:101–123.

Ziprasidone

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
PO: 59	<1[a]	99.9 ± 0.08	11.7	2.3[b]	2.9[c]	PO: 4 ± 1[d]	PO: 68 ± 20 ng/mL[d]
↑ Food						IM: 0.7[e]	IM: 156 ng/mL[e]
IM: 100							

[a]Recovery following oral administration. [b]Approximately one-third of the dose is oxidized by CYP3A4, and the remainder undergoes reduction. [c]A longer $t_{1/2}$ after oral dosing is rate limited by absorption; food decreases apparent $t_{1/2}$. In the elderly, the $t_{1/2}$ is slightly longer. [d]Following a 20-mg oral dose given twice daily for 8 days. [e]Following a single 10-mg IM dose.

References: Gunasekara NS, et al. Ziprasidone: a review of its use in schizophrenia and schizoaffective disorder. *Drugs,* **2002**, *62*:1217–1251. Miceli JJ, et al. Single- and multiple-dose pharmacokinetics of ziprasidone under nonfasting conditions in healthy male volunteers. Single- and multiple-dose pharmacokinetics of ziprasidone in healthy young and elderly volunteers. *Br J Clin Pharmacol,* **2000**, *49*(suppl):15S–20S.

Zolpidem

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
72 ± 7	<1	92	4.5 ± 0.7[a]	0.68 ± 0.06	1.9 ± 0.2	1.0–2.6[b]	76–139 ng/mL[b]
		↑ RD, LD	↓ LD, Aged	↑ RD	↑ Aged, LD		
			↑ Child		↓ Child		

[a]Metabolically cleared predominantly by CYP3A4. [b]Following a single 10-mg oral dose given to young adults. No accumulation of drug with once-daily dosing.

References: Greenblatt DJ, et al. Comparative kinetics and dynamics of zaleplon, zolpidem, and placebo. *Clin Pharmacol Ther,* **1998**, *64*:553–561. Patat A, et al. EEG profile of intravenous zolpidem in healthy volunteers. *Psychopharmacology (Berl),* **1994**, *114*:138–146. Salva P, et al. Clinical pharmacokinetics and pharmacodynamics of zolpidem. Therapeutic implications. *Clin Pharmacokinet,* **1995**, *29*:142–153.

Zonisamide

BIOAVAILABILITY (ORAL) (%)	URINARY EXCRETION (%)	BOUND IN PLASMA (%)	CLEARANCE (mL/min/kg)	VOL. DIST. (L/kg)	HALF-LIFE (h)	PEAK TIME (h)	PEAK CONCENTRATION
—[a]	29–48[b]	38–40[c]	0.13[d,e]	1.2–1.8[f]	63 ± 14	1.8 ± 0.4[g]	28 ± 4 μg/mL[g]

[a]Absolute bioavailability is not known; minimum equal to urine recovery after an oral dose. [b]Recovery following an oral dose. [c]Concentrates in erythrocytes to as much as 8-fold. [d]Primary routes of metabolism involve reductive cleavage of the isoxazole ring (CYP3A4) and *N*-acetylation. [e]Steady-state *CL/F* for a 400-mg once-daily dose reported. AUC increases disproportionately when the dose is increased from 400 to 800 mg. [f]*V/F* for a single dose is reported; decreases as the dose is increased from 200 to 800 mg. [g]Following a 400-mg oral dose given once daily to steady state in healthy adults.

References: Kochak GM, et al. Steady-state pharmacokinetics of zonisamide, an antiepileptic agent for treatment of refractory complex partial seizures. *J Clin Pharmacol,* **1998**, *38*:166–171. Peters DH, et al. Zonisamide. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in epilepsy. *Drugs,* **1993**, *45*:760–787. *PDR*58, **2004**, p. 1232.

Appendix II

Drug-Drug Interactions

Isabelle Ragueneau-Majlessi, Jingjing Yu, and
Nina Isoherranen

Tables AII–1 and AII–2 present a perspective on drug-drug interactions as examined by the effect that one drug (the perpetrator) can have on the area under the plasma concentration versus time curve (AUC) of another drug (the victim). The cutoff value of 5-fold change in the AUC of the victim drug was chosen in order to focus these tables on clinically significant drug-drug interactions that would generally contraindicate the coadministration of the drugs or at least require dosage adjustment.

Table AII–1 lists *sensitive substrates,* which are drugs for which at least a 5-fold increase in their AUC is observed in humans when coadministered with an inhibitor of drug metabolism or transport.

Table AII–2 lists *strong inhibitors* and *strong inducers*, which are drugs that cause at least a 5-fold increase or 80% decrease in the AUC of a victim drug, respectively. The therapeutic classes for the substrates and inhibitors are also provided.

The *enzyme/transporter implicated* section refers to the main clearance pathway of the victim drug that is likely impacted by the drug-drug interaction. In the majority of cases, this assignment is based on information collected from *in vitro* experiments demonstrating metabolism of the victim drug via the implicated pathway or inhibition or induction of the target enzyme or transporter.

TABLE AII–1 ■ DRUG-DRUG INTERACTIONS WITH AT LEAST 5-FOLD CHANGE IN EXPOSURE OF VICTIM DRUG: SENSITIVE SUBSTRATES

SENSITIVE SUBSTRATE (victim drug)	THERAPEUTIC CLASS	CYP or TRANSPORTER IMPLICATED	AUCR[a]	INHIBITOR	ORAL DOSE[b] of INHIBITOR (perpetrator)	REFERENCE
Alosetron	Gastrointestinal agents	CYP1A2	6.0	Fluvoxamine	50–200 mg/day	1
Atazanavir	HIV protease inhibitors	CYP3A	25.9	Ritonavir	100 mg QD	2
Atorvastatin	Statins	CYP3A	5.6	Itraconazole	200 mg QD	3
		OATP1B	8.5	Rifampin	600 mg SD	4
Buspirone	Anxiolytics	CYP3A	19.2	Itraconazole	100 mg BID	5
Duloxetine	SNRIs	CYP1A2	5.6	Fluvoxamine	100 mg QD	6
Eletriptan	Triptans	CYP3A	5.9	Ketoconazole	Not available	7
Lopinavir	HIV protease inhibitors	CYP3A	≤30	Ritonavir	Not available	8
Lovastatin	Statins	CYP3A	36.4	Itraconazole	200 mg QD	9
Metoprolol	β adrenergic antagonists	CYP2D6	5.1	Pridopidine	45 mg BID	10
Midostaurin	Protein kinase inhibitors	CYP3A	10.4	Ketoconazole	400 mg QD	11
Quetiapine	Antipsychotics	CYP3A	6.2	Ketoconazole	200 mg QD	12
Ramelteon	Hypnotics-sedatives	CYP1A2	189.9	Fluvoxamine	100 mg BID	13
Repaglinide	Meglitinides	CYP2C8	8.3	Gemfibrozil	900 mg SD	14
Rosuvastatin	HMG-CoA reductase inhibitors (statins)	OATP1B, BCRP	7.1	Cyclosporine	75–200 mg BID	15
Sildenafil	Erectile dysfunction treatments	CYP3A	9.9	Ritonavir	300–500 mg BID	16
Simvastatin	Statins	CYP3A	16.1	Grapefruit juice	200 mL TID	17
Sirolimus	Immunosuppressants	CYP3A	10.9	Ketoconazole	200 mg QD	18
Tacrolimus	Immunosuppressants	CYP3A	78.0	Telaprevir	750 mg TID	19
Tolterodine	Muscarinic Antagonists	CYP2D6	14.9	Fluoxetine	20 mg/day	20
Valsartan	AngII receptor blockers	OATP1B	5.5	Rifampin	600 mg SD	21
Vardenafil	Erectile dysfunction treatment	CYP3A	49.1	Ritonavir	300–600 mg BID	22
Venetoclax	Cancer therapy	CYP3A	7.9	Ketoconazole	400 mg QD	23
Venlafaxine	SNRIs	CYP2D6	0.17 (CL ratio)	Quinidine	100 mg BID	24

AngII, angiotensin II; CL, clearance; HIV, human immunodeficiency virus; SNRIs, serotonin-norepinephrine reuptake inhibitors.

[a] AUCR is the ratio of the AUC of the sensitive substrate in the presence of inhibitor to the control AUC (absence of inhibitor):

$$\mathrm{AUCR}=\frac{\mathrm{AUC}_{\mathrm{inhib}}}{\mathrm{AUC}_{\mathrm{control}}}$$

[b] QD, once daily; BID, twice daily; TID, thrice daily; SD, single dose.

The magnitude of the alteration in the AUC of the victim drug AUC is provided in the column labeled AUCR, the *AUC ratio* (mean $AUC_{inhibited}$ divided by the mean $AUC_{control}$). In this column, the most severe observed drug-drug interaction magnitude is listed (worst-case scenario). In reports in the biomedical literature, the magnitude of drug-drug interactions varies considerably for the same perpetrator (inhibitor) with different victim drugs and with the same victim with different perpetrators.

For sensitive substrates (Table AII-1), the inhibitor listed refers to the coadministered inhibitor that resulted in the *AUCR* provided for the sensitive substrate. For strong inhibitors/inducers (Table AII-2), the substrate listed refers to the victim drug that was coadministered in the clinical studies to observe the *AUCR* provided. The *oral dose of the perpetrating inhibitor or inducer* is listed in order to provide some context to the reported findings, since the magnitude of a drug-drug interaction is often dependent on the dose of the perpetrator and its circulating concentrations.

TABLE AII–2 ■ DRUG-DRUG INTERACTIONS WITH AT LEAST 5-FOLD CHANGE IN EXPOSURE OF VICTIM DRUG: STRONG INHIBITORS AND INDUCERS

INHIBITOR OR INDUCER	THERAPEUTIC CLASS	CYP or TRANSPORTER IMPLICATED	AUCR[a]	VICTIM DRUG	ORAL DOSE[b] of INHIBITOR or INDUCER	REFERENCE
Strong Inhibitors						
Ciprofloxacin	Antibiotics	CYP1A2	9.7	Tizanidine	500 mg BID	25
Clarithromycin	Antibiotics	CYP3A	8.4	Midazolam	500 mg BID	26
Clopidogrel	Anticoagulants/antiplatelets	CYP2C8	5.1	Repaglinide	300 mg SD	27
Fluconazole	Antifungals	CYP2C19	13.5	Omeprazole	200 mg QD	28
Fluoxetine	SSRIs	CYP2D6	27.2	Dextromethorphan	60 mg QD	29
		CYP2C19	7.1	Omeprazole	60 mg QD	29
Gemfibrozil	Fibric acid derivatives	CYP2C8	11.3	Dasabuvir	600 mg BID	30
		CYP2C8	8.3	Repaglinide	900 mg SD	31
Itraconazole	Antifungals	CYP3A	10.8	Midazolam	200 mg QD	32
		Pgp	6.9	Dabigatran	200 mg QD	33
Nelfinavir	HIV protease inhibitors	CYP3A	5.3	Midazolam	1250 mg BID	34
Paroxetine	Antidepressants	CYP2D6	14.4	Dextromethorphan	20 mg (2 doses)	35
Posaconazole	Antifungals	CYP3A	6.2	Midazolam	400 mg BID	36
Ribociclib	Protein kinase inhibitors	CYP3A	5.2	Midazolam	600 mg QD	37
Rifampin	Antibiotics	OATP1B	7.25	Atorvaststin	600 mg SD (IV)	38
Ritonavir	HIV protease inhibitors	CYP3A	26.4	Midazolam	100 mg TID	39
Telithromycin	Antibiotics	CYP3A	6.2	Midazolam	800 mg QD	40
Voriconazole	Antifungals	CYP3A	9.6	Midazolam	200 mg BID	41
Strong Inducers						
Carbamazepine	Anticonvulsants	CYP3A	0.13	Quetiapine	200 mg TID	42
		CYP2B6	0.10	Bupropion	942 ± 254 mg/day	43
Phenytoin	Anticonvulsants	CYP3A	0.10	Nisoldipine	200–450 mg/day	44
Rifampin	Antibiotics	CYP2C19	0.07	Omeprazole	600 mg QD	45
		CYP3A	0.003	Budesonide	600 mg QD	46
Ritonavir	HIV Protease Inhibitors	CYP2C19	0.16	Voriconazole	400 mg BID	47

Pgp, P-glycoprotein; SSRI, selective serotonin reuptake inhibitor.

[a] AUCR is the ratio of the AUC of the sensitive substrate in the presence of inhibitor or inducer to the control AUC (absence of inhibitor/inducer):

$$AUCR = \frac{AUC_{inhib/inducer}}{AUC_{control}}$$

[b] QD, once daily; BID, twice daily; TID, thrice daily; SD, single dose.

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